Editors
Lala Behari Sukla
Sandeep Panda
Jacintha Esther

Applied & Industrial Biotechnology

Applied & Industrial Biotechnology
Lala Behari Sukla • Sandeep Panda • Jacintha Esther

Applied & Industrial Biotechnology

Editors
Lala Behari Sukla
Sandeep Panda
Jacintha Esther

Printed By :

Reproprint Pvt. Ltd., I.R.C. Village, Nayapalli, Bhubaneswar-15

Published By :

Applied Environmental Biotechnology Innovation, Bhubaneswar

Price : ₹ 800.00

Preface

In the recent years, importance of biotechnology has been realised worldwide. Biotechnology has a tremendous potential in terms of educational, scientific and industrial implementation. A lot of further studies are needed to be done in these areas to identify organisms, optimise and develop processes for industrial applications. The emphasis on increasing the efficiency of energy utilization and decreasing environment pollution from process will continue. Technical innovations, in the form of new methods and procedures based on engineering and specific principles, gave new impetus to the biotechnology. As the non-renewable resources are on the verge or almost exhausted with several increasing environmental problems, there is an invitation for global efforts to develop cleaner technologies based on renewable natural resources. Articles were invited from scientists and academicians working in the various fields of microbiology, biotechnology, environmentalists, chemists and related areas; were compiled and presented in the present book as an edited volume. It is our proud privileged to be associated with experts in the field whose significant contributions have taken environment related biotechnology related to greater height. The main advantages of biotechnology processes lie in their economic viability and environmental

friendliness. In all these processes microorganisms play a key role with physiological characters, growth, development and efficiency contributing to the activity like isolation, development or strains, process optimisation, scale up studies, pilot studies, designing of plants are equally important. The book also contains a set of review papers mainly on topics which are new application for bioleaching, bio remediation, bio surfactants, bio fuels from lignocelluloses biomass, different aspects of environmental biotechnology that are already practised by industries, chemical industries in particular, GIS based mapping, and common scientific principles etc.

The book is coincidentally being published on the 'World Environment Day' 2015 and is our immense pleasure to release the book on this momentous occasion.

Lala Behari Sukla
Sandeep Panda
Jacintha Esther

Editors

Lala Behari Sukla

Er. Lala Behari Sukla is presently Director of the Institute for Applied Environmental Biotechnology Innovation (IAEBI) Bhubaneswar, Odisha, India. He is also at present the Emeritus Professor, AcSIR, New Delhi. He has completed B.Sc (Engg.) in Metallurgy from NIT, Rourkela in the year 1971, M.Tech in Metallurgy from NIT, Rourkela in the year 1973. He has also worked as CSIR-Emeritus Scientist at CSIR-IMMT, Bhubaneswar after superannuation as Chief Scientist on 2011. He has more than 39 years of R&D experience in the area of Biomineral Processing, Hydrometallurgy and contributed over 171 papers in International & National Journals in the area of Bio Science and other related fields. He has published 2 books & 11 patents and seven students have been awarded Ph.D degree under his guidance. He is now supervising several students from different Universities for Ph.D in the area of bioleaching. He has co-edited book on Mineral Biotechnology 2002, Mineral Biotechnology 2007, and Environmental Microbial Biotechnology (SPRINGER) 2015. The books has

been widely acclaimed by the readers in the field. He was also leader of several national and international R&D projects in the area of bio-minerals, being pursued at CSIR-IMMT. He has visited several Institutes like Higher Institute of Mining and Geology, Sofia, Bulgaria, Department of Metallurgy, National Technical University of Athens, Greece, Institute of Environmental Engineering, Polish Academy of Science, Poland and Stolberg Engineer Beratung GmbH Germany. Er. Sukla is the recipient of several prestigious awards that include Prof. S.R. Vyas Memorial Award for the year 2010 by Association Of Microbiologists of India (AMI) towards his significant contribution for the Development of Microbiology in India, IIME Mineral Beneficiation Award: Academic / R&D for the year 2009 for his outstanding professional contribution to Mineral Engineering and Sita Ram Rungta Memorial Award - 2007 of the Society of Geoscientists and Allied Technologists (SGAT) for the year 2007 for outstanding work in the field of bio-mineral processing. Er. Sukla has become Editorial Board Member of Scientific Reports, a journal from Nature Publishing Group, the publishers of Nature. Er. Sukla is the editorial board member of the World Environment journal (Scientific &Academiv Publishing) and International Journal of Nonferrous Metallurgy. Er. Sukla is the National Expert in Envis Centre on Environmental Biotechnology (MOEF), New Delhi. Er. Sukla has implemented projects of Alcoa, USA, Ministry of Mines, DST, DBT, MOE and Govt of Odisha.

❐❐

Editors

Dr. Sandeep Panda

Dr. Sandeep Panda was a former CSIR - Senior Research Fellow (CSIR - SRF) at the Bioresources Engineering Department of CSIR - Institute of Minerals and Materials Technology, Bhubaneswar, India. Post completion of his master's degree in Biotechnology in the year 2008, Dr. Panda's current research area and interest includes applied environmental microbiology and industrial biotechnology. He has published over 23 well reputed international publications in SCI journals with nearly 35 impact factor which has received over 150 citations with an h-index of 8 and i-10 index of 5. In addition, to his credit he has also published 6 book chapters and a reputed book entitled "Environmental Microbial Biotechnology" published by Springer publications, Switzerland. Dr. Panda has been awarded with the Best Research Scholar Award of CSIR-IMMT in the year, 2010 and has received a National level (MISRA) award by the Indian Institute of Minerals Engineers (IIME) in 2013. Presently he is serving as the editorial board member of two international journals and is the reviewer of over 12 international journals of good reputation.

❐❐

Editors

Jacintha Esther

Ms. Jacintha Esther was a former Senior Research Fellow at CSIR-Institute of Minerals and Materials Technology (CSIR-IMMT), Bhubaneswar, Odisha. Her research area of interest is Mineral Bio-processing and Environment Biotechnology. To her credit she has published over 6 papers in reputed, peer-reviewed national and international journals.

❐❐

Contents

❐❐

ENVIRONMENTAL BIOTECHNOLOGY: INDUSTRIAL PRACTICES & PROSPECTS

Rati Ranjan Nayak and Pradosh Prasad Chakrabarti*

Centre for Lipid Research, CSIR-Indian Institute of Chemical Technology
Uppal Road, Tarnaka. Hyderabad 500007. Telangana, India.

*Corresponding Author: pradosh@iict.res.in / rrnayak@iict.res.in

Abstract

Sustainability is the most recent buzz-word and sustainable growth is the model followed by modern industries. In this model, major thrust is given on growth without disturbing the natural biotic systems. The general perception of the common people is that the industrial growth will always be coupled with environmental degradation. The indiscriminate industrial growth ignoring the environment in last few decades added fuel to this argument. However, as the environment protection agencies are becoming more and more stringent and as industries are looking towards sustainable growth, optimum utilization of natural resources, less emission/generation of wastes have become major objectives for all types of industries. Biotechnology has emerged as a key tool for achieving these targets for many industries and this can be judiciously utilized for economic, social and environmental benefits. Starting from traditional fermentation technologies for food industries to most recent recombinant DNA technology or cellular and molecular biology, biotechnology has established itself as a versatile technique. Biotechnology can also be used to develop processes that will significantly reduce the generation of contaminants, enzymatic degumming of rice bran oil may be one example for the same. This tool can also be utilized to degrade and remove the contaminants already generated by industries. This review will try to outline different aspects of environmental biotechnology that are already practised by industries, chemical industries in particular. This review will also highlight some of the emerging applications of biotechnology that may provide new solutions in otherwise highly polluting industries.

Keywords: Indu.strial Biotechnology, Pollution Prevention, Wastewater, Microorganism, Enzymes, Bioremediation

1. Introduction

Biotechnology involves processing of materials using biological agents. The application of biotechnology could be traced even in 6000 B.C. as the people of Neolithic era were found to have the knowledge to prepare wine by fermentation of grapes and the Babylonians were found to have the technique for preparation of beer using yeasts. Though the processes were known, the science behind these processes was explored much later. In the early nineteenth century, Louis Pasteur discovered that the fermentation processes are initiated and controlled by microorganisms that are not visible with naked eyes. In 1897, Eduard Buchner discovered enzyme that converts sugars to alcohol. Later, in early twentieth century, Sir Alexander Flemming extracted penicillin from mold and thereafter, large scale fermenters were developed to produce this drug. Since then, biotechnology has developed many folds and the modern biotechnology include genetic engineering, recombinant techniques, protein engineering, genomics in one side and bioelectronics, nanobiotechnology, bioseparation and bioreactor technologies on the other. The application of biotechnology, however, was not that widespread in industries barring a few sectors until few years back. The benefits and economics are yet to be judged completely by industry, policy makers and consumers.

In recent years, industries have focussed on sustainable developments. According to the World Commission on Environment and Development, the definition of sustainable development is "... a process of change in which the exploitation of resources, the direction of investments, the orientation of technological developments and institutional change are all in harmony and enhance both current and future potential to meet human needs and aspirations.." (Brundtland, 1987). The main aim is to prepare products that have all desirable properties and have more durability, less toxicity and more biodegradability compared to the conventional products. For the production of such items, processing technologies are to be developed that require optimum use of materials and energy, less emission of pollutants and

efficient use of renewable resources (Gavrilescu and Nicu, 2004; Gavrilescu and Chisti, 2005). Biotechnology has become an important tool to achieve all these aspects and chemical and environmental scientists, design engineers, biotechnologists and biologists are trying hard to bridge the gap between the basic knowledge of biotechnology to industrial scale operations for many emerging areas. Industrial biotechnology, also known as white biotechnology, is the application of modern biotechnology to the sustainable production of chemicals, materials, and fuels from renewable sources, using living cells and/or enzymes. This field is widely regarded as the third wave of biotechnology, following the first two; healthcare (red) biotechnology and agricultural (green) biotechnology. Industrial biotechnology is often associated with reduced energy consumption, greenhouse gas emissions, and waste generation, and also may enable the paradigm shift from fossil fuel-based to bio-based production of value-added chemicals. The reasons behind the rapid development/progress on Industrial biotechnology are as follows: (a) highly efficient processes at lower operating and capital expenditures, (b) political and societal demands for sustainability and environment-friendly industrial production systems, (c) depletion of crude oil reserves, (d) growing world demand for raw materials and energy (Soetaert and Vandamme, 2006; Tang and Zhao H. 2009).

Environmental biotechnology is comparatively a new domain that deals with the application of biotechnology for pollution prevention and handling of wastes already generated by industrial processes. In this, review, the recent advances of biotechnology based industrial applications for pollution control and remediation are discussed with some practical examples. It is shown with the examples of textiles, polymers, pharmaceuticals and vegetable oil industries how biotechnological interventions can reduce environmental pollution significantly with efficient and economical use of energy and renewable resources. It is also discussed how enzyme based industrial production of many important products are actually reducing the pollution load. The use of different types of microorganisms for bioremediation including the role of different species of microorganisms in bioremediation of specific pollutants is also discussed. The role of biotechnology of renewable

resources itself is a vast subject and hence, kept out of the scope of this review.

2. Applications of Biotechnology for Prevention of Pollution

Environmental pollution can be controlled by two means. Processes for manufacturing the desired products can be chosen in such a way that less amount of pollutants are generated. On the other hand, pollutants can be treated to convert into some less toxic or safe components before discharging to the environment. Industry has already adopted biotechnological processes for various production units. These processes are economic, produce better quality of products, and more importantly reduces the environmental pollution quite significantly compared to the conventional processes. In this section, some of these case studies are presented in short to show how biotechnological intervention plays an important role in protection of the environment. Moreover, these processes primarily use renewable resources and can be easily commercialised in different climatic areas. In a report prepared by Biotechnology Industry Organization (BIO), detailed case studies in different industrial sectors were presented where biotechnological application were used for pollution prevention, resource conservations and cost reductions (Erickson et al., 2004).

2.1. Pharmaceutical Industries

After the discovery of *penicillin,* biotechnology was used successfully for the production of many products in pharmaceuticals and health care industries. In some cases, the conventional processes were replaced by biotechnology based processes due to economic benefits, ease of operation, relatively less complications of technologies and primarily, due to huge reduction in environmental pollution. The benefits of implementing biotechnological approaches in manufacturing pharmaceutical products are described here with two examples.

2.1.1. Vitamin Production

In the conventional process Riboflavin (Vitamin B2) is manufactured from glucose following six chemical steps using hazardous reactants and the process generates harmful wastes.

This process was replaced by biotechnological process where crude Riboflavin is obtained from glucose with the help of genetically modified strain of a gram-positive bacterium *Basillus subtilies*. This process is based on a renewable material glucose and significantly reduces generation of hazardous waste. This also reduces wastewater discharge by 66%, air emission by 50% and cost by 50% (OECD, 2001). After the switching over to this process, in hardly twelve years of time the market share of biotechnologically processed Vitamin B2 increased from 5% to 75%.

2.1.2. Cephalosporin C Production

A complex ten step chemical route of production Cephalosporin C, an antibiotic against some gram-negative bacteria was replaced by a single fermentation process. It eliminated the use of various highly toxic chemical and reduce the audity of the wastewater produced (OCED, 2001). The emission of carbon dioxide was reduced to 50%, energy requirement was reduced to 20% and water usage was reduced to 75% compared to that of the conventional chemical process.

2.2. Food Processing Industry

Food technology is the sector where biotechnology has been used traditionally. Fermentation processes were immensely developed to produce food products like cheese, yogurt, vinegar etc. using different types of microorganisms and units with very high capacities were established for manufacturing of these products throughout the world. With the advent of commercial enzymes, biotechnology based approaches were attempted for other food items also. Food processing industries require huge amount of water and in some of the processes significant amount of organic waste is generated. The shift to biotechnology in some of these processes may result in considerable reduction in both water and waste.

2.2.1. Enzymatic Degumming of Vegetable Oils

Vegetable oil is one of the largest producing commodities. The benefit of biotechnology was successfully implemented for

enzymatic degumming of vegetable oils. In the conventional process, degumming is done by using phosphoric acid, citric acid and this is followed by caustic soda treatment and requires large quantity of water for the whole process, partially for washing of oil after acid degumming. This process cannot remove the gums completely from the vegetable oil. This process were replaced by a biotechnology based process where Phospholipase A1 or Phospholipase A2 enzymes were used that can remove gums completely irrespective of gums nature, whether hydratable or non-hydratable. The degummed oil thus produced, can be subjected to physical refining route without the addition of caustic soda, thus without producing any soapstock. As no soapstock is generated the pollution load is enormously reduced and a much better quality of nutritionally rich vegetable oil is produced. A Germany based company, Cereol Deutschland implemented the enzyme based degumming process and reported to reduce the water usage by 92%, waste sludge generation by 88% and overall cost by 43% (OECD, 2001). CSIR- Indian Institute of Chemical Technology, Hyderabad has developed a process using commercially available Phopholipase A1 enzyme (Chakrabarti et.al. 2009) for the degumming of rice bran oil. If the total production of rice bran oil in India is processed by CSIR-IICT process, the following advantages will be achieved (Chakrabarti, 2015) (Table 1).

Table 1 : Advantages of CSIR-IICT

Extra Oil Recovery @ 1 to 1.5%	9,500 to 14,250 tons p.a. (Rs 48 to 70 Crores)
Extra Fatty Acid Recovery@ 0.3 to 0.6%	2,850 to 5,700 tons p.a. (Rs 8 to 16 Crores)
Minimum Water Savings	More than 200,000 KL p.a. (compared to caustic refining)
Waste Sludge Generation	Negligible compared to conventional process
Quality of Oil	Most of the anti-oxidants like oryzanol, phytosterols, tocols, squalene etc. are retained whereas in conventional process of caustic refining considerable amount of these anti-oxidants are removed

2.3. Plastic and Chemical Production

The plastic manufacturing sector has tremendous scope for utilization of biotechnological processes for a variety of products. Bioprocesses where raw materials are primarily the agrochemicals from the renewable resources, and can replace petroleum as feedstock. Biotechnology has potential to reduce energy required to produce plastic and has huge potential to reduce the waste. In the estimate made in 2001, it was projected that USA produced around 80 billion pounds of plastics, out of which 1 billion pounds were bio-based (BRDB, 2001). Cargill-Dow was pioneer to establish world's first commercial biorefinery to produce bio based plastics. Composting of food items into food service items were used in Utah winter Olympic in 2002 at Utah and the rough estimation showed that the cost of food waste management could be reduced to 35% (Energetics, 2003).

DuPont had developed along with Genecor International, a biotechnology based process to create polymer from renewable resource. A micro-organism that can generate tri-methylene glycol, a precursor of a 1, 3 propane diol was used in the process. Similarly industrial biotechnology was used to produce ethylene, one of the versatile chemical feedstock for production of plastic, solvent, anti-freeze agents etc. Replacing petrochemical feedstock by the raw materials obtained from renewable resources like corn and other agro products, 80% demand of petrochemicals can be reduced (Erickson et al., 2004). Products prepared from Bioplastics like polylactic acid (PLA) can be composted and are recyclable. As the plastic made from PLA are biodegradable, they can eliminate the solid waste landfills and incinerations otherwise performed for plastics produced from petroleum based products. There are many such examples where biotechnology has proven to have key roles to develop greener technologies for prevention of environmental pollution.

2.4. Pulp and Paper Industry

Conversion of wood and recycled paper to pulp and freshly prepared paper is an energy, water and chemical intensive process.

The chemical process involves treatment of wood chips in sulphide and sulphate solution at around 170°C under high pressure (Erickson et al., 2004). In the next step, the pulp is bleached using chlorine or chlorine di-oxide to remove the lignin. Large amount of pollutants are produced during the pulping and bleaching stages (EPA, 2002). Methanol, chlorinated compounds and sulphuric acid are the major contaminants. In the biotechnological process Xylanase enzyme is employed to reduce chlorine chemicals necessary for bleaching by 10 to 15%. In an estimate it was projected that potential energy savings in European paper industries by shifting to biotechnological processes would lead to CO2 savings in between 155,000 and 270,000 tons per annum (Vigsoe et.al., 2002). In another biotechnological process white rot fungus was used to breakdown the lignin present in wood cell wall. This eases the bleaching operation with the reduced amount of bleaching agent. This process may further improve the bleaching related energy savings to another 40% and thus would reduce additional pollution pressure (Erickson et al., 2004).

2.5. Textile Industry

Biotechnology has many industrial applications in this sector. Industrial enzymes found many applications in textile processing industry. Hydrogen peroxide is generally used for bleaching of textiles and this is followed by repeated hot water washing at around 95°C (OECD, 2001). This process is energy and water intensive. Stone-washed jeans require the crushing of the fabric with acid or pumice stones. Hence, this process requires huge energy, produces highly contaminated wastewater and requires open pit mining of pumice. Another highly polluting step in textile processing is the traditional method of preparing cotton fibre, yarns and fabrics. This is performed by treating the cotton with hot alkali and subsequent water washing steps. This process generates huge amount of wastewater polluted with salts, acids, alkali, starch, surfactants, biocides, chelants, lubricants, metals, dyes, formaldyde based resins, metal catalysts, softeners, etc. depending on the process. This highly contaminated wastewater required elaborate and expensive treatment before disposal pressure (Erickson et al., 2004).

With the advent of biotechnology, the textile processing became more simpler (OCED, 2001). An enzyme is used to degrade residual peroxide into water and oxygen and thus reduces the temperature of second wash. Industry-wide use in USA would save 3 trillion BTU of energy per year. Cellulase enzyme can be used for softening and fading of jeans in place of pumic stone/ acid washing. This would reduce the environmental cost of stone washing up to 50%. The intervention of biotechnology would reduce energy demand by 10-14% and water usage by around 20%. Using a trademark enzyme Bioprep 3000L cotton fibre can be treated under mild conditions, thus, cutting the effluent load significantly and can reduce water usage by 30-50% pressure (Erickson et al., 2004).

There are many other industrial processes where intervention of biotechnology could reduce energy requirement, cost of production and the pollution load. Because of paucity of space those examples are not cited in this review.

3. Biotechnology in Waste Management

The last few decades have witnessed rapid and unplanned urbanization, indiscriminate industrialization and as a result, huge quantities of industrial pollutants have been released into the environment. There were paradigm shifts in human activities also, particularly in transport sector and agricultural practices. These factors also contributed into increased soil, air and water pollution. In this chapter, it has already been discussed how biotechnology can be used to intervene the production process and can prevent generation of pollutants. However, in many industrial processes, pollutants are bound to get generated. CO_2, NO_x, green house gases are generated that pollute the air; disposal of hazardous wastes, spraying pesticides, insecticides etc., use of non-biodegradable materials that pollute soil and chemical and biological contaminants, oil spillages create water pollution. The major pollutants generated by industry are shown in Figure 1 (Gavrilescu, 2010). Once these pollutants are formed, these are to be treated to minimize the health hazards - both for human being and other living organisms including the aquatic animals. The conventional

methods of treating these contaminants result in production of other hazardous contaminants - the landfills and incinerators have their own demerits and many a times the conventional processes are not efficient for complete removal of the pollutants and are not economically feasible.

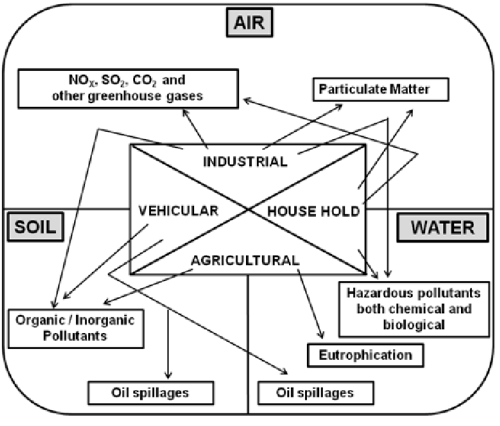


Figure 1: Sources of environmental pollution (Adapted from EIBE 2000 and Gavrilescu, 2010)

Environmental biotechnology - a combination of biochemistry, microbiology, molecular biology and chemical and environmental engineering has significantly emerged as a tool to remove/reduce the environmental pollution caused by these contaminants (Hashim and Uijang, 2004). Environmental biotechnology refers the use of microorganisms for safeguarding the environment. Natural microorganisms collectively can degrade a very wide range of chemicals and they have significant evolutionary capabilities to adapt to a wide variety of chemicals. In fact, the use of activated sludge and the anaerobic digestion was started in early 20^{th} century using a consortium of naturally occurring microorganisms. However, introduction of modern microbiology and molecular biology has given the scientists more options in handling contemporary environmental problems through bioremediation (Chen et al., 2005). According to the definition given by the Environmental Protection Agency of United States of America (USEPA), bioremediation means "A managed or spontaneous

practice in which microbiological processes are used to degrade or transform contaminants to less toxic or non-toxic forms, thereby remediating or eliminating environmental contaminations" (USEPA, 1994). The major factors that control the bioremediation process include, firstly, the prevailing conditions - moisture content, temperature, pH, presence of nutrients etc., secondly, the nature of the contaminants - toxicity, solubility in water, amount of contaminants present etc. and thirdly, the nature of the microorganisms that are capable of degrading the contaminants (Beaudette et al., 2002; Bitton, 2005 and Sasikumar and Papinazath, 2003).

There are mainly four strategies to consider for the treatment of the wastes: (i) Removal of contaminants where the pollutants are physically removed from the place without separating from the host media, (ii) Separation of contaminants from the medium (soil or water), (iii) Degradation of contaminants either by chemical or by biological methods to lesser toxic/non-toxic compounds and (iv) Containment or Immobilization of the contaminants to prevent their migration from the site (Gavrilescu, 2010; Gavrilescu, 2006; Doble and Kumar 2005; Khan et al. 2004 and Asante-Duah, 1996). In this chapter, primarily the third aspect, the degradation of contaminants by using biological means in different industries will be discussed. The degradation in this process is generally carried out using individual microorganisms and/or their consortia. The microorganisms may be either eukaryotes like yeasts, fungi, protozoa, unicellular plants and rotifars or these may be prokaryotes like bacteria and arcaea. Most of these microbes are isolated from hostile environments (e.g. from hot springs) and have the capability to degrade even most hazardous chemicals. These microbes can be utilized for biotreatment of soil, air and water.

3.1. Biological Treatment of Soil

Microorganisms can be used to degrade the soil contaminants either adopting an in situ process or using a process where the treatment is done in another place. Microorganisms generally react on the contaminants by enzymatic activity and degrade

them to less harmful or safe products. In soil composting process, in a very controlled manner, decomposition is done by the bacteria and fungi and produces humus-like products (Gavrilescu, 2010). This can be done in ex situ system. On the other hand, a carbon source or manure can be added for the in situ biological treatment of soil. Both the processes are practised by the industry for soil treatment (Langwaldt and Puhakka, 2000). The success of both of these processes depends on proper monitoring of the reaction conditions and availability of food source for the microorganisms (Saval, 1999). The major limitation of these processes are time dependence, environmental constraints and space requirements and in some cases lack of bioavailability (Gavrilescu, 2010).

3.2. Biological Treatment of Gaseous Stream

Biotechnology is being utilized for the treatment of mal-odourous emissions from the industries (Le Cloirec et al., 2005; Penciu and Gavrilescu, 2003). The emission of gaseous components having pungent and obnoxious smell coming out of the industries poses serious threat to the near-by locality and it becomes more evident for the composting plants where waste materials are treated. The weather and the topography of the region play an important role and the smell may be carried over to a long distance and industries have to treat the gaseous stream to avoid any complaint (Heroux et al., 2004). Herold and his co-workers had analyzed the exhaust gas of a composting plant and found that the major composition is alcohols, esters, ketones, aldehydes and terpenes (Herold et al., 2002). In another communication, similar composition was reported mentioning these components as the major contaminants (Schlegelmilch et al., 2005). These contaminants are captured in industrial practises by using biofilters and trickling filters and using biosorption techniques (Gadd, 2008; Andres et al., 2006). There are many matured technologies using this principle (Cohen, 2001; Penciu et al., 2004; Cox et al., 2001). Consortia of many microorganisms are used in the trickling biofilters and these microbes generally degrade the pollutants to a safe product before discharge to air (Penciu and Gavrilescu, 2004; Kennes and Thallasso, 1998).

3.3. Biological Treatment of Wastewater Stream

In every habitat of mankind fresh drinking water is constantly required and on the other hand, wastewater is continuously generated. Providing fresh drinking water is a major challenge and starting from ancient Roman aqueducts to modern dams, tremendous efforts are being made by the authorities to supply drinking water to the people. Despite the efforts made, one billion people in the world are not getting proper drinking water. Barring a few, in all chemical or other major industry, fresh water is required and most of these industries generate huge amount of wastewater. In majority of the cases, the contaminated water can't be even discharged to the environment without any treatment. The mother nature supply the fresh water for the mankind, whereas, for treatment of wastewater human beings are dependent on different types of microorganisms. These microbes can degrade a variety of chemicals to harmless products and can help in cycling the nitrogen, phosphorus and other elements. This particular nature of some microorganisms is being exploited by the industry as one of the most important application of biotechnology in industry (Rittmann, 2006; Rittmann et al., 2006). One of the primary criteria for biological treatment is that the wastewater stream should have more amount of such compounds that can be degraded to stable compounds like CO, CO_2, NH_3, CH_4, H_2S etc (Cheremsinoff, 1996; Dun et al., 2003).

The biological treatment can be achieved by either aerobic or anaerobic or combination of both aerobic and non aerobic oxidation processes. Aerobic processes are generally used for waste streams that can be easily biodegradable, municipal wastewater may be the ideal target (Doble and Kumar, 2005; Roussel, 2006). This is also suitable for low biomass concentration - an example being the domestic wastewater where the contaminants are primarily proteins, oils and fats, carbohydrates,, urea, pesticides, surfactants etc (Bitton, 2005). Wastewater streams with higher amounts of contaminants are generally subjected to anaerobic oxidation. This process may generate lesser amount of sludge and the biogas generated in this process can be recovered for free energy (Gallert and Winter,

1999). The development work in the engineering aspects is also huge in volume and the monitoring and control systems and the operating parameters also play a key role for the success of these processes. However, these aspects are not considered in the scope of this review. A detailed list of the microorganisms used for waste management systems are given in Table 2 (Gavrilescu, 2010).

Table 2: Microbial groups involved in environmental remediation (adapted from Gavrilescu M. (2010)

Microorganism	Type	Shape	Example	Abilities	References
Bacteria	cocci	Spherical	Streptococcus	Hydrocarbon degradation bacteria	Atlas 1981
				Heavy Oil	Leahy and Colwell 1990
				Degrade dairy industry waste (whey)	Ince 1998 Donkin 1997 Grady et al 1999 Mohana et al 2007 Xu et al 2009
	bacilli	rod	Bacillius subtilis	Degrade crude oil	Gallert and Winter 1999
				Bioremediation of chlorpyrifos contaminated soil	Eglit 2002 Das and Mukherjee 2007 Lakshmi et al 2008
		spiral form	Vibrio cholera	Heavy metals	Bitton 2005
	Sheated bacteria	Filamentous (gram-negative rods that become flagellated)	Sphaeratilus Leptothrix Crenothrix	Reduced iron to ferric hydroxide (Sphaeratilus natans, Crenothrix) Reduced manganese to manganese oxide (Leptothrix) Found in polluted streams and waste water treatment plants	Sukla and Panchanadikar 1993 Smith et al 1994 Sasaki et al 2001 Gray 2004 Bitton 2005 Fitzgiblon et al 2007
	Ptalked bacteria	flagellated	Caulobacter	Aerobic, aquatic environments with low organic content	Poindexter et al 2000 Bitton 2005
			Gallioionella	G. ferruginea, present in iron rich waters and oxidizes Fe^{2+} to Fe^{3+}	Benz et al 1998 Blanco 2000
	Budding bacteria	Filaments or hyphae	Hyphomicrobium	Soil and aquatic environments requires one-carbon compounds to grow (e.g. methanol)	Trejo and Quintero 1999 Gallert and Winter 2005 Burton et al 2002 Duncan and Horan 2003
			Rhodomicrobium	Phototrophic	Bitton 2005

	Gliding bacteria	Filamentous (gram-negative)	Beggiatoa	Oxidize H_2S to S^0	Droste 1997
	Bdellovibrio	Flagellated (predatory)	Thiothrix B. bacteriovorus	Grow independently on complex organic mdia	Guest and Smith 2002 Reddy et al 2003 Bitton 2005 Saratale et al 2009
	actinomycetes	Filamentous (gram-positive) Mycelial growth	Micromonospora Streptomyces Nocordia (Gordonia)	Most are strict aerobes Found in water, wastewater treatment plants, soils (neutral and alkaline) Degrade polysaccharides (starch, cellulose), hydrocarbons, lignin Can produce antibiotics (streptomycin, tetracycline, chloramphenicol) Gordonia is significant constituent of foams in activated sludge units	Grady et al 1999 Lema et al 1999 Olguin 1999 Saval 1999 Duncan and Horan 2003 Gavrilescu 2004 Bitton 2005 Dash et al 2008 Joshi et al 2008
	Cyanobacteria (blue green algae)	Unicellular, colonial or filamentous organisms	Anabaena	Prokaryotic organisms	Blanco2000
				Able to fix nitrogen	Burton et al 2002
				Have a high resistance to extreme environmental conditions (temperature, dessication) so that are found in desert soil and hot springs Responsible for algal blooms in lakes and other aquatic environments Some are quite toxic	Bitton 2005 Brinza et al 2005a El-Sheekh et al 2009
Archea	crenarchaeotes	Extremophyles	thermophiles	Prokaryotic cells	Eglit 2002
	Euryarchaeotes Korarchaeotes (more closely related to eukaryotes than to bacteria)		Hyperthermophiles Psychrophiles Acidophiles Alkaliphiles halophiles	Use organic compounds as a source of carbon and energy (organotrophs)	Burton et al 2002 Gavrilescu 2002 Dunn et al 2003 Bitton 2005 Doble and Kumar 2005

Eukaryotes	Fungi	Long filaments (hiphae) which form a mass called		Use organic compounds as carbon source and energy, and play	Hamer 1997 Burton et al 2002 Brinza and Gavrilescu 2003
		mycellium		an important role in nutrient recycling in aquatic and soil environments Some form traps that capture protozoa and nematodes Grow under acidic conditions in foods, water or wastewater (pH 5) Implicated in several industrial application (fermentation processes and antibiotic production	Gupta et al 2004 Bitton 2005
			Phycomycetes (water molds)	Occur on the surface of plants and animals in aquatic environments some are terrestrial (common bread mold, rhizopus)	Duncan and Horan 2003 Bitton 2005
			Ascomycetes (Neurospora crassa, Saccharomyces cerevisiae)	Some yeasts important industrial microorganisms involved in bread, wine, beer making	Bitton 2005
			Basidiomycetes (mushrooms Agaricus, Amanita (poisonous))	Wood-rotting fungi play a significant role in the decomposition of cellulose and lignin	Hernandez-Luna et al 2007 Bitton 2005
			Fungii imperfecti (eg. Penicillium)	Can cause plant diseases	Gadd 2007
	Algae	Floating unicellular microorganisms	phyloplankton	Play the role of primary producers in aquatic environments (oxidation ponds for waste water treatment)	Chavan and Mukherji 2010
		Filamentous	Uhlothrix	Carryout oxygenic photosynthesis and grow in mineral media with vitamin supplements (provide by some bacteria) and with CO_2 as the carbon source	Tuzen et al 2009 Duncan and Horan 2003 Feng and Aldrich 2004

		Colonial	Volvox	Some are the heterotrophic and use organic	
			Phylum Chlorophyta (green algae) Phylum Chrysophyta (golden-bown algae) Phylum Pyrrophyta (dinoflagellates) Phylum Rhodophyta (red algae) Phylum Phaeophyta (brown algae)		Bitton 2005 Gadd 2007
	Protozoa	Unicellular organisms		Important for public health and process microbiology in water and wastewater treatment	
			Sarcodina (amoeba) Mastigophora (flagellates) Ciliophora (ciliates) Sporozoa	resistant to desiccation, starvation, high temperature, lack of oxygen, disinfection in waters and wastewaters found in soils and aquatic environments some are parasitic to animals and humans	Bitton 2005
Viruses	Belong neither to prokaryotes nor to eukaryotes (carry out no catabolic or anabolic functions)		Animal viruses Algal viruses Bacterial phages	Some are indicators of contamination Distruct host cells Infect a wide range of organisms (animals, algae, bacteria)	Duncan and Horan 2003

3.3.1. Recent Developments in Wastewater Treatment

In the conventional process of biological treatment of wastewater, microorganisms obtained by isolation of pure cultures from natural resources are used. At present around 7000 bacterial species are known (DSMZ, 2005; http://www.dsmz.de). However, the real number may be much higher than this and are yet to be

explored. Molecular biology has contributed significantly by revealing the key microbes that degrade contaminants present in different types of wastewater. Recent developments of some techniques like cloning, creation of gene library, DGGE, FISH etc. has contributed a lot in microbial ecology research (Sanz and Kochling, 2007). Modern molecular techniques including the environmental genomics could identify otherwise unexplored microorganisms for nutrient removal and sludge bulking and foaming (Daims et al., 2006). It was understood from molecular biology studies that for direct combination of ammonia and nitrite into nitrogen gas can be performed with chemolithoautotrophic bacteria of phylum *Planctomycetes* (Storus M, et al., 1999). These anammox bacteria can be used more effectively in large scale wastewater treatment plants for nitrogen removal in place of conventional nitrogen removing *Nitrobacter* species (Daims et al., 2006). Using designer microorganisms with enhanced biodegradation ability is another trend of research. A hybrid strain was made by redesigning the metabolic pathway of *Pseudomonas putida* for demineralization of benzene, toluene and p-xylene (Lee et al., 1994). Another strategy is to use metal-binding peptides for enhanced biosorption. Scientists are also working on enzyme engineering for improved biodegradation of recalcitrant pollutants (Chen et al., 2005). Combination of all these biotechnological approaches would lead to more efficient industrial wastewater treatment processes.

4. Microorganisms for Treatment of Specific Pollutants

Environmental biotechnology has already worked on series of microorganisms and found some specific microbes that act on specific contaminants. Both soil and water sometimes get contaminated with pollutants of petroleum origin containing hydrocarbons due to accidental spillages. A large number of microorganisms like bacteria, yeasts, filamentous fungi etc. have already been identified for biodegradation of hydrocarbons. A list of microorganisms that can biodegrade hydrocarbons is tabulated in Table 3 (Adapted from Gavrilescu, 2010 and Van Hamme et al., 2003). However, this is not a comprehensive list and many

more new microorganisms, particularly genetically engineered ones are being studied. As discussed earlier, there are specific microorganisms that can remove/recover heavy metals from the wastewater streams coming out from various industries. These microbes are helping the industry to avoid heavy metal contamination and protecting the aquatic life from high level of toxicity. In Table 4, common organisms used for treatment of metal-contaminated wastewater are listed (Adapted from Gavrilescu, 2010). Apart from these two major areas, scientists are also working on genome sequencing of microorganisms like Nitrosomonus europaea, *Nitrosomonas eutropha, Nitrobacter hamburgensis, Nitrospira marina* etc. for nitrogen removal or *Gemmatimonas aurantiaca, Candida Accumulibacter phosphatis* for phosphorus removal and *Acidovorax temperans* and *Herpetosiphon aurantiacus* for floc/biofilm formation or bulking in wastewater treatment (Daims et al., 2006). There are many more examples of microorganisms for treating specific contaminants and the ambit of microorganisms either new or genetically engineered are increasing for specific uses.

Table 3: Petroleum compounds and fuel components degrading microorganism (adapted from Gavrilescu, 2010 and Van Hamme, 2003) Please check this table carefully for the names of microorganisms against their domain name (ex-bacteria, yeast, algae). (Checked and found OK)

Microorganism		Compound	Reference
Bacteria	*Blastochloris sulfoviridis* ToP1	Toluene	Zengler et al. 1999
	Azoarcus sp. strain EB1	Ethylbenzene	Ball et al. 1996
	Azoarcus sp. strain T	Toluene, *m*-xylene	Dolfing et al.1990
	Azoarcus tolulyticus Td15	Toluene, *m*-xylene	Fries et al 1994
	Azoarcus tolulyticus To14	Toluene	Zhou et al. 1995
	Dechloromonas sp. strain JJ	Benzene, toluene	Coates et al. 2001[a]
	Dechloromonas sp. strain RCB	Benzene, toluene	Coates et al. 2001[a]
	Pseudomonas sp. strain NAP-3	Naphthalene	Rockne et al. 2000
	Thauera aromatica K172	Toluene	Anders et al. 1995
	Thauera aromatica T1	Toluene	Evans et al. 1992
	Vibrio sp. strain NAP-4	Naphthalene	Rockne et al. 2000
	Geobacter grbiciae TACP-2^T	Toluene	Coates et al. 2001[b]
	Geobacter grbiciae TACP-5	Toluene	Coates et al. 2001[b]
	Geobacter metallireducens GS15	Toluene	Lovely and Lonergan 1990
	Desulfobacula toluolica To12	Toluene	Rabus et al. 2001
	Desulfobacterium cetonicum	Toluene	Harms et al. 1999

Yeasts	*Thrichosporon, Pichia rhodosporidium, Rhodotorula, Debraryomyces, Endomycopsis, Candida parapsilasis, C. tropicalis, C. guilliermondii, C.lipolytica, C.maltosa, Debaramyces hansenii, Trichosporon sp., Rhodosporium taruloidles*	Hexadecane and Kerosene (naphthalene, biphenyl, benzo(α)pyrene)	Riser-Roberts 1998
Algae	*Selanastrum capricornatum,* Cyanobacteria *Microcystis aeruginosa,* Mixed Cultures (yeasts, molds, protozoa, bacteria, activated sludge) Activated sludge, Sewage sludge, *Acinetobacter calcoaceticus, Pseudomonas putida, Trichosporon pullulans, Aeromonium sp., Mycobacterium sp.*	Benzene, toluene, naphthalene, phenanthrene, pyrene, petroleum derivatives, phenol cresols, paraffins, Total petroleum hydrocarbons, n-undecane, Acrylonitrile	

Table 4: Organism involved in treatment of metal contaminated wastewater (adapted from Gavrilescu, 2010)

Metal	Organism	
Cd(II)	Yeasts	*Saccharomyces cerevisiae, A. pullulans, Cr. Laurentii, Cy.capitatum, H. Anomala, P.fermentans, R.rubra, S.cerevisiae, Sp.roseus*
	Living microalgae free in solution	*Chlorella vulgaris, Chlorella salina, Chlorella homospaera, Scenedesmus obliquus, Chlamydomonas reinhardtii, Asterionella Formosa, Fragilaria crotonensis, Thalassiosira rotula, Cricosphaere elongate*
	Macro algal Biomass	*Sargassum natans, Ascophyllum nodosum, Halimeda opuntia, Fucus vesiculosus*
Cr(VI)	Yeasts	*S.cerevisiae, Candida utilis,*
Pb(II)	Living microalgae free in solution	*Chlorella vulgaris, Euglena sp.*
	Macro algal Biomass	*Sargassum natans, Sargassum fluitans, Sargassum vulgaris. Ascophyllum nodosum. Palmaria palmate. Chondrus Crispus, Fucus vesiculosus, Padina gymnospora, Codium taylori*
Zn(II)	Living microalgae free in solution	*Chlorella vulgaris, Chlorella regularis, Chlorella salina, Chlorella homospaera, Euglena sp.*
	Macro algal Biomass	*Sargassum natans*
Au(I)	Living microalgae free in solution	*Chlorella vulgaris*
	Macro algal Biomass	*Sargassum natans, Ascophyllum nodosum, Palmaria palmate, Chondrus Crispus, Porphyra palmata*

U(II)	Living microalgae free in solution	*Chlorella vulgaris, Chlorella* sp., *Scenedesmus obliquus, Scenedesmus* sp., *Chlamydomonas* sp., *Dunaliella tertiolecta, Ankiistrodesmus* sp., *Selenastrum* sp.
	Macro algal Biomass	*Sargassum natans*
Cu(I)	Living microalgae free in solution	*Chlorella vulgaris, Euglena* sp., *Cricosphaere elongate*
	Macro algal Biomass	*Sargassum natans, Vaucheria*
Co(II)	Living microalgae free in solution	*Chlorella regularis, Chlorella salina*
	Macro algal Biomass	*Sargassum natans, Ascophyllum nodosum, Halimeda opuntia, Chondrus Crispus, Porphyra palmata*
Ni(I)	Living microalgae free in solution	*Chlorella regularis, Thalassiosira rotula*
Mn(II)		*Chlorella regularis, Chlorella salina, Euglena* sp.
Mo(I)		*Chlorella regularis, Scenedesmus* sp., *Chlamydomonas reinhardtii*
Tc(II)		*Chlorella emersonii, Scenedesmus obliquus, Chlamydomonas reinhardtii*
Zr(II)		*Chlorella emersonii, Scenedesmus obliquus, Chlamydomonas* sp.
Hg(II)		*Chlorella* sp.
Al(III)		*Euglena* sp.
Ag(I)	Macro algal Biomass	*Sargassum natans*
Sr(II)		*Vaucheria*

5. Role of Enzymes in Protection of Environment

Enzymes are proteins produced from living organisms and acts as catalyst for various biochemical reactions. The last few decades have witnessed application of enzymatic processes in many industries. The major advantages being these enzymes are specific to certain reactions, the rate of reactions are fast and can be controlled very easily. The enzymatic reactions save raw materials, energy and chemicals and also require less water. Enzymes react under milder conditions compared to the conventional chemical processes and they are easily biodegradable and do not pose any environmental problem once they reach the environment after industrial use (Kirk-Othmer, 2005; Soetaert and Vandamme, 2010). Enzymes are used in a large number of

industrial processes like in paper and pulp industry (Nguyen et al., 2008) where cellulase, laccase, xylynase, lipase, esterase enzymes are used. In leather industry (Kandaswami et al., 2012) proteases and lipases are used. Similarly,, in textiles industry (Zhou et al., 2008) pectate lyase, catalase, arylstearases etc. are used. Enzymes are used in detergent production (Saeki *et al.,* 2007) where lipases and proteases are the predominant enzymes. In food and beverage production (Ramos and Malcata, 2011; Okamura-Matsui, 2003) enzymes like lipases, phospholipases, amylase, protease pectinases etc. are used whereas, in animal feed industries (Gado et al., 2009; Zhu et al., 2011) the uses of xylenase, phytase are well known. Pharmaceuticals industries (Woodley, 2008) also use enzymes like peniciline amidase, glutaryl acylase etc for production of different medicines. In fine chemicals and cosmetics industries (Gavrelescu and Chisti, 2005; Lods et al., 2001), enzymes like tolune ortho monooxygenase are used. Biodiesel industries also use lipase catalysed trans-esterification reactions (Harnandez-Martin and Otero, 2008). All these reactions result in various advantages including the benefit of less use of raw materials, energy and less generation of pollutants. In a recent report, all these environmental benefits were described (Jegannathan and Nielsen, 2013). The main features of that report are given in Table 5.

Table 5: Environmental assessments of enzymatic and conventional processes in various industries. Greenhouse gas (GHG) savings reported in the table refer to the specific production conditions addressed in the actual studies and specific figures cannot necessarily be generalized. (Jegannathan and Nielson 2013)

Industry	Application process	Enzyme	Function of enzyme	Savings obtained by enzyme use	Functional unit, FU	GHG saving, kg CO_2 equivalents/ FU (unless otherwise noted)	Impact assessment method	Scale of production	References
Pulp and paper	Thermo-mechanical Pulping	Cellulase	Softens wood chips	Energy	1 ton pulp	145	EI'95a	Full-scale	Skals et al., 2008
	Deinking	Cellulase	Acts on recycled fibers and facilitates ink loosening	Chemicals	1 ton recycled pulp	25 5	IPCC[b] EI'95	Full-scale Full-scale	Kallioinen et al., 2003, Skals et al., 2008
	Bleaching	Laccase	Oxidizes lignin and enhances lignin removal	Bleaching chemicals, energy	1 ton bleached pulp	–9%	EI'95	Full-scale	Fu et al., 2005
		Xylanase	Hydrolyzes xylan and enhances lignin extraction	Bleaching chemicals	1 ton virgin pulp	37	EI'95	Lab-scale	Skals et al., 2008
	Pitch control	Lipase	Hydrolyzes pitch	Cleaning agent, talc, energy	1 ton virgin paper	9	EI'95	Full-scale	Skals et al., 2008
	Stickies control	Esterase	Hydrolyzes glue and controls stickies	Talc, solvent, energy	1 ton recycled paper	13	EI'95	Full-scale	Skals et al., 2008
Leather	Beam house	Protease, lipase	Facilitates hair and fat removal from hides	Chemicals, energy	1 ton hide	97	EI'95	Full-scale	Nielsen, 2006
Textile	Scouring	Pectate lyase	Degrades pectin and assist in removal of	Energy, water, chemicals, cotton	1 ton yarn	990	EI'95	Full-scale	Nielsen and Høier, 2009

			wax etc. from raw cotton						
	Bleaching	Catalase	Hydrolyzes hydrogen peroxide to oxygen and water	Heat, electricity, water	1 ton yarn	410	EI'95	Full-scale	Nielsen and Høier, 2009
	Bleaching	Arylesterase	Perhydrolyzes to form peracetic acid (bleaching activator)	Chemical, cotton, energy	1 kg cotton fabric	1.3	IMPACT 2002+	Full-scale	Dettore, 2011
Detergent	Laundry washing	Protease, lipase, amylase, cellulase	Removes stains from laundry	Chemicals, energy	3 kg laundry	0.3	EI'95	Model detergent	Nielsen and Skagerlind, 2007
Food and beverage	Degumming of soybean oil	Phospolipase	Hydrolyzes phospholipids	Oil, chemicals	1 ton oil	45	EI'95	Full-scale	Cowan et al., 2008
	Hard stock production	Lipase	Interesterification of vegetable oil	Chemicals, energy	1 ton hard stock	340	EI'95	Lab/full-scale	Holm and Cowan, 2008
	Fruit juice production	Pectinase	Breaks down pectin in fruit	Fruits	1 L juice	CO2 emission reduced	IPCC EI'99	Full-scale	DSM, 2009
	White bread production	Amylase	Degrades starch and delays hardening of bread	Bread	100 breads sold	5.4	EI'95	Full-scale	Oxenbøll and Ernst, 2008
	Steamed bread production	Amylase, lipase			1 ton bread sold	39	CML 2 baseline 2000		Oxenbøll et al., 2010
	Mozzarella cheese production	Phospho-lipase	Hydrolyzes phospholipids in milk	Milk	1 ton cheese	220	EI'95	Full-scale	Nielsen and Høier, 2009
	100%	Amylase,	Converts	Barley,	100 L beer	2.5	CML 2	Full-scale	Kløverpris and

	Barley beer production	protease etc.	starch to fermentable sugars	energy			baseline 2000		Spillane, 2010
		Amylase, protease		Energy		4.7	EI'99		Yon-Miaw, 2011
Animal feed	Pig feed production	Xylanase	Depolymerizes xylans and enables better digestion	Feed	1 ton pig feed	78	EI'95	Full-scale	Nielsen et al., 2008
	Pig feed production	Phytase	Hydrolyzes phytate and releases phosphorus bound in feed	Inorganic phosphorus	1 kg phytase product	30	EI'95	Full-scale	Nielsen and Wenzel, 2006
	Poultry feed production	Phytase			1 ton poultry feed	7.0	CML baseline 2000	Full-scale	Nagaraju and Nielsen, 2011
	Poultry feed production	Protease	Hydrolyzes protein in the feed	Feed protein	1 ton poultry	11	CML baseline 2000	Full-scale	Oxenboll et al., 2011
Fine chemicals	Amino-butanoic acid production	Lipase	Aminolysis in (S)-3-aminobutanoic acid production	Chemicals, waste	1 kg (S)-3-aminobutanoic acid production	Environmental Factor reduced 8.8 times	F-Factor[e]	Lab-scale	Weiß et al., 2010
	α-Naphthol production	Toluene orthomonooxygenase	Oxidizes naphthalene to alpha-naphthol	Chemical, waste	NA[d]	Yield improvement: 4.5 times	N/A[d]	Lab-scale	Osborne-Lee et al., 2008
Pharmaceuticals	(S)-2-3-Dihydro-1Hindole-2-carboxlic acid production	Phenyl-alanine ammonia lyase	Formation of C–N bond	Chemical, energy	1 kg product	155	IPCC	Lab-scale	Poechlauer et al., 2010
	γ-Amino-	Lipolase	Resolution of	Chemical,	1 ton product	Environment	F-Factor	Full-scale	Dunn, 2011

	butyric acid production		cyanodiester	energy, waste		al Factor reduced 5.1 times			
	6-Aminopenicillanic acid production	Penicillin amidase	Deacylates penicillin molecule	Chemical, energy	1 kg product	Environmental Index reduced 16 times	EI[f] (SuperPro designer)	Lab scale	Biwer and Heinzle, 2004
	7-Aminopenicillanic acid production	D-Amino acid oxidase, glutaryl 7-ACA acylase	Oxidizes cephalosporin C salt and deacylates gluiaryl 7-aminocephalosporic acid	Chemical, energy	1 kg product	270	FLASC[g]	Full-scale	Henderson et al., 2008
Cosmetics	Oleo chemical ester production	Lipase	Transesterification of vegetable oil	Chemical, energy, raw material	5 ton myristyl myristate	940	EI'95	Full-scale	Thum and Oxenboll, 2008
Biodiesel	Methyl ester production	Lipase	Catalyzes the reaction of triglyceride and menthol to form methyl ester	Energy, chemicals, raw material	1 ton biodiesel	100	CML base 2000	Lab-scale	Harding et al., 2007
					1 ton biodiesel	2.5%	EI'99	Lab-scale	Jeganmathan et al., 2011

Conclusions

Even in recent past, it was generally accepted by common men that industrial growth will always be accompanied with deterioration of environment. The aggressive expansion of industrial activities had worsened the environmental conditions and the Environment Protection Agencies throughout the world had to take corrective measures by issuing stringent regulations. Reviewing the situations, industries decided to go for sustainable growth where not only the present needs of the society but also the needs of the future generations are prioritized. The economic and environmental benefits offered by different biotechnological processes attracted the attention of many industries and conventional processes were gradually replaced by bio based processes. This review described various aspects of commercial biological processes, particularly, the role in pollution prevention, waste management and applications of microorganisms and enzymes for pollution control and remediation. It is quite evident from the discussions presented in this review that the environmental biotechnology that deals with application of biotechnology in pollution prevention/remediation has emerged as a matured area of science and its areas are continuously expanding. The role of microorganisms and also the uses of enzymes for bioremediation, greener production and profit generation are being explored by industries. This review has presented some of the case studies and thrown light on some emerging industrial applications by citing some examples. Undoubtedly, there are far more areas where industrial applications of environmental biotechnology are in practice or at final stages of implementations.

The data available on these industrial processes are insufficient and more systematic studies are to be performed to assess the potential economic benefits of biotechnology in specific industries. New initiatives should also be taken to understand and quantify the health benefits that can be achieved by the industry if traditional processes are replaced by biotechnological processes. Some of the industries possess the data required for these studies.

However, these are not available in the public domain and refrains one from having better understanding of the problem. The research on engineering aspects for the development of the processes is also important and initiatives like reaction in membrane bioreactors are also to be studied with economic benefits keeping in mind. The advances in research on microbial ecology, the genetically modified microorganisms and modified biosorbents also should be explored carefully to extract the maximum benefits.

References

Anders H.J., Kaetzke A., Kaempfer P., Ludwig W., Fuchs G. (1995) Taxonomic position of aromatic-degrading denitrifying pseudomonad strains K 172 and KB 740 and their description as new members of the genera Thauera, as Thauera aromatica sp. nov., and Azoarcus, as Azoarcus evansii sp. nov., respectively, members of the beta subclass of the Proteobacteria. *Int. J. Syst. Bacteriol.* 45, 327.

Andres Y., Dumont E., Le Cloirec P., Ramirez-Lopez E. (2006) Wood bark as packing material in a biofilter used for air treatment. *Environmental Technology* 27, 1297.

Asante-Duah D.K. (1996) Managing Contaminated Sites: Problem Diagnosis and Development of Site Restoration, John Wiley and Sons, New York, pp. 254.

Atlas R.M. (1981) Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological Reviews* 45, 180.

Ball H.A., Johnson H.A., Reinhard M., Spormann A.M. (1996) Initial reactions in anaerobic ethylbenzene oxidation by a denitrifying bacterium, strain EB1. *J. Bacteriol.* 178, 5755.

Beaudette L.A., Cassidy M.B., England L., Kirk J.L., Habash M., Lee H., Trevors J.T., (2002) Bioremediation of Soils. In: Bitton G. (Ed) Encyclopedia of Environmental Microbiology, Wiley Interscience, New York, pp. 722-737.

Benz M., Brune A., Schink B. (1998) Anaerobic and aerobic oxidation of ferrous iron at neutral pH by

chemoheterotrophic nitrate-reducing bacteria. *Archives of Microbiology* 169, 159.

Biomass Research and Development Board, "Fostering the Bioeconomic Revolution in Biobased Products and Bioenergy" (2001) Available electronically at http:// www.bioproducts-bioenergy.gov. pp.1-30.

Bitton G. (2005) Wastewater Microbiology, Wiley-Liss, JohnWiley and Sons, New Jersey, USA, pp. 766.

Biwer A., Heinzle E. (2004) Environmental assessment in early process development. *J. Chem. Technol. Biotechnol.* 79, 597.

Blanco A. (2000) Immobilization of non-viable cyanobacteria and their use for heavy metal adsorption from water. In: Olguin E.J., Sanchez G., Hernandez E. (Eds) Environmental Biotechnology, Philadelphia, pp.135-151.

Brinza L., Dring M., Gavrilescu M. (2005) Ability of different algal species to take up heavy metals from wastewater: A review. *The Phycologist* 68, 30.

Brinza L., Gavrilescu M. (2003) pH effect on the biosorption of cu2+ from aqueous solution by *Saccharomyces cerevisiae. Environmental Microbiology* 57, 1956.

Brundtland G. Our common future. Oxford: Oxford University Press; 1987.

Burton F.L., Stensel H.D., Tchobanoglous G. (2002) Wastewater Engineering: Treatment and Reuse, Metcalf and Eddy Inc., McGraw-Hill Professional, pp.1848.

Chakrabarti P.P. (2015) Newer Processing Methodologies and Value Addition to By-products, Paper presented in IICT Business meet held at CSIR-IICT, Hyderabad, India on 17th April.

Chakrabarti P.P., Rao B.V.S.K., Roy S.K., Prabhavathidevi B.L.A., Prasanna Rani K.N., Vandana V., Kalyani C., Gaddam K., Kale V., Prasad R.B.N. (2009) An Improved process for the pre-treatment of vegetable oils for physical refining by, US patent No. 7494676.

Chavan A., Mukherji S. (2010) Response of an algal consortium to diesel under varying culture conditions. *Applied Biochemistry and Biotechnology* 160, 719.

Chen W., Mulchandani Ashok, Deshusses M.A. (2005), Environmental Biotechnology: Challenges and Opportunities to Chemical Engineers, *AIChE Journal,* 51, 690.

Cheremsinoff N.P. (1996) Biotechnology for Waste and Wastewater Treatment, Noyes Publications, Westwood, New Jersey, pp.231.

Coates J.D., Chakraborty R., Lack J.G., OConnor S.M., Cole K.A., Bender K.S., Achenbach L.A. (2001b) Anaerobic benzene oxidation coupled to nitrate reduction in pure cultures by two strains of Dechloromonas. *Nature* 411, 1039.

Coates J.D., Bhupathiraju V.K., Achenbach L.A., McInerney M.J., Lovely D.R. (2001a). Geobacter hydrogenophilus, Geobacter chapellei, and Geobacter grbiciae, three new, strictly anaerobic, dissimilatory Fe(III)-reducers. *Int. J. Syst. Bacteriol.* 51, 581.

Cohen Y. (2001) Biofiltration - the treatment of fluids by microorganisms immobilized into the filter bedding materials: a review. *Bioresource Technology* 77, 257.

Cowan D., Oxenbøll K.M., Holm H.C. (2008) Enzymatic bioprocessing of oil and fats. *Inform* 4, 210.

Cox H.H.J., Deshusses M.A., Converse B.M., Schroeder E.D., Patel D.D., Vosooghi D., Iranpour R. (2001) Odor and VOC treatment by biotrickling filters: pilot scale studies at the Hyperion Treatment Plant. *Water Environment Research* 74, 557.

Daims H., Taylor M.W., Wagner M. (2006) Wastewater treatment: a model system for microbial ecology. *Trends in Biotechnology* 24, 483.

Das K., Mukherjee A.K. (2007) Crude petroleum oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum

oil contaminated soil from North-East India. *Bioresourse Technology* 98, 1339.

Dash R.R., Majumder C.B., Kumar A. (2008) Treatment of metal cyanide bearing wastewater by simultaneous adsorption and biodegradation (SAB). *Journal of Hazardous Materials* 152, 387.

Dettore C. (2011) Comparative life-cycle assessment (LCA) of textile bleaching systems: gentle power bleach vs. conventional bleaching System. In: Scientific Poster. AATCC International Conference, 22-24 March.

Doble M., Kumar A. (2005) Biotreatment of Industrial Effluents, Elsevier-Butterworth-Heinemann, pp.337.

Dolfing J., Zeyer J., Binder-Eicher P., Schwarzenbach R.P. (1990) Isolation and characterization of a bacterium that mineralizes toluene in the absence of molecular oxygen. *Arch. Microbiol.* 154, 336.

Donkin J. (1997) Bulking in aerobic biological systems treating dairy processing wastewater. *International Journal of Dairy Technology* 50, 67.

Droste R.L. (1997) Theory and Practice of Water and Wastewater Treatment, John Wiley and Sons Inc., New Jersey, pp.816.

DSM 2009. DSM - Annual Report 2009. Royal DSM N.V., Heerlen, The Netherlands.

Duncan M., Horan N.J. (2003) Handbook of Water and Wastewater Microbiology, Elsevier, pp.832.

Dunn I.J., Heinzle E., Ingham J., Prenosil J.E. (2003) Biological Reaction Engineering. Dynamic Modelling Fundamentals with Simulation Examples, Wiley-VCH, Weinheim, pp.532.

Dunn P.J. (2011) The importance of green chemistry in process research and development. *Chem. Soc. Rev* 41, 1452.

Eglit T. (2002) Microbial degradation of pollutants at low concentrations and in the presence of alternative carbon substrates: emerging patterns. In: Agathos S.N., Reineke W. (Eds) Biotechnology for the Environment: Soil Remediation, Springer, Berlin, pp.131.

EIBE (2000) Biotechnology and Environment. European Initiative for Biotechnology Education. Available on line at: http://www.ipn.uni-kiel.de/eibe/UNIT16EN.PDF.

El-Sheekh M.M., Gharieb M.M., Abou-El-Souod G.W. (2009) Biodegradation of dyes by some green algae and cyanobacteria. *International Biodeterioration and Biodegradation* 63, 699.

Energetics, Incorporated, Report for the Department of Energy, "Industrial Bioproducts: Today and Tomorrow," Columbia, MD (2003) 41.

EPA, Environmental Protection Agency, Office of Compliance, Sector Notebook Project: Profile of the Pulp and Paper Industry, Washington, DC (2002) 19.

Erickson B., Hessler C.J., Doerr P., Higgins M., Pannenbacker L., Rupp J., Wade J. (2004) New Biotech Tools for a cleaner Environment. Dickson J., Strickland D. (Eds) Biotechnology Industry Organization's (BIO's) Industrial and Environmental Section report. www.bio.org. pp1-76.

Evans P.J., Ling W., Goldschmidt B., Ritter E.R., Young L.Y. (1992) Metabolites formed during anaerobic transformation of toluene and oxylene and their proposed relationship to the intial steps of toluene mineralization. *Appl. Environ. Microbiol.* 58, 496.

Feng D., Aldrich C. (2004) Adsorption of heavy metals by biomaterials derived from the maine alga Ecklonia maxima. *Hydrometallurgy* 73, 1.

Fitzgiblon F.J., Nigam D.P., Singh D., Marchant R. (2007) Biological treatment of distillery waste for pollution-remediation. *Journal of Basic Microbiology* 35, 293.

Fries M.R., Zhou J., Chee-Sanford J.C., Tiedje J.M. (1994) Isolation, charcterization, and distribution of denitrifying toluene degraders from a variety of habitats. *Appl. Environ. Microbiol.* 60, 2802.

Fu G.Z., Chan A.W., Minns D.E. (2005) Preliminary assessment

of the environmental benefits of enzyme bleaching for pulp and paper making. *Int. J. Life Cycle Assess* 10, 136.

Gadd G.M. (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research* 111, 3.

Gadd G.M (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. *J Chem Technol Biotechnol* 84, 13.

Gado H.M., Salem A.Z.M., Robinson P.H., Hassan M. (2009) Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. *Anim. Feed Sci. Technol.* 154, 36.

Gallert C., Winter J. (1999) Bacterial metaboloism in wastewater treatment system. In: Rehm H-J, Reed G. (Eds) Biotechnology (2nd Edn), Wiley-VCH Verlag, GmbH, Weinheim, pp.17.

Gallert C., Winter J. (2005) Perspectives of wastewater, waste, off-gas and soil treatment. In: Jordening H-J, Winter J. (Eds) Environmental Biotechnology Concept and Applications, Wiley-VCH, Weinheim, pp.439.

Gavrilescu M, Nicu M. (2004) Source reduction and waste minimization. Iasi, Romania7 Ecozone Press.

Gavrilescu M. (2002) Engineering concerns in anaerobic waste-water treatment. *Clean Technology and Environmental Policy* 3, 346.

Gavrilescu M. (2004) Cleaner production as a tool for sustainable development. *Environmental Engineering and Management Journal* 3, 45.

Gavrilescu M. (2006) Overview of in situ remediation of technologies for sites and groundwater. *Environmental Engineering and Management Journal* 5, 79.

Gavrilescu M. (2010) Environmental Biotechnology: Achievements,

Opportunities and Challenges. *Dynamic Biochemistry, Process Biotechnology and Molecular Biology* 4, 1.

Gavrilescu M., Chisti Y. (2005) Biotechnology - a sustainable alternative for chemical industry. *Biotechnology Advances* 23, 471.

Grady L.J.R., Daigger G.T., Lim H.C. (1999) Biological Wastewater Treatment, Marcel Dekker, New York, pp.1076.

Gray N.F. (2004) Biology of Wastewater Treatment (2nd Edn), World Scientific Publishing, New Jersey, pp.1444.

Guest R.K., Smith D.W. (2002) A potential new role for fungi in a wastewater MBR biological nitrogen reduction system. *Journal of Environmental Engineering and Science 1*, 433.

Gupta S.K., Gupta S.K., Hung Y-T. (2004) Treatment of pharmaceutical waste. In: Wang L.K., Hung Y-T., Lo H.H., Yapijiakis C. (Eds) Handbook of Industrial and Hazardous Waste Treatment (2nd Edn), Marcel Dekker, pp.63.

Hamer G. (1997) Microbial consortia for multiple pollutant biodegradation. Pure and Applied Chemistry 69, 2343.

Harding K.G., Dennis J.S., Von Blottnitz H., Harrison S.T.L. (2007) A life-cycle comparison between inorganic and biological catalysis for the production of biodiesel. J. Clean. Prod. 16, 1368.

Harms G., Rabus R., Widdel F. (1999) Anaerobic oxidation of the aromatic plant hydrocarbon p-cymene by newly isolated denitrifying bacteria. *Arch. Microbiol.* 172, 303.

Hernández-Martín E., Otero C. (2008) Different enzyme requirements for the synthesis of biodiesel: Novozym® 435 and Lipozyme® TL IM. *Bioresour. Technol.* 99, 277.

Hashim M.A., Uijang Z. (2004) Environmental Biotechnology: Its Relevance and Prospects for Developing Countries. In: Ujang Z., Henze M. (Eds). Environmental Biotechnology, IWA Publishing, London, UK, pp. 7-12.

Henderson R.K., Jiménez-González C., Preston C., Constable D.J.C., Woodley J.M. (2008) EHS & LCA assessment for 7-ACA synthesis. *Ind. Biotechnol 4,* 180.

Hernandez-Luna C.E., Gutierrez-Soto G., Salcedo-Martinez S.M. (2007) Screening for decolorizing basidiomycetes in Mexico. *World Journal of Microbiology and Biotechnology* 24, 465.

Herold T., Biedermann W., Schlegelmilch M., Hensel A. (2002) Einfluss verschiedener Filtermaterialien auf den Wirkungsgrad von Biofiltern zur Reinigung von Rotteabluft einer Kompostierungsanlage. *Gefahrstoffe - Reinhaltung der Luft* 62, 147.

Heroux M., Page T., Gelinus C., Guy C. (2004) Evaluating Odour Impacts from a Landfilling and Composting Site: Involving Citizens in the Monitoring. *Water Science and Technology* 50, 131.

Holm H.C., Cowan W.D. (2008) The evolution of enzymatic interesterification in the oils and fats industry. *Eur. J. Lipid Sci. Technol* 110, 679.

Ince O. (1998) Potential energy production from anaerobic digestion of diary waste water. Journal of Environmental Science and Health 33, 1219.

Jegannathan K.R., Nielsen P.H. (2013) Environmental assessment of enzyme use in industrial production a literature review. *Journal of Cleaner Production* 42, 228.

Jegannathan K.R., Wang Ting V.F., Pogaku R. (2011) LCA of biodiesel production using alkali, soluble and immobilized enzyme catalyst processes. *Biomass Bioenergy* 35, 4221.

Joshi T., Iyengar L., Singh K., Garg S. (2008) Isolation, identification and application of novel bacterial consortium TJ-1 for the decolorization of structurally different azo dyes. *Bioresource Technology* 99, 7115.

Kallioinen A., Pere J., Siika-aho M., Lehtilä A., Mälkki H., Syri S., Thun R. (2003) Biotechnical Methods for Improvement

of Energy Economy in Mechanical Pulping. Espoo. VTT Tiedotteita Research Notes 2183. VTT Technical Research Centre, Finland.

Kandasamy N., Velmurugan P., Sundarvel A., Raghava R.J., Bangaru C., Palanisamy T. (2012) Eco-benign enzymatic dehairing of goatskins utilizing a protease from a Pseudomonas fluorescens species isolated from fish visceral waste. *J. Clean. Prod.* 25, 27.

Kennes C., Thalasso F. (1998) Waste gas biotreatment technology. *Journal of Chemical Technology and Biotechnology* 72, 303.

Khan F.I., Hussain T., Hejazi R. (2004) An Overview and Analysis of Site Remediation Technologies, *Journal of Environmental Management* 71, 95.

Kirk-Othmer (2005) Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons, New York.

Kløverpris J.H., Spillane D. (2010) Comparative LCA of malt-based beer and 100% barley beer. *Brewing Beverage Industry International* 2, 34.

Lakshmi C.V., Kumar M., Khann S. (2008) Biotransformation of chlorpyrifos and bioremediation of contaminated soil. *International Biodeterioration and Biodegradation* 62, 204.

Langwaldt J.H., Puhakka J.A. (2000) On-site Biological Remediation of Contaminated Groundwater: A Review. *Environmental Pollution* 107, 187.

Le Cloirec P., Andres Y., Gerente C., Pre P. (2005) Biological Treatment of Waste Gases Containing Volatile Organic Compounds. In: Shareefdeen Z., Singh A. (Eds.) Biotechnology for Odour and Air Pollution Control, Springer, Berlin, pp. 3-16.

Leahy J.G., Colwell R.R. (1990) Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews* 54, 305.

Lee J.Y., Roh J.R., Kim H.S. (1994) Metabolic engineering of *Pesudomonas Putida* for simultaneous biodegradation of benzene, toluene and p-xylene mixture. *Biotechnol Bioengg* 4, 1146.

Lema J.M., Moreira M.T., Palma C., Feijoo G. (1999) Clean biological bleaching processes in the pulp and paper industry. In: Olguin E.J., Sanchez G., Hernadez E. (Eds) Environmental Biotechnology and Cleaner Bioprocesses, Taylor and Francis, Boca Raton, pp. 211.

Lods L.M., Dres C., Johnson C., Scholz D.B., Brooks G.J. (2001) The future of enzymes in cosmetics. *Int. J. Cosmet. Sci.* 22, 85.

Lovely D.R., Lonergan D.J. (1990) Anaerobic oxidation of toluene, phenol, and p-cresol by the dissimilatory iron-reducing organism GS-15. *Appl. Environ. Microbiol.* 56, 1858.

Mohana S., Desai C., Madammar D. (2007) Biodegradation an decolorization of anaerobically treated distillery spent wash by a novel bacterial consortium. *Bioresource Technology* 98, 333.

Nagaraju R.K., Nielsen P.H. (2011) Environmental advantages of phytase over inorganic phosphate in poultry feed. *Poultry Punch* 27, 57.

Nguyen D., Zhang X., Jiang Z., Audet A., Paice M.G., Renaud S., Tsang A. (2008) Bleaching of kraft pulp by a commercial lipase: accessory enzymes degrade hexenuronic acids. *Enzym. Microb. Technol.* 43, 130.

Nielsen P.H. (2006) Environmental assessment of enzyme application in tanning industry. *Leather International* (August/September), 18.

Nielsen P.H., Dalgaard R., Korsbak A., Pettersson D. (2008) Environmental assessment of digestibility improvement factors applied in animal production: use of Ronozyme WX CT xylanase in Danish pig production. *Int. J. Life Cycle Assess* 13, 49.

Nielsen P.H., Høier E. (2009) Environmental assessment of yield improvements obtained by the use of the enzyme phospholipase in mozzarella cheese production. *Int. J. Life Cycle Assess* 14, 137.

Nielsen P.H., Skagerlind P. (2007) Cost-neutral replacement of surfactants with enzymes. *Household and Personal Care Today* 4, 3.

Nielsen P.H., Wenzel H. (2006) Environmental assessment of Ronozyme P5000 CT phytase as an alternative to inorganic phosphate supplementation to pig feed used in intensive pig production. *Int. J. Life Cycle Assess* 12, 514.

OECD (2001) The Application of Biotechnology to Industrial Sustainability. Organisation for Economic Co-operation and Development Publications. *Sustainable Development*, France.

Okamura-Matsui T., Tomoda T., Fukuda S., Ohsugi M. (2003) Discovery of alcohol dehydrogenase from mushrooms and application to alcoholic beverages. *J. Mol. Catal. B Enzym.* 23, 133.

Olguin E.J. (1999) Cleaner bioprocesses and sustainable development. In: Olguin E.J., Sanchez G., Hernadez E. (Eds) Environmental Biotechnology and Cleaner Bioprocesses, Taylor and Francis, Boca Raton, pp. 3-18.

Osborne-Lee I., Ajiboye G., Deason J. (2008) Economic and environmental impact assessment of alpha-naphthol production by traditional and enzymatic routes. In: Proceedings of AIChE Annual Meeting, 16 21 Nov, Philadelphia.

Oxenbøll K., Ernst S. (2008) Environment as a new perspective on the use of enzymes in the food industry. *Food Sci. Technol* 22, 45 47.

Oxenbøll K.M., Li Y., Axelsen A.G. (2010) Life Cycle Assessment of Using Novamyl Steam for Industrial Production of Steamed Bread in China. Novozymes A/ S, Krogshoejvej 36, 2880 Bagsvaerd, Denmark.

Oxenbøll K.M., Pontoppidan K., Fru-Nji F. (2011) Use of a protease in poultry feed offers promising environmental benefits. *Int. J. Poult. Sci* 10, 842.

Penciu O.M., Gavrilescu M. (2004) Biodegradation - Innovative technology for treating gaseous fluxes containing VOCs. *Environmental Engineering and Management Journal* 3, 737.

Penciu O.M., Ungureanu F., Gavrilescu M. (2004) Modelling and simulation of three phase bioreactors applied to the depollution of gaseous streams containing volatile organic compounds - A comparison between fixd bed and fluidized bed reactors. *Environmental Engineering and Management Journal* 3, 177.

Penciu O.M. and Gavrilescu M. (2003) Survey on the Treatment of Gaseous Streams Containing Volatile Organic Compounds, *Environmental Engineering and Management Journal* 2, 77.

Poechlauer P., Braune S., De Vries A., May O. (2010) Sustainable route design for pharmaceuticals. *Chemistry Today* 28, 14.

Poindexter J.S., Pujara K.P., Staley J.T. (2000) In situ reproductive rate of freshwater *Caulobacter spp. Applied Environmental Microbiology* 66, 4105.

Rabus R., Wilkes H., Behrends A., Armstroff A., Fischer T., Pierik A.J., Widdel F. (2001) Anaerobic initial reaction of n-alkanes in a denitrifying bacterium: evidence for (1-methylpentyl)succinate as initial product and for involvement of an organic radical in n-hexane metabolism. *J. Bacteriol.* 183, 1707.

Ramos O.S., Malcata F.X. (2011) Food-grade enzymes. In: Moo-Young M., Butler M., Webb B.C., Moreira A., Grodzinski B., Cui Z.F., Agathos S. (Eds.), Comprehensive Biotechnology, second ed. Academic Press, Burlington.

Reddy C.S.K., Ghai R., Rashmi Kalia V.C. (2003) Polyhydroxyalkanoates: an overview. *Bioresource Technology* 87, 137.

Riser-Roberts E. (1998) Remediation of petroleum contaminated soils, biological, physical and chemical processes, Lewis Publishers, CRC Press, pp. 542.

Rittmann B.E. (2006) Microbial ecology to manage processes in environmental biotechnology. *Trends Biotechnol.* 24, 261.

Rittmann B.E., Hausner M., Loffler F., Love N.G., Muyzer G., Okabe S., Oerther D.B., Peccia J., Raskin L., Wagner M. (2006) A vista for microbial ecology and environmental biotechnology. *Environ. Sci. Technol.* 40, 1096.

Rockne, K.J., Chee-Sanford J.C., Sanford R.A., Hedlund B.P., Staley J.T. (2000) Anaerobic naphthalene degradation by microbial pure cultures under nitrate-reducing conditions. *Appl. Environ. Microbiol.* 66, 1595.

Russell D.L. (2006) Practical Wastewater Treatment, John Wiley & Sons, Hoboken, New Jersey, pp.288.

Saeki K., Ozaki K., Kobayashi T., Ito S. (2007) Detergent alkaline proteases: enzymatic properties, genes, and crystal structures. *J. Biosci. Bioeng.* 103, 501.

Sanz J.L., Kochling T. (2007) Molecular biology techniques used in wastewater treatment: An overview. *Process Biochemistry* 42, 119.

Saratale R.G., Saratale G.D., Kalyani D.C., Chang J.S., Govindwar S.P. (2009) Enhanced decolorization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium GR. *Bioresource Technology* 100, 2493.

Sasaki T., Kajino T., Li B., Sugiyama H., Takahasi H. (2001) New pulp biobleaching system involving manganese peroxidise immobilized in a silica support with controlled pore sizes. *Applied and Environmental Microbiology* 67, 2208.

Sasikumar C.S., Papinazath T. (2003) Environmental Management: Bioremediation of Polluted Environment, In: Bunch M.J., Suresh V.M., Kumaran T.V. (Eds) Proceedings of the Third International Conference on Environment and Health,

Chennai, India, 15-17 December, Department of Geography, University of Madras and Faculty of Environmental Studies, York University, pp. 465-469.

Saval S. (1999) Bioremediation: Clean-up biotechnologies for soils and aquifers. In: Olguin E.J., Sanchez G., Hernadez E. (Eds) Environmental Biotechnology and Cleaner Bioprocesses, Taylor and Francis, Boca Raton, pp. 155-166.

Schlegelmilch M., Streese J., Biedermann W., Herold T., Stegmann R. (2005) Reducing odorous emissions from biowaste composting plants by means of biological waste gas treatment systems. Proceedings (CD-ROM) of Sadinia 2005 - 10th International Waste Management and Landfill Symposium. Cossu R., Stegmann R. (Eds) Session G4: Odour analyses, Cagliari, Italy, 3-7 October 2005, CISA, Environmental Sanitary Engineering Centre, Italy.

Skals P.B., Krabek A., Nielsen P.H., Wenzel H. (2008) Environmental assessment of enzyme assisted processing in pulp and paper industry. *Int. J. Life Cycle Assess* 13, 124.

Smith L.A., Alleman B.C., Copley-Graves L. (1994) Biological treatment options. In: Means J.L., Hinchee R.E. (Eds) Emerging Technology for Bioremediation of Metals, Lewis Publishers, New York, pp.1-12.

Soetaert W., Vandamme E.J. (2010) The scope and impact of industrial biotechnology. In: Soetaert,W., Vandamme, E.J. (Eds.), Industrial Biotechnology. WILEYVCH Verlag GmbH and Co, Weinheim, pp. 1-16.

Soetaert W., Vandamme E. (2006) The impact of industrial biotechnology. *Biotechnol. J. 1,* 756.

Strous M, Fuerst J.A., Kramer E.H.M., Logemann S., Muyzer G., Pas-Schoonen K.T.V.D., Webb R., Kuenen J.G., Jetten M.S.M. (1999) Missing lithotroph identified as new planctomycete. *Nature* 400, 446.

Sukla L.B., Panchanadikar V. (1993) Bioleaching of lateric nickel ore using a heterotrophic microorganism. *Hydrometallurgy* 32, 373.

Tang W.L., Zhao H. (2009) Industrial biotechnology: Tools and applications. *Biotechnol. J.* 4, 1725.

Thum O., Oxenbøll K.M. (2008) Biocatalysis e a sustainable method for the production of emollient esters. *Int. J. Appl. Sci* 134, 44.

Trejo M., Quintero R. (1999) Bioremediation of contaminated soils. In: Olguin E.J., Sanchez G., Hernadez E. (Eds) Environmental Biotechnology and Cleaner Bioprocesses, Taylor and Francis, Boca Raton, pp. 179-190.

Tuzen M., Saria A., Mendil D., Uluozlu O.D., Soylak M., Dogan M. (2009) Characterizaion of biosorption process of As(III) on green algae *Ulothrix cylindricum. Journal of Hazardous Materials* 165, 566.

USEPA, (1994) Assessment and Remediation of Contaminate Sediments (ARCS) Program., Final Summary Report, EPA-905-S-94-001, USEPA, Chicago.

Van Hamme J.D., Singh A., Ward O.P. (2003) Recent Advances in Petroleum Microbiology. *Microbiology and Molecular Biology Reviews* 67, 503.

Vigsoe, D., Jürgensen, E., Kvistgaard, M. (2002) The Assessment of Future Environmental and Economic Impacts of Process-Integrated Biocatalysts. European Commission, Joint Research Centre. Institute for prospective technological studies.

Weiß M., Brinkmann T., Groger H. (2010) Towards a greener synthesis of (S)-3-aminobutanoic acid: process development and environmental assessment. *Green Chem.* 12, 1580.

Woodley J.M. (2008) New opportunities for biocatalysis: making pharmaceutical processes greener. *Trends Biotechnol.* 26, 321.

Xu T., Chen C., Liu C., Zhang S., Wu Y., Zhang P. (2009) A novel way to enhance the oil recovery ratio by Streptococcus sp. BT-003. *Journal of Basic Microbiology* 49, 477.

Yon-Miaw S. (2011) Barley brewing e an opportunity for choice and sustainability. In: Brewing Conference, 15 June, Bangkok.

Zengler K., Heider J., Rosello´-Mora R., Widdel F. (1999) Phototrophic utilization of toluene under anoxic conditions by a new strain of Blastochloris sulfoviridis. *Arch. Microbiol.* 172, 204.

Zhou Y., Nie H., He Z., Zhu L., 2008. Immobilization of dual enzyme systems on magnetic chitosan nanoparticles for textile industry. *J. Biotechnol.* 136, S307.

Zhu D., Wu Q., Wang N. (2011) Industrial enzymes. *Compr. Biotechnol.* 3, 3.

Zhou J., Fries M.R., Chee-Sanford J.C., Tiedje J.M. (1995) Phylogenetic analyses of a new group of denitrifiers capable of anaerobic growth on toluene and description of Azoarcus tolulyticus sp. nov. *Int. J. Syst. Bacteriol.* 45, 500.

❐❐

BIOMETALLURGY: GREENER TECHNOLOGY FOR MINERAL RECOVERY FROM WASTES

A.P. Das[1*], S. Ghosh[1], S. Mohanty[1], L.B. Sukla[2]

[1]Centre of Biotechnology, Bioengineering and Bio-mineral Processing Laboratory

Siksha O Anusandhan University, Bhubaneswar 751003, India

[2]Institute for Applied Environmental Biotechnology Innovation, Bhubaneswar

[*]Corresponding Author: alok1503@gmail.com

Abstract

The abilities of microorganisms to accelerate the oxidative dissolution of minerals have been exploited in the progress and application of biotechnology for extracting metals from mining effluents. Biometallurgy process is presently used mostly to leach copper sulphides and as an oxidative pre-treatment method for refractory gold ores, though it is also used to recover other base metals, such as manganese, cobalt, nickel and zinc. Modern developments for the selective recovery of heavy metals from waste water treated with minerals or to extract metals from oxidized low grade ores and electronic waste includes use of acidophilic microorganisms. This encapsulation signifies a physical recovery of the metal ion via conventional methods for recovery of heavy metals. On contrast, the biometallurgy research look for to add a better scope in framing environmental friendly development with regards to hydrometallurgical process in future and create a certified technology for the benefit human beings. However this technology has developed over the past 50 years, and relates the challenges and opportunities for mineral biotechnological industries for economic recovery on a large scale. This review illustrates the involvement of commercial biometallurgical operations concerning diverse group of microorganism and mechanism of mineral solubilisation.

Keywords: Biometallurgy, Bioleaching, Microbe interaction, Extraction, Reuse

1. Introduction

Biometallurgy is a more general term that is used to refer technologies utilizing biological systems mainly to the application of microbial process in the mining industry to facilitate the extraction and recovery of metals from ores and waste materials (Brierley & Brierley, 2012; Rawlings, 2002; Das & Mishra, 2010; Rawlings et al., 2007). The term biometallurgy ideologically relates with bioleaching, biomining processes which refers to situations where the target metals are solubilized during bio-processing. Researchers in diverse countryside have explored the application of biotechnology in mining over the last 40 years, in some commercial engineered systems that can be considered under the term 'biometallurgy'. Biometallurgy or bioleaching is the interaction between metals ions and cultivable microbes with specific aim of converting insoluble metal sulphides to soluble metal sulphates.

Biomining is considered as a generalized term that relates both bioleaching and biooxidation to metal ores and compounds in functioning essential application of microbial process in the mining industry. Bio-oxidation technique is another closely related method to bioleaching/ biomining that explains the microbial oxidation of metals which include recovery of metal of interest (Das et al., 2012). Microorganisms are used to remove minerals targeting valuable metals which are being solubilized by extracting with cyanide or thiosulphate as in the case of gold recovery from arsenopyrite ores (Rawlings & Johnson, 2007).An estimated 15% of copper, 5% of gold, 20% of Mn and some other Cr heavy metal (Mohanty et al., 2014) by biometallurgy recovering process and smaller amounts of other metals (such as nickel and zinc) are presently produced using biometallurgy technology (Brierley et al., 2013).

In mining industries, pyrometallurgical technologies (roasting or smelting ore) have been precisely used as often major investments though others use it by non-biological innovations technique or by pressure leaching (Berezowsky et al., 1991).The mentioned characteristics describe by biometallurgy process to attain economic height of metal extraction depend on time period

which ranges from days, in the case of stirred tanks concerning with the biological system. The recovery of metals by biometallurgy is supposed to be much more environmentally benign involving less energy supplying a detailed analysis of major proposed processes using various applied techniques. On the other hand, the latter statement is justified in that the main microorganisms involved in mineral oxidation processes are autotrophs, which distinguish with smelting procedure emitting huge amounts of CO_2. Bioprocessing also work at atmospheric pressure and at moderately low temperatures (20^0C-80^0C). Though it is an exothermal process, it doesn't need external heat, indeed it generates excess of heat increasing the rate of oxidation intensity as operated in stirred tank and technical systems need to be cooled maintaining appropriate temperatures (40^0C -45^0C) for the mineral degradation via microorganisms (Rawlings et al., 2003; Olsonet et al., 2003).

However, present procedure of biometallurgy still depends on the blasting and grinding of ore particles, which is considered to consume about 5% of total global energy production.It was thus highly desirable to develop a novel process so-called bio-processing which has advantage in containing major quantities of arsenic released during smelting emissions but retained in liquid and solid phases in bio-hydrometallurgical processing and for processing low-grade or polymetallic ores (Brierley, 2008). Biometallurgy also permits metal by-products to the readily recover metals from processed ores with waste slags generated by pyrometallurgy (Johnson & Hallberg 2009). The first documented biometallurgy operation which was set up within 20 years of the discovery of biometallurgy technologies (Temple et al., 1951) observed the first demonstrated bacterial species to oxidise the pyrite and other base metal containing sulphide raw materials in acidic medium (Bryner et al., 1958). The same procedure had been used to extract metals at mine sites in China, Spain, and United Kingdom for several years using *in situ* bioleaching (Wang et al., 2011; Rawlings 2002; Johnson et al., 2010). Targeted biomineralization process is used in some active and abandoned mine sites to isolate microbial diversity for

recovering valuable metals from processed waste water sludge.

Low grade manganese ore deposits are usually of sedimentary origin, with oxide ore layers interbedded with iron-rich formations. The manganese ores are mostly low to medium grade type with low phosphorous content in contrast to the other manganese deposits in Odisha. Over the last few decades, selective mining of better grade manganese ores from this area has generated a huge quantity of low grade ores which is considered as mining waste as deposited in Sanindipur Manganese mines of National enterprises of Bonai Tahasil in Sundargh district of Barbil in Odisha, India (Figure 1).

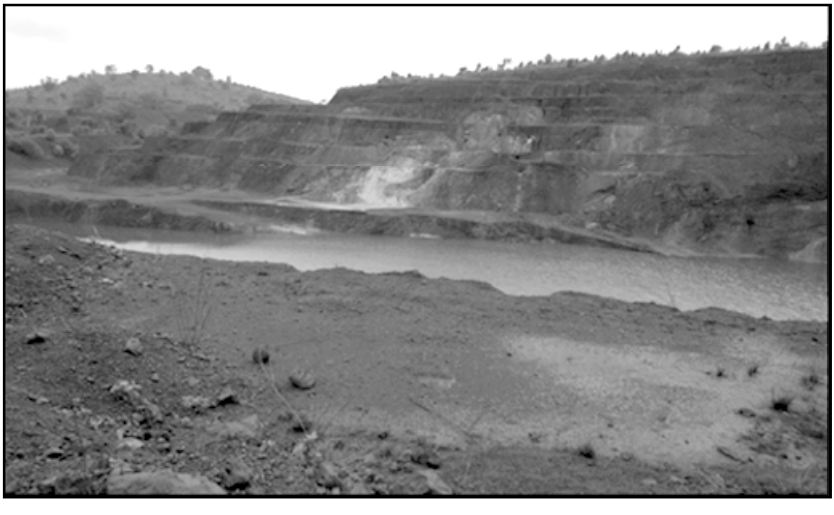


Fig. 1: Low grade ore deposits at mining sites.

The recent biotechnology with new application was established in the 1960s by the Kennecott Copper Corporation to extract copper from waste rock dumps at the Bingham Canyon mine in Utah, and later at the Chino mine in New Mexico (Olson et al., 2003; Johnson et al., 2010). Related species of *Thiobacillus ferrooxidans,* and *Thiobacillus ferridurans* grow autotrophically by oxidizing ferrous iron, elemental sulphur and various reduced forms of sulphur, or hydrogen (Johnson and Hallberg 2009). The

capability of the iron-oxidizing *Acidithiobacilli species* to produce both ferric ion, oxidative sulphide mineral and sulphuric acid creates an acidic tolerating environment and accelerate mineral dissolution. The excessive acidity environment causes the majority of metals released from the degraded sulphide minerals to be retained in solution (Das et al., 2015a). The traditionally bioleaching operations, which have been replicated in many other 'dump leaching' operations (Wu et al., 2008), involved stacking waste rock into huge mounds. Bioleaching recommends various attractive characteristics, being cost efficient, and more environmentally friendly compared to their chemical counterparts, which may involve the utilization of tough chemicals under elevated temperature conditions (Das et al., 2011).

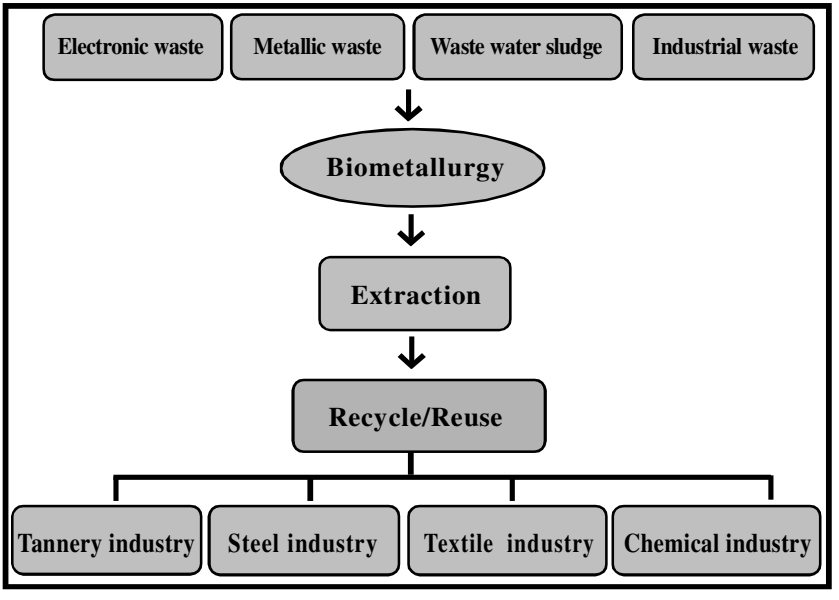


Fig. 2: Schematic diagram of waste recycling by biometallurgy process.

2. The Microbiology of Biometallurgy

The biomining relevant for oxidizing bacteria and archaea with high efficiency of recovering metals are mostly extremophiles

(can survive in extremes of temperature, salinity or acidity). Some species of mesophilic bacteria also survive with temperature ranging from 20-50°C. However most hyperthermophilic species of biomining organisms tend to be achaea (Mikkelsen et al., 2006), which can live at temperatures up to 90°C.These microbial species present some advantages over enhanced reaction speeds, with apparent cost benefits to companies increasingly looking for to develop yields from low grade ores. Utilization of leaching additives and assistance to extreme physico-chemical nature of bioleach liquors at low pH, elevates the concentration of toxic heavy metals, metalloids and other solutes, and highly positive redox potential (Eh values exceeding +900 mV) concluding that they are extremely toxic to the majority of life forms, including microorganisms. However pure cultures of bacteria or archaea are supposed to degrade sulphide elements, the method of bioleaching and bio-oxidation is highly conventional in biometallurgy operations mediated by acidophilic prokaryotes (Rawlings et al., 2007).

Biomining organisms have been categorized as first ferric iron generating autotrophs (primary prokaryotes) producing mineral oxidant and sulphuric acid-generating autotrophs (secondary prokaryotes), which maintain the low pH environment required for degrading organic compounds. Biometallurgy can function over a wide range of temperature as acidophilic mineral-degrading prokaryotes differ significantly in the range of optimised temperature at which they grow and survive (Dopson et al., 2006; Norris et al., 2013). It is known that the formation of extracellular polymeric substances plays an important role in the attachment of thiobacilli to mineral surfaces e.g., sulphur, pyrite, or covellite. Extraction or loss of these exo-polymers prevents cell attachment resulting in decreased metal leaching efficiencies (Escobar et al., 1997; Gehrke et al., 1998). It was concluded that a direct contact between bacterial cells and solid surfaces is needed and represents an important prerequisite for an effective metal mobilization (Ostrowski&Sklodowska, 1993). Interactions between microorganisms and the mineral surface occur on two levels (Barrett et al.,1993).

Metal recovery from aqueous stream by the process of biosorption and bioaccumulation has gained attention recently and is a very economic process for removal of metals. These processes involve ion exchange method where the metal ion is exchanged for a counter-ion attached to biomass. The ability to suitably transform Mn (II) to either Mn (III) or Mn (IV) has been found in a various isolated groups of bacteria including Proteobacteria, Actinobacteria, and Firmicutes (Tebo et al., 2008) and from diverse environments (Templeton et al., 2005). Bacteria capable of oxidizing Mn (II) have been isolated from diverse phylum, the physiological role of Mn oxidation is still unclear. The pathway for biotic manganese oxidation is an aerobic process demanding presence of oxygen. Among the prokaryotes, the ability to oxidize Mn is also quite widespread including members of many phylogenetic and physiological groups, e.g., cyanobacteria strains related to *Pseudomonas* species.

Bioleaching or biometallurgy has been investigated for the extraction of valuable metals from various solid industrial wastes including fly ash, water sewage sludge, spent batteries, tannery and electronic scrap materials, which can provide as derived raw materials for important metals(Bogdanor et al., 1986; Aung et al., 2005). (Table 1) lists some previous reports microbial species for bioleaching.

Table 1. Microbial Diversity of leaching species.

Domain	Organism	Site	Leaching Agent	Reference
Bacteria	Stilbellaaciculosa Pithomyceschartarum	Coal Mine Drainage	Manganese	Santelli et al., 2010
	Staphylococcus epidermidis	Low grade ore	Mn	Das et al., 2012
	Leptospirillumferrooxidans Acidithiobacillusferrooxidans	Polymetallic Sulphide Ore	Gold	Spasova et al., 2008
	Acidithiobacillusthiooxidans Thiobacillusprosperu	Waste water sludge	Copper	Donati& Sand 2007
	Bacillus megaterium	Wetlands	Chromium	Basu et al., 2014
	Enterobacteragglomerans	Low grade ore	Iron	Rossi 1990
Fungi	Aspergillusniger Aspergillusterreus	Uraniferous sedimentary rocks	Uranium	Amin et al., 2013
	Bacillus subtilis Cladosporiumresinae	Sewage sludge.	Copper and Zinc	Narasimhulu et al., 2012
	Paraphaeosphaeria sp. Coniothyriumsp.	Aquatic environments	Manganese	Takano et al., 2006
	Penicilliumsimplicissimum	Spenthydrocracking catalyst	Tungsten	Amiri et al., 2011

3. Mechanism of Bacterial Oxidation

An indiscriminate reaction of the biological oxidation involved in leaching of a mineral sulphide is two major mechanisms involved in microbial metal solubilization of sulphide minerals. One is a direct mechanism that involves physical contact of the organism with the insoluble sulphide. Microorganisms oxidize the metal sulphides obtaining electrons directly from the reduced minerals. Another mechanism, involves the ferric-ferrous cycle. The oxidation of reduced metals is mediated by the ferric (III) ion and this is formed by microbial oxidation of ferrous (II) ion present in the minerals. Ferric (III) ion acts as an oxidant and oxidizes metal sulphides and is reduced to ferrous (II) ion that, in turn, can be microbially oxidized. Both direct and indirect mechanisms of bacterial leaching are shown schematically in Figure 3.

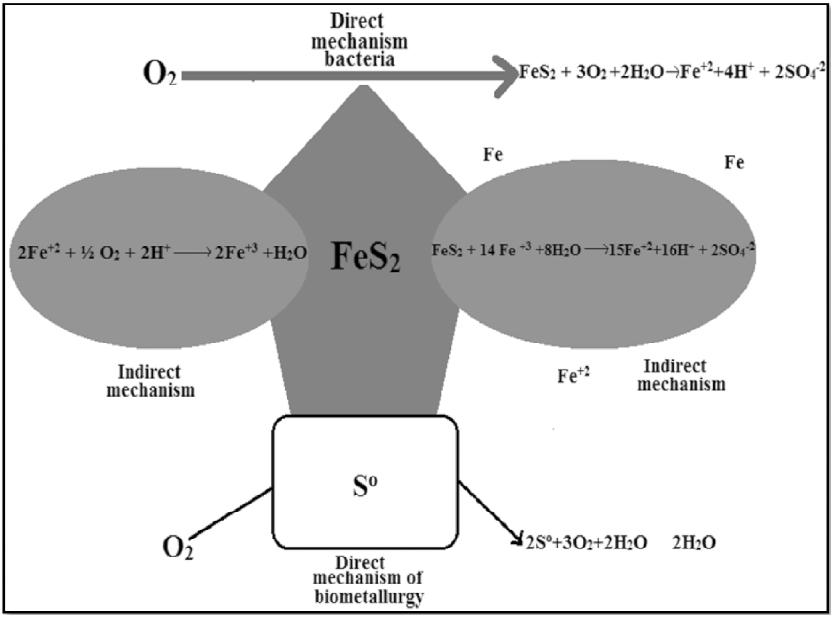


Fig. 3: Schematic digram presenting direct and indirect mechanism of pyrite biometallurgy.

The significance of the occurrence and attachment of microbial species and their dynamic involvement has been confirmed for the leaching of heavy metals (Das et al., 2014). In many cases it was concluded that the direct mechanism dominates over the indirect due to the fact that direct is equated with direct physical contact. The oxidation of pyrite in studies utilizing mesophilic and thermophilic bacteria such as *T. ferrooxidans* and *Acidianus brierleyi* in bioreactors which consisted of chambers separated with dialysis membranes to avoid physical contact (Tian et al., 2010).In most cases, bio- leaching witnessed nearly about 89% for extraction of Mn. Acidic leaching could significantly recover extraction of Mn by addition of inorganic compounds such as H_2O_2, SO_2 or organic compound such as glucose, sucrose, lactose, oxalic acid, citric acid, tartaric acid, formic acid and triethanolamine (Sayilgan et al., 2009).

Research in the field of biometallurgy is still continuing to solve the inhibitory effect of copper recovery in a heap leaching by biological methods. Use of extreme thermophiles in bioleaching is making a leading progress to solve the mystery behind the scaling up technique, which could be possible to be solved in future. Bioleaching with other sulphide minerals together with Acid Mine Drainage (AMD), which is a serious concern today, is taking figure to perform the inadequate needs of the mankind.

Conclusions

Biometallurgy is well established as a novel technology for extracting metals from low-grade and polymetallic metal ores by most widely used acidophilic iron-oxidizing bacteria were utilized for bioleaching. In order to further demonstrate the role of acidophilic organisms in biometallurgy process the and environmental constraints become more demanding to recover and recycle metals from electronic and other metallic wastes while in situ biometallurgy could permit metal compounds to be economically exploited in recent years. Although several unresolved challenges in recovering precious metals conventionally by biometallurgy process, such as bioleaching chalcopyrite and using brackish and saline water for bio-processing minerals suggest

that new opportunities for developing biotechnological techniques in the mining and mineral sectors will emerge in the near future. Thus biometallurgy or biomining could be significantly beneficial for them use of synthetic consortia (of naturally occurring species) for economic recovery of metals on a big scale.

References

Amiri F., Yaghmaei S., Mousavi S.M. (2011) Bioleaching of tungsten-rich spent hydrocracking catalyst using *Penicilliumsimplicissimum*. Bioresour. Technol. 102, 1567-1573.

Amin M.M., Elaassy I.E., El-feky M.G., Sallam A.M., Talaat M.S., Kawady N.A. (2013) Bioleaching of uranium by *Aspergillusniger* and *Aspergillusterreus* isolated from uraniferous sedimentary rocks, southwesternsinai, Egypt. Romanian J. Biophys. Vol. 23, No. 4, P 231-247.

Aung K.M.M., Ting Y.P. (2005) Bioleaching of spent fluid catalytic cracking catalyst using *Aspergillusniger*. J. Biotechnol. 116, 159-170.

Behera K.B., Panda P.P., Saini S.K., Pradhan N., SuklaL.B., Mishra B.K. (2013) Recovery of nickel from chromite overburden, Sukinda using *Aspergillusniger* supplemented with manganese.

Basu S., Dasgupta M., &Chakraborty B. (2104) Removal of Chromium (VI) by *Bacillus subtilis* isolated from east calcutta wetlands, West Bengal, India. Int J BioscBiochBioin, Vol. 4, No. 1.

Brierley C.L., Brierley J.A. (2013) Progress in bioleaching. Part B. Applications of microbial processes by the minerals industry. ApplMicrobiolBiotechnol. 97:7543-7552.

Brierley C.L.(2008) How will biomining be applied in future? Trans Nonferrous Met Soc. China, 18:1302-1310.

Brierley J.A. (2008) A perspective on developments in biohydrometallurgy. Hydrometallurgy 2008, 94:2-7.

Berezowsky R.M.G.S., Collins M.J., Kerfoot D.G.E., Torres N. (1991) The commercial status of pressure leaching technology. J Min Met Mater Soc. 43:9-15.

Bryner L.C., Jameson A.K. (1958) Microorganisms in leaching sulphide systems. ApplMicrobiol. 6:281-287.

Bogdanor J.M., Rase H.F. (1986) Characteristics of a commercially aged nickel molybdenum-alumina hydrotreating catalyst: component distribution, coke characteristics, and effects of regeneration. Ind. Eng. Chem. Prod. Res. Dev. 25, 220-230.

Donati E.R., Sand W. (2007) Microbial processing of metal sulphides. ISBN 978-1-4020-5589-8.

Das AP., Mishra S. (2010) Biodegradation of the metallic carcinogen hexavalent chromium Cr (VI) by an indigenously isolated bacterial strain. J Carcinog. 9:6.

Das A.P., Sukla L.B., Pradhan N., Nayak S. (2011) Manganese biomining: A review. Bioresour Technol. 102, 7381-7387.

Das A.P., Ghosh S., Mohanty S., Sukla L.B. (2015a) Advances in Manganese pollution and its bioremediation. Environmental Microbial Biotechnology. Soil biology, Springer (In Press).

Das A.P., Ghosh S., Mohanty S., Sukla L.B. (2014) Consequences of manganese compounds: a review. Toxicol Environ Chem. Volume 96, Issue 7.

Das A.P., Sukla L.B., Pradhan N. (2012) Microbial Recovery of Manganese Using *Staphylococcus epidermidis*. Int J Non Met. 1, 9-12.

Dopson M., Halinen A.K., Rahunen N., Ozkaya B., Sahinkaya E., Kaksonen A.H., Lindstrom E.B., Puhakka J.A. (2006) Mineral and iron oxidation at low temperatures by pure and mixed cultures of acidophilic microorganisms. BiotechnolBioeng. 2006, 97:1205-1215.

Johnson D.B., Hallberg K.B.(2009) Carbon, iron and sulphur

metabolism in acidophilic micro-organisms. AdvMicrob Physiol. 54:202-256.

Johnson D.B. (2010) The biogeochemistry of biomining. In Geomicrobiology: Molecular and Environmental Perspective. Edited by Barton L, Mandl M, Loy A. Heidelberg, Germany: Springer. 401-426.

Mohanty S., Bal B., Das A.P. (2014) Adsorption of hexavalent chromium onto activated Carbon. Austin Journal of Biotechnology & Bioengineering.

Mikkelsen D., Kappler U., McEwan A.G., Sly L.I.(2006) Archaeal diversity in two thermophilic chalcopyrite bioleaching reactors. Environ Microbiol. 8:2050-2055.

Narasimhulu K., Setty Y.P. (2012) Biosorption of copper from wastewater by Bacillus subtilis in a packed bed bioreactor. J Biodiver Environ Scie (JBES) Vol. 2, No. 11, p. 23-30.

Norris P.R., Burton N.P., Clark D.A. (2013) Mineral sulphide concentrate leaching in high temperature bioreactors. Miner Eng. 48:10-19.

Olson G.J., Brierley J.A., Brierley C.L. (2003) Bioleaching review. Part B. Progress in bioleaching: applications of microbial processes by the minerals industry. ApplMicrobiolBiotechnol. 63:249-257.

Rawlings D.E. (2002) Heavy metal mining using microbes. Annu Rev Microbiol. 56:65-91.

Rawlings D.E., Dew D., du Plessis C. (2003) Biomineralization of metal-containing ores and concentrates. Trends Biotechnol. 21:38-44.

Rawlings D.E., Johnson D.B. (2007) (Eds): Biomining. Hedelberg, Germany: Springer-Verlag.

Rawlings D.E., Johnson D.B. (2007) The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia. Microbiol. 153:315-324.

Rossi G.(1990) Biohydrometallurgy. McGraw-Hill, Hamburg.

Spasova I., Nicolova M., Veglio F., Groudev S. (2006) Leaching of gold from a polymetallic sulphide ore. Geology and Geophysics. Vol. 49.

Santelli C.M., Pfister D.H., Lazarus D., Sun L., Burgos W.D., Hansel C.M. (2010) Promotion of Mn(II) Oxidation and Remediation of Coal Mine Drainage in Passive Treatment Systems by Diverse Fungal and Bacterial Communities. Appl Environ Microb p. 4871-4875.

Sayilgan E., Kukrer T., Civelekoglu G., Ferella F., Akcil A., Veglio F., Kitis M.(2009) A review of technologies for the recovery of metals from spent alkaline and zinc-carbon batteries. Hydrometallics. 97, 158-166.

Takano K., Itoh Y., Ogino T., Kurosawa K., Sasaki K. (2006) Phylogenetic analysis of manganese-oxidizing fungi isolated from manganese-rich aquatic environments in Hokkaido, Japan. Limnology 7:219-223 doi: 10.1007/s10201-006-0177-x

Tian X., Wen X., Yang C., Liang Y., Pi Z., Wang Y. (2010). Reductive leaching of manganese from low-grade manganese dioxide ores using corncob as reductant in sulfuric acid solution. Hydrometallurgy. 100(3-4), 157-160.

Templeton A.S., Staudigel H., Tebo B.M., (2005). Diverse Mn (II)-oxidizing bacteria isolated from submarine basalts at Loihi Seamount. Oeomicrobiol J. 22, 127-139.

Wang D., Qui G., Liu X. (2011) The progress of biohydrometallurgy in China. In Biohydrometallurgy: Biotech Key to Unlock Mineral Resources Value. Edited by Qui G, Jiang T, Qin W, Liu X, Yang Y, WangH. Changsha, China: Central South University Press; 13-20.

Wu A., Yin S., Wang H., Qin W., Qiu. (2008) Technological assessment of a mining-waste dump at the Dexing copper mine, China, for possible conversion to an in situ bioleaching operation. Bioresour Technol. 100:1931-1936.

❐❐

A REVIEW ON USE OF GOLD NANOPARTICLES FOR DETECTION OF TOXIC METAL POLLUTANTS IN WATER

E. Priyadarshini[1,2*] and N. Pradhan[2]

[1]Ph.D. Scholar, AcSIR, CSIR-Institute of Minerals and Materials Technology Bhuabneswar-751013

[2]Bioresources Engineering Department, CSIR-Institute of Minerals and Materials Technology, Bhubaneswar 751013, India

[*]Corresponding Author: lisa.eepsita@gmail.com, eepsita03@immt.res.in

Abstract

Gold nanoparticles because of their intriguing optical and electrochemical properties have been of paramount interest in sensing and detection applications. During the last decade researchers have greatly focused on the fabrication and functionalization of gold nanoparticles so as to enable target specific detection of toxic contaminants and metal ions. Compared to traditional used detection techniques, nanotechnology, specifically the use of gold nanoparticles as sensors presents a rapid and cost-effective solution towards the detection of toxic heavy metal ions. Additionally, it overcomes the use of costly instrumentation and lengthy protocols making on-site analysis feasible. The review discusses the synthesis and functionalization of gold nanoparticles and the current state of applicability of gold nanoparticles as sensors for detection of metal ions in aqueous system.

Keywords: Toxic metal ions, Gold nanoparticles, Colorimetric, Biosensors, Detection

1. Introduction

With rapid globalization, industrialization has been of prime focus as it significantly contributes to the financial backbone of the country. One of the major problems associated with industrialization is environmental pollution, contributed by the

improper disposal and dumping of industrial effluents in nearby water bodies that posses a significant threat to human health. A major issue being faced today is the scarcity of pure drinking water and contaminated water supply that is responsible for the widespread infectious diseases thereby impeding sustainable development of society. Hence, there is an immense need to monitor the presence of toxic chemical compounds and heavy metals in water bodies and uphold the supply of clean drinking water. Recently, numerous physical, chemical and biological approaches have been used to facilitate sensitive detection of toxic metal ions in water bodies. Nanotechnology has also significantly contributed in the field of environmental monitoring, wherein metal nanoparticles have emerged as sensing probes for detecting metal ions (Wang et al., 2010; Wang and Ma 2009; Wang and Yu, 2013). Since then, nanotechnology based sensing approach is gaining attention and has provided promising advancements in the detection of various types of pollutants present in water. Nanoparticles possess intriguing properties compared to their bulk counterparts, owing to the physical confinement of electrons and large surface to volume ratio of particles at nanoscale (Eustis and El-Sayed, 2000).

Amongst metal nanoparticles, gold nanoparticles (GNP) have gained tremendous interest in the area of sensing and detection because of its unique optical properties and molecular recognization potential. The fascinating optical property of GNPs arises because of the phenomenon termed as Surface Plasmon Resonance (SPR). At nanoscale, the surface electrons undergo a coherent and collective oscillation when irradiated with light of a particular wavelength, the frequency of which resonant with the visible region of electromagnetic radiation (Atwater, 2007; Ozbay 2006). Any alteration in the size, shape, aggregation state or dielectric constant of the medium leads to alteration of the electron charge density and change in the resonant of a particular light wavelength. This is responsible for the variation in colour of GNPs colloidal solution and corresponding shift in SPR peak position (Wang and Yu, 2012; Wang et al., 2010). This phenomenon form the basis of colorimetric sensing systems, wherein binding

of the target molecule/compound to functionalized GNP induces a change in colour of the colloidal GNP solution and corresponding SPR peak shift. Additionally, GNPs offer the advantages of high stability and easy surface functionalization enabling conjugation to target specific-ligands molecules. Researchers are gaining renewed interest towards utilizing GNPs as effective probes for detection of various chemicals, toxic compounds and metal ions. These advantages of GNPs have made them emerge as excellent detection probes, in form of biosensors.

In this review we discuss the potential applicability of functionalized GNPs as nanosensors. More specifically, we focus on the use of GNPs as sensing agents to detect toxic metal ions and contaminants in water bodies. The unique optical properties of GNPs favour their exploration as colorimetric detection agents. Briefly, the initial sections discuss the synthesis and functionalization of GNPs focusing on the need of conjugating target specific ligand molecules prior to detection. The latter section focuses on the use of the functionalized GNPs as sensors to detect toxic metal ions.

2. Synthesis and Functionalization of Gold Nanoparticles (GNPs)

During the last decade, synthesis and fabrication of nanoparticles has been an important area of interest amongst researchers because of their diverse application in electronics, photonics, detection, imaging, catalysis and biomedicines (Satuby and Orenstein, 2007; Herderick et al., 2007; Huang et al., 2006; Wang and Yu, 2013; Durr et al., 2007). The special properties and resultant use exhibited by nanoparticles has led to the exploration of every possible aspect for nanoparticle synthesis giving way to a limitless number of synthesis methods. Numerous methods have been developed to synthesize both isotropic and anisotropic particles focusing on controlled size and shape (Melosh et al., 2003; Zhao et al., 2004; Ahmadi et al., 1996). Basically three different approaches are used to synthesize metal nanoparticles, namely physical, chemical and biological methods. While physical and chemical methods are the traditional and

popularly used methods, biological method represents an eco-friendly and green technological method of synthesis (Priyadarshini et al., 2014). These methods generally follow a bottom-up approach of synthesis wherein the metal salt is reduced to form metal atoms, which then form small nuclei particles. Subsequent growth of these nuclei particles by the deposition of reduced metals ions result in production of metal nanoparticles which thereby are capped by stabilizing agents leading to production of nanoparticles of defined size and shape (Wang and Yu, 2013). Nanoparticles synthesized by physical and chemical methods require supplementation of additional stabilizing molecules that stably cap the synthesized particles, preventing aggregation of particles among themselves and assist in efficient synthesis of colloidal nanoparticle solution. However, biological synthesis does not require supplementation of additional capping molecules since the biological/microbial (fungus, bacteria, algae, yeast) extracts contain both reducing and stabilizing agents. Certain biomolecules also serve the dual purpose of reduction and stabilization, thereby helping in synthesis of ready to use nanoparticles in a single step. Hence, the application of biologically synthesized metal nanoparticles as sensors or detecting agents eliminates the requirement of conjugating an additional ligand molecule specific for the target molecule of interest. However, nanoparticles synthesized by physico-chemical means are generally tagged with a molecule, specific for the target. The process of conjugating the surface of synthesized nanoparticles with an additional ligand molecule is termed as functionalization and is a basic requirement when nanoparticles are used for detection/sensing applications.

Various functionalized GNPs have been used for targeting biomolecules as DNA, RNA, protein, metal ions and small organic molecules. Basically GNPs are functionalized with thiolated compounds because of the strong affinity of Au-S bond with the free end of the compound available for binding with the target molecule of interest. Allylmercaptan modified GNPs have been used for high sensitive detection of dopamine (Matusi et al., 2005). GNPs modified with mercaptoal-kyloligonucleotide molecules were also observed to increase the detection sensitivity

of oligonucleotide up to fmols (Elghanian et al., 1997). GNPs self assembled on thiol terminated silicate networks were found potential of oxidising NADH and also showed a high sensitivity and minimized the limit of detection to 5 nM (Jena et al., 2006). Gold nanorods conjugated with 11-mercaptoundecanoic acid (MUDA) have been reported to be used for binding of specific antibodies (Yu et al., 2007). Other than thiol compounds, many other functionalizing molecules have been used. For example, primary amines have been used for the preparation of amine capped GNPs. Gold- amine precursors have been reported to study the decomposition of tetrahydrofuran (THF) (Gomez et al., 2000; Heath et al., 1997). Sulfur containing ligands as disulfides, di and trithiols, thioethers and xanthates (Shelley et al., 2002; Resch et al., 1999; Li et al., 2002; Li et al., 2001; Tzhayik et al., 2002) have been reported to be used as GNP functionalizing agents. Maleic acid functionalized GNPs have been used for high selective detection of lead (Ratnarathorn et al., 2014). A disulfide, phenylboronic acid functionalized GNPs have been used for high sensitive colorimetric detection of the bacteria, *Staphylococcus aureus* (Wang et al., 2012).

3. Optical Properties of Gold Nanoparticles (GNPs)

The fascinating optical properties of GNPs has favoured its use as nanosensors in detection and sensing applications. Colloidal metal nanoparticle solution feature surface plasmon resonance (SPR) activity when light of a particular wavelength resonates with the collective oscillation of conduction band electrons. In case of GNPs this resonance condition occurs at the visible region and thus is responsible for the blue, green, violet or pink colour of colloidal solution (Halas et al., 2011). SPR in nanometer-sized structures is called localized surface plasmon resonance (LSPR). The SPR of nanoparticles greatly depends on its size, shape, aggregation state and local refractive index. Any change in size, shape or aggregation results in alteration of surface geometry of nanoparticles leading to a shift in electric field density which is reflected by a change in colour of solution and corresponding shift in absorption maxima (Jain et al., 2006). The binding of

nanoparticles to target molecule induces aggregation among themselves thereby inducing inter-particle surface plasmon coupling and consequent red shift and broadening of SPR peak and change in colour of solution (Su et al., 2003; Srivastava et al., 2005). This forms the basis of colorimetric based sensing and therein nanoparticles have evolved as excellent nanosensor candidates. The use of nanosensors for detection provides a cost-effective, rapid and promising alternative compared to the conventionally used detection techniques.

4. Application of Gold Nanoparticles in Detection of Metal Ions

Heavy metal ions as Pb, Hg, Cd, etc. hold deleterious effect on human health and welfare. Hence, researchers have focused on a rapid and high sensitive detection of these heavy metal ions using GNPs as colorimetric sensors. Lin et al., 2006 reported the use a mixed monolayer-protected GNPs functionalized with carboxylate and 15-crown-5 for colorimetric detection of Pb^{2+}. The system is based on generation of GNPs aggregates by formation of hydrogen bonds between carboxylic acid residues in methanol/water system, which disrupts on addition of Pb^{2+} ions. This results in an electrostatic repulsion between GNPs resulting in a change in colour from blue to red. Recently, Ratnarathorn et al., 2014 reported a simple and high sensitive detection method for Pb^{2+} ions using maleic acid functionalized GNPs. The colorimetric detection method was based on the binding of the free -COOH group of maleic acid to Pb^{2+} inducing aggregation and resultant change in colour from red to blue. Mao et al., 2011 reported the use of L-glutathione conjugated GNPs as sensors for detection of Pb^{2+} ions.

Colorimetric detection of Hg^{2+} ions has been achieved using GNPs functionalized with a peptide moiety (Si et al., 2007). Rex et al., 2005 reported the use of gold nanorods for detection of Hg^{2+} ions in water. Nanorods enhance the lower detection limit as the longitudinal SPR peak is more sensitive to any change in refractive index. On addition of Hg^{2+} they observed distinct shifts in longitudinal peak, attributed to changes in aspect ratio of gold

nanorods. An innovative column detection approach was developed by Lisha et al., 2009, wherein GNPs with aluminium was used for adsorption of Hg^{2+}. However, the method suffered from a major disadvantage of reducing Hg^{2+} to Hg0 state prior to adsorption. Simple colorimetric sensor using GNPs functionalized with quaternary ammonium has been utilized to detect Hg^{2+}, based on induction of aggregation of GNPs on addition of Hg^{2+} (Xue et al., 2010). DNA-functionalized GNPs were used by Mirkin et al., 2007 for high selective and sensitive detection of Hg^{2+}. The method relies on Hg^{2+}- thymidine coordination and melting temperature (Tm) of the nano aggregates. They conjugated GNPs with two different thiolated DNA sequences (probe 1 and 2), which when mixed formed aggregates with lower Tm value due to T–T mismatches in their base sequence. On adding Hg^{2+}, it forms stable T–Hg^{2+}–T base pairs by selectively coordinating with the T–T mismatches. Thus the presence of Hg increases the Tm of GNPs thereby providing low sensitive detection down to 100 nM. As the method relies on electronic heating coupled with sensing, latter Liu et al., 2008 reported a method, wherein they optimized the number of thymidine units in DNA thereby overcoming the need for heating and allowed ambient temperature sensing of Hg^{2+} ions.

A simple colorimetric method has been reported to detect Cu^{2+} ions using citrate capped GNPs of 12.2±1.65 nm size. Treatment with Cu^{2+} ions lead to a red shift of SPR peak from 520 nm to 650 nm which was due to aggregation of GNPs confirmed from TEM analysis (Salcedo and Sevilla III, 2013). Similarly, Yang et al., 2007 reported the use of cysteine stabilized GNPs as colorimetric sensors for detection of Cu^{2+} ions. Biologically synthesized GNPs using the extracts of *Solanum lycopersicums* have also been used for detection of Cu^{2+} ions (Bindhu et al., 2014). The use of biologically synthesized GNPs provides the advantages of eliminating the need of conjugation of additional functional groups as the biological extract itself contains biomolecules that naturally stabilize and cap the synthesized nanoparticles.

Neeley et al., 2011 used a two-photon lightscattering (TPS) assay method to detect As^{3+} in waste water using GNPs conjugated with glutathione (GSH) and dithiothreitol (DTT). As^{3+} is known to show a high affinity for sulphur compounds and hence was detectable by the functionalized GNPs. Electrochemical detection of As^{3+} was investigated using a nano gold-CRV (Crystal violet) film with glassy carbon electrode (GCE). Rajkumar and co-wokers reported that they successfully detected As^{3+} down to micro molar concentration in drinking water samples (Rajkumar et al. 2011). Kalluri et al. (2009) reported a simple colorimetric method for the selective detection of As^{3+} in contaminated water samples. They used GNPs functionalized with thiol compounds, glutathione (GSH), dithiothreitol (DTT), and cysteine (Cys). The detection principle was based on aggregation of As^{3+} with the modified GNPs leading to a change in colour and corresponding red shift in SPR peak. While GNP-DTT binds to As^{3+} by means of As-S linkage, Cys and GSH modified GNPs bind by means of As-O bonds. The MDL was observed to be 5 ppb, 20 ppb, and 25 ppb for DTT, GSH, and Cys-conjugated GNPs respectively. MDL further decreased to 10 ppt by using Dynamic light Scattering (DLS) analysis, suggesting DLS to be more sensitive towards detection. Citrate synthesized GNPs were also used for As^{3+} detection after conjugating with DTT and DTT-Cys-PDCA. PDCA was used to avoid interference from mercury ions. Addition of As^{3+} induces aggregation of GNPs leading to red shift of SPR peak and corresponding change in colour. DTT assists in the transformation of As(V) to As(III) thereby helping in determination of arsenic concentration in water. GNPs functionalized with GSH-DTT-Cys-PDCA were observed to be comparatively better in detection than the GNP-DTT. The former showed a MDL of 2.5µg/l with a minimum quantification limit of 8.4 µg/l (Dominguez-Gonzalez et al., 2014).

GNPs have also been reported to detect alkaline earth metals. Selective detection of alkaline earth metals calcium, stronium and barium was reported for the first time by Zhang et al., 2011 using 2-mercaptosuccinic acid conjugated GNPs. Mercaptosuccinic acid (MSA) binds to the surface of citrate

capped GNPs by means of thiol group and on interaction with these metals induces a bathochromic sift in peak and change in colour of the colloidal solution. Aggregation was because of the interaction of the divalent cations with the carboxylic groups of MSA and interaction of calcium with carbohydrate. This was confirmed by addition of EDTA, a chelating agent, in whose presence no such aggregation or change in colour of assay solution was observed. Minimum visible detection limit of the assay solution was 20, 8 and 2.5 mM for Ca^{2+} , Sr^{2+} and Ba^{2+} respectively, suggesting the affinity of the functionalized GNPs in the order of Ca< Sr< Ba. They thus inferred that the sensitivity of the system is dependent on the cation size. Tiopronin functionalised GNPs was also reported to detect barium ions. Tiopronin conjugates with GNPs by means of sulphur atoms and upon addition of barium resulted in its aggregation leading to red shift of SPR peak (Bai et al., 2011).

Tetramethylmalonamide (TMMA) functionalized GNPs were reported by Lisowski and Hutchison, 2009 to serve as selective sensors for trivalent lanthanide (Ln^{3+}) ions. Treating with Ln^{3+} ions results in the formation of TMMA-Ln^{3+} chelating complex inducing aggregation of GNPs and concomitant red to blue colour change of assay solution. The system showed a MDL of ~50 nM for Eu^{3+} and Sm^{3+}. Ali et al., 2014 reported the use of a highly selective ligand 1, 4-bis-(8-mercaptooctyloxy)-benzene (I) conjugated to GNPs for the detection of Ce^{3+}. Recently, biogenic GNPs synthesized using a fungal extract was reported for high specific detection of Ce^{3+} ions. The method overcome the two-step procedure of functionalization as it makes use of as-synthesized GNPs and was based on the reversible cross linking of GNPs on treatment with Ce^{3+} (Priyadarshini et al., 2015)

5. Conclusions and Future perspective

The fascinating optical properties, high stability and easy surface modification ability of GNP has made them evolve as useful candidates over the traditionally used detection and sensing techniques. These fundamental advantages of GNPs has made them emerge as promising nanosensor probes in the recent past

and will continue to revolutionize and dominate the area of sensing and detection study in near future. We envision that in forthcoming years, there will be a tremendous growth in the applicability of GNPs in environmental monitoring. Manufacturing of stable, multifunctional and reproducible GNP sensors will favour high throughput, sensitive detection and quantification of potential toxic agents and industrial effluents. The technology would thus help in minimizing environmental toxicity and safeguard human health and welfare.

References

1. Ahmadi T.S., Wang Z.L., Green T.C., Henglein A., El-Sayed M.A. (1996) Shape controlled synthesis of colloidal platinum nanoparticles. *Science* 272, 1924.
2. Ali T.A., Mohamed G.G., Azzam E.M.S., Abd-elaal, A.A. (2014) Thiol surfactant assembeled on gold nanoparticles ion exchanger for screen-printed electrode fabrication. Potentiometric determination of Ce (III) in environmental polluted samples. *Sens. Acutators, B.* 192-203.
3. Atwater H. A. (2007) The promise of plasmonics. *Sci. Am.* 296, 56 - 63.
4. Bai L., Zhang Y, Chen N., Chen J., Zhou X., Hu, L. (2011) Visual detection of barium ions using tiopronin functionalised gold nanoparticles. *Micro & Nano Letters.* 6(6), 337-341.
5. Bindhu M.R., Umadevi M. (2014) Silver and gold nanoparticles for sensor and antibacterial applications. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 128, 37-45.
6. Dominguez-Gonzalez R., GonzalezVarela L., Bermejo-Barrera P. (2014) Functionalizedgoldnanoparticles for the detection of arsenic in water. *Talanta.1*18, 262-269.
7. Durr N. J., Larson T., Smith D. K., Korgel B. A., Sokolov K., Ben-Yakar A. (2007) Two-photon luminescence imaging of cancer cells using molecularly targeted gold nanorods. *Nano Lett.*7,941 - 945.

8. Elghanian R., Storhoff J.J., Mucic R.C., Letsinger R.L., Mirkin C.A. (1997) Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* 277, 1078-1081.

9. Eustis S., El-Sayed M.A. (2006) Why gold nanoparticles are more precious than pretty gold: Noble metalsurface plasmon resonance and its enhancement of the radiative and nonradiative properties of nanocrystals of different shapes. *Chem. Soc. Rev.* 35, 209-217.

10. Gomez S., Philippot K., Colliere V., Chaudret B., Senocq F., Lecante P. (2000) Gold nanoparticles from self-assembled gold(I) amine precursors. *Chem. Commun.* 1945-46.

11. Halas N. J., Lal S., Chang W. S., Link S., Nordlander P. (2011) Plasmons in strongly coupled metallic nanostructures. *Chem. Rev.* 111, 3913.

12. Heath J. R., Knobler C. M., Leff D. V. J. (1997) Pressure/ temperature phase diagrams and supperlattices of organically functionalized metal nanocrystal monolayers: The influence of particle size, size distribution and surface passivant. *Phys. Chem. B.* 101, 189-97.

13. Herderick E. D., Tresback J. S., Vasiliev A. L., Padture N. P. (2007) Template-directed synthesis, characterization and electrical properties of Au - TiO 2 - Au heterojunction nanowires. *Nanotech.* 18, 155204.

14. Huang X., El-Sayed I. H., Qian W., El-Sayed M. A. (2006) Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods. *J. Am. Chem. Soc.* 128, 2115 - 2120.

15. Jain P. K., Lee K. S., El-Sayed I. H., El-Sayed M. A. (2006) Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. *J. Phys. Chem. B.* 110, 7238-7248.

16. Jena B.K., Raj C.R. (2006) Electrochemical biosensor based on integrated assembly of dehydrogenase enzymes and gold nanoparticles. *Anal Chem* 78(18), 6332-6339.

17. Kalluri J.R., Arbneshi T., Khan S.A., Neely A., Candice P., Varisli B., Washington M., McAfee S., Robinson B., Banerjee S., Singh A.K., Senapati D., Ray P.C. (2009) Use of Gold Nanoparticles in a Simple Colorimetric and Ultrasensitive Dynamic Light Scattering Assay: Selective Detection of Arsenic in Groundwater. *Angew. Chem. Int. Ed.* 48, 1-5.

18. Li X. M., de Jong M. R., Inoue K., Shinkai S., Huskens J., Reinhoudt D. N. J. (2001) Formation of gold colloids using thioether derivatives as stabilizing ligands. *Mater. Chem.* 11, 1919-1923.

19. Li Z., Jin R. C., Mirkin C. A., Letsinger R. L. (2002) Multiple thiol-anchor capped DNA-gold nanoparticle conjugates. *Nucleic Acids Res.* 30, 1558-1562.

20. Lin S. Y., Wu S. H., Chen C. H. (2006) A simple strategy for prompt visual sensing by gold nanoparticles: general applications of interparticle hydrogen bonds. *Angew. Chem., Int. Ed.* 45, 4948.

21. Lisha K.P., Anshup, Pradeep T., (2009) Towards a practical solution for removing inorganic mercury from drinking water using gold nanoparticles. *Gold Bulletin Volume.* 42(2), 144-152.

22. Lisowski C. E., Hutchison J. E. (2009) Malonamide-functionalized gold nanoparticles for selective, colorimetric sensing of trivalent lanthanide ions. *Anal. Chem.* 81, 10246-53.

23. Liu D. B., Qu W. S., Chen W. W., Zhang W., Wang Z., Jiang X. Y. (2010) Highly Sensitive, Colorimetric Detection of Mercury(Ii) in Aqueous Media by Quaternary Ammonium Group-Capped Gold Nanoparticles at Room Temperature. *Anal. Chem.* 82 (23), 9606-9610.

24. Mao S., Yu K., Lu G et al (2011) Highly sensitive protein sensor based on thermally-reduced grapheme oxide field-effect transistor. *Nano Res* 4(10), 921-930.

25. Matsui J., Akamatsu K., Hara N., Miyoshi D., Nawafune H., Tamaki K., Sugimoto N. (2005) SPR sensor chip for detection of small molecules using molecularly imprinted polymer with embedded gold nanoparticles. *Anal Chem,* 77, 4282-4285.

26. Melosh N.A., Doukai A., Diana F., Gerardot B., Petroff P.M. (2003) Ultra High Density Nanowire Lattices and Circuits. *J.R. Heath, Science.* 300,112.

27. Mirkin C. A., Lee J. S., Han M. S. (2007) Colorimetric detection of mercuric ion (Hg2+) in aqueous media using DNA functionalized gold nanoparticles. *Angew. Chem., Int. Ed.* 46, 4093.

28. Neeley A., Khan S.A., Beqa L., Fan Z., Singh A.K., Lu W., Senapati D., Arbneshi T., Lee E., Anderson Y., Banerjee S., Mao J., Dubey M., Amirtharaj P., Ray P. (2011) Selective Detection of Chemical and Biological Toxins Using Gold-Nanoparticle-Based Two-Photon Scattering Assay. *IEEE Transactions on Nanotechnology.* 10(1).

29. Ozbay E. (2006) Plasmonics: merging photonics and electronics at nanoscale dimensions. *Science.* 311, 189 - 193.

30. Priyadarshini E., Pradhan N., Panda P.K., Mishra B.K. (2015). Biogenic unmodified gold nanoparticles for selective and quantitative detection of cerium using UV-vis spectroscopy and photon correlation spectroscopy (DLS). Biosensors and Bioelectronics. 68, 598-603.

31. Priyadarshini E., Pradhan N., Sukla L.B., Panda P.K., Mishra B.K. (2014). Biogenic synthesis of floral-shaped gold nanoparticles using a novel strain, Talaromyces flavus. *Ann. Microbiol.* 64, 1055-1063.

32. Rajkumar M., Thiagarajan S., Chen. S. (2011) Electrochemical Detection of Arsenic in Various Water Samples. *Int. J. Electrochem. Sci.,* 6, 3164 - 3177.

33. Ratnarathorn N., Chailapakul O., Dungchai W. (2014) Highly

sensitive colorimetric detection of lead using maleic acid functionalized gold nanoparticle. *Talanta.* 10, 24.

34. Resch R., Baur C., Bugacov A., Koel B. E., Echternach P. M., Madhukar A., Montoya N., Requicha A. A. G., Will P. J. (1999) Linking and Manipulation of Gold Multi-Nanoparticle Structures using Dithiols and Scanning Force Microscopy. *Phys. Chem. B.* 103, 3647-3650.

35. Rex M., Hernandez F. E., Campiglia A.D. (2005) Pushing the Limits of Mercury Sensors with Gold Nanorods. *Anal Chem.* 78 (2), 445-451.

36. Salcedo A. R.M., Sevilla III, F.B. (2013) Citrate-Capped Gold Nanoparticles as Colorimetric Reagent for Copper(II) Ions. *Philippine Science Letters.* 6(1), 90-96.

37. Satuby Y., Orenstein M. (2007) Surface-plasmon-polariton modes in deep metallic trenches- measurement and analysis. *Opt. Express.* 15, 4247 - 4252.

38. Shelley E. J., Ryan D., Johnson S. R., Couillard M., Fitzmaurice D., Nellist P. D., Chen Y., Palmer R. E., Preece J. A. Dialkyl sulfides : novel passivating agents for gold nanoparticles. (2002) *Langmuir.* 18, 1791-1795.

39. Si S., Kotal A., Mandal, T. K. (2007) One-dimensional Assembly of Peptide functionalized Gold Nanoparticles: An Approach Towards Mercury Ion Sensing. *J. Phys. Chem. C.* 111, 1248.

40. Srivastava S., Frankamp B. L., Rotello V. M. (2005) Controlled plasmon resonance of gold nanoparticles self-assembled with PAMAM dendrimers. *Chem. Mater.* 17, 487-490.

41. Su K. H., Wei Q. H., Zhang X., Mock J. J., Smith D. R., Schultz S. (2003) Interparticle Coupling Effects on Plasmon Resonances of Nanogold Particles. *Nano Lett.* 3, 1087.

42. Tzhayik O., Sawant P., Efrima S., Kovalev E., Klug J. T. (2002) Xanthate capping of silver, copper and gold colloids. *Langmuir* 18, 3364-3369.

43. Wang C., Yu C. (2013) Detection of chemical pollutants in water using gold nanoparticles as sensors: a review. *Rev Anal Chem* 32(1), 1-14.

44. Wang J., Gao J., Liu D., Han D., Wang Z. (2012) Phenylboronic acid functionalized gold nanoparticles for highly sensitive detection of *Staphylococcus aureus*. *Nanoscale*. 4, 451.

45. Wang L., Ma W., Xu L., Chen W., Zhu Y., Xu C., Kotov N. A. (2010) Nanoparticle-based environmental sensors. *Mater. Sci. Eng. R.* 70 , 265 - 274.

46. Wang Q., Yu C., (2012) Chemical and biological sensing and imaging using plasmonic nanoparticles and nanostructures. In: Biomedical nanosensors. Boca Raton: Taylor and Francis, *CRC Press, pp.* 59-96.

47. Wang Z., Ma L. (2009) Gold nanoparticle probes. *Coord. Chem. Rev.* 253 , 1607 - 1618.

48. Xue X. J., Wang F., Liu X. G., (2008) One-step, room temperature colorimetric detection of mercury (Hg2+) using DNA/nanoparticle conjugate. *J. Am. Chem. Soc.* 130, 3244-3245.

49. Yang W. R., Gooding J. J., He Z. C., Li Q., Chen G. N. J. (2007) Fast colorimetric detection of copper ions using L-cysteine functionalized gold nanoparticles. *J. Nanosci. Nanotechnol.* 7, 712-716.

50. Yu C., Vahgese L., Irudayaraj J. (2007) Surface modification of CTAB capped gold nanorods. *Langmuir*. 23, 9114 - 9119.

51. Zhang J., Wang Y., Xu X., Yang X. (2011) Specifically colorimetric recognition of calcium, strontium, and barium ions using 2-mercaptosuccinic acid-functionalized gold nanoparticles and its use in reliable detection of calcium ion in water. *Analyst.* 136 (19), 3865-8.

52. Zhao Y., Zhang Z., Liu W., Dang H., Xie Q., (2004) Controlling synthesis of Bi In dendritic nanocrystals by solution dispersion. *J. Am. Chem. Soc.* 126, 6854.

❐❐

GIS BASED MAPPING OF SUITABLE AGRO CLIMATIC ZONES FOR GINGER CULTIVATION IN ODISHA

Enketeswara Subudhi*, Champakraj Kar, Aradhana Das, Mahendra Gaur, Rajesh Kumar Sahoo

Center of Biotechnology, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, Pin-751003

*Corresponding Author: enketeswarasubudhi@soauniversity.ac.in

Abstract

India accounted 30% of the global share for ginger production with 7.453 lakh tons from an area of 1.578 lakh hectares during 2012-13. Odisha is the largest producer of ginger in the country in terms of area of cultivation but lagging much behind in productivity and marketability, probably because of lack of information on land suitability and suitability of prevailing agro climatic conditions for the crop. It shows from statistical analysis of last two decades of ginger production in Odisha that the increase in area is not having significant effect over the ginger production rate. In the present paper the suitability maps and area productivity graphs are given to show the position of ginger in the important ginger growing districts. It was found that site suitability is an important factor to determine the productivity of the crop. A highly suitable location may not result in larger yields than suitable or marginally suitable areas. Suitability maps are useful to determine areas which will have the greatest success for growing a particular crop like ginger in a region.

Keywords: Ginger, Zingiber officinale, Suitability of crops, GIS mapping, Agro climatic zone.

1. Introduction

Ginger *(Zingiber officinale Rosc)* is a great herb having a pan-tropical distribution with abundant diversity in Southeast Asia. Ginger is not known to occur in truly wild state and is believed

to have originated in South East Asia but has been under cultivation from ancient times in India as well as in China (Bailey, 1949; Purseglove, 1972). Because of the easiness with which ginger rhizomes can be transported to long distances, it has spread throughout the tropical and sub-tropical regions in both hemispheres. In India, ginger is produced in Kerala, Orissa, Karnataka, Arunachal Pradesh, West Bengal, Sikkim and Madhya Pradesh (Chhetri & Gudade, 2013). In India, about 75 named varieties of ginger are under cultivation in various parts besides large number of local varieties (500) and two exotic cultivars viz; Rio-de-Janeiro and China (Rattan, 1994).

Rhizome of ginger has extraordinary medicinal properties as well as got popular use in food preparation which creates a very good national and international market for India. India accounted 30% of the global share for ginger production with 7.453 lakh ton from an area of 1.578 lakh hectares during 2012-13 (Das et al., 2014). Ginger essential oil is valued for its pleasant aroma and pungency which also decides its quality and industrial usage (Gupta et al., 2011). Ginger oil is used primarily as food flavouring agent in soft drinks, as spice and preservative in bakery products, confectionary items, pickles and sauces etc. It is used for treating nausea, morning sickness (Sultan et al., 2005; Raja et al., 2011) and chemotherapy supplement (Liu et al.,2013).

India, although a leader in ginger production, occupies 17th position world-wide in terms of productivity (Das et al., 2014). Similarly, Odisha is the largest producer of ginger in the country in terms of cultivation area but lagging much behind in productivity and marketability, probably because of lack of information on land suitability and suitability of prevailing agro climatic conditions for the crop. It has been realised that the increase in area of ginger cultivation is not having a significant effect on the production rate. As ginger is an important crop of the country and state as well, it is essential to study the distribution of the crop in the state and to find out the areas of suitability for ginger cultivation at varied climatic condition at block level. Environmental

suitability is an important aspect which has a direct impact on the productivity of the crop. Climatic variables are the principle drivers of geographic distribution. Agricultural crop distribution is rarely limited to a crop's native range. Increased crop range is largely the result of the introduction of crops into new areas which may not provide optimum growing conditions (Walker & Cocks, 1991; Guisan & Zimmerman, 2011). Ginger is one such crop which propagated by vegetative means using its rhizomes are robust and easily transportable to distant places from the place of origin and thus has been ended up with overall low productivity rate. Therefore, land suitability analysis is a prerequisite for sustainable agricultural production. The process of decision making for selection of suitable land involves a number of criteria involving the range of factors that influence crop production. Many factors, such as soil fertility and pH, vary continuously with space and time, and it is not possible to incorporate them into a large scale suitability model. The process of land suitability classification requires the evaluation and grouping of specific areas of land for a defined use. The main objective of the land evaluation is the prediction of the inherent capacity of a land unit to support a specific land use for a long period of time without deterioration, in order to minimize the socio-economic and environmental costs. Land suitability analysis is an interdisciplinary approach by including the information from different domains, such as soil science, crop science, meteorology, social science, economics and management. Being interdisciplinary, land suitability analysis deals with information, which is measured in different scales including ordinal, nominal and ratio scales (Parthsarathi et al., 2007).

Geographic information system (GIS) integrates hardware, software, and data for capturing, managing, analysing, and displaying all forms of geographically referenced information. GIS can perform complicated analytical functions and then present the results visually as maps, tables or graphs, allowing decision makers to virtually see the issues before them and then select

the best course of action.GIS is playing an increasing role in agriculture production throughout the world by helping farmers increase production, reduce costs, and manage their land more efficiently. Balancing the inputs and outputs on a farm is fundamental to its success and profitability. The ability of GIS to analyse and visualize agricultural environments and workflows has proven to be very beneficial to those involved in the farming industry. Making decisions on farming based on geography is important criteria for crop productivity development initiatives (Parthasarthy et al., 2007). Before cultivation of any crop including ginger the geography of the locality should be taken into consideration so as to find its land suitability aiming at commercial scale production.

Ginger can be grown best in warm and humid climate in diverse tropical and sub-tropical conditions at 300-900 m above mean sea level (MSL) up to 1500 m elevation, within a temperature range of 20-30^{0}C and should not fall below 25°C (77°F) and temperature of 28^0 to 35^{0}C for about a month before harvesting. It grows well in areas with annual rainfall between 1250 to 2500mm with an optimum of 1500 mm under natural rainfall or irrigated conditions. Though it can be grown on different types of soils, it thrives best in well-drained sandy or clay loam soils. Odisha has ten identified agro climatic zones covering 30 districts and 314 blocks basing on variation in temperature, rainfall, humidity and soil types which probably affects the productivity of ginger crop of the state.

In this study, more than 19 years of ginger productivity data across the number of blocks in the state along with the their respective agro climatic factors like; temperature (min and max), annual rainfall were used to study the trend of productivity over the environmentally suitable regions of Odisha, to assess the impact of climatic suitability of ten agro climatic zones on ginger production and to categorise each agro climatic zone to four prevailing types (most suitable, suitable, marginally suitable, unsuitable) below district or below level.

2. Materials and Methods

Odisha has ten identified agro climatic zones basing on variation in temperature, rainfall, humidity and soil types which decides probably the productivity of cultivation of a crop including ginger. The area and production of ginger with respect to its agro climatic condition is given along with the climatic conditions of each zone in table 1. Data on area and production of different ginger growing sites of Odisha for 19 years were collected from the Directorate of Horticulture, Odisha, Centre for environmental studies, Odisha and Meteorological department, Bhubaneswar. Area and production curves for all these districts were prepared using 19 years of data to understand the dissimilarity of compound growth rate of area and production.

2.1 Site Suitability Criteria

Optimum climatic conditions for ginger cultivation were determined from a literature search. The most variable climatic factors prevailing in the state as considered as suitable criteria to categorise the blocks of each zone into four types in this model are temperature and rainfall; i) Most suitable: average temperature and rainfall at which ginger grows at higher optimum level, ii) Suitable: average temperature and rainfall at which ginger grows at lower optimum level, iii) Marginally suitable: average temperature and rainfall at which ginger grows, and iv) Unsuitable: average temperature and rainfall at which ginger ceases to grows.

2.2 Suitability Maps for Agro Climatic Zones

Site suitability maps of the state and individual agro climatic zone were prepared on the maximum diversity of the climatic factors such as temperature and rainfall using EcoCrop predictions of ArcGIS, release 10 (ESRI, 2011), putting the values of minimum and maximum temperature and rainfall.

Table 1: Climatic conditions and broad soil types of 10 agro climatic zone of Odisha.

Agro Climatic Zone	Districts (Included in zone)	Climate	Broad Soil groups	Annual average of the zone						
				Area of cultivation (Hectare)	Productivity (Kg /Hec)	Temp. Max. (°C)	Temp. Min. (°C)	Humidity (%)	Rainfall (MM)	Alltitude (Meters)
[1] NORTH WESTERN PLATEAU	Sundargarh, parts of Deogarh, Sambalpur & Jharsuguda	Hot & moist sub-humid	Red, Brown forest, Red & Yellow, Mixed Red & Black	1224.00	4910.00	39.25	12.28	82.00	1586.00	252.00
[2] NORTH CENTRAL PLATEAU	Mayurbhanj, major parts of Keonjhar, (except Anandapur & Ghasipura block)	Hot & moist sub-humid	Lateritic, Red & Yellow, Mixed Red & Black	1452.00	6830.00	38.06	11.80	83.00	1772.00	260.00
[3] NORTH EASTERN COASTAL PLAIN	Balasore, Bhadrak, parts of Jajpur & hatdihi block of Keonjhar	Moist sub-humid	Red, Lateritic, Deltalc alluvial, Coastal alluvial & Saline	1185.00	4340.00	42.10	13.90	72.00	1311.60	28.00
[4] EAST AND SOUTH EASTERN COASTAL PLAIN	Kendrapara, Khurda, Jagatsinghpur, part of Cuttack , Puri, Nayagarh & part of Ganjam	Hot & Humid	Saline, Lateritic, Alluvial, Red & Mixed red & Black	1390.00	5238.99	41.90	13.80	72.00	1357.00	24.66

Table 1: Continued...

[5] NORTH EASTERN GHAT	Phulbani, Rayagada, Gajapati, part of Ganjam & small patches of Koraput	Hot & moist, sub-humid	Brown forest, Lateritic Alluvial, Red, Mixed Red & Black	4803.16	26630.00	39.70	12.40	82.00	1620.20	234.00
[6] EASTERN GHAT HIGH LAND	Major parts of Koraput, Nabarangpur	Warm & humid	Red, Mixed Red & Black, Mixed Red & Yellow	1849.00	8210.00	39.70	11.40	83.00	1763.20	647.16
[7] SOUTH EASTERN GHAT	Malkangiri & part of Koraput	Warm & humid	Red, Lateritic, Black	1561.00	8930.00	37.40	11.70	86.00	1795.90	757.00
[8] WESTERN UNDULATING ZONE	Kalahandi & Nuapada	Hot & moist sub-humid	Red, Mixed Red & Black and Black	256.00	3536.17	43.30	14.30	68.83	1195.60	286.00
[9] WESTERN CENTRAL TABLE LAND	Bargarh, Bolangir, Boudh, Sonepur, parts of Sambalpur & Jharsuguda	Hot & moist sub-humid	Red & Yellow, Red & Black, Black, Brown forest, Lateritic	1467.00	5670.00	42.30	13.70	75.00	1309.60	203.00
[10] MID CENTRAL TABLE LAND	Angul, Dhenkanal, parts of Cuttack & Jajpur	Hot & moist sub-humid	Alluvial, Red, Lateritic, Mixed Red & Black	1459.00	4430.00	42.54	13.70	74.00	1324.30	113.00

3. Result and Discussions

Optimum utilisation of the available natural resources for efficient crop production leads to sustainable farming which in turn results in production of quality products in an eco-friendly and economically efficient method. Growing crop at its best suitable locality is another complementary principle in sustainable agriculture as it involves the local need of the crop and depends on local ago-climatic conditions.

Thirty districts with 314 blocks of Odisha is classified into ten zones basing on varying range climatic and agronomic factors across the state *viz;* temperature (average minimum12.8°C to average maximum 40.6 °C), rainfall (1195.5mm to 1795.9mm), humidity (72 to 86%) and various soil types which decides productivity of a crop. Table-1 represents the agro climatic zones, the districts and blocks included in it, respective annual agro climatic conditions, area of ginger cultivated and the average productivity of the zone.

Maximum diversity of climatic factors is represented by temperature and rainfall and thus further these two parameters are taken into account while evaluating the site suitability for ginger basing on four criteria designed from the literature. All 314 blocks of Odisha are classified into four major suitability types of locations with 90 blocks showing most suitable sites, 89 blocks showing suitable, 77 marginally suitable blocks and 58 unsuitable blocks accounting 25.6%, 26.4%, 25.5% and 21.6% respectively of the total sites (Table 2).

Table 2: Classification of range of suitability at block level for each agro climatic zone

Agro Climatic Zone	Most Suitable (%)	Suitable (%)	Marginally Suitable (%)	Not Suitable (%)	Total
[1] North Western Plateau	8 (30.77)	8 (30.77)	7 (26.92)	3 (11.54)	26
[2] North Central Plateau	15 (41.67)	10 (27.78)	4 (11.11)	7 (19.44)	36
[3] North Eastern Coastal Plain	10 (33.33)	7 (23.33)	6 (20.00)	7 (23.33)	30
[4] East And South Eastern Coastal Plain	14 (20.59)	19 (27.94)	27 (39.71)	8 (19.05)	68
[5] North Eastern Ghat	20 (47.62)	17 (40.48)	3 (7.14)	2 (4.76)	42
[6] Eastern Ghat High Land	8 (44.44)	6 (33.33)	2 (11.11)	2 (11.11)	18
[7] South Eastern Ghat	2 (20.00)	3 (30.00)	3 (30.00)	2 (20.00)	10
[8] Western Undulating Zone	0 (0.00)	1 (5.56)	6 (33.33)	11 (61.11)	18
[9] Western Central Table Land	11 (25.58)	12 (27.91)	10 (23.26)	10 (23.26)	43
[10] Mid Central Table Land	2 (8.70)	6 (26.09)	9 (39.13)	6 (26.09)	23
Total	**90**	**89**	**77**	**58**	**314**

Zone 1: North western plateau (Sundergarh and Deogarh districts) has got 8 most suitable (30.77%), 8 suitable (30.77%), 7 (26.92%) marginally suitable and 3 (11.54%) not suitable out of total 26 blocks and over all taken 5th best zone for ginger cultivation from GIS study (Table 2). However very poor average ginger productivity 2.7 tons/hectare (6th in position) is recorded from 19 years statistics, from area of 1.6 thousand hectares and the average production recorded to be 5.2 thousand tons of ginger. The prevalent performance can be explained due to the lack awareness of poor tribal people about good cultivation practises and small land holding capacity (Fig. 1(A) and 1(B)).

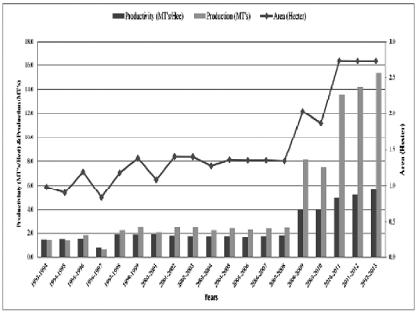


Fig. 1(A): Average ginger production, productivity and area for 19 years of Zone 1.

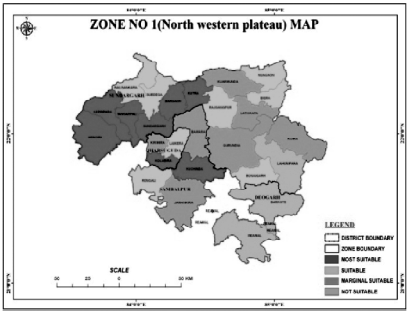


Fig. 1(B): Site suitability of ginger cultivation at block level of Zone 1.

Zone 2: North central plateau covering Mayurbhanj and Keonjhar districts stands third best suitable site as analysed for climatic condition by GIS study having 15 most suitable block (41.67%), 10 suitable block (27.78%), 6 marginally suitable blocks (20%) and 7non-sutable blocks (19.44%) out of total 36 blocks of the zone (Table 2). From 19 years secondary ginger cultivation data it is found to occupy 5th position in terms of productivity (3.1 tons/hectares) from an area of (1.6 thousand hectares) with average annual production of 4.9 thousand tons. Relatively poor performance is probably due to the lack awareness on use of elite high yielding cultivars and good cultivation practises by the farmers (Fig. 2(A) and 2(B)).

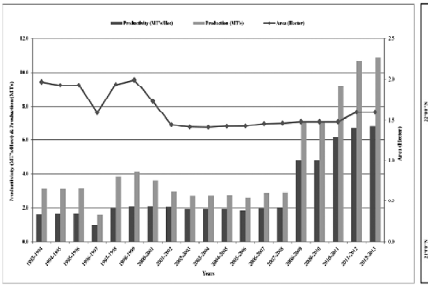


Fig. 2(A): Average ginger production, productivity and area for 19 years of Zone 2.

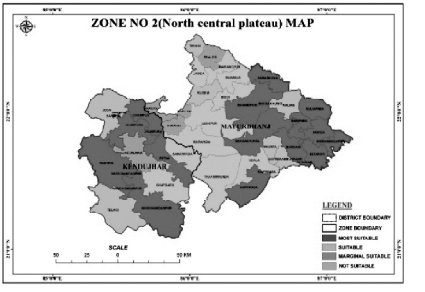


Fig. 2(B): Site suitability of ginger cultivation at block level of Zone 2.

Zone 3: (Northern eastern coastal plain covering districts Bhadrak, Balasore and Jajpur districts) stands fourth best suitable site as analysed from climatic condition by GIS study but it occupies 7th position in terms of productivity (2.7 tons/hectare) from area of (5 thousand hectares) with average annual production of 16.6 tons/hectares. This zone has been classified to have 10 (33.33%) most suitable, 7 (23.33%) suitable, 6 (20%) marginally suitable and 7 (23.33%) not suitable from our analysis (Table 2, Fig. 3(A) and 3(B)).

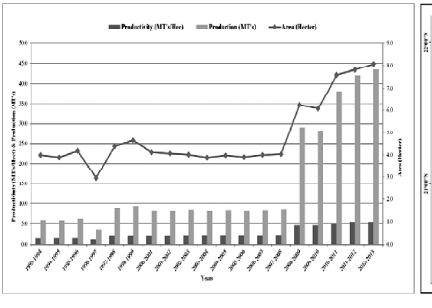


Fig. 3(A): Average ginger production, productivity and area for 19 years of Zone 3.

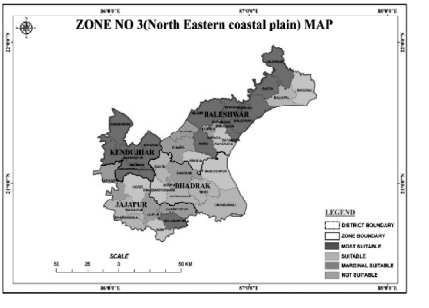


Fig. 3(B): Site suitability of ginger cultivation at block level of Zone 3.

Zone 4: East & south eastern coastal plain having in it Jagatsinghpur, Kendrapada, Cuttack, Khorda, Puri districts stands eighth in position in suitable site as analysed for climatic condition by GIS study having 14 most suitable block (20.59%), 19 suitable

block (27.94%), 27 marginally suitable blocks (39.71%) and 8 non-suitable blocks (11.76%) out of total 68 blocks of the zone (Table 2). From 19 years secondary ginger cultivation data it is found to occupies 8th position in terms of productivity (2.8 tons/ hectares) from an area of (1.7 thousand hectares) with average annual production of 5.0 thousand tons. Poor performance is justified because of non-suitability of the zone from the agroclimatic conditions prevailing in the zone in spite it covers largest area (Fig. 4(A) and 4(B)).

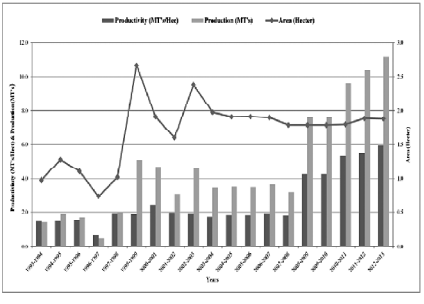


Fig. 4(A): Average ginger production, productivity and area for 19 years of Zone 4.

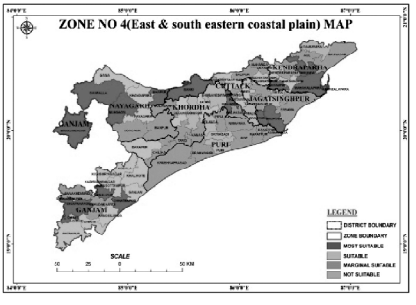


Fig. 4(B): Site suitability of ginger cultivation at block level of Zone 4.

Zone 5: North eastern ghat covering districts of Phulbani, Rayagada, Gajapati, part of Ganjam & small patches of Koraput ranks highest suitable for ginger production with 20 blocks under most suitable conditions (47.62%), 17 blocks under suitable (40.48%),3 blocks (7.14%) as marginally suitable and 2 blocks as not suitable (4.76%) out of total 42 blocks of the zone (Table 2). From 19 years of ginger cultivation data it was found that zone 5 stands highest in the state in terms of ginger production with an average of 5.2 tons from highest area of cultivation 11.2 thousands hectares with the second highest production rate 3.1 thousand tons per hectare (Fig. 5(A) and 5(B)).

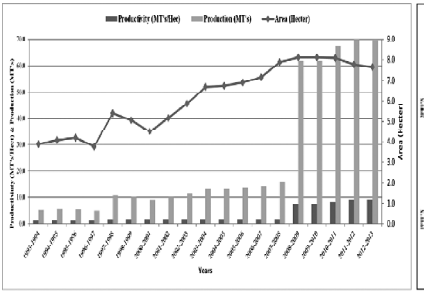


Fig. 5(A): Average ginger production, productivity and area for 19 years of Zone 5.

Fig. 5(B): Site suitability of ginger cultivation at block level of Zone 5.

Zone 6: Eastern ghat high land encompassing Nabarangpur and Malkangiri district ranks second highest suitable for ginger production with 8 blocks under most suitable conditions (44.44%), 6 blocks under suitable (33.33%), 2 blocks (11.11%) as marginally suitable and 2 blocks as not suitable (11.11%) out of total 18 blocks of the zone (Table 2). From 19 years of ginger cultivation data it was found that zone 6 stands second highest in the state in terms of ginger production with an average of 10.7 tons from area of cultivation 2.1 thousands hectares with the highest average production rate 3.9 thousand tons per hectare (Fig. 6(A) and 6(B)).

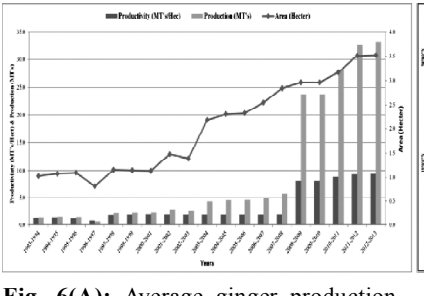


Fig. 6(A): Average ginger production, productivity and area for 19 years of Zone 6.

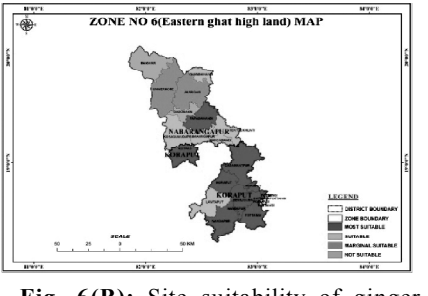


Fig. 6(B): Site suitability of ginger cultivation at block level of Zone 6.

Zone7: GIS analysed site suitable for ginger keeps zone 7 at 7th position because it has 2 most suitable, 3 suitable, 3 marginally suitable and 2 not suitable blocks but from the 19 years secondary data collection from Department of Horticulture, Odisha it is found that productivity is relatively high 8.9 tons/hectare from small area of 3.6000 hectares with a production of 10.3 tons of ginger. The reason may be due to awareness created by the nearest research station HARS, Potttangi on good cultivation practises for ginger and providing suitable seed material from high yielding varieties of ginger Suruchi, Suprabha and Surabhi etc. released and maintained by them for cultivation (Fig. 7(A) and 7(B)).

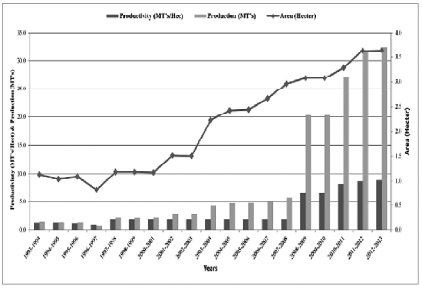


Fig. 7(A): Average ginger production, productivity and area for 19 years of Zone 7.

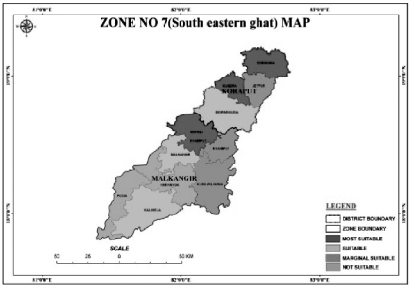


Fig. 7(B): Site suitability of ginger cultivation at block level of Zone 7.

Zone 8: Western undulating zone covering Kalahandi & Nuapada was found to be the least suitable for ginger production having no most suitable block (0.0%), 1suitable block (5.56%), 6 marginally suitable blocks (33.33%) and 11non-sutable blocks(61.11%) out of total 18 blocks of the zone (Table 2).19 years of ginger cultivation shown zone 8 as lowest in the state in terms of ginger production with lowest average of 0.6 tons from an area of cultivation 0.2 thousands hectares only with the average production rate of 2.8 tons per hectare (Fig. 8(A) and 8(B)).

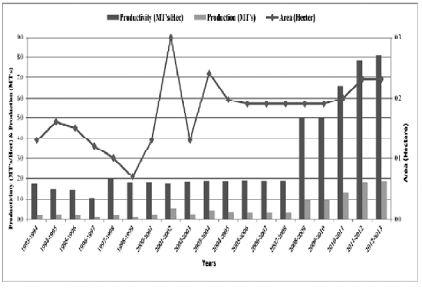


Fig. 8(A): Average ginger production, productivity and area for 19 years of Zone 8.

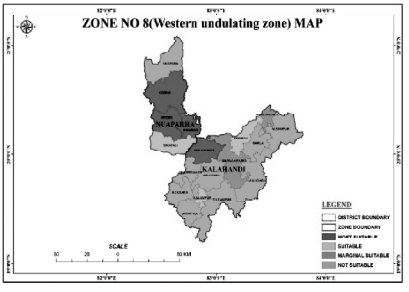


Fig. 8(B): Site suitability of ginger cultivation at block level of Zone 8.

Zone 9: Western central table land covers districts like, Bolangir Boudh, Sonepur, Deogarh. GIS analysed site suitable for ginger keeps zone 9 at 6th position because it has 11 most suitable, 12suitable, 10 marginally suitable and 10 not suitable blocks but from the 19 years secondary data collection from Department of Horticulture, Odisha it is found that productivity is relatively moderate2.7 tons/hectare from small area of 1.3 thousand hectares with a production of 3.9 tons of ginger (Fig. 9(A) and 9(B)).

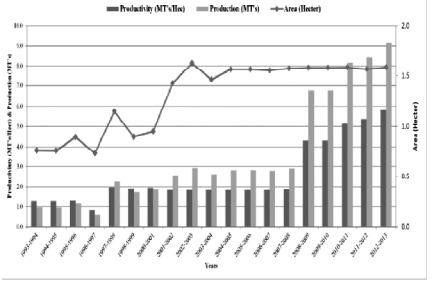


Fig. 9(A): Average ginger production, productivity and area for 19 years of Zone 9.

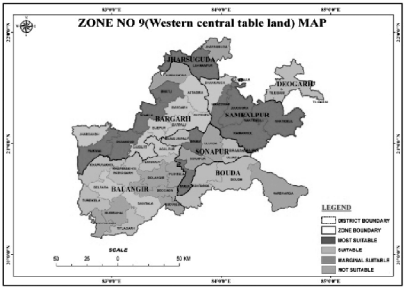


Fig. 9(B): Site suitability of ginger cultivation at block level of Zone 9.

Zone 10: Mid central table land encompassing Angul, Cuttack and Denkanal district ranks second least suitable for ginger production with 2 blocks under most suitable conditions (8.7%), 6 blocks under suitable (26.09%), 9 blocks (39.13%) as marginally

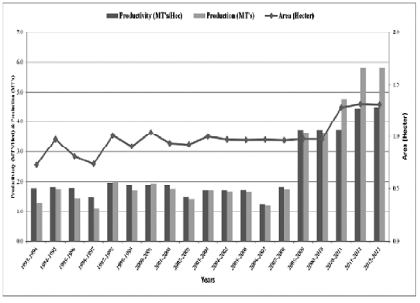


Fig.10(A): Average ginger production, productivity and area for 19 years of Zone 10.

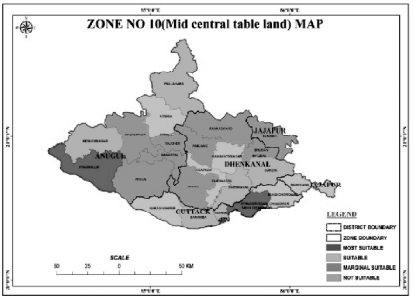


Fig. 10(B): Site suitability of ginger cultivation at block level of Zone 10.

suitable and 6 blocks as not suitable (26.09%) out of total 23 blocks of the zone (Table 2). From 19 years of ginger cultivation data it was found that zone 9 stands second least in the state in terms of ginger production with an average of 2.6 tons from area of cultivation 1.0 thousands hectares with the least average production rate 2.4 thousand tons per hectare (Fig. 10(A) and 10(B)).

Conclusions

GIS study of crop production and site suitability study based on climate and soil conditions gives sufficient indication of suitability of a location for specific crops and provides information on the area that will have the greatest likelihood of success of growing a particular crop in a region. However final identification of a site requires an in depth study of other environmental factors and infrastructure like irrigation, modern technology, marketing and storage facility that may limit crop growth and production. A personal small scale or single state survey may provide much more information. Precision farming involves the use of most advanced technologies like GPS, GIS, Remote Sensing and VRT (Variable Rate Technologies). Such systems are designed to monitor, analyse and control plant production parameters with the aim to optimize expense and ecological effects and to increase the income. To fulfil such contrasting aims the first prerequisite

is to select the best suitable crop for the area. The land suitability analysis will best suffice such a basic need.

Present study indicates that most of the zones/blocks which are naturally suitable for ginger cultivation are otherwise cultivating high quality varieties and having good production. But the zones/ block having better condition but where good quality varieties are not cultivating, hence productivity level is low. The reasons may be many including lack of awareness on good cultivation practices and use of elite cultivars, small land holding capacity of poor farmers.

References

Bailey, L.H. (1949) Manual of Cultivated Plants, 2nd Edn. Macmillan, New York.

Chhetri, P., Gudade, B.A. (2013) Organic Production of Ginger: A Prominent Sub-Himalayan Spice, Pop. Kheti (Special Issue on Organic Farming), 1 207-210.

Das, A., Kar, C., Subudhi, E. (2014) Diversity of exportable phytochemical traits in ginger from 10 agro climatic zones of Odisha. *JCHPS* Special Issue 2: 16-22.

ESRI (2011) Arc GIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

Guisan, A., Zimmerman, N.E. (2011) Predictive habitat distribution models in ecology. *Ecol. Modell.* 135, 147-186.

Gupta, S.I., Pandotra, P., Ram, G., Anand, R., Gupta, A.P., Husain, K., Bedi, Y.S. (2011)Mallavarapu GR Composition of a monoterpenoid-rich essential oil from the rhizome of Zingiber officinale from north western Himalayas. *Nat. Prod. Commun.* 6, 93-96.

Liu, Y., Whelan, R.J., Pattnaik, B.R., Ludwig, K., Subudhi, E., Rowland, H., Claussen, N., Zucker, N., Uppal, S., Kushner, D.M., Felder, M., Patankar, M.S., Kapur, A. (2013) Terpenoids from Zingiber officinale (Ginger) induce apoptosis in endometrial cancer cells through the activation of p53. *Plos one.* 7, e53178.

Parthasarthy, U., Johny, A.K., Jayarajan, K., Parthasarathy, V.A. (2007) Site suitability for turmeric production in India; A GIS interpretation. *Nat. Prod. Radiance* 6, 142-147.

Purseglove, J.W. (1972) Tropical Crops. Monocotyledons Band 1 und 2, 1. Aufl. 1972, zusammen 607 Seiten und 32 Abb., Leinen, Preis: 11,00 £ Tropical Crops, Dicotyledons, 3. Aufl. 1974, 702 Seiten, 102 Abb., kartoniert, Preis: 4,95 £. Verlag Longman Limited, London, Beccles, Colchers. Nahrung, 19: 519.

Raja, R.D.A., Jeeva, S., J.W., Prakash, Antonisamy, J.M., Irudayaraj, V. (2011) Antibacterial activity of selected ethnomedicinal plants from South India, *Asian Pacific Journal of Tropical Medicine,* 4 , 375-378,

Rattan, R.S. (1994) Improvement of ginger, In: Chadha, K.L., Rethinam, P. (Eds.), Advances in horticulture, vol. 9 - Plantation and spice crops, Part -1. Malhotra publishing house, New Delhi, India, pp 333-344.

Sultan, M., Bhatti, H.N., Iqbal, Z. (2005) Chemical analysis of essential oil of ginger (Zingiber officinale). *Pak J Biol Sci.* 8, 1576-1578.

Walker, P.A., Cocks, K.D. (1991) HABITAT: A Procedure for Modelling a Disjoint Environmental Envelope for a Plant or Animal Species. *Glob. Ecol. Biogeogr. Lett.* 1, 108-118.

❐❐

BIOFUELS FROM LIGNOCELLULOSIC BIOMASS

Shuvashish Behera[1], Ramesh C. Ray[2*]

[1]Biochemical Conversion Division, Sardar Swaran Singh National Institute of Renewable Energy, Kapurthala, Punjab-144601, India

[2]Microbiology Laboratory, Central Tuber Crops Research Institute (Regional Centre), Bhubaneswar-751019, Orissa, India

[*]Corresponding Author: rc.ray6@gmail.com, rc_rayctcri@rediffmail.com

Abstract

The progressive depletion of non-renewable energy sources worldwide has resulted in environmental deterioration and has led to the development of renewable energy harvesting technologies. It is a great challenge for finding different alternative resources, which can replace fossil fuels. Lignocellulosic biomasses have been considered as the major or the sole components in different sectors from various industries, agriculture, forestry, and municipalities and acts as the better alternative resource to conventional petroleum fuels. It contains various untapped source of fermentable sugars for significant industrial use for the production of different biofuels. Many physicochemical, structural and compositional factors hinder the hydrolysis of components present in the biomass to sugars and other organic compounds that can later be converted to biofuels. Lignocellulosic biomass has been investigated for the implementation of economic conversion processes producing different biofuels such as bioethanol, biobutanol, biohydrogen, biogas, and other valuable co-products. A cost-effective and energy efficient method is required with low energy input for an economic process development in comparison to others. Therefore, in the present chapter, the recent findings and advance developments in lignocellulosic biomass for improved biofuel production have been explored. Further, this chapter discusses about the importance of the biomass pretreatment, different strategies for product formation through various conversion technologies, and its future scope as an energy security.

Keywords: Biofuels, bioenergy, bioethanol, biobutanol, biohydrogen, biogas

1. Introduction

The global energy requirement through the use of fossil fuels creates an urgency to search for a cost effective and environmentally benign alternative energy resources. Today, there is an interest in producing certain fuels using microorganisms by fermentation focusing on the environmental aspects and renewable nature of this mode of production (Rankovic et al., 2009; Behera et al., 2014). Bioenergy is defined as one of the most important components which can provide the final solution for emission control and energy supply by continually developing four generations biofuels: first generation- food crops for grain ethanol; second generation- residues of food crops/non-food plants for cellulosic ethanol; third generation- algal/cyanobacteria for ethanol and biodiesel and fourth generation- photosynthetic machinery into artificial systems for biohydrogen and others (Peng & Gutterson, 2011). Further, selection of energy crops offers promising solutions for large-scale biofuel production. Currently, a new manufacturing concept has been raised from the leading advances of microbial, biotechnology, and genetic engineering for converting renewable biomass to valuable products. The integration of these manufacturing technologies will provide the possibility for the development of sustainable model for the production of commodity products from biomasses (Damisha et al., 2008). In this respect, lignocellulosic biomass has contributed to the recently renewed interest as an alternative to petroleum-based transportation fuel (Taherzadeh & Karimi, 2007).

Various liquid transport fuels derived from renewable lignocellulosic resources offer unique and desirable features: lower fossil fuel inputs, contribute little net CO_2 accumulation to the atmosphere, decreased urban air pollution, improved energy security, reduced trade deficits, limited conflict with land use for food and feed production. Lignocellulosic wastes (LCW) refer to the agricultural biomass wastes that are composed of mainly cellulose, hemicellulose and lignin (Dashtban et al., 2009).

Pretreatment is necessary to make these carbohydrates available for enzymatic hydrolysis and fermentation due to the close association of cellulose and hemicellulose with lignin in the plant cell wall (Radeva et al., 2012; Behera et al., 2014). Also, small amounts of other materials such as ash, proteins and pectin are also present in the lignocellulosic residues, in different degrees based on the source. They may be grouped into different categories such as agricultural residues (including straw, stover, peelings, cobs, stalks, nutshells, non-food seeds, bagasse), domestic wastes (lignocelluloses garbage and sewage), wood residues (including sawdust and paper mill discards), grasses, waste papers, municipal solid wastes and food industry residues (Kalogo et al., 2007; Chang, 2007; Rodriguez et al., 2008; Talebnia et al., 2010; Kumar et al., 2009). Moreover, the biomass is renewable, inexpensive, environment friendly and is widely available. Extensive research has been carried out on biofuel production from lignocellulosics in the past two decades (Binod et al., 2010). Production of biofuels from lignocellulosic biomass, improvement in fermentation strain through genetic engineering for better conversion of biomasses and development of consolidated bioprocessing for process development is a major focus; with additional biotechnological paths towards energy production are the highlights of current research (Roberts et al., 2010; Argyros et al., 2011; Hasunuma & Kondo, 2012).

Therefore, this book chapter is based on various biofuel types, use of technologies for conversion of various lignocellulosic biomasses for the industrial applications in terms of the mechanisms involved, advantages and disadvantages and economic assessment.

2. Lignocellulose: Source of Biofuels

Lignocellulosic biomass serves as the cheap and abundant feedstock required to produce certain fuels at reasonable costs. Based on the type of resource, these materials can be classified into four groups: (1) forest residues, (2) municipal solid waste,

(3) waste paper, and (4) crop residue resources. Rice straw is one of the abundant lignocellulosic waste materials in the world. About 731 million tons are produced annually which is distributed in Africa (20.9 million tons), Asia (667.6 million tons), Europe (3.9 million tons), America (37.2 million tons) and Oceania (1.7 million tons) (Bohlmann, 2006; Balat et al., 2011). Chemical composition of lignocellulosic materials is a key factor affecting efficiency of biofuel production during conversion processes. It mainly consists of cellulose, hemicellulose and lignin; these components constitute approximately 90% of the dry matter in lignocelluloses, and the rest is composed of extract and ash (Dehkhoda, 2008).

The major component of the plant biomass is cellulose containing 30-60% of total feedstock dry matter which is a homopolysaccharide composed of *â*-D-glucopyranose units, linked by *â*-(1-4)-glycosidic bonds. Cellobiose is the smallest repetitive unit of cellulose and can be converted into glucose residues. The orientation of the linkages and additional hydrogen bonding make the polymer rigid and difficult to break. Hence, the polysaccharide is broken down to free sugar molecules during hydrolysis by the addition of water (Hamelinck et al., 2005).

Hemicellulose, consisting 20-40% of total feedstock dry matter is a short, highly branched polymer of both five-carbon (pentoses) and six-carbon (hexoses) sugars. Specifically, hemicellulose contains xylose and arabinose (five-carbon sugars) and galactose, glucose, and mannose (six-carbon sugars). Hemicellulose is more readily hydrolyzed compared to cellulose because of its branched, amorphous nature (Lee et al., 2007). The dominant sugars in hemicelluloses are mannose in softwoods and xylose in hardwoods and agriculture residues.

Lignin is an aromatic polymer consisting 15-25% of total feedstock dry matter which is one of the drawbacks of using lignocellulosic-biomass materials in fermentation, as it makes lignocellulose resistant to chemical and biological degradation

(Demirbas, 2008). It consists of phenylpropane units joined together by different types of linkages. The polymer is synthesized by the generation of free radicals, which are released in the peroxidase-mediated dehydrogenation of three phenyl propionic alcohols (ligols): trans-p-coniferyl alcohol (guaiacy1 propano1), trans-p-coumary1 alcohol (p-hydroxypheny1 propano1), and trans-p-sinapy1 alcohol (syringy1 propano1), derived from p-cinnamic acid. The final result of this polymerization is a heterogenous structure whose basic units are linked by C-C and ary1-ether linkages, with ary1-glycero1 *â*-ary1 ether being the predominant structure.

3. Necessity for Pretreatment

Pretreatment process is required to break the lignin structure and disrupt the crystalline structure of cellulose in lignocellulosic biomass which enhances the accessibility of acids or enzymes to hydrolyze into monomers (Harmsen et al., 2010; Brodeur et al. 2011). This process allows change in the structure of lignocelluloses through increase in surface area and improved porosity of biomass which modifies and removes the lignin; partially polymerizes and removes the hemicelluloses and reduces the crystallinity of cellulose (Zhang et al., 2009). The pretreatment processes solely or in combination with other pretreatment methods can enhance the bio-digestibility of the wastes for biofuel production, and increase accessibility to the enzymes (Taherzadeh & Karimi, 2008; Mirahmadi et al., 2010). It results in increase in digestibility of the difficult biodegradable materials, and improves the product yield from the wastes. Pretreatment methods can be divided into different categories: physical (milling and grinding), physico-chemical (steam explosion, hydrothermolysis, wet oxidation, etc.), chemical (alkali, dilute acid, oxidizing agents and organic solvents), biological, electrical, or a combination of these (Alvira & Tomas-Pejo, 2010; Chiaramonti et al., 2012). The schematic configuration of pretreatment is shown in the Figure 1.

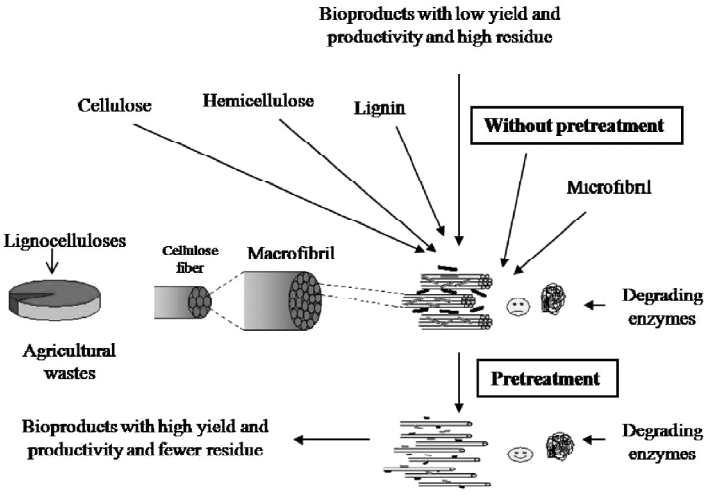


Fig. 1: Schematic representation of the pretreatment process of lignocellulosic biomass.

Pretreatment is a most expensive process in biomass-to-fuels conversion. However, there is great potential for improvement in the efficiency of the process and lowering the cost through further investigations (Hendriks & Zeeman, 2009). Recent investigations have clearly proven that there is a direct correlation between the removal of lignin and hemicelluloses and the digestibility of cellulose. Theoretically, fractionation of any biomass species allows to solubilize the majority of the hemicelluloses into the solution, and leaves the cellulose fraction intact (Amidon et al., 2011).

4. Bioethanol Production

Cellulose and hemicellulose, which typically make up two thirds of cell wall dry matter, are polysaccharides that can be hydrolyzed to sugars and then fermented to bioethanol. Process performance, i.e. Bioethanol yield from biomass, is directly related to cellulose, hemicellulose, and individual sugar concentration in the feedstock (Karunanithy et al., 2008). Glucose is the primary

abundant sugar in lignocelluloses which can be easily metabolized for the production of ethanol. *Saccharomyces cerevisiae* is the most famous glucose fermenting yeast with highest ethanol yield and tolerance. The second most abundant sugar xylose is not easily fermentable by industrial strains (Olofsson et al., 2008). There are different specific xylose fermenting yeast such as *Candida shehatae, C. tenuis, C. lyxosophila, C. intermedia, C. prachuapensis, C. jeffriesii, Pichia segobiensis, P. stipitis, Scheffersomyces stipitis,* etc. which has been reported by different researchers (Lorliam et al., 2013). But the rate of xylose consumption is reported to be very low in these strains.

The biological process for converting lignocellulose to fuel ethanol requires: (1) delignification to liberate cellulose and hemicellulose; (2) depolymerization of carbohydrate polymers to produce free sugars; and (3) fermentation of mixed hexose and pentose sugars to produce ethanol (Kumar et al., 2009). Hydrolysis of these polysaccharides (cellulose and hemicellulose) is usually accomplished by acid and/or enzymatic treatment. There are cellulose-hydrolyzing enzymes (i.e. cellulases) which are divided into three major groups: endoglucanases, cellobiohydrolases (exoglucanases), and *â*-glucosidases. The endoglucanases catalyze random cleavage of internal bonds of the cellulose chain, while cellobiohydrolases attack the chain ends, releasing cellobiose. *â*-glucosidases are only active on cello-oligosaccharides and cellobiose, and release glucose monomer units from the cellobiose. There are various enzymes responsible for the degradation of hemicellulose. In xylan degradation, for instance, endo-1,4-*â*-xylanase, *â*-xylosidase, *â*-glucuronidase, *â*-L-arabinofuranosidase and acetylxylan esterase act on the divergent heteropolymers available in nature. In glucomannan degradation, *â*-mannanase, and *â*-mannosidase cleave the polymer backbone. The figure describing enzymatic hydrolysis of cellulose has been shown in the Figure 2.

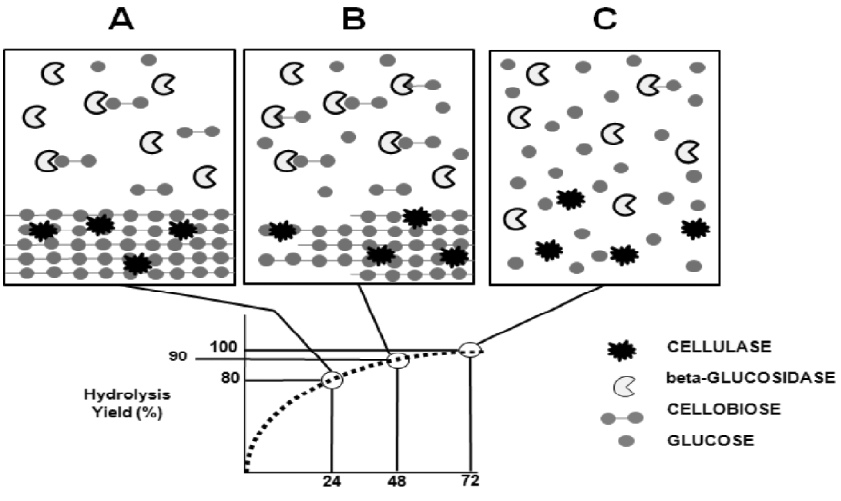


Fig. 2: Enzymatic hydrolysis of cellulose (Ruffell, 2006).

Like cellulose, hemicellulose is also an important source of fermentable sugar for biorefining applications (Kumar et al., 2008). The utilization of both cellulose and hemicellulosic sugars like hexose, pentose etc. present in a typical biomass hydrolysate is essential for the economical production of ethanol. Therefore, microorganisms which are able to ferment both glucose and xylose are required for an efficient bioconversion of biomass to ethanol production (Han & Chen, 2007; Lu et al., 2007; Hou et al., 2007). Lignin contains no sugar, and therefore does not undergo any breakdown. Lignin is therefore a residue in ethanol production, and it represents a big challenge to convert it into a value-added product. The schematic configuration of ethanol production has been shown in the Figure 3.

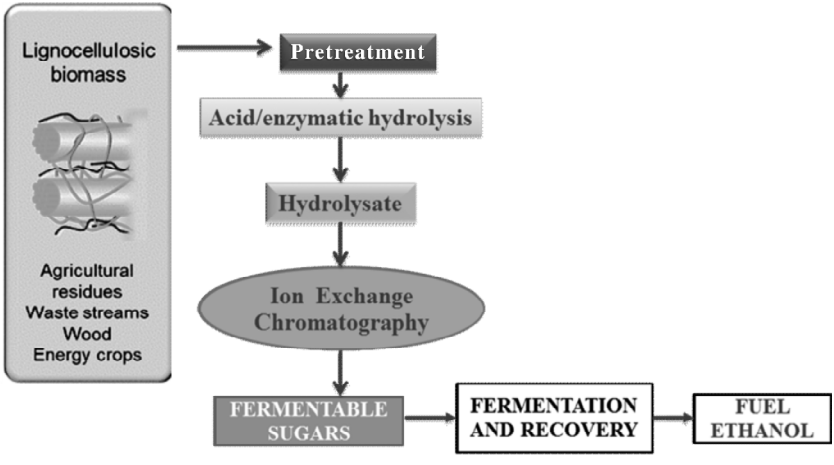


Fig. 3: Schematic representation of the ethanol production process from lignocellulosic biomass.

5. Biobutanol Production

Biobutanol has many better properties than bioethanol when used as fuel, such as higher octane number, higher energy content, higher blending rate with gasoline without engine modification, less corrosive and lower solubility in water (Durre, 2007; Lee et al., 2008). Therefore, demand on biological butanol production has increased considerably in recent years which has certain applications as a solvent for the production of antibiotics, vitamins, hormones, inorganic synthesis, chemical intermediate, processing of paint thinner and hydraulic and brake fluids (Lee et al., 2008; Zheng et al., 2015).

Butanol can be produced either from fossil fuel by chemical synthesis (as petro-butanol) or from biomass by microbial fermentation (as biobutanol). However, butanol through chemical synthesis requires many fossil oil-derived raw materials. Therefore, butanol production through ABE fermentation using some microbial species has regained much attention recently (Garcia, 2011; Lehmann & Lutke-Eversloh, 2011; Abd-Alla & El-Enany, 2012). Butanol is a superior fuel to ethanol and an

industrial solvent that can be produced from renewable resources (mentioned above) employing a number of organisms including *Clostridium acetobutylicum* and/or *Clostridium beijerinckii* which are classified as gram-positive, strictly anaerobic and spore forming bacteria (Qureshi et al., 2006). Lignocellulosic biomasses need to be hydrolyzed prior to fermentation using a combination of pretreatment (acid, alkali, or ammonia explosion) and hydrolysis (enzymes: cellulase, *â*-glucosidase, and xylanase) techniques. It should be noted that in contrast to ethanol production by yeasts; hexose and pentose sugars obtained as a result of pretreatment and hydrolysis of these residues can be used by butanol-producing cultures. Developing co-culture systems and improving cellulolytic and xylanolytic activities might be alternative approaches to utilize cellulose and hemicelluloses more effectively. Pretreatment and hydrolysis are generally performed in two separate reactors due to different and/or adverse conditions. Following pretreatment and hydrolysis, fermentation is carried out (Qureshi & Blaschek, 2005; Qureshi et al., 2007).

The high accumulations of butanol in the fermentor have the inhibition effect on the microbial cell which further inhibits fermentation process. Therefore, various alternative techniques to recover butanol from the fermentation broth have been investigated. These techniques include adsorption, liquid-liquid extraction, perstraction, pervaporation, reverse osmosis, and gas stripping. The application of these techniques helps in the use of a concentrated sugar solution in the fermentor and further reduction in the butanol inhibition. Butanol inhibition decreases with the recovery of the product (butanol). Therefore, more concentration of sugar could be used in the fermentor. Gas stripping is one of the techniques, which can be used to remove liquid fuels such as butanol which is simple and does not require expensive apparatus. Gas can be sparged through the fermentor/ bioreactor and volatile butanol can be condensed and recovered from the condenser (Qureshi & Blaschek, 2001). The schematic configuration of butanol production has been shown in the Figure 4.

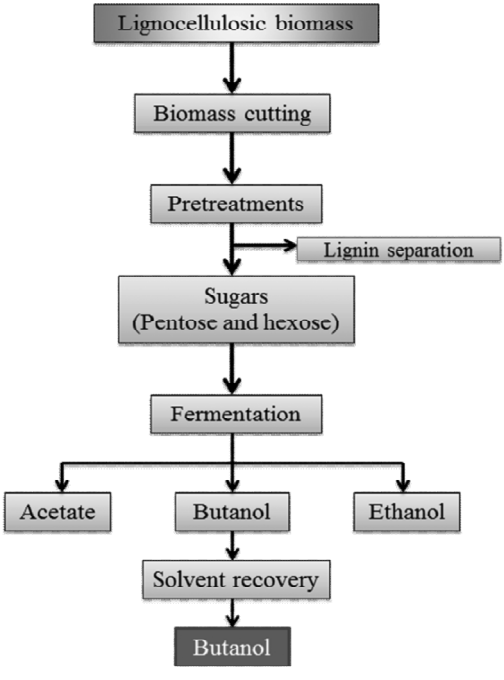


Fig. 4: Schematic representation of the butanol production process from lignocellulosic biomass.

6. Biohydrogen Production

Lignocellulosic waste biomass from forestry, agriculture, and municipal sources is a potential feedstock for the synthesis of biofuels like hydrogen (H_2), which could replace fossil fuels and reduce greenhouse gas emissions. The selection of feedstocks is mainly influenced by the feedstock availability. Moreover, each feedstock has different characteristics ;(composition and texture) thus handling of each feedstock in anaerobic digestion process varies. In addition to the aforementioned feedstock, lignocellulosic biomass has lately gained more attention as a suitable substrate for anaerobic digestion. Pretreatment methods, feedstock composition (lignin, lignocellulose and extractive), particle size, harvesting season substantially affect the methane production. The reactor system configurations, amount and type of inoculum,

pH adjustment, nutrients (macronutrients and micronutrients) supplementation and digestion duration (ranged from 20 to 150 days) are the operating parameters affecting the performance of anaerobic digestion (Hallenbeck, 2005; Levin et al., 2007).

Lignocellulosic material can be efficiently utilized during fermentation processes which require breaking of cellulose and hemicellulose to their corresponding monomers. This can be done with several different methods, including mechanical pretreatment such as grinding, milling, chipping, shredding, extrusion and combinations of these. Although the complex structures of lignocellulosic biomasses are not ideal for fermentative hydrogen production, some pretreatment methods allow this biomass to be easily used by hydrogen-producing bacteria (Wang et al., 2009; Wang & Zhao, 2009; Lakaniemi et al., 2011; Nazlina et al., 2011). Also, some pretreatments such as hot liquid water treatment, steam explosion, ammonia fiber explosion and carbon dioxide explosion and chemical methods such as acid hydrolysis, alkaline hydrolysis, hydrogen peroxide and organosolv process, as well as biological pretreatment and enzymatic pretreatment have been used to break the lignin seal and disrupt the crystalline structure of cellulose, increase the porosity and surface area of lignocelluloses (Taniguchi et al., 2005; Hongzhang & Liying, 2007; Silverstein et al., 2007).

Hydrolysis can be used after pretreatment to increase the sugar yield from cellulose and hemicellulose. For example, steam explosion and hydrothermal treatments result in cellulose-rich solid fraction that can be further hydrolyzed into sugars (Nissila et al., 2014). Dark fermentative hydrogen production from lignocellulosic hydrolyzates containing sugars is an appealing method for renewable energy. Hydrogen production via fermentation involves either facultative or strict anaerobic bacteria. In anaerobic digestion, organic carbon is degraded in the absence of oxygen, into the most reduced state (methane) and the most oxidized state (carbon dioxide) (Chong et al., 2009). Fig. 5 shows a schematic diagram of possible hydrogen production route from lignocellulosic materials.

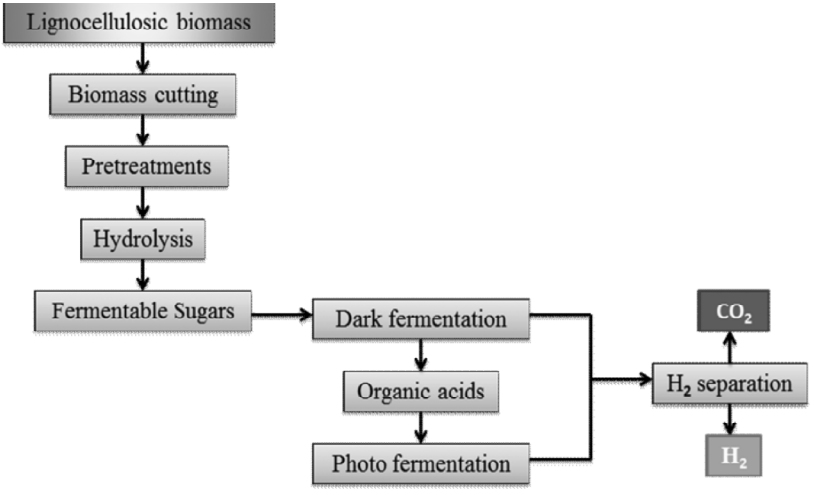


Fig. 5: Schematic representation of the biohydrogen production from lignocellulosic biomass

7. Biogas Production

Biogas can be produced by anaerobic digestion of lignocellulosic biomass which includes agricultural wastes, energy crops, wood residues and municipal paper. These bio-wastes also contain lignin rendering its anaerobic digestion slow with conventional digestion methods. As a result these bio-wastes cannot be directly used for biogas production. Another disadvantage of lignocellulosic substrates for anaerobic digestion is their poor nutrients content, which is necessary for microbial growth with a supplementing co-substrate (Nkemka & Murto, 2013). This has to be compensated by co-fermentation to break the lignin content. Various pretreatment methods can be applied which includes mechanical/physical, chemical and biological pretreatment (Sims & Kristen, 2013). The ratio between carbon and nitrogen (C:N) has an important in biogas production. Ghatak & Mahanta (2013) also characterized lignocellulosic biomass such as bamboo dust, sugarcane bagasse, saw dust and rice straw and found that the C:N ratio of the biomasses varied widely from 32 to 82:1.

Biogas is formed naturally in different natural environments,

such as swamps, the rumen of ruminants, rice fields, landfills, and other anaerobic environments. In anaerobic digestion, organic carbon is degraded in the absence of oxygen, into the most reduced state (methane) and the most oxidized state (carbon dioxide). Trace gases such as hydrogen sulfide, nitrogen, ammonia, and hydrogen are also formed in the same process. The process can be divided into four phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 6); in each individual phase, different groups of facultative or obligatory anaerobic microorganisms work together. The microorganisms use their substrate for a source of energy as well as a carbon source for growth (Angelidaki et al., 2009; Gerardi, 2003).

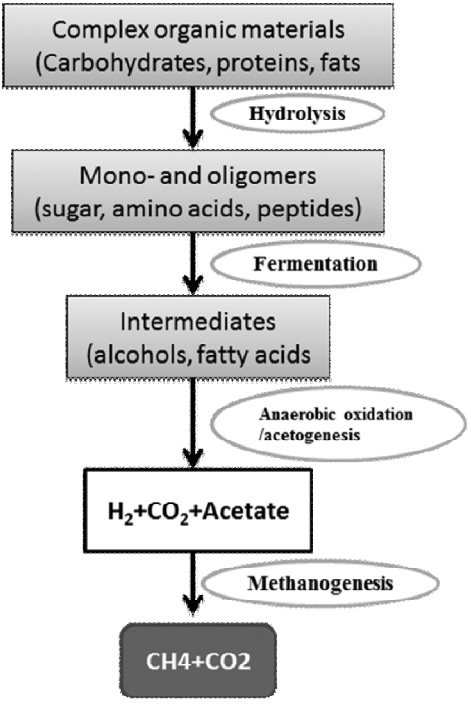


Fig. 6: Schematic representation of the biogas production process from lignocellulosic biomass.

Hydrolysis is the first step in the anaerobic digestion. During this phase, undissolved compounds, such as polysaccharides, proteins, and fats get degraded into their monomers, such as sugars, amino acids, and fatty acids. This is performed by extracellular hydrolytic enzymes including cellulases, hemicellulases, amylases, lipases, and proteases. Many cellulose-degrading organisms have their enzymes in exoenzymecomplexes, called cellulosomes. These complexes are attached to the cellular wall and simultaneously they attach to the substrate for a more effective degradation (Bayer et al., 2008; Parawira et al., 2008). In acidogenesis, monomers produced in the hydrolysis phase are further degraded by fermentative bacteria into short-chain organic acids, with one to five carbons (valeric acid, butyric acid, propionic acid, acetic acid, and formic acid), alcohols, hydrogen, ammonia, and carbon dioxide. The fatty acids longer than two carbon atoms, alcohols longer than one carbon atom, and branched chained and aromatic fatty acids are degraded further into acetic acid, hydrogen, and carbon dioxide in the acetogenic phase. The last step in the anaerobic digestion is the methanogenesis. The methanogenic microorganisms, work under strictly anaerobic conditions. The methanogens mainly use acetate, carbon dioxide, and hydrogen, but also methylamines, alcohols, and formate for the production of methane. The methanogens have the longest generation times (2-25 days) of the microorganisms in the reactor, which makes this step the most time-limiting step for easily hydrolyzed materials (Chen et al., 2008).

Conclusions and Future Perspectives

Development of multiple biofuels based biorefinery from lignocellulose is seen as an important possibility to increase the efficiency for materials and energy, and reduce the costs of biomass options to mitigate green house gas emissions. Sugars released from lignocellulosic biomass by various pretreatment and hydrolysis steps could be used for biofuels (bioethanol, biobutanol, biohydrogen and biogas) production based biorefinery successfully. Although pretreatment systems and the concomitant release of bio-products from lignocellulose have been greatly

improved by new technologies, there are still challenges that need further investigations. These challenges include development of more efficient pretreatment and production technologies, bioprospecting and development of stable genetically engineered microorganisms, improved gene cloning and sequencing technologies and enhancement of productions based on economies of scale for more efficient and cost effective conversions of lignocellulosic biomass into value-added products.

References

Abd-Alla M.H., El-Enany A.W.E. (2012) Production of acetone-butanol-ethanol from spoilage date palm *(Phoenix dactylifera L.)* fruits by mixed culture of *Clostridium acetobutylicum* and *Bacillus subtilis. Biomass Bioenergy* 42,172-178.

Alvira P.E., Tomas-Pejo (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour Technol* 101,4851-4861.

Amidon T.E., Bujanovic B., Liu S., Howard J.R. (2011) Commercializing biorefinery technology: a case for the multi-product pathway pathway to a viable biorefinery. *Forests* 2, 929-947.

Angelidaki I., Alves M., Bozonella D., Borzacconi L., Campos J.L., Guwy A.J., Kalyuzhnyi S., Jenicek P., vanLier J.B. (2009) Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. *Water Sci Technol* 59 (5), 927-935.

Argyros D.A., Tripathi S.A., Barrett T.F., Rogers S.R., Feinberg L.F., Olson D.G., Foden J.M., Miller B.B., Lynd L.R., Hogsett D.A., Caiazza N.C. (2011) High ethanol titers from cellulose by using metabolically engineered thermophillic, anaerobic microbes. *Appl Environ Microbiol* 8288-8294.

Balat M. (2011) Production of bioethanol from lignocellulosic

materials via the biochemical pathway: A review. *Energy Conversion Management* 52,858-875.

Bayer, E.A., Henrissat, B. Lamed, R. (2008) The cellulosome: a natural bacterial strategy to combat biomass recalcitrance. In: Himmel M.E. (Eds). Biomass Recalcitrance, Blackwell Publishing, pp. 407-435.

Binod P., Sindhu R., Singhania R.R., Vikram S., Devi L., Nagalakshmi S. (2010) Bioethanol production from rice straw: an overview. *Bioresour Technol* 101,4767-4774.

Behera S., Arora R., Nandhagopal N., Kumar S. (2014) Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. *Ren Sust Energy* Rev 36,91-106.

Bohlmann G.M. (2006) Process economic considerations for production of ethanol from biomass feedstocks. *Ind Biotechnol* 2,14-20.

Brodeur G., Yau E., Badal K., Collier J., Ramachandran K.B., Ramakrishnan R. (2011) Chemical and physiocochemical pretreatment of lignocellulosic biomass: a review. *Enz Res* DOI: 10.4061/2011/787532.

Chang M.C. (2007) Harnessing energy from plant biomass. *Current Opinion in Chem Biol* 11,677-684.

Chen Y., Cheng, J.J. Creamer K.S. (2008) Inhibition of anaerobic digestion process: A review. *Bioresour Technol* 99(10),4044-4064.

Chong M.L, Sabaratnam V., Shirai Y., Hassan M.A. (2009). Biohydrogen production from biomass and industrial wastes by dark fermentation. *Int J Hyd Energy* 34,3277-3287.

Ghatak DM., Mahanta P. (2013) Biogas production from lignocellulosic biomasses. Proceedings of the 22nd National and 11th International ISHMT-ASME heat and mass transfer Conference, 28th to 31st Dec organized by IIT Kharagpur, India.

Dehkhoda A. (2008). Concentrating lignocellulosic hydrolysate by evaporation and its fermentation by repeated fedbatch using flocculating *Saccharomyces cerevisiae*. Master thesis, Industrial Biotechnology Boras University and SEKAB E-Technology, Sweden.

Damisa D., Ameh J., Umoh V.J. (2008) Effect of chemical pretreatment of some lignocellulosic wastes on the recovery of cellulose from *Aspergillus niger* AH3 mutant. *Afr J Biotechnol* 7,2444-2450.

Dashtban M., Schraft H., Qin W. (2009) Fungal bioconversion of lignocellulosic residues: opportunities and perspectives. *Int J Biol Sci* 5,578-595.

Demirbas A. (2008) Heavy metal adsorption onto agro-based waste materials: a review. *J Haz Mat* 157,220-229.

Durre P. (2007) Biobutanol: an attractive biofuel. Biotechnol J 2,1525-1534.

Garcia V., Pakkila J., Ojamo H., Muurinen E., Keiski R.L. (2011) Challenges in biobutanol production: How to improve the efficiency?. *Ren Sus Energy Rev* 15, 964-980.

Gerardi M.H. (2003) The Microbiology of Anaerobic Digesters.: John Wiley & Sons, Inc.

Hallenbeck P.C. (2005) Fundamentals of the fermentative production of hydrogen. *J Water Sci Technol* 52,21-29.

Hamelinck C.N., Van H.G., Faaij A.P.C. (2005) Ethanol from lignocellulosic biomass: techno-economic performance in short, middle and long-term. *Biomass Bioenergy* 28,384-410.

Han Y., Chen H. (2007) Synergism between corn stover protein and cellulase. Enz Microbial Technol 41, 638-645.

Harmsen P., Huijgen W., Bermudez-Lopez L., Bakker R. (2010) Literature review of physical and chemical pretreatment processes for lignocellulosic biomass, 1st ed. Netherlands, pp. 857-570.

Hasunuma T., Kondo A. (2012) Consolidated bioprocessing and simultaneous saccharification and fermentation of lignocellulose to ethanol with thermotolerant yeast strains. *Process Biochem* 47(9),1287-1294.

Hendriks A., Zeeman G. (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour technol* 100,10-18.

Hongzhang C., Liying L. (2007) Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction. *Bioresour Technol* 98(3),666-676.

Hou J., Shen Y., Li X.P., Bao X.M. (2007) Effect of the reversal of coenzyme specificity by expression of mutated *Pichia stipitis* xylitol dehydrogenase in recombinant Saccharomyces *cerevisiae. Lett Appl Microbiol* 45,184-189.

Kalogo Y., Habibi S., MacLean H.L., Joshi S.V. (2007) Environmental implications of municipal solid waste-derived ethanol. Environ *Sci Technol* 41,35-41.

Karunanithy C., Muthukumarappan K., Julson J.L. (2008). Enzymatic hydrolysis of corn stover pretreated in high shear bioreactor. In: ASABE Annual international meeting, June 29-July 2, Paper No. 084114, Rhode Island.

Kumar R., Singh S., Singh O.V. (2008) Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. *Ind J Microbiol Biotechnol* 35,377-391.

Kumar S., Singh S.P., Mishra I.M., Adhikari D.K. (2009) Recent advances in production of bioethanol from lignocellulosic biomass, *Chem Eng technol* 32 (4),517-526

Lakaniemi A.M, Koskinen P.E.P, Nevatalo L. M., Kaksonen A.H., Puhakka J.A. (2011). Biogenic hydrogen and methane production from reed canary grass. *Biomass Bioenergy* 35,773-780

Lee D., Owens V.N., Boe A., Jeranyama P. (2007) Composition of herbaceous biomass feedstocks. South Dakota State

University Publication, SGINC1-07, Brookings.

Lee S.Y., Park J.H., Jang S.H., Nielsen L.K., Kim J., Jung K.S. (2008) Fermentative butanol production by clostridia. *Biotechnol Bioeng* 101,209-228.

Lehmann D., Lutke-Eversloh T. (2011) Switching Clostridium acetobutylicum to an ethanol producer by disruption of the butyrate/butanol fermentative pathway. *Metabolic Eng* 13,464-473.

Levin D.B., Zhu H., Beland M., Cicek N., Holbien B.E. (2007) Potential for renewable hydrogen and methane biogas energy production from residual biomass in Canada. *Bioresour Technol* 98,654-660.

Lorliam W., Akaracharanya A., Jindamorakot S., Suwannarangsee S., Tanasupawat S. (2013) Characterization of xylose-utilizing yeasts isolated from herbivore faeces in Thailand, *Sci Asia,* 39,26-35.

Lu X.B., Zhang Y.M., Yang J., Liang Y. (2007) Enzymatic hydrolysis of corn stover after pretreatment with dilute sulfuric acid. *Chem Eng Technol* 30, 938-944.

Mirahmadi K., Kabir M.M., Jeihanipour A., Karimi K., Taherzadeh M.J. (2010) Alkaline pretreatment of spruce and birch to improve bioethanol and biogas production. *Bioresources* 5 (2),928-938.

Nazlina H.M.Y.N.H., Nor'Aini A.R., Man H.C., Yusoff M.Z.M., Hassan M.A. (2011) Microbial characterization of hydrogen-producing bacteria in fermented food waste at different pH values. *Int J Hyd Energy* 36,9571-9580.

Nissila M.E., Lay C.H., Puhakka J.A. (2014) Dark fermentative hydrogen production from lignocellulosic hydrolyzates-A review. *Biomass Bioenergy* 67,145-159.

Nkemka V.N., Murto M. (2013) Biogas production from wheat straw in batch and UASB reactors: the roles of pretreatment and seaweed hydrolysate as a cosubstrate. *Bioresour Technol* 128,164-172.

Olofsson K., Bertilsson M., Liden G. (2008) A short review on SSF-an interesting process option for ethanol production from lignocellulosic feedstocks. *Biotechnol for Biofuels* 1:7.

Parawira W., Read J.S., Mattiasson B., Bjornsson L. (2008) Energy production from agricultural residues: High methane yields in pilot-scale two-stage anaerobic digestion. *Biomass Bioenergy* 32(1), 44-50.

Peng L., Gutterson N. (2011) Energy crop and biotechnology for biofuel production. *J Integ Plant Biol* 53 (3), 253-256.

Qureshi N., Blaschek H.P. (2005) Butanol production from agricultural biomass. In: Shetty K, Paliyath G., Pometto A., Levin R.E., (Eds.) Food biotechnology, Taylor & Francis, 2nd ed. New York. pp. 525-549.

Qureshi N., Blaschek H.P. (2001) Recovery of butanol from fermentation broth by gas stripping. *Ren Energy* 22,557-564.

Qureshi N., Li X.L., Hughes S., Saha B.C., Cotta M.A. (2006) Butanol production from corn fiber xylan using Clostridium *acetobutylicum. Biotechnol Progress* 22,673-680.

Qureshi N., Saha B.C., Cotta M.A. (2007) Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii:* Part II-Fed batch fermentation. *Biomass Bioenergy* 32, 176-183.

Radeva G., Valchev I., Petrin S., Valcheva E., Tsekova P. (2012). Comparative kinetic analysis of enzyme hydrolysis of steam-exploded wheat straw. Cellulose *Chem Technol* 46,61-7.

Rankovic J., Dodic J., Dodic S., Popov S. (2009) Bioethanol production from intermediate products of sugar beet processing with different types of *Saccharomyces cerevisiae. Chem Ind Chem Eng* 15,13-16.

Roberts S.B., Gowen C.M., Brooks J.P., Fong S.S. (2010) Genome-scale metabolic analysis of Clostridium

thermocellum for bioethanol production. *BMC Systems Biol* 4,31.

Rodríguez G., Lama A., Rodríguez R., Jiménez A., Guilléna R., Fernández-Bolaños J. (2008) Olive stone an attractive source of bioactive and valuable compounds. *Bioreasour Technol* 99,5261-5269.

Ruffell J. (2006) Pretreatment and hydrolysis of recovered fibre for ethanol production. M.Sc. Thesis, The University of British Columbia.

Silverstein R.A., Chen Y., Sharma-Shivappa R.R., Boyette M., Osborne J. (2007) A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresour Technol* 98(16),3000-3011.

Sims K.M. (2013) Strategies to enhance conversion of lignocellulosic biomass to fermentable sugars and to enhance anaerobic digestion of algal biomass for biomass production, All Graduate Plan B and other Reports. Paper 256. http://digitalcommons.usu.edu/gradreports/256

Taherzadeh M.J., Karimi K. (2007) Acid based hydrolysis processes for ethanol from lignocellulosic materials: A review. *Bioresources* 2,472-499.

Taherzadeh M.J., Karimi K. (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *Int J Mol Sci* 9,1621-1651.

Talebnia F., Karakashev D., Angelidaki I. (2010) Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. *Bioresour Technol* 101,4744-4753.

Taniguchi M., Suzuki H., Watanabe D., Sakai K., Hoshino K., Tanaka T. (2005) Evaluation of pretreatment with Pleurotus ostreatus for enzymatic hydrolysis of rice straw. *J Biosci Bioeng* 100(6),2026-2032.

Wang Y., Wang H., Feng X., Wang X., Huang J. (2009) Biohydrogen production from cornstalk wastes by anaerobic fermentation with activated sludge. *Int J Hyd Energy* 24,1-8.

Wang, X., Zhao, Y. (2009) A bench scale study of fermentative hydrogen and methane production from food waste in integrated two-stage process. *Int J Hyd Energy* 34,245-254.

Zhang Y.H., Berson E., Sarkanen S., Dale B.E. (2009) Pretreatment and biomass recalcitrance: Fundamentals and progress. *Appl Biochem Biotechnol* 153,80-83.

Zheng J., Tashiro Y., Wang Q., Sonomoto K. (2015) Recent advances to improve fermentative butanol production: Genetic engineering and fermentation technology. *J Biosci Bioeng* 119,1-9.

❐❐

MICROBES-THE SHIELD OF THE PLANET

S. Singh

CSIR-Institute of Microbial Technology, Chandigarh-160036

Corresponding Author: singhsradha@gmail.com

Abstract

"We cannot fathom the marvellous complexity of an organic being; but on the hypothesis here advanced this complexity is much increased. Each living creature must be looked at as a microcosm--a little universe, formed of a host of self-propagating organisms, inconceivably minute and as numerous as the stars in heaven". Charles Darwin

1. Introduction

Planet Earth is four-and-a-half-billion-years-old. Life exists on it for nearly, 3.8 billion years ago. Life begins from a single cell, from where the multicellular life evolves giving rise to complex creatures in the sea and on the land. As time passes, the great sweeping ages of animals, the amphibians, the dinosaurs, at last the mammals, each one enduring millions of years,. The great dynasties of creatures rising, flourishing, dying away - all this is occurring due to continuous and violent upheaval. Mountain ranges thrust up, erodd away, cometary impacts, volcanic eruptions, oceans rising and falling, whole continents moving, an endless, constant, violent change, colliding and buckling to make mountains over millions of years.

This is the Earth- Our mother planet, a unique planet, restless and dynamic where life born, prosperous and flourish-!! Holding the colours of life in her arms and spreading its rays for our growth and survival. A long yet amusing journey of a single cell to the most complicated organism- 'human beings'. The terminology "cell" needs no more introductions leading to the ultimate creation on Earth. Human threaten the Mother Nature by damaging

habitats, producing wastes and as a consequence too many species diminishes without giving the nature a chance to regenerate. However, this so called ultimate creation 'Human' and its activities have made this planet excruciating for survival. In this era of rejuvenation and invention, the human race have forgotten the most crucial role played by our mother nature and its creatures leading to several queries in mind- what will happen if everything comes to an end? Will life still exist/continue? And so on and so forth.....

These imperative questions are worthwhile to consider and the answers lies in it.

Beautifully explained by Michael Crichton -***"Earth has survived everything in its time. Even if all the plants, the animals are died and the earth remains blistering hot for a hundred thousand years, life would survive, somewhere: under the soil, frozen in Arctic ice. Sooner or later, when the planet was no longer inhospitable, life would spread again"***. The evolutionary process would begin again. It might take a few billion years for life to regain its present variety. Of course, it would be very different from what it is now, but the earth would survive. To this he added-What if the ozone layer gets thinner, ultraviolet radiation sears the earth? He says-*"Ultraviolet radiation is good for life. It's powerful energy and it will bring mutations/changes"*. Many forms of life will thrive with more UV radiation while many others will die out. Further he added-Do you think this is the first time that's happened ? Think about oxygen!! Necessary for life, but oxygen is actually a metabolic poison, a corrosive gas, like fluorine. When oxygen was first produced as a waste product by certain plant cells some three billion years ago, it created a crisis for all other lives on earth. Those plants were polluting the environment, exhaling a lethal gas. Hence, *Earth eventually had an atmosphere incompatible for life. Nevertheless, life on earth took care of itself.* According to the human being a hundred years is a long time but for earth even a million years are nothing.*"This planet lives and breathes on a much wider scale. We can't imagine*

its slow and powerful rhythms, and we haven't got the humbleness to try". We've been residents here for the blink of an eye. *If we're gone tomorrow, the earth will not miss us."*

Life originated from single cells archaea and bacteria which thrive in extreme environmental conditions like- lack of oxygen, ozone layer, UV radiation, heavy rain, lighting and volcanic activities. Over a million years of evolution different forms of living organisms originated and their presence changed the earth dramatically. These tiny, minuscule life supporters super singly play the crucial role and helped in establishing a stable atmosphere and produced enough oxygen that eventually helped in evolution of different life forms. This new atmospheric condition calmed the weather and helped other life forms to flourish. This process is one of the greatest wonders of nature. In due course, they grew and function in colonies generally referred as microbial communities. Moreover, they have a huge impact on humans which is hard to envisage. Humans depends on microbes in many ways such as - microbes:

- Make air breathable
- Provide sources of new drugs
- Clean up hazardous chemicals
- Keep us healthy
- Helps us digest food
- Support and protect crops

Most of the things microbes do for our world could never be done by a single type of microbe alone, but require a complex community working together. These communities are like a "bucket brigade"-each individual does just one part to help the functioning of whole group. With evolution, microorganisms have developed effective mechanism that helps them to perform the cellular functions in response to change of their microenvironment. Once these survival strategies get implemented they become the perfect tool for maintaining a balance between nature and mankind. Microbes, being the pioneer colonizers on earth, underpin all the life processes and an essential component in biogeochemical processes and element cycling, maintaining the functioning of the global ecosystem by physiological and functional diversity.

Although these tiny bugs are the gifts given to us by our mother nature and they can do wonders unless their importance are genuinely considered and implemented. Nonetheless, it's a vast subject area encompassing several fields but here in this chapter we emphasize on discussing the ***"Role of microbes in keeping our planet clean"*** although other roles are also discussed briefly.

a) Microbes foster life on earth

The vast majority of microbes, which inhabit in nearly every corner of Earth, are no threat to human life. Many actually benefit human health directly by defending us against pathogens, by providing sources of new drugs, and even by helping us to digest food. Amazingly, only about 1 out of 10 cells in the human body is actually a human cell: most of the cells that make up our bodies are microbes! Is it strange to know!!!! Think about it.

In fact, humans couldn't have evolved without microbes. Billions of years ago, microbes converted the Earth's entire atmosphere from nitrogen-based to oxygen-based, making it possible for larger forms of life to evolve. Human evolution has been inextricably linked with the microbes that have surrounded us from the very beginning.

Being a microbiologist, the microbial world is nothing new to me but it's hard for the layman to understand & be aware of the facts, figures and the need to utilize these wonder bugs for the betterment of society & human kind. In 2005, at the National Institutes of Health lead up to the landmark, Human Microbiome Project by David Relman, a microbiologist at Stanford, grabed the attention by *quoting* ***"humans were made up of communities."*** *By peering deep into what he called "inner space," scientists discovered that we were never* alone: Our bodies have 10 trillion cells, but we are host to 100 trillion microbes. "In other words," Relman said, "we are ten parts microbe, and one part human. We are clearly outnumbered." The figure has been repeated by researchers in scientific journals, books, and TED talks. Earlier this year, Judah L. Rosner, a researcher at the National Institutes of Health, suggests that the

human gut contains between 30 trillion and 400 trillion microorganisms, whereas the human body has an estimated 37 trillion cells-with a considerable range that goes from 5 to 724 trillion. Based on these approximations, the human body could have nearly the same number of cells as microbes or, at the more extreme end; nonhuman cells might outnumber our own almost 100 to 1. Luckey, a biochemist who spent much of his career at the University of Missouri, did far more in his unusual life than set this fact in motion. `He explained that he'd been a farm boy, an expert milker of rattlesnakes, a doll collector, and the first scientist ever to demonstrate that farm animals grew faster when given low doses of antibiotics-a practice that has since become widespread in agriculture. He did some of extraordinary work which were least mentioned but he predicted -ten parts microbe, one part man-nonetheless persevered. Peter Turnbaugh, a microbiologist at the University of California San Francisco says "The most important thing is that much of what makes us human-many of important aspects of health and the predisposition to disease and recovery-depends on metabolic activity of these microbes."

b) Microbes keeps us 'hale and hearty'

Our interactions with microbes actually do a lot more good for human health than harm. Some of the microbes living in our bodies actually help us to fight against pathogens by competing against them for space. Both of them get mutually benefited by protecting the humans from diseases at the same time giving the microbes a place to live. An article was published by the National Academies-National Academy of Sciences, National Academy of Engineering, Institute of Medicine and National Research council for awareness says -'From the moment we were born, microbes began living in and on our bodies. These early colonizers helped to "educate" our immune systems to differentiate good microbial partners from pathogenic microbes'. Many of the foods we eat would be indigestible without the 10-100 trillion microbes living within our guts. Microbes also play a major role in creating many of the foods we love, such as cheese, yogurt and bread.

Microbes used in food and brewing industries are very well known and are established processes. Many microbes living within our intestines actually help us to keep us healthy. Bacteroides thetaiotaomicron, for example, helps process complex sugars in our bodies.

Hundreds of drugs available today were derived from chemicals that were first found in microbes. Scientists can use the amazing variety of chemicals microbes naturally produce to create new medicines. Major scientific advances are opening new doors to explore how microbes can benefit human health and the environment. The emerging field of metagenomics allows scientists to study how whole communities of microbes function without any need to separately culture individual species, thus making more microbes accessible to science than ever before.

c) Microbes keeps environment clean

Industrialization along with its benefits brought up pollution issues and environmental awareness. Its strict rules and regulations imposed several norms and conditions prior to its safe disposal. Microbes being the bioremediator, makes the task easier and safer which otherwise would have not been possible for us to deal with it. All the conventional processes do come with some consequences that drastically change the scenario of the environment. Without microbes, we wouldn't have oxygen to breathe. Plants aren't the only things that carry out photosynthesis: photosynthetic microbes are responsible for about half of the photosynthesis on Earth, simultaneously increasing the amount of oxygen and decreasing the amount of carbon dioxide in the air. Through this process, microbes are helping to mitigate some of the greenhouse gasses that cause global warming. These microbial processes are often regarded as inexpensive or economic, safe, efficient and eco-friendly. The ubiquitous nature and their physiological and metabolic capabilities are unique which helps them to use this contaminant as a carbon and energy source.

Another aspect is- they reside in the soil providing the plants with natural protection from pests and diseases. Eventually

essential for converting nitrogen and other nutrients into more soluble forms that the plants can utilize for growth. Their extraordinary and distinctive adaptation strategies makes them 'wonder bugs' that can degrade-and there by render harmless-chemicals that are extremely dangerous to humans. These microbes can help clean up gasoline leaks, oil spills, sewage, nuclear waste, and many other types of pollution.

Several bioprocesses in recent years have emerged out which could otherwise poise ecological threats causing carcinogenic, mutagenic and teratogenic effects to human and environment. Serious pollutions such as ***nitro-toluenes, perchlorates, PAH's, heavy metals, organic pollutants, dyes, oil spill, petroleum catalysts etc….***can be efficiently degraded or utilized by these microbes. However, several emerging remediation technologies underlying bioremediations are available and employed worldwide.

Humans aren't the only ones that depend on microbes for digesting our food, fighting disease, and maintaining a liveable planet-no plants or animals could live without microbes. A growing number of researchers are looking at bacteria that can remediate inorganic substances, especially heavy metals. A special issue was published in The Scientist magazine entitled 'Today's Microbiologists Put Microbes to Work in Cleanup' where Derek Lovley, a senior microbiologist of the United States Geological Survey in Reston, Va., talks about an anaerobic bacterium that survives in a high uranium environment. The bacterium oxidizes the soluble form of uranium into an insoluble form that precipitates out of solution. Lovley and his co-workers have designed special bioreactors containing the bacterium to specifically precipitate and concentrated uranium into a compact solid from contaminated water and soil. He also confirmed that by using this approach other radioactive metals, like plutonium and technetium can be precipitaed. Brian Klubek and David Clark, microbiologists at Southern Illinois University in Carbondale, showed that by altering nutrients and temperature conditions, bacterial growth and metabolism boost up while Clark characterizes the genes that degrade thiophene--an organic sulfur-containing compound.

In 2014, a new alert was published by Science for Environment Policy by European Commission- 'Some microorganisms are equipped with enzymes that allow them to degrade, and even live on chemicals that are found toxic by other species. For example, many bacteria in the genus *Rhodococcus* are able to break down polychlorinated biphenyl (PCB), an industrial chemical and environmental pollutant linked to cancer in humans. In a process called 'bioremediation', humans can use these micro-organisms to break down hazardous chemicals and clean up contaminated environments' says Dellagnezze et al.(year).

In August 29, 2011, Environment magazine published an article where they reported that deploying microbes to the purge sites of contaminants such as PCBs, oil, radioactive waste, gasoline and mercury appears as a breakthrough in scientific research. It was Oscar Ruiz, researchers at the Inter American University of Puerto Rico showed that the transgenic *E. coli* bacterial strain (a common lab bacteria) with a gene that produces proteins called metallothionein and polyphosphate kinase allow the microorganisms to not only survive in presence of mercury but to remove it from waste sites. Later on it was published in the journal BMC Biotechnology. Kenneth Lee, a researcher with Canada's Department of Fisheries and Oceans who has extensive experience researching bioremediation of oils spills says- "Bioaugmentation in the open environment really isn't effective," 'microbes that aren't adapted to the environment die quickly, simply providing more nutrients for the indigenous bacteria to feed on can help to grow their populations'- also says Terry Hazen, the head of the Center for Environmental Biotechnology at UC Berkeley.

31 August 2013, Jen Wiltshire, a Doctorial Research Scientist in the Department of Microbiology at La Trobe University in an interview on The Science Show, ABC Radio National spoke about the microbial processes that could significantly improve phytoremediation as a technology and established methods to detect these microbial plant growth promoting activities and

determine the contribution of each process to the plants metal uptake ability. March 16th, 2010, Tina Casey wrote an article in Clean Technica on "Billions of Tiny Bugs Have Green Jobs Cleaning Up Polluted Sites" saying 90% of all domestically produced perchlorate are used by US military as a rocket fuel additive, explosive etc. causing severe pollution as perchlorate is highly mobile in nature and can enter into groundwater, which means that cleanup can be extremely difficult and expensive. They applied a pilot-test plant called 'microbe-based green remediation system' for perchlorate at a defence industry site in California.

The Chemical technology news from across RSC published a new article on 1st July 2010 'Cleaning up organic pollutants' by Sara Bilmes and colleagues at the University of Bunenos Aires, developed a material called 'hydrogel-fungi system' that could help break up organic pollutants without releasing the microorganisms. Bilmes also added this system could be tailored to a wide range of organic pollutants if the right kind of microorganism is chosen. Recently after 7 years of hard labour and patience, in 18th, May 2015, the researchers from the NUS Environmental Research Institute (NERI) and the Singapore Centre for Environmental Life Sciences Engineering (SCELSE) at Nanyang Technological University (NTU Singapore) have discovered that the untapped natural ability of microbial communities could be harnessed to treat raw water even before undergoing treatment. However, the generations of wastes are not limited for the human beings. We hardly learn any lesson from our past experiences which were indeed devastating. We have conceptualized the necessities but haven't customized the crucial role of the minuscule in making us and our nature safe and sound for future generation. The xenophobia blinds us to a more fundamental insight: the health of our environment, and our bodies, depends on bacterial communities. Indeed, they are responsible, as ancestors, for our very existence.

2. My Thinking

There is no end if we go on discussing the pros and cons;

being a civilized animal, we have to have an immediate and stringent action to protect our mother nature. The roles of microbes are evitable and incomparable, the only thing which we need is to choose the right kind of microbes at right place and exploring their unmatched competence for the fostering the planet to its original form- "a form where all can equally survive and prosperous". Their existence, need and significance in the survival of human life are commendable. The problems might be big but solutions dwells in a very small thing-'The microbes'. This is the world of microbe where nothing expire rather regenerates!! Lynn Margulis and Emily Case have beautifully written -"Humans have nonetheless found no shortage of ways to foul communities, cause extinctions, and threaten our own existence in the process. But bacteria wouldn't miss us. They have run the planet for most of its history, and our rush to indiscriminately kill them only reveals our own naïveté. The bacteria, with their complex history and virtuoso performances in energy and food recycling, will easily endure our assault. But our own survival depends on a revolution in human attitudes toward - and ability to learn from - our microbial ancestors" - Orion Magazine.

The sole motto of writing this chapter was to share some of the breakthrough scientific research which can really brings revolution in human thinking that - it would be difficult even to envisage life without tiny bugs. Although lots of other examples are available and well known but few things written above can be consider as major vital problem which the entire world is facing now-a-days.

References

www.bostonglobe.com/ideas/2014/09/13/your-body.../story.html/Is your body mostly microbes? Actually, we have no idea, by Peter Andrey Smith.

dels.nas.edu/resources/static-assets/materials.../microbes_brochure.pdf/viTAL roLe Microbes eArTH - Division on Earth and Life, www.aaas.org/event/microbiomes-built-environment, 27 march 2014 AAAS

Symposium, AAAS Headquarters, 1200 New york avenue, NY, Washington, DC-20005.

www.popularmechanics.com/.../environment/.../ how Microbes Clean Up Our Environmental Messes, Aug 29, 2011 by Mary Beth Griggs.

www.latrobe.edu.au › Home/../Microbes help to clean up polluted soils - La Trobe University, Aug 31, 2013 by Jen Wiltshire Robyn Williams.

newsfee.com/.../scientists-discover-tiny-microbes-with-potential-to-cleans, by Nanyang Technological University,NTU Singapore.

www.rsc.org/Publishing/.../Volume/.../ cleaning_up_organic_pollutants- Royal Society of Chemistry.

❐❐

IMPORTANCE OF BIOSURFACTANTS FOR INDUSTRIAL APPLICATIONS: A REVIEW

Pratyush Behera, Rachna Mund*

National Institute of Technology, Rourkela, Odisha, India

*Corresponding Author: mund.rachna91@gmail.com

Abstract

Biosurfactants are gaining widespread importance in the industrial sector owing to their diversified applications and superior surfactant activities. Their compatibility with the environment, high biodegradability and low toxicity allow them to be a suitable candidate in the race for becoming a better surfactant. This literature presents a short description on the importance of biosurfactants, their applications in industrial scale, various types available, their limitations and recent development in technologies to modify them for their up-gradation and effective utilisation.

1. Introduction

Biosurfactants are surface active substances synthesized by the living cells to reduce the surface tension between two liquids or between a liquid and a solid. They are usually organic compounds and amphiphilic in nature with both hydrophilic and hydrophobic moieties where their hydrophobic groups are made up of long aliphatic tails. They show a preferential partition in the interfaces of the fluid phases owing to their presence of both the hydrophilic and hydrophobic domain that allow the biosurfactants to acquire different degrees of polarity and formation of H-bonds (Cameotra and Makkar,1998). They diffuse in between air and water very easily and are potential substitutes for various synthetic surfactants used in industrial processes for wetting, lubrication, fixing dyes, softening, making emulsions, stabilizing dispersions, foaming, and preventing foaming. They

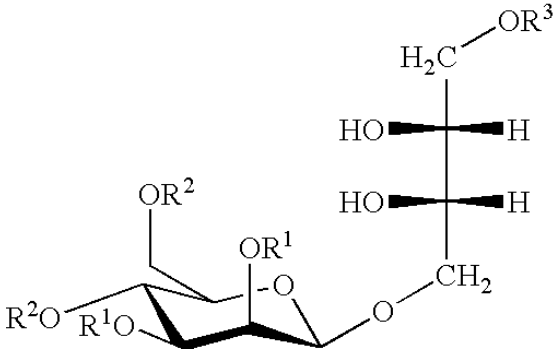
also find their application in food, biomedical and pharmaceutical industries and also in the bioremediation of organic- or inorganic-contaminated sites (Mulligan and Gibbs,2004).

It is a matter of great importance to achieve dispersion of colloidal particles to foster their use in cosmetics, pharmaceutical, paint and ceramic industries. The desired stability of dispersion depends on the surface chemistry of the particles. Biosurfactants provide the desired properties to these dispersed media prior to the tasks like slip casting, gel casting, etc (Mulligan and Gibbs, 2004; Urum and Turgay, 2004). The problem is the aggregation of colloidal particles due to strong inter-atomic attractive forces which is overcome with the addition of biosurfactants that lower this attraction. A good dispersion is obtained when the particles are separated from each other in suspension and are stable for long periods of time.

2. Types of Biosurfactants

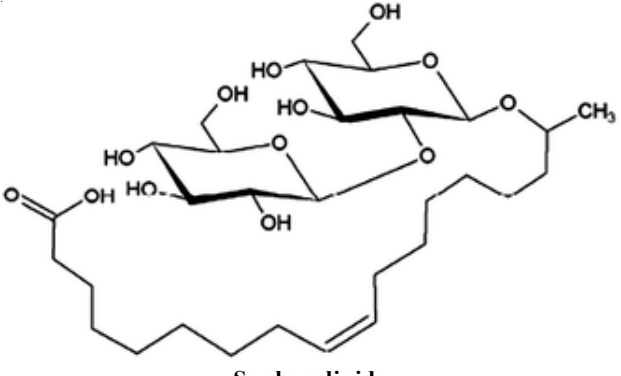
Although the biosurfactants can be categorized into non-ionic or anionic, organic and inorganic, they are specifically grouped as glycolipids, phospholipids, polysaccharide-lipid complexes, lipoproteins-lipopetides, hydroxylated and cross-linked fatty acids. The best-studied microbial surfactants are glycolipids (Mulligan, 2005; Kitamoto et al., 2009). Among these, the best-known compounds are rhamnolipids, trehalolipids, sophorolipids and mannosylerythritol lipids (MELs) which contain mono- or disaccharides, combined with long-chain aliphatic acids or hydroxyaliphatic acids.

(a) ***Mannosylerythritol lipid:*** This type of glycolipidic biosurfactants occurs in two forms of MEL-A and MEL-B. Although their hydrophobic parts contain medium chain fatty acids, these still exhibit exceptional surface and interfacial tension-lowering actions and low critical aggregate concentrations (CAC). Recently MEL-C has been discovered that possesses a different fatty acid profile compared to MEL-A or -B. MEL-C displays a higher CAC and hydrophilicity compared to conventional MELs, with excellent surface-tension lowering activity (Kitamoto et al., 2009).



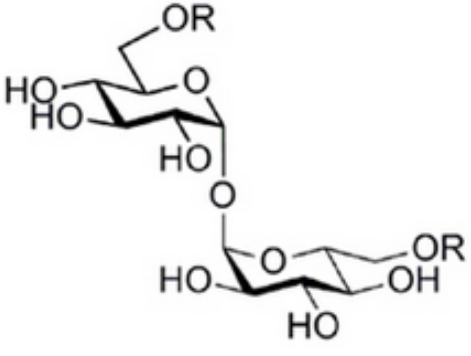
Mannosylerythritol lipid

(b) ***Sophorolipids:*** Mostly, Candida bombicola is related to the production of sophorolipids. Although sophorolipids lower the aqueous surface tension, these are not much effective for stabilizing oil in water. The modified form of sophoroplipds possesses hydrophilic-hydrophobic balance. Their derivatives show a wide range of surface activities such as emulsifying, wetting, cleaning, and solubilising. Adducts like propyleneglycol shows excellent hygroscopic activities and is commercialized as a skin moisturizer and softener in cosmetics (Kitamoto et al., 2009). Interestingly, these surfactants absorb strongly on alumina but weakly on silica. They reduce the surface tension of water. These lipids have an excellent packing property despite their bulky and complicated structure.



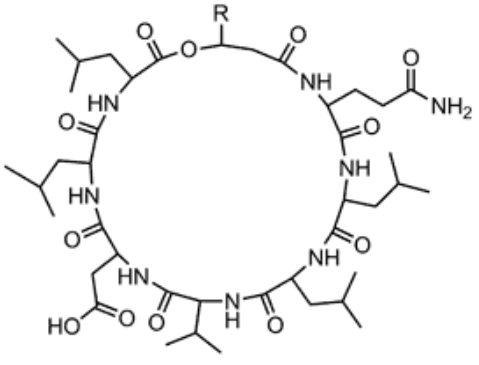
Sophorolipids

(c) ***Trehalose lipids:*** Trehalose lipids are chemically stable compounds with their surface activities not varying with temperature, pH values and salt concentration. The presence of carboxyl and hydroxyl groups in the molecule facilitates in desired surface phenomenon like dispersing and dispersion-stabilizing activities towards solid particles such as red iron oxide (α-Fe_2O_3), carbon black and α-copper phthalocyanine blue. Currently, "sugar-based surfactants" such as alky(poly)glycosides and sucrose fatty acid esters are being manufactured, and play important roles in the surfactant & detergent industry (Kitamoto et al., 2009). The industrial preference to these surfactants is due to the fact that they can be synthesized using renewable resources like glucose and fatty alcohol. In addition to their functionality and environmental compatibility, glycolipid biosurfactants are directly produced by microbial processes from renewable resources (Karanth et al., 1999). Thus, they hold great potential for "environmentally advanced surfactants" as well as sugar-based surfactants.



Trehalose lipids

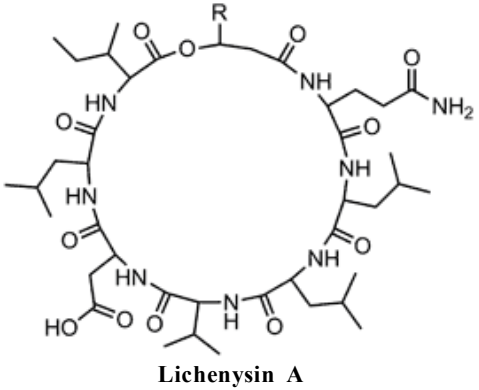
(d) ***Surfactin:*** Surfactin refers to a cyclic lipopeptide. These biosurfactants are known for their exceptional surfactant power, since it lowers the surface tension of water from 72 to 27 mN/m at a very low concentration (Kitamoto et al., 2009). This was discovered about 30 years ago, as a biologically active compound in the culture broth of the Bacillus subtilis strain (Karanth et al., 1999; Banat et al., 2000). Due to the rising demands for effective biosurfactants to deal with various ecological problems, the study



Surfactin

of this chemical compound has been invoked. The presence of two charges due to glutamic and aspartic amino acids as part of its peptide structure imparts extra benefits to its functionality and biodegradability.

(e). ***Lichenysin:*** This lipopeptidic biosurfactant has been isolated recently from Bacillus licheniformis. Lychenysin A is a mixture of lipopeptides with the major components ranging between 1006 and 1034 Da. Lichensysin is capable in reducing the surface tension of water from 72 to 28 mN/m with Critical Micelle Concentration (CMC) as little as 12 lM (Mulligan, 2005; Kitamoto et al., 2009). Lichenysin A consists of a heterogeneous lipophilic chain, represented by one of the 14 C12-C17 linear and branched b-hydroxy fatty acids linked to a peptide sequence similar to that of surfactin.



Lichenysin A

These varieties of biosurfactants provide them with widespread applications in specific industrial fields. Their optimisation and characterization are essential prior to their use. This would help determine the various physic-chemical properties of the biosurfactants. In the below tabulation, the types and methods of characterisations of biosurfactants are lined out.

Table 1: Characterisation of biosurfactants

Types	Method
Thin-Layer Chromatography	Use of chemicals
Fourier Transform Infrared Spectroscopy	Infrared spectral analysis
Calcein Dye Assay	Use of Calcein dye on bacterial culture with glass surface
Crystal Violet Dye Assay	Use of Calcein dye on bacterial culture with plastic surface
Nuclear Magnetic Resonance Analysis	Use of proton NMR and carbon NMR

3. Biosurfactants in Industries

The major use of biosurfactants in industrial sector is the extraction of oil and its further processing. A further application comes in breaking emulsions owing to the de-emulsifying property of the biosurfactants. Bioremediation is a process that aims the detoxification and degradation of toxic pollutants, through microbial assimilation or enzymatic transformation, to less toxic compounds. This process is incorporated with soil washes, in situ flushing and using biosurfactants as dispersants (Urum and Turgay, 2004). The solubility factor plays a crucial role in soil washing when dealing with poorly soluble hazards. Hydrophobic contaminants generally require detergents or dispersants, both in soil or aquatic environment, and the process is often followed by their biodegradation. Some metals including heavy metals cannot be degraded and they are just transformed into less toxic forms (Mulligan et al., 2001; Zouboulis et al., 2003). The microbial production of biosurfactants leads to the enhancement of potential

biodegradable nutrients and the success of microbes in colonizing a nutrient-restricted environment is often related to their capacity of producing polymers with high surfactant activity (Banat et al., 2010).

Dispersion of metal particles like alpha alumina are useful in numerous applications like anti-static materials, surface coatings, wall coatings, formation of aqueous pseudoplastic system, etc. (Manjula et al., 2005). The aqueous dispersion of alpha alumina particles is obtained from the alpha alumina monohydrate. The preparation method of alpha alumina monohydrate is immaterial with respect to their ability to form an aqueous dispersion. One method consists in homogeneously precipitating the basic aluminium sulphate from an aluminium sulphate solution by the in situ decomposition of an ammonia yielding compound contained in the solution. After precipitation, a basic solution is added to convert the basic aluminium sulphate in to the desired alpha alumina monohydrate. Another procedure is the hydrolysis of aluminium alcoholates that also yields aluminium monohydrates. Aluminium alkoxides are hydrolyzed to yield an alcohol-solvent stream and an alumina-water stream. The alumina-water segment is then treated to remove organic impurities that might remain in the stream. These may be completely crystalline or may be a mixture of crystalline and amorphous materials. The formation of colloidal dispersion is easily accomplished using alumina of crystal sizes less than 35Å while alumina with sizes greater than 90Å give unsatisfactory results. Also, the variations in surface area, particle size and pore volumes of alumina affect their dispersion (Manjula et al., 2005).

The settling rate of alumina dispersion varies with the type and concentration of biosurfactants. Due to the soapy nature of the dispersant, foaming occurs during stirring. The sedimentation tests demonstrated that the biosurfactant stabilizes the dispersion to a very large extent (Manjula et al., 2005). Also, a minimum amount of dispersant is required to achieve stable dispersion. Beyond the point of optimum dosage, increase in the concentration

of dispersant have no significant effects on the dispersion stability.

Chemical dispersants like Darvan, ammonium polycarboxylate Tiron, dibasic ammonium citrate, albumin, organic dispersants, citric acid, and ammonium polyacrylate are used in dispersing alumina quite effectively (Raichur, 2007). Copolymers, polar organic solvents, polyelectrolytes are also used for the dispersion. Polyelectrolytes are the most widely used dispersants. Rhamnolipids containing biosurfactant has been used in bioremediation treating contaminated soils. There these biosurfactants make a complex to remove the heavy metals from water. With recent raise in environmental concerns, new dispersants are being developed which are biodegradable as well as meets the environmental regulations (Mulligan, 2005). Any ideal dispersant will be able to carry out degradation naturally without yielding any harmful by-products.

4. Limitations of using Biosurfactants

Biosurfactants have become the backbone of various industries owing to their delivery of several benefits. However, there are still some aspects that are to be considered like their production costs which are quite high for task specific biosurfactants. Problems related to safety and yields are also a major concern (Langer et al., 2006). The use of biosurfactant also affects the immune system. The producer organism can be a pathogen and may give rise to several diseases. The ambient conditions for proper functioning of biosurfactants are not universal and vary with respect to the system (Pradhan et al., 2014). The different conditions for the production of biosurfactants cause them to produce different yields and their applications vary accordingly. The carbon source and the water-immiscible substrates give in for an induced biosurfactant production. Even the nitrogen content affects its production. Studies have showed that limiting the nitrogen concentration not only alleviated the production yield but also changed the composition of bio surfactants for the better efficacy. Ammonium is more preferred

to nitrogen now a days; even using urea results in the production of a good biosurfactants (Cameotra and Makkar, 1998). Large scale production is achieved by fermentation that improves the efficiency of the biosurfactants and overcomes most of the economic limitations.

Conclusions

According to a recent data, global biosurfactant market was worth USD 1.7 billion in 2011 and it is expected to reach USD 2.2 billion in 2018, based on a growth rate of 3.5% per annum. The global biosurfactant market volume is expected to reach 476,512.2 tons by 2018, due to increasing demand from the Asia, Africa and Latin America, which should account for 21% of it. The production of biosurfactants is linked to several regulatory factors that respond to environmental inputs such as population density, nutrient availability and diverse stresses. They enhance biodegradation by influencing the bioavailability of the contaminant. Their biodegradability and low toxicity make them very attractive resources for industrial applications. The development of more sophisticated genetically modified microorganisms producing required biosurfactants is hoped to solve or lessen their drawbacks. New techniques are to be employed with innovative ideas to make these biosurfactants available for common use.

References

Cameotra, S. S., and R. S. Makkar. "Synthesis of biosurfactants in extreme conditions." Applied Microbiology and Biotechnology 50.5 (1998): 520-529.

Mulligan, Catherine N., and BERNARD F. Gibbs. "Types, production and applications of biosurfactants." Proceedings-Indian National Science Academy Part B 70.1 (2004): 31-56.

Urum, Kingsley, and Turgay Pekdemir. "Evaluation of biosurfactants for crude oil contaminated soil washing." Chemosphere 57.9 (2004): 1139-1150.

Mulligan, Catherine N. "Environmental applications for biosurfactants."Environmental pollution 133.2 (2005): 183-198.

Kitamoto, Dai, et al. "Self-assembling properties of glycolipid biosurfactants and their potential applications." Current Opinion in Colloid & Interface Science 14.5 (2009): 315-328.

Karanth, N. G. K., P. G. Deo, and N. K. Veenanadig. "Microbial production of biosurfactants and their importance." Current Science 77.1 (1999): 116-126.

Banat, Ibrahim M., Ravinder S. Makkar, and S. S. Cameotra. "Potential commercial applications of microbial surfactants." Applied Microbiology and Biotechnology 53.5 (2000): 495-508.

Mulligan, Catherine N., Raymond N. Yong, and Bernard F. Gibbs. "Heavy metal removal from sediments by biosurfactants." Journal of Hazardous Materials 85.1 (2001): 111-125.

Zouboulis, A. I., et al. "The use of biosurfactants in flotation: application for the removal of metal ions." Minerals Engineering 16.11 (2003): 1231-1236.

Banat, Ibrahim M., et al. "Microbial biosurfactants production, applications and future potential." Applied Microbiology and Biotechnology 87.2 (2010): 427-444.

Manjula, S., et al. "A sedimentation study to optimize the dispersion of alumina nanoparticles in water." Cerâmica 51.318 (2005): 121-127.

Raichur, Ashok M. "Dispersion of colloidal alumina using a rhamnolipid biosurfactant." Journal of Dispersion Science and Technology 28.8 (2007): 1272-1277.

Langer, Olaf, et al. "Production and modification of bioactive biosurfactants." Process Biochemistry 41.10 (2006): 2138-2145.

Pradhan, Sukla, et al. "Inhibition of pathogenic bacterial biofilm by biosurfactants produced by Lysinibacillus fusiformis S9." Bioprocess and Biosystems Engineering 37.2 (2014): 139-149.

❐❐

WETLANDS FOR REHABILITATION OF METAL MINE WASTES

Gobinda Nanda Pujari

Institute for Applied Environmental Biotechnology Innovation (IAEBI)
Bhubaneswar, Pin-751016, Odisha, India

*Corresponding Author: gnpujari@gmail.com

Abstract

Wastes from mining activities generally contain high concentrations of heavy metals which is toxic to natural ecosystem as they bioaccumulate. Reclamation methods to treat these wastes include the use of wetlands, for revegetation of mine tailings under flooded conditions and for the treatment of tailings water. Both natural and constructed wetlands are frequently employed for the treatment of mine wastes. The metal removal mechanisms in wetland waters are adsorption to fine grained sediments and organic matters, precipitation as insoluble salts and adsorption and induced changes by plants and bacteria. Through a complex array of plant, soil and microbial interactions, contaminants, such as heavy metals and sulfates, can be successfully removed from wastewater. Suitable vegetation can stabilize the tailings sediment, thereby preventing it from being dust-blown or leached into the surrounding environment. A review of acid-mine drainage and their treatment using both natural and constructed wetland has been discussed in this chapter.

1. Introduction

Wetlands are defined as having (1) a water table above or at the soil surface for a significant proportion of the year, which is a determining factor in their make-up of the ecosystem, (2) an emergent vegetation characteristic of wet biotopes (often containing a large proportion of helophytes), and (3) a soil characteristic of wet biotopes (anoxic, chemically reduced) (Mitch and Gosselink, 1986).

Wetlands are attractive as an endpoint in the rehabilitation of mine wastes, such as tailings and tailings water, for two reasons. First, pollutants originating from mining activities, such as metals and sulphur, are relatively immobile when present under waterlogged conditions (Gambrell, 1994). Second, pollutants are retained by the wetlands from water passing through it (Hammer, 1989; Keita et al., 2009). Both characteristics are largely due to the same process. Permanently waterlogged wetland soils are generally anaerobic, because of the relatively low diffusion rate of oxygen through water compared to air. Constructed wetland have been used successfully to improve the quality of contaminated waters and waste waters for at least two decades and half (Murray-Gulde et al., 2005a; Maine et al., 2009; Zhang et al., 2010) while 'natural volunteer' wetland have been improving water over million of years.

Some macrophytes have accumulator phenotypes for one or several metals (Kamal et al., 2004). These plants can accumulate metals in concentrations 100,000 times greater than the associated water, and therefore have been used for metal removal from a variety of sources (Mishra and Tripathy, 2008). Hyperaccumulators can tolerate, take up and translocate high levels of certain metals. They are defined as plants that complete their life cycle with foliar metal concentrations exceeding (mgL^{1} Dry Weight) Cd>100, Ni and Cu>1000 and Mn>10,000 (Zavoda et al., 2001). However most of works on metal hyperaccumulators has been done on dryland plants. To date no emergent wetland plants have been identified as hyperaccumulators. Only submerged elodeids wetland plant like *Hydrilla verticillata* which is invasive and distributed widely in Europe, Asia, Australia, New Zealand, the Pacific islands, Africa, South and North America shows faster growth and potential to accumulate various metals (Lee et al, 1991 and Gupta et al., 1998). Wetland plants provide organic matter needed to perpetuate the biogeochemical processes in the substrate through die-back, and to provide organic compounds via exudation from the roots (Jenssen et al., 1993). Thus the functions of macrophytes include production of organic matter,

pollution uptake and bioengineering of the rhizosphere, e.g. maintenance of habitats for micro-organisms.

Micro-organisms present in wetland soils respire using terminal electron acceptors other than oxygen. For example, many members of Protobacteria utilize nitrate (NO_3-) as terminal electron accepter in denitrification process. Nitrate, like oxygen, has a high reduction potential. This process is widespread in nature. Many denitrifying bacteria can also use ferric iron ($Fe3^+$) and different organic electron accepters. Sulfate reduction uses sulfate (SO_2^{-4}) as the electron accepter, producing hydrogen sulfide (H_2S) as a metabolic end product. Sulfate reduction requires the use of electron donors, such as carbon compound lactate and pyruvate (organotrophic reducers), or hydrogen gas (lithotropic reducers). Some unusual species use phosphate (HPO_3-) as electron donor. Others such as certain *Desulfovibrio* species, are capable of sulphur disproportionation (splitting one compound into an electron donor and electron accepter) using elemental sulfur (S^0), sulfite (SO_3^{-2}), and thiosulfate ($S_2O_3^{2-}$) to produce both hydrogen sulfide (H_2S) and sulphate (SO_2-). Ferric iron (Fe_3^+) is a widespread anaerobic terminal electron accepter used by both autotrophic and heterotrophic organisms. Electron flow in these organisms is similar to those in electron transport, ending in oxygen or nitrate, except that in ferric iron-reducing organisms the final enzyme in this system is a ferric iron reductase. Since some ferric iron-reducing bacteria (e.g. *G. metallireducens*) can use toxic hydrocarbons (e.g. toluene) as a carbon source, there is significant interest in using these organisms as bioremediation agent in ferric iron contaminated aquifers. Other inorganic electron accepters include the reduction of Manganic ion (Mn_4^+) to manganous (Mn_2^+), Selenate (SeO_4^{2-}) to selenite (SeO_3^{2-}) to selenium (Se), Arsenate (AsO_4^{3-}) to arsenite (AsO_3^{3-}), and Uranyl (UO_2^{2+}) to uranium dioxide (UO_2). The formation of highly insoluble sulphide from soluble sulfate in particular is important. Not only does that process lead to the precipitation of sulphur, but also co-precipitation of metals, including iron, zinc, lead and cadmium. Once metal sulphides have precipitated, they are stable and insoluble provided the soil remains anaerobic (McIntire and

Edenborn 1990; Dvorak *et al.,* 1992). Wetlands can therefore be used in several aspects of rehabilitation of mine wastes. First, mine tailings can be rehabilitated under wetland conditions, using wetland plants, and second, the quality of water originating from mining operations can be improved by passing it through wetlands, whether they are naturally-occurring or constructed specifically for that purpose (Hammer, 1989).Wetland soil processes, interactions with the vegetation, and the application of wetlands for the rehabilitation of metal mine tailings are among the subjects discussed in this chapter.

Wetland vegetation has been successfully established on mine tailings (Nawrot, 1994; Beckett *et al.,* 1997). Characteristically, mine tailings have a low nutrient content and high concentrations of potentially toxic metals and sulphur compounds, both of which can be problematic for the successful establishment of plants. Nutrient supply to the plants can be improved by adding fertilizer. Alternatively, plants that have low nutrient requirements can be used. The latter solution is more attractive as it reduces the cost of the reclamation process. In addition, plants that are used for rehabilitation purposes can survive higher metal concentrations than plants that are not accustomed to such conditions.

Beining and Otte (1996) observed that the amphibious floating sweetgrass *(Glyceria fluitans)* was growing very well on tailings in a pond near the abandoned lead-zinc mine at Glendalough, Co. Wicklow, Ireland. This was the first time that this species was reported to grow under such conditions and a study was initiated to investigate whether the species was suitable for revegetation purposes (McCabe, 1998).

In a short-term (five weeks) greenhouse experiment and a longer term (fourteen months) field experiment, G. *fluitans* (R. Br.) from a metal-contaminated site (Glendalough tailings pond) and a non-metal contaminated site (Lough Dan) grew equally well on metal mine tailings from Glendalough under flooded conditions (McCabe and Otte, 1997). However, growth of both populations was significantly reduced under non-flooded conditions compared to flooded conditions.

Glyceria fluitans from Lough Dan also grew successfully on lead-zinc tailings from the active tailings pond at Outokumpu Zinc-Tara Mines in an outdoor microcosm experiment in Ireland. Findings of the short-term (five weeks) experiment (McCabe and Otte, 1997) suggested that treatment of G. *fluitans* with NPK fertilizer was of no measurable benefit to the plants. However, the long-term (thirteen months) experiment indicated that treatment of tailings with NPK fertilizer (700kg ha-1) significantly improved growth and biomass production of G. *fluitans*. It appears that growth of G. *fluitans* responds slowly (after a period of three-four months) to treatment with fertilizer.

The minimal fertilizer requirements of *G. fluitans* and the ability to grow vigorously on mine tailings of elevated metal concentrations (220-360mmol g^{-1} Zn; 10-120mmol g^{-1} Pb; 310-410mmol g^{-1} Fe) favor the use of this wetland plant for revegetation purposes. It is essential, however, that a cover of standing water is maintained over the tailings in order to ensure that G. *fluitans* is continually grown under flooded conditions.

2. Filtering of Metals from Contaminated Water Passing through a 'Volunteer' Wetland

Wetlands can also be used for quality improvement of contaminated water (Brix and Schierup, 1989; Hammer, 1989). Biogeochemical and physical processes, as well as uptake by plants, lead to reduced concentrations of contaminants, including nitrogen, phosphorus and metals, as the water passes through the wetlands. Naturally occurring, so-called 'volunteer' wetlands, as well as constructed wetlands, can be used for the treatment of polluted water. Many studies have shown the effectiveness of such systems in reducing concentrations of contaminants in water (Walski, 1993). For example, in Glendalough, Co. Wicklow, a marsh adjacent to an abandoned lead-zinc mine, which had its peak activity during the 1880s and finally closed in the 1950s, still receives metal contaminated water. This water, having passed through the marsh, enters the Upper Lake.

3. Sulfate Retention by a Constructed Wetland

Retention of substances in wetlands, including metals and sulphur, from water passing through them is accommodated by the physico-chemical characteristics of the wetland components (Dvorak *et al.,* 1992). In theory, the prevailing anaerobic, chemically-reduced conditions in wetland soils lead to the reduction of sulfate to sulphide, which may precipitate with metals to form insoluble metal sulphides, or may evolve as hydrogen sulphide. Therefore, levels of sulfate and metals tend to be reduced in water after passage through wetlands. These processes are mediated by interactions between micro-organisms, soil and plants (Hammack and Hedin, 1991; Ledin and Pederson, 1996; Roane *et al.,* 1996).

The design of wetland for treatment of the sulfate-rich tailings water originating from Outukumpu Zinc-Tara Mines Ltd, Meath, Ireland is described in this chapter. Typically the untreated tailing water contains 300-2200mg SO_4^{2-} L^{-1}, compared to background levels of 70mg SO_4^{2-} L^{-1} in the nearby Yellow River (Knight Piesold, 1996). The two separate systems each comprise of a pond receiving untreated tailings water (P1), a wetland compartment (W) and a pond containing treated (i.e. after passage through the wetland compartment) water (P2) (Fig.1). All three compartments in each system measure 4m×4m×1m (l×w×d) with a 30° slope in each. Header tank 1 (HT1) receives water pumped from the interceptor ditch, while HT2 receives run-off from a vegetated tailings pond (Fig. 1).

The wetland compartment of each system is filled with approximately 50cm depth of a mixture of Spent Mushroom Compost (25%) and fine grit (75%). This mixture was chosen because pilot experiments had shown that it combined good permeability with optimal growth of plants. Mushroom compost is also regarded as one of the best organic substrates for sulfate-reducing micro-organisms (Ledin and Pederson, 1996). At the bottom of the inflow and outflow ponds in each system, a layer of about 25cm of a 1:6 mixture of Spent Mushroom Compost and fine grit was deposited to provide a substrate for the invertebrate species that spontaneously inhabit the systems.

The planting density chosen was based on similar research on constructed wetlands (Szczepanska and Szczepanska, 1982; Kadlec and Alvord, 1989). The wetland compartments of each system were planted with *Typha latifolia* (four plants per m^2) and *Phragmites australis* (nine plants per m^2). Glyceria fluitans (seven plants per m^2) was added a year later. Flow rates were set at 300-500mL min^{-1}. These rates were adapted to fit the size of the systems based on the values given for other operational systems as described by Crites (1994).

Parameters measured were volunteer species (invaders), pH, redox potential, conductivity and sulfate concentrations in water. The plant species introduced to the systems-*Phragmites australis, Typha latifolia* and *Glyceria fluitans*-were rapidly established. Some *P. australis* flowered, but *T. latifolia* was particularly successful, growing to 164cm tall in some cases and with at least ten inflorescences in each of the wetland compartments.

The pH of the water passing through the systems remained in the neutral to alkaline range, with values varying from 6.8 to 9.1. Observations of redox potential indicated that the wetland soil environment is chemically reduced, with values generally lower than 100mV. Average concentrations of sulphate varied between 1300mg L-1 in the interceptor ditch in September, 1998 and 250mg L-1 in the outflow in November 1998.

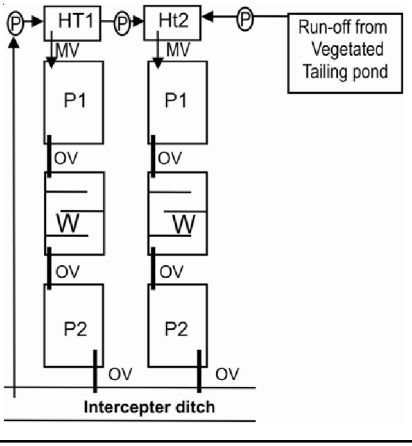


Fig. 1. Design of the experimental set-up at the Randalstown tailings facility of Outukumpu Zinc-Tara Mines Ltd, Co. Meath. MV, manual valve; OV, overflow. Waterproof baffles in each wetland compartment serve to increase the flow path of the water, thereby increasing the potential for sulfate retention.

The data illustrate that between the interceptor ditch and the outflow, sulfate concentrations were reduced by 64% in September, 14% in October, and 62% in November of 1998.

References

Beckett, P., Willianen, S.P., Vivyurka, A. and Courtin, G. 1997. Establishment of deep water aquatic plants in permanently flooded pyretic uranium tailings-a field test. *Proceedings of the Fourth International Conference on Acid Rock Drainage, Vancouver, BC, June* 1997, 1-11.

Beining, B.A. and Otte, M.L. 1996. Retention of metals from an abandoned lead-zinc mine by a wetland at Glendalough, Co. Wicklow, Ireland. *Biology and Environment: Procedings of the Royal Irish Academy* 96B (2), 117-26.

Brix, H. and Schierup, H. 1989. The use of aquatic macrophytes in water-pollution control. *Ambio* 18, 100-107.

Crites, R.W. 1994. Design criteria and practice for constructed wetlands. *Water, Science and Technology* 29, 1-6.

Dvorak, D.H., Hedin, R.S., Edenborn, H.M. and McIntire, P.M. 1992. Treatment of metal-contaminated water using bacterial sulfate reduction: results of pilot-scale reactors. *Biotechnology and Bioengineering* 40, 609-16.

Gambrell, R.P. 1994. Trace and toxic metals in wetlands-a review. *Journal of Environmental Quality* 23, 883-91.

Gupta, M., Tripathi, R.D., Rai, U.N., Chandra, P. 1998. Role of glutathione and phytochelatin in *Hydrilla verticillata* (L.f.) Royle and *Vallisneria spiralis* L. Under mercury stress. Chemosphere, 37, 785-800.

Hammack, R.W. and Hedin, R.S. 1991. Microbial sulfate reduction

for the treatment of acid mine drainage: a laboratory study. *US Bureau of Mines Publication,* p.13. Pittsburgh Research Center. US Department of the Interior.

Hammer, D.A. 1989. *Constructed wetlands for wastewater treatment: municipal, industrial and agricultural.* Chelsea, MI. Lewis Publishers.

Jenssen, P.D., Maehlum, T., Krogstad, T., 1993. Potential use of constructed wetlands for waste-water treatment in Northern environments. Water Science and Technology, 28, 149-157

Kadlec, R.H. and Alvord, H. 1989. Mechanisms of water quality improvement in wetland treatment systems. In D.W. Fisk (ed.), *Wetlands: concerns and successes,* 489-98. Florida. American Water Resources Association.

Kamal, M., Ghaly, A.E., Mahmoud, N., Cote, R., 2004. Phytoaccumulation of heavy metals by aquatic plants. Environmen International 29, 1029-1039.

Keita, S., Tang, Z., Dong, S. And Jiang, 2009. Evaluation of some organic pollutants transport into the shallow groundwater and surface water of jiaxing landfill area. Am. J. Applied Sci., 6, 2010-2017.

Knight Piesold 1996. Randalstown Tailings Facility-Stage IV. Environmental Impact Statement. Ashford, Kent. Knight Piesold.

Ledin, M. and Pederson, K. 1996. The environmental impact of mine wastes-roles of micro-organisms and their significance in treatment of mine wastes. *Earth Science Reviews* 41, 67-108.

Lee, C.K.; Low, K.S.; Hew, N.S. 1991. Accumulation of arsenic by aquatic plants. Sci. Total Environ. 103, 215-227

Maine, M.A., Suné, N., Hadad, H., Sanchez, G. , Bonetto, C., 2009. Influence of the vegetation on the removal of heavy metals and nutrients in a constructed wetland. Journal of Environmental Management 90, 355-363.

McCabe, O.M. 1998. Wetland plants for revegetation of metal mine tailings. Unpublished Ph.D. thesis, University College Dublin.

McCabe, O.M. and Otte, M.L. 1997. Revegetation of mine tailings under wetland conditions. In J.E. Brandt, J.R. Galevotic, L. Kost and J. Trouart (eds), *Proceedings of the Fourteenth Annual National Meeting-Vision* 2000: *An Environmental Commitment, May* 10 -16, 1997, 99-103. Austin, Texas. American Society for Surface Mining and Reclamation.

McIntire, P.E. and Edenborn, H.M. 1990. The use of bacterial sulfate reduction in the treatment of drainage from coal mines. *Proceedings of the Mining and Reclamation Conference and Exhibition, Charlestown, WV, April* 23 -6, 1990, 409-15.

Mishra, V.K., Tripathi, B.D., 2008. Concurrent removal and accumulation of heavy metals by the three aquatic macrophytes. Bioresource Technology, 99, 7091-7097.

Mitch, W.J. and Gosselink, J.G. 1986. *Wetlands*. New York. Van Nostrand Reinhold.

Murray-Gulde, C.L., Huddleston, G.M., Garber, K.V., Rodgers, J.H., 2005a. Contributions of *Schoenoplectus californicus* in a constructed wetland system receiving copper contaminated wastewater. Water Air Soil Pollution 163, 355-378.

Nawrot, J.R. 1994. Mined land wetlands establishment principles and guidelines. In *Proceedings of the International Reclamation and Mine Drainage Conference And Third International Conference on Abatement of Acid Drainage, Pittsburg, PA, April* 24 -9, 1994, 1-8.

Roane, T.M., Pepper, I.L. and Miller, R.M. 1996. Microbial remediation of metals. In R.L. Crawford and D.L. Crawford (eds), *Bioremediation, principles and applications,* 312-40. Cambridge University Press.

Szczepanska, W. and Szczepanska, A. 1982. Interactions between

Phragmites australis (Cav.) Trin. Ex. Steud. and *Typha latifolia L. Ekologia Polska* 30, 1165-86.

Walski, T.M. 1993. Long-term effects of wetland treatment of mine drainage. Journal of *Environmental Engineering* 119, 1004-1005.

Wharton, L. 1999. Investigation of sulphate-reducing bacteria in the constructed wetlands at Tara Mines and the environmental factors affecting their growth. Unpublised B.Sc. thesis, University College Dublin.

Zavoda, J., cutright, T., szpak, J., Fallon, E., 2001. Uptake, selectivity, and inhibition of hydroponic treatement of contaminants. Journal of Environmental Engineering, 127, 502-508.

Zhang, C.B., Wang, J., Liu, W.L., Zhu, S.X., Liu, D., Chang, S.X., Chang, J., Ge, Y., 2010. Effects of plant diversity on nutrient retention and enzyme activities in a full scale constructed wetland. Bioresource Technology 101, 1686-1692

❐❐

PRETREATMENT: AN INEVITABLE STEP FOR UTILIZATION OF LIGNOCELLULOSIC WASTE TO WEALTH

Sunil Kumar Behera[1], Sandeep Kumar Panda[2], Sudharshan Sekar[2] and Antoine Floribert Mulaba-Bafubiandi[1]

[1]Mineral Processing and Technology Research Center,
Department of Metallurgy,
[2]Department of Biotechnology and Food Technology
P. O. Box 17911. Doornfontein Campus, 2028, University of Johannesburg, South Africa
*Corresponding Author: skbehera2020@gmail.com

1. Introduction

Lignocellulosic wastes (LW) are plant biomass origin; bio-chemically these are composed of cellulose, hemicellulose, and lignin. The plant biomass based LW are generated from wood residues (including sawdust and paper mill discards), grasses, waste paper, agricultural residues (including straw, peelings, cobs, stalks, nutshells, non food seeds, bagasse), domestic wastes (lignocellulose garbage and sewage), food industry residues, municipal solid wastes and the like (Qi et al., 2005; Roig et al., 2006; Rodríguez et al., 2008). The lignocellulosic biomass wastes represent the largest renewable reservoir of potentially fermentable carbohydrates on earth which are mostly generated during pre-harvest and post-harvest agricultural losses and wastes in food processing industries (Mtui and Nakamura, 2005). Due to the abundance and renewability potential of lignocellulosic wastes it draw attention for their exploitation in the production and recovery of many value-added products (Das and Singh, 2004; Foyle et al., 2007). The prominent value added products recovered from LW include enzymes, reducing sugars, furfural, ethanol, protein and amino acids, carbohydrates, lipids, organic acids, phenols, activated carbon, degradable plastic composites, cosmetics, bio-sorbent, resins, medicines, foods and feeds, methane, bio-pesticides, bio-

promoters, secondary metabolites, surfactants, fertilizer and other miscellaneous products (Tengerdy and Szakacs, 2003; Mtui, 2007; Swain and Ray, 2007; Demirbas, 2008; Swain and Ray, 2008).

Recently biofuel is gaining momentum as an alternative to conventional petroleum fuel. Large scale biofuel production started with the first generation biofuels i.e. fuel production from food crops. For example ethanol production from sugarcane in Brazil, corn ethanol in US and palm oil biodiesel in Malaysia were popular biofuels. Though the first generation biofuels could reduce the greenhouse gas emissions and was beneficial in energy balance, still some drawbacks were observed. The most important hindrance to the first generation biofuel was food security, as it was a competitor with food crops. Hence the focus of biofuel production shifted to second generation biofuels, known as advanced biofuels. The second generation biofuel does not depend on agricultural/horticultural crops rather depends on lignocellulosic biomass, woody crops, agricultural and horticultural residues etc. regarded as waste. However the large scale production of biofuel is becoming a threat for food security in society. The existing techniques used for bioconversion of LW to value added products has long been considered to be very expensive. But recent increase in grain prices have influenceding the biofuel producing industries to utilize LW instead of food grains for production of biofuel. As a consequence the production of second generation bio-products such as biofuels from LW will reduce influx of food grains for biofuel production. Technologies that will allow cost effective conversion of biomass into fuels and chemicals consider economy of scale, low-cost pretreatment systems and highly effective and efficient biocatalysts (Schneider and McCar, 2003; Gray et al., 2006).This chapter represents the recent developments in LW pretreatment, value addition and techno-economic considerations.

2. Pretreatment Technologies for Lignocellulosic Wastes

Pretreatment of LW is the most essential and major steps in bioconversion process for development of value added products. Because, the lignocellulose present in LW chemically consist of

cross linking linked between the polysaccharides (cellulose and hemicellulose) and the lignin via ester and ether linkages, form strong bonds and thus they create barrier for enzymatic degradation (Xiao et al., 2007). Cellulose, hemicellulose and lignin are organized into micro fibrils that provide structural stability to the plant cell (Rubin, 2008). The main objective for any pretreatment process is to alter or remove structural and compositional impediments by hydrolysis and subsequent degradation processes in order to enhance digestibility, improve the rate of enzyme hydrolysis and increase yields of intended products (Mosier et al., 2005; Hendriks and Zeeman, 2009). These pretreatment processes cause mechanical, physical chemical or biological alterations in the plant biomass in order to achieve the desired products.

2.1 Mechanical Pretreatment

Mechanically based pretreatment technologies are aimed at reducing the size of LW to facilitate subsequent treatments. Reduction of biomass size below ~20 sieves shows the best mechanical performance (de Sousa et al., 2004). Mechanical pretreatment technologies increase the digestibility of cellulose and hemicellulose in the lignocellulosic biomass. The use of mechanical chopping (de Sousa et al., 2004); hammer milling (Mani et al., 2004); grind milling (Mtui and Nakamura, 2005); roll milling (Qi et al., 2005); vibratory milling (Guerra et al., 2006) and ball milling (Inoue et al., 2008) have been adopted as low cost pretreatment strategies. The mechanically treated pulverized LW materials (corn stover, barley straw, sugar cane baggage, wheat straw, wood waste and municipal solid waste) have increased surface area and further these are effectively processed by physicochemical and biochemical pretreatments routes. The mechanicallly pretreatment improves the cellulose and hemi-cellulose enzymatic digestibility with lower enzyme loads. Mechanical pretreatment also result into substantial lignin de-polymerization via the cleavage of uncondensed-aryl ether linkages (Inoeu et al., 2008). Solubility and fermentation efficiency of the natural lignocellulosic residues is also substantially improved by

mechano-physicochemical pretreatment, leading to value-added utilization of these residues (Qi et al., 2005). However the need for high capital investment for mechanical pretreatment setup makes it difficult for medium and small enterprises.

2.2 Physical Pretreatment

Thermal pretreatment and radiation exposure are the most successful physical treatments steps in the processing of LW. Thermogravimetric treatment of wood waste under both inert and oxidant atmospheres from room temperature up to 1100 K leads to dehydration and decomposition of hemicellulose, cellulose and lignin (Lapuerta et al., 2004). On the other hand, pyrolysis of nutshells, straws, sawdust and municipal solid wastes at temperatures of 600 - 1200 K results in to yields of char, liquid and gaseous products of up to 55% of the original LW (Demirbas, 2002; Bonelli, 2003; Álvarez et al., 2005; Phan et al., 2008). Irradiation can cause significant breakdown of the structure of LW. Microwave irradiation at a power of up to 700 W at various exposure times resulted to weight loss due to degradation of cellulose, hemicellulose and lignin, and the degradation rates are significantly enhanced by the presence of alkali (Zhu et al., 2005, 2006). Also it has been reported that, the gamma radiation cause significant breakdown of the structure in the powdered (140 mesh) wheat straw, leading to weight loss and glucose yield of 13.40% at 500 kGy (Yang et al., 2008). Although the process is proved successful environmental issues raised during the scaling up of the thermal pretreatment is a matter of concern.

2.3 Physicochemical Pretreatment

Hemicellulose dissolution and alteration in lignin structure induced by the combined effort of chemical and physical pretreatment systems providing an improved accessibility for the cellulose for hydrolytic enzymes (Hendriks and Zeeman, 2009). The most successful physicochemical pretreatments include thermochemical treatments such as steam explosion or (steam disruption), liquid hot water (LHW), ammonia fiber explosion (AFEX) and CO_2 explosion (Sun and Cheng, 2002). In these

processes, chipped biomass is treated with high-pressure saturated steam, liquid ammonia or CO_2 and then the pressure is swiftly reduced, making the materials to undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160 - 260°C (corresponding pressure of 0.69 - 4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure. The processes favors degradation of hemicellulose and lignin transformation due to high temperature, thus it enhance the pace of cellulose hydrolysis. Supplement of H_2SO_4 (or SO_2) or CO_2 in steam explosion of LW can effectively improve enzymatic hydrolysis, decreasinge the production of inhibitory compounds, and lead to more complete liquefaction of hemicellulose, glucan, xylan, mannan, galactan, and arabinan (Jeoh and Agblevor, 2001; Sun and Cheng, 2002). Such pretreatments also lead to higher digestion efficiencies during production of monosaccharides, oligosaccharides, lactic acid, antibacterial violet pigments and methane gas (Asada et al., 2005; Wang and Chen, 2007; Öhgren et al., 2007)). Wet oxidation pretreatment at 200 - 210°C in the presence of alkali or Na_2CO_3 leads to LW solubilization and better enzymatic convertibility to value-added products (Lissens et al., 2004; Martín et al., 2008). Liquid hot water (LHW) pretreatment utilizes pressurized hot water at pressure less than 5 MPpa and temperature range of 170 - 230°C for several minutes followed by decompression up to atmospheric pressure. Bagasse, corn stalk and straws of wheat, rice and barley pretreated by LHW have been reported to effect 80 - 100% hemicellulose hydrolysis, resulting to 45-65% of xylose release (Sun and Cheng, 2002; Sánchez and Cardona, 2008). On the other hand, in AFEX treatment, the dosage of liquid ammonia ranging from 1-2 kg ammonia/kg dry biomass, temperature 90°C, and residence time of 30 min can significantly improve the saccharification rates (Thomsen and Belinda, 2007). On CO_2 explosion, 75% of the theoretical glucose released during 24 h of the enzymatic hydrolysis has been reported (Sun and Cheng, 2002). Ethanol yield of up to 83% of the theoretical value has been obtained when LW were subjected to physicochemical treatment (Jeoh and Agblevor, 2001).

2.4 Chemical Pretreatment

Various chemicals reagents such as oxidizing agents, alkali, acids and salts can be used to degrade lignin, hemicellulose and cellulose from LW. Use of powerful oxidizing agents such as ozone and H_2O_2 have been reported to effectively removed lignin. Also they do not produce toxic residues for the downstream processes; and the reactions need only ambient temperature and pressure (Sun and Cheng, 2002). Alkali (NaOH, $Ca(OH)_2$, NaOH-urea, Na_2CO_3) hydrolyses of rice straw (Carrillo et al., 2005); spruce wood waste (Zhao et al., 2007); sugarcane, cassava and peanuts wastes (Thomsen and Belinda, 2007); corn cob (Torre et al., 2008); organic fraction of municipal solid waste (Torres and Lloréns, 2008) have been investigated. When these pretreatments are performed by using 0.5 - 2 M alkali at 120 - 200°C, they substantially facilitate saccharification and improve enzymatic hydrolysis of LW. Dilute and concentrated acids at high temperature are suited for hydrolysis of LW. Studies by del Campo et al. (2006) and Karimi et al. (2006) have established that 0.5% H_2SO_4 is optimal for treatment of wastes from vegetables and rice straw, respectively. More concentrated H_2SO_4 (up to 2.5 M) has been shown to be able not only to hydrolyse cellulose and hemicellulose, but also in separating lignin and other organic components from LW (Alma and Acemioglu, 2004; Okafoagu and Nzelibe, 2006; Rahman et al., 2007). SO_2 and fly ash in flare gas; HNO_3, HCl and polyhydric alcohol in the presence of sulfuric acid are also useful in LW pretreatment (Herrera et al., 2004; Hassan and Shukry, 2008): Recent studies have shown that when acids are combined with alkali, they play a more effective role in LW pretreatment than acids and alkalis alone (Damisa et al., 2008). Organic acids such as oxalic, acetylsalicylic and salicylic acid can be used as catalysts in the organosolv process whereby an organic or aqueous organic solvent mixture with inorganic acids (HCI or H_2SO_4) are used to break the internal lignin and hemicellulose bonds. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol (Sun and Cheng, 2002). The use of a dicarboxylic acid catalyst, maleic acid, for

hemicellulose hydrolysis in corn stover overcomes the technical and economic hurdle of hemicellulose hydrolysis (Lu and Mosier, 2007). Excessive use of acids and hazardous chemical reagents makes the process more complicated as exposure to chemical reagents have ill effects on the workers as well as on the environment.

In addition to the description of each pre-treatment methods, the advantages and dis-advantages of each method will help readers conclude the better treatment method.

2.5 Ionic liquids as a Tool for Lignocellulosic Biomass Fractionation

Lignocellulosic biomass is highly recalcitrant and various pretreatment techniques are needed to facilitate its conversion to value added products. Ionic liquids (ILs) are of interest in pretreatment because of their potential to dissolve lignocellulosic materials including crystalline cellulose (John et al., 2014). Deconstruction and fractionation of lignocellulosic biomass, in ionic liquids focuses on the solubility of lignocellulosic biomass along with the other biopolymers present within it (Brandt, Agnieszka et al., 2013).

The deconstruction of the lignocellulosic biomass makes these fractions susceptible for easier transformation to large number of commodities including energy, chemicals and material within the concept of bio refinery. Generally, the biomass pre-treatment depends on the final goal in the biomass processing. The recalcitrance of lignocellulose materials is the main limitation in their processing. Furthermore, none of the currently known processes is highly selective and efficient for the satisfactory and versatile use, thus, there is inevitable need for new methodologies. The ionic liquid technology on biomass processing is relatively recent and first studies were focused on the lignocellulosic biomass dissolution in different ionic liquids (ILs). The dissolution in IL drives to the structural changes in the regenerated biomass by reduction of cellulose crystallinity and lignin content contrasting to the original biomass. These findings

provided ILs as tools to perform biomass pre-treatment and the advantageous use of their specific properties over the conventional pre-treatment processes. (da Costa Lopes, 2013).

2.6 Biological Pretreatment

Biological treatment refers to the use of microorganisms or enzymes derived from microbes in pretreatment of LW. A wide range of microbes belong to fungi and bacteria have been used for pretreatment of LW. Biological pretreatment is considered as "green technology" as it does not have any harmful effects on the environment and has no toxic emission. Fungal pretreatment of agro-industrial residues is a new method for improvement of digestibility (Sinegani et al., 2005). White-, brown- and soft-rot fungi are used to degrade lignin and hemicellulose in waste materials whereby brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Sun and Cheng, 2002). Recent studies have shown that *Aspergillus terreus* (Emtiazi et al., 2001); *Trichoderma* spp (Pérez et al., 2002); *Cyathus stercoreus* (Keller et al., 2003); *Lentinus squarrosulus* (Shide et al., 2004); *Lentinus edodes* (Brienzo et al., 2007); *Trametes pubescens* (Melamane et al., 2007); *Penicillium camemberti* (Taeli, 2008), *Phanerochaete chrysosporium* (Shi et al., 2008) grown at 25 - 35°C for 3 - 22 days resulted to 45 - 75% and 65 - 80% hemicellulose and lignin degradation, respectively. The post-treatment by anaerobic bioprocesses of LW effluents that have been pretreated with fungi can lead to higher biogas than the original effluents (Coulibaly et al., 2003). Recombinant strains of *Saccharomyces cerevisiae* have been genetically engineered to carry out simultaneous saccharification and fermentation (SSF) to produce extracellular endoglucanase and _-glucosidase that are able to ferment cellulose and hemicellulose to 6-carbon and 5-carbon sugars and subsequent fermentation to ethanol (Sedlak and Ho, 2004; Wisselink et al., 2007). In bioorganosolv process, fungal *(Ceriporiopsis subvermispora)* pretreatment of wood waste for 2 - 8 weeks followed by organic solvent treatment at

140-200°C for 2 h has achieved considerable energy efficient delignification and hemicellulose hydrolysis (Itoh et al., 2003; Sánchez and Cardona, 2008). Bacterial pretreatment of LW involves both anaerobic and aerobic systems. Anaerobic degradation utilizes mainly mesophillic, rumen derived bacteria (Han and Shin, 2002; Neves et al., 2006; Yue et al., 2008). Aerobic-anaerobic systems have an upper hand when it comes to degradation of LW richer in lignin content (Ammary, 2004; Mshandete et al., 2008) while in aerobic system alone, actinomycete *Streptomyces griseus* is able to produce high levels of extracellular hydrolytic enzyme that degrade lignocellulose (Arora et al., 2005). *Escherichia coli* and *Klebsiella oxytoca* strains have been genetically engineered to produce microbial biocatalysts that produce bioethanol from lignocellulosic materials (Jarboe et al., 2007; Peterson and Ingram, 2008). Enzymatic pretreatment of LW utilize hydrolytic and oxidative enzymes which are mainly derived from fungi and bacteria. Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase (endo-1,4-glucanohydrolase) which attacks regions of low crystallinity in the cellulose fiber, creating free chainends; (2) exoglucanase or cellobiohydrolase (CBH) (1,4-glucan cellobiohydrolase) which degrades the molecule further by removing cellobiose units from the free chainends and (3) glucosidase which hydrolyzes cellobiose to produce glucose (Sun and Cheng, 2002). In addition, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetylesterase, feruloylesterase, xylanase, xylosidase, galactomannanase and glucomannanase (Mtui and Nakamura, 2005, Roman et al., 2006; Georgieva et al., 2008). During the enzymatic hydrolysis, cellulose is degraded by cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol. Ligninolytic enzymes are primarily involved in lignin degradation in oxidative reactions that are mainly free radical driven in the presence (or sometimes absence) of mediators. The main enzymes involved are lignin peroxidase, manganese peroxidase and laccase (Mtui and Masalu, 2008). The hydrolytic

and oxidative enzymatic reactions are mainly carried out at 30 - 45°C with low enzyme loading rate at reaction time of 6 - 26 h.

3. Processes of Lignocellulosic Wastes into Value added Product Production

Advances in industrial biotechnology offer potential opportunities for economic utilization of agro-industrial residues. Keeping in view the demand of the biological products, researchers are interested to develop them from renewable resource like LW (Howard et al., 2003).

3.1 Enzymes

Enzymes are bio-catalysts responsible for various metabolic activities that maintain life. Knowledge is established to produce important enzymes from cheaper substrates such as LW.

Lignocellulosic enzymes, mainly derived from fungi and bacteria, are important commercial products of LW bioprocessing used in many industrial applications including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture (Howard et al., 2003). Overall, extracellular enzymes are secondary metabolic products released in the presence of inducers at N-limited media (Mtui and Nakamura, 2007). They include hydrolytic enzymes such as cellulases; hemicellulases and pectinases; degradative enzymes like amylases, proteases; and ligninolytic enzymes like laccases, peroxidases and oxidases. Cellulases production from LW has been extensively studied (Ojumu et al., 2003; Wen et al., 2005; Muthuvelayudham and Viruthagiri, 2006; Gao et al., 2008, Fujii et al., 2015). Phytases, mannanases and amylases are also produced by microorganisms using LW as the main feedstock (Bhavsar et al., 2008; Mabrouk et al., 2008). On the other hand, hemicellulolytic enzymes, mainly xylanases, are produced from a wide range of LW biomass (Abdel-Sater and El-Said 2001; Dobrev et al., 2007; Mohana et al., 2008). Pectinases such as endopolygalacturonase (endo-PG), exo-polygalacturonase (exo-PG) and pectinliase are mainly produced from solid state fermentation processes utilizing agricultural residues (Silva et al., 2005; Botella et al., 2007),

while protease has been produced by *Penicillium janthinellum* in submerged cultures (Oliveira et al., 2006). Among the ligninases produced from LW, laccases are the mostly studied (Nazareth and Sampy, 2003; Couto et al., 2006, Majumdar et al., 2014), followed by Manganese peroxidase and lignin peroxidase (Couto and Sanromána, 2005; Songulashvili et al., 2007; Elisashvili et al., 2008). Very high enzyme activities (31,786 U/L) have been reported when the experiments are carried out under optimal conditions (pH 5.5-6: temperature 30-45°C) (Rosales et al., 2007). Recovery of pure enzymes is achieved through 50 - 80% $(NH_4)_2SO_4$ saturation followed by chromatographical purification techniques (Mtui and Nakamura, 2008). Several efforts have been made to increase the production of enzymes through strain improvement by mutagenesis and recombinant DNA technology. Cloning and sequencing of the various genes of interest could economize the enzymes production processes (Kumar et al., 2008).

3.2 Biofuels

Worldwide, fossil oil prices increase dramatically and it induces negative impact on the environment, particularly greenhouse gas emissions, for which there is a growing concern over for use of fossil fuel (Hahn-Hägerdal et al., 2006). Conversion of LW to biofuels provides the best economically feasible and conflict-free second generation renewable alternatives (Rubin, 2008, Gong et al., 2014). Significant advances have been made towards bioconversion of plant biomass wastes into bioethanol, biodiesel, biohydrogen, biogas (methane). Production of ethanol from sugars or starch from sugarcane and cereals, respectively, impacts negatively on the economics of the process, thus making ethanol more expensive compared with fossil fuels. Hence, the technology development focus for the production of ethanol has shifted towards the utilization of residual lignocellulosic materials to lower production costs (Howard et al., 2003). Currently, research and development of saccharification and fermentation technologies that convert LW to reducing sugars and ethanol, respectively, in eco-friendly and profitable manner

have picked tempo with breakthrough results (Lin and Tanaka, 2006; Prasad et al., 2007; Sánchez and Cardona, 2008). Ethanol yield of 6-21% has been obtained through fermentation of agricultural and municipal residues (Sjöde et al., 2007; Cara et al., 2008). While microaeration enhances productivity of bioethanol from LW using ethanologenic *E. coli* (Okuda et al., 2007), simultaneous saccharification and fermentation (SSF) using recombinant *Saccharomyces cereviasiae* result to as high as 62% of the theoretical value (Itoha et al., 2003). The principal benefits of performing the enzymatic hydrolysis together with the fermentation, instead of in a separate step after the hydrolysis, are the co-fermentation of both hexoses and pentoses during SSF which resulted in reduced end-product inhibition of the enzymatic hydrolysis and the reduced investment costs (Olofsson et al., 2008). Life cycle assessment (LCA) shows that bio-ethanol from LW results to reductions in resource use and global warming (von Blottnitz and Curran, 2007). The long-term benefits of using waste residues as lignocellulosic feedstocks will be to introduce a sustainable solid waste management strategy for a number of lignocellulosic waste materials; contribute to the mitigation in greenhouse gases through sustained carbon and nutrient recycling; reduce the potential for water, air, and soil contamination associated with the land application of organic waste materials; and to broaden the feedstock source of raw materials for the bio-ethanol production industry (Champagne, 2007). Bio-diesel is a renewable fuel conventionally prepared by transesterification of pre-extracted vegetable oils and Mtui 1403 animal fats of all resources with methanol, catalyzed by strong acids or bases (Liu and Zhao, 2007). They are fatty acid methyl or ethyl esters used as fuel in diesel engines and heating systems (Ito et al., 2005). Production of biodiesel from lignocellulosic residues such as olive oil wastes has been a subject of research towards improving the thermal waste treatment systems and cleaner energy production. Since the current supplies from LW based oil crops and animal fats account for only approximately 0.3%, biodiesel from algae is widely regarded as one of the most efficient ways of generating biofuels and also appears to represent the only current renewable

source of oil that could meet the global demand for transport fuels (Schenk et al., 2008). Hydrogen has been considered a potential fuel for the future since it is carbon-free and oxidized to water as a combustion product (Najafpour et al., 2004). While conventional burning or composting seem to be the most cost-effective hydrogen production methods, bacteria such as *Enterobacter aerogenes* and *Clostridium* sp isolates can convert saccharified LW biomass into biohydrogen (Ito et al., 2003). Biohydrogen production from agricultural residues such as olive husk pyrolysis (Çalar and Demirba, 2002); conversion of wheat straw wastes into biohydrogen gas by cow dung compost (Fan et al., 2006); bagasse fermentation for hydrogen production (Singh et al., 2007) generate up to 70.6% gas yields. System optimization for accessibility of polysaccharides in LW and the use of genetically efficient bacterial strains for agro waste-based hydrogen production seem to be the ideal option for clean energy generation. Hydrogen generation from inexpensive abundant renewable biomass can produce cheaper hydrogen and achieve zero net greenhouse emissions (Zhang et al., 2007). Biogas production from lignocellulosic materials is a steady anaerobic process where methane rich biogas comes mostly from hemicellulose and cellulose. Anaerobic biomethane production is an effective process for conversion of a broad variety of agricultural residues to methane to substitute natural gas and medium calorific value gases (Demirbas and Ozturk, 2005).

Biogas containing 55 - 65% methane can be produced from jute caddis - a lignocellulosic waste of jute mills by anaerobic fermentation, using cattle dung as sole source of inoculum (Banik, 2004). Anaerobic digestion of poultry droppings, cow dung and corn stalk can give up to 137.16 L of biogas from 0.28 m3 digester (Anozie et al., 2005). Mesophilic aerobic pretreatment to delignify sisal pulp waste prior to its anaerobic digestion has been shown to improve methane yields (Mshandete et al., 2008). Overall, the success of biofuels production from LW is dependent on the optimal performance and cost effectiveness of pretreatment and product generation processes.

3.3 Organic Acids

Organic acids are some of the products of ligninolytic residues fermentations via environmentally friendly integrated processes. Volatile fatty acids including acetic acid, propionic acids and butyric acid are produced from a wide range of LW such as cereal hulls (Jin et al., 2006); bagasse residues (Henrique et al., 2005); food wastes (Lim et al., 2008) and sisal leaf decortications residues (Mshandete et al., 2008). In addition, lactic acid is produced from waste sisal stems (Muruke et al., 2006), sugarcane bagasse (Adsul et al., 2007) and kitchen waste (Ohkouchi and Inoue, 2007) by using Lactobacillus isolates. Furthermore, formic acid, levulinic acid, citric acid, valeric acid, caproic acid and vanillinic acid are obtainable from bioprocessing of LW (Chaudhary and Sharma, 2005; Mshandete et al., 2008). Overall, organic acids production requires batch or continuous incubation conditions, the average reaction parameters being 35°C, pH 6.0, hydraulic retention time (HRT) of up to 8 days and organic loading rates of 9 g/l d. Product yields of up to 39.5 g/l have been reported (Lim et al., 2008).

3.4 Compost

Compost, a nutrient-rich, organic fertilizer and soil conditioner, is a product of humification of organic matter. This process is aided by a combination of living organisms including bacteria, fungi and worms which transform and enhance lignocellulosic waste into humic-like substances (Eyheraguibel et al., 2008). Vermicomposting is the bio-oxidation and stabilization of organic matter involving the joint action of earthworms and microorganisms, thereby turning wastes into a valuable soil amendment called vermicompost (Benitez et al., 2005). Substrates suitable for making humus-rich compost include cereal straw and bran (Hart et al., 2003); urban wastes (Taiwo and Oso, 2004); water hyacinth (Chatterjee et al., 2005); lemon tree prunings, cotton waste and brewery waste (García-Gómez et al., 2005); horticultural wastes (Lopez et al., 2006); olive, palm and grape wastes (Alburquerque et al., 2006). While bacteria inoculants such as *Bacillus shackletonni, Streptomyces thermovulgaris*

and *Ureibacillus thermosphaericus* are used to improve the composting process (Vargas-Garci et al., 2007), ligno-cellulolytic fungi inocula (*e.g. Trichurus spiralis*) may also be used in a pretreatment process before composting in order to reduce the resistance of the substrate to biodegradation (Vargas-García et al., 2007). A new earthworm strain of *Perionyx sansibaricus* is able to humify a substrate combination of guar gum industrial waste, cow dung and saw dust (Suthar, 2007). Composting can, therefore, be considered as a low-cost technology to convert agro industrial LW into value-added biofertilizers.

3.5 Biosorbents

Adsorbents obtained from plant wastes are feasible replacements for costly conventional methods of removing pollutants such as heavy metals ions, dyes, ammonia and nitrates from the environment. The use of lignocellulosic agrowastes is a very useful approach because of their high adsorption properties, which results from their ion-exchange capabilities. Agricultural wastes can be made into good sorbents for the removal of many metals, which would add to their value, help reduce the cost of waste disposal, and provide a potentially cheap alternative to existing commercial carbons (Krishnani and Ayyappan, 2006). Chemically modified plant wastes such as rice husks/rice hulls, spent grain, sugarcane bagasse/fly ash, sawdust, wheat bran, corncobs, wheat and soybean straws, corn stalks, weeds, fruit/ vegetable wastes, cassava waste fibres, tree barks, azolla (water fern), alfalfa biomass, coirpith carbon, cotton seed hulls, citrus waste and soybean hulls show good adsorption capacities for Cd, Cu, Pb, Zn and Ni (Dupont et al., 2005; Harman et al., 2007; Siban et al., 2008). They are usually modified with formaldehyde in acidic medium, NaOH, KOH/K_2CO_3 and CO_2, or acid solution or just washed with warm water (Siban et al., 2008). Scanning electron micrographs with energy spectra shows that heavy metals are immobilized via two possible routes: adsorption and cation exchange on hypha, and the chelation by fungal metabolite (Huang et al., 2008). LW have also been shown to be able to adsorb dyes from aqueous solutions. Adsorption of reactive dyes by

sawdust char and activated carbon (Gan et al., 2004); ethylene blue by waste *Rosa canina* sp. seeds (Gurses et al., 2006); anionic dyes by hexadecyltrimethylammonium modified coir pith (Namasivayam and Sureshkumar, 2006); and methylene red by acid-hydrolysed beech sawdust (Batzias and Sidiras, 2007) have been reported. Ammonia and nitrate removal by using agricultural waste materials as adsorbents or ion exchangers have also been studied (Orlando et al., 2002; Kishore et al., 2006). Prehydrolysis enhances the adsorption properties of the original LW material due to the removal of the hemicelluloses during sulphuric acid treatment, resulting in the 'opening' of the lignocellulosic matrix's structure, the increasing of the surface area and the activation of the material's surface owing to an increase in the number of dye binding sites (Batzias and Sidiras, 2007).

4. Future Prospects

Pretreatment of LW is a committed step to produce superior products of demand. Improved technology has also added one further step. With the increase in population and demand of biological products such as enzymes, organic acids, polymers etc. are increasing day by day. Furthermore, the limited resource of fossil fuel on the planet has compelled the researchers to plan for alternative fuels from cheaper substrate like LW. Abuse of plastic is alarming on environmental point of view, hence biodegradable plastic from LW is gaining momentum. It is envisaged that LW will be one of the prime substrates for production of value added biological products in future.

Conclusions

Application of modern knowledge of biotechnology in LW utilization can produce alternatives of fossil fuel, thereby reducing the greenhouse gas emission. The LW are abundant for processing into value added products without affecting the food chain. Improvement in pretreatment conditions can produce simple sugars from the LW to be processed further. The advent of modern genetic engineering along with the knowledge of waste management can be useful tools for over expression of superior

products from LW. Use of ionic liquids to yield fermentable sugars from lignocellulose is a new development in this field of biofuel production.

Although pretreatment systems and the concomitant release of bio-products from LW have been greatly improved by new technologies, there are still challenges that need further investigations. These challenges include development of more efficient pretreatment and production technologies, bioprospecting and development of stable genetically engineered microorganisms, improved gene cloning and sequencing technologies and enhancement of productions based on economies of scale for more efficient and cost effective conversions of LW into value-added products. So far, lignocellulosic biomass has been the most promising economically viable and renewable source of biohydrogen and biofuel. Therefore, extensive research is now being directed toward that end. Plant fibers as fillers and reinforcements for polymers are currently the fastest-growing type of polymer additives. Nanobiotechnology seems to take charge as far as the use of LW nanofibres in plastic composites is concerned. (Alemdar and Sain, 2008, Iwamoto et al., 2014). It is envisaged that nano materials from renewable biowastes will be the main focus of future research.

References

Abdel-Sater MA, El-Said AHM (2001). Xylan-decomposing fungi and xylanolytic activity in agricultural and industrial wastes. Int. Biodeterioration Biodegrad. 47(1): 15-21.

Adsul MG, Varma AJ, Gokhale DV (2007). Lactic acid production from waste sugarcane bagasse derived cellulose. Green Chem. 9: 58-62.

Alburquerque JA, Gonzálvez J, García D, Cegarra J (2006). Effects of bulking agent on the composting of "alperujo", the solid by-product of the two-phase centrifugation method for olive oil extraction. Process Biochem. 41(1): 127-132.

Alemdar A, Sain M (2008). Isolation and characterization of nanofibers from agricultural residues - Wheat straw and

soy hulls. Bioresour. Technol. 99(6): 1664-1671.

Alma MH, Acemioglu B (2004). A kinetic study of sulfuric acid-catalyzed liquefaction of wood into phenol Chem. Eng. Comm. 191(7): 968-980.

Álvarez P, Santamaría R, Blanco C, Granda M (2005). Thermal degradation of lignocellulosic materials treated with several acids. J. Analyt. Appl. Pyrolysis 74(1-2): 337-343.

Ammary BY (2004). Nutrients requirements in biological industrial wastewater treatment. Afr. J. Biotechnol.3 (4): 236-238. and mineral nutrition of maize. Bioresour. Technol. 99(10: 4206-4212.

Anozie AN, Layokun SK, Okeke CU (2005). An evaluation of a batch pilot-scale digester for gas production from agricultural wastes. Energy Sources, Part A: Recovery, Util. Environ. Effects 27(14):1301-1311.

Arora A, Nain L, Gupta JK (2005). Solid-state fermentation of wood residues by Streptomyces griseus B1, a soil isolate, and solubilization of lignins. World J. Microbiol. Biotechnol. 21(3): 303-308.

Asada C, Nakamura Y, Kobayashi F (2005). Waste reduction system for production of useful materials from un-utilized bamboo using steam explosion followed by various conversion methods. Biochem. Eng. J. 23(2): 131-137

Banik S (2004). Jute caddis: A new substrate for biogas production. J. Sci. Ind. Res. 63(9): 747-751.

Batzias FA, Sidiras DH (2007). Dye adsorption by prehydrolysed beech sawdust in batch and fixed-bed systems. Bioresour. Technol. 98(6): 1208-1217.

Benitez E, Sainzh H, Nogales R (2005). Hydrolytic enzyme activities of extracted humic substances during the vermicomposting of a lignocellulosic olive waste. Bioresour. Technol. 96(7): 785-790

Bhavsar K, Shah P, Soni SK, Khire JM (2008). Influence of pretreatment of agriculture residues on phytase production

by Aspergillus niger NCIM 563 under submerged fermentation. Afr. J. Biotechnol. 7(8): 1101-1106.

Bonelli PR (2003). Slow pyrolysis of nutshells: characterization of derived chars and of process kinetics . Energy Sources, 25(8): 767- 778.

Botella C, Diaz A, Ignacio de Ory I, Webb C, Blandino A (2007). Xylanase and pectinase production by *Aspergillus awamori* on grape pomace in solid state fermentation. Process Biochem. 42(1): 98-101.

Brandt A, Grasvik J, Hallett J. P, Welton T (2013) Deconstruction of lignocellulosic biomass with ionic liquids. Green chemistry 15 (3): 550-583.

Brienzo M, Silva EM, Milagres AMF (2007). Degradation of eucalypt waste components by *Lentinula edodes* strains detected by chemical and near-infrared spectroscopy methods. J. Appl. Biochem. Biotechnol. 141(1): doi 10.1007/ s12010-007-9209.

Ça lar A, Demirba A (2002). Hydrogen rich gas mixture from olive husk via pyrolysis. Energy Conversion Manag. 43(1): 109-117

Cara C, Ruiza E, Ballesteros M, Manzanares P, Negro MJ, Castro E (2008). Production of fuel ethanol from steam-explosion pretreated olive tree pruning. Fuel 87(6): 692-700.

Carrillo F, Lisa MJ, Colom X, López-Mesas M, Valldeperas J (2005). Effect of alkali pretreatment on cellulase hydrolysis of wheat straw: Kinetic study. Process Biochem. 40(10): 3360-3364.

Champagne P (2007). Feasibility of producing bio-ethanol from waste residues. Resourc. Conserv. Recycling 50:(3) 211-230.

Chatterjee P, Metiya G, Saha N, Haldar M, Mukherjee D (2005). Methods and substrate oriented composting of lignocellulosic materials for the production of humus rich composts. Environ. Ecol. 23(1): 55-60.

Chaudhary N, Sharma, CB (2005). Production of citric acid and single cell protein from agrowaste. Nat. Acad. Sci. Lett. 28(5/6): 189-193.

Coulibaly L, Gourene G, Agathos NS (2003). Utilization of fungi for biotreatment of raw wastewaters. Afric. J. Biotechnol.2 (12): 620-630

Couto SR, Sanromán MA (2005). Application of solid-state fermentation to ligninolytic enzyme production. Biochem. Eng. J. 22(3): 211-219

Couto SR, Sanromán MÁ (2006). Effect of two wastes from groundnut processing on laccase production and dye decolourisation ability. J. of Food Eng. 73(4): 388-393

da Costa Lopes A. M, Karen G Joao K. G, Morais A. R. C, Bogel-Lukasik E, Bogel-Lukasik R (2013). Ionic liquids as a tool for lignocellulosic biomass fractionation. Sustain. Chem. Process 1 (3): 1-31.

Damisa D, Ameh J, Umoh, VJ (2008). Effect of chemical pretreatment of some lignocellulosic wastes on the recovery of cellulase from *Aspergillus niger* AH3 mutant. Afr. J. Biotechnol. 7 (14): 2444-2450.

Das H, Singh S (2004). Useful by-products from cellulosic wastes of agriculture and food industry - A critical appraisal. Crit. Rev. Food Sci. Nutr. 44(2): 77-89

De Sousa MV, Monteiro SN, d'Almeida JRM (2004). Evaluation of pretreatment, size and molding pressure on flexural mechanical behavior of chopped bagasse-polyester composites. Poly. Testing 23 (3): 253-258.

Del Campo I, Alegría I, Zazpe M, Echeverría M, Echeverría I (2006). Diluted acid hydrolysis pretreatment of agri-food wastes for bioethanol production. Industrial Crops Products 24(3): 214-221.

Demirbas A (2002). Utilization of urban and pulping wastes to produce synthetic fuel via pyrolysis. Energy Sources Part A: Recovery, Util. Environ. Effects, 24(3): 205-213.

Demirbas A (2008). Products from lignocellulosic materials via degradation processes. Energy Sources Part A: Recovery, Utilization, and Environmental Effects 30(1): 27 - 37.

Demirbas A, Ozturk T (2005). Anaerobic digestion of agricultural solid residues. Int. J. Green Energy 1(4): 483-494

Dobrev GT, Pishtiyski IG, Stanchev VS, Mircheva R (2007). Optimization of nutrient medium containing agricultural wastes for xylanase production by *Aspergillus niger* B03 using optimal composite experimental design. Bioresour. Technol. 98(14): 2671- 2678.

Dupont L, Bouanda J, Dumonceau J, Aplincourt M (2005). Biosorption of Cu(II) and Zn(II) onto a lignocellulosic substrate extracted from wheat bran. J. Environ. Chem. Lett. 2(4): 165-168.

Elisashvili V, Kachlishvili E, Penninckx MJ (2008). Lignocellulolytic enzymes profile during growth and fruiting of *Pleurotus ostreatus* on wheat straw and tree leaves. J. Acta Microbiologica et Immunologica Hungarica 55(2): 157-168.

Emtiazi G, Naghavi N, Bordbar A (2001). Biodegradation of lignocellulosic waste by *Aspergillus terreus*. Biodegradation 12(4): 257-261.

Eyheraguibel B, Silvestre J Morard P (2008). Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. Bioresour. Technol. 99(10: 4206-4212.

Fan YT, Zhang YH, Zhang SF, Hou HW, Ren BZ, (2006). Efficient conversion of wheat straw wastes into biohydrogen gas by cow dung compost. Bioresour. Technol. 97(3): 500-505.

Foyle T, Jennings L Mulcahy P (2007). Compositional analysis of lignocellulosic materials: Evaluation of methods used for sugar analysis of waste paper and straw. Bioresour. Technol. 98(16): 3026- 3036.

Fujii T, Koike H, Sawayama S, Yano, S, Inoue H (2015). Draft Genome Sequence of *Talaromyces cellulolyticus* Strain Y-94, a Source of Lignocellulosic Biomass-Degrading

Enzymes. Genome Announc. 3(1): 14-15.

Gan Q, Allen SJ, Matthews R (2004). Activation of waste MDF sawdust charcoal and its reactive dye adsorption characteristics. Waste Management. doi: 10.1016/j. wasman. 2004.02.010. 24(8): 841-848.

Gao J, Weng H, Zhu D, Yuan M, Guan F, Xi Y (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. Bioresour. Technol. 99(16): 7623-7629.

García-Gómez A, Bernal MP, Roig A (2005). Organic matter fractions involved in degradation and humification processes during composting. Compost Sci. Util. 13:(2) 127-135.

Georgieva TI, Hou X, Hilstrøm1 T, Ahring BK (2008). Enzymatic hydro lysis and ethanol fermentation of high dry matter wet-exploded wheat straw at low enzyme loading. J. Appl. Biochem. Biotechnol. 148(1-3): 35-44.

Gong Z, , Wang Q, Shen H, Wang, L, Xie H, Zhao Z K (2014). Conversion of biomass-derived oligosaccharides into lipids. Biotechnology for Biofuels 7:13

Gray KA, Zhao L, Emptage M (2006). Bioethanol. Curr. Opin. Chem. Biol. 10(2): 141-146.

Guerra A, Filpponen I, Lucia LA, Saquing C, Baumberger S, Argyropoulos DS (2006). Toward a better understanding of the lignin isolation process from wood. J. Agric. Food Chem., 54(16): 5939-5947.

Hahn-Hägerdal B, Galbe M, Gorwa-Grauslunda MF, Lidén G, Zacchi G (2006). Bio-ethanol- the fuel of tomorrow from the residues of today. Trends Biotechnol. doi: 10.1016/ j.tibtech.2006.10.004. 24(1): 549- 556.

Han SK, Shin HS (2002). Enhanced acidogenic fermentation of food waste in a continuous-flow reactor. Waste Manag. Res. 20(2): 110- 118.

Harman G, Patrick R, Spittler T (2007). Removal of heavy metals

from polluted waters using lignocellulosic agricultural waste products. Ind. Biotechnol. doi:10.1089/ind.2007.3.366. 3(4): 366-374.

Hart TD, Lynch JM,De Leij FAAM (2003). Production of Trichurus spiralis to enhance the composting of cellulose-containing waste. Enz. Microbial. Technol. 32(6):745-750.

Hassan EM, Shukry N (2008): Polyhydric alcohol liquefaction of some lignocellulosic agricultural residues. Ind. Crops Products. 27(1): 33-38.

Hendriks ATWM, Zeeman G (2009). Pretreatments to enhance the digestibility of lignocelluloses biomass. Bioresour. Technol. .100: (1)10-18.

Hendriks ATWM, Zeeman G (2009). Pretreatments to enhance the digestibility of lignocelluloses biomass. Bioresour. Technol. 100(1)10-18.

Henrique M, Baudel HM, Zaror C, de Abreu CAM (2005). Improving the value of sugarcane bagasse wastes via integrated chemical production systems: An environmentally friendly approach. Ind. Crops Products, 21 (3): 309-315.

Herrera A, Téllez-Luis SJ, González-Cabriales JJ, Ramírez JA, Vázquez M (2004): Effect of the hydrochloric acid concentration on the hydrolysis of sorghum straw at atmospheric pressure. J. Food Eng. 6(1): 103-109.

Howard RL, Abotsi E, Jansen van Rensburg EL, Howard S (2003). Lignocellulose biotechnology: Issues of bioconversion and enzyme production. Afr. J. Biotechnol. 2(12): 602-619.

Huang DL, Zeng GM, Feng CL, Hu S, Jiang XY, Tang L, Su FF, Zhang, Y, Zeng W, Liu HL (2008). Degradation of lead-contaminated lignocellulosic waste by *Phanerochaete chrysosporium* and the reduction of lead toxicity. Environ. Sci. Technol. 42(13): 4946-4951.

Hydrolysis of sugar cane bagasse using nitric acid: a kinetic assessment. J. Food Eng. 61(2): 143-152.

Inoue H, Yano S, Endo T, Sakaki T, Sawayama S (2008).

Combining hot-compressed water and ball milling pretreatments to improve the efficiency of the enzymatic hydrolysis of eucalyptus. Biotechnol Biofuels. 2008; 1(2): 1-9.

Ito T, Nakashimada Y, Senba K, Matsui T, Nishio N (2005). Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process. J. Biosci. Bioeng. 100(3): 260-265.

Itoh H, Wada M, Honda Y, Kuwahara M, Watanabe T (2003). Bioorganosolve pretreatments for simultaneous saccharification and fermentation of beech wood by ethanolysis and white rot fungi. J. Biotechnol. 103(3): 273-280.

Iwamoto S, Yamamoto S, Lee S, Ito H, Endo T (2014). Mechanical and Thermal Properties of Polypropylene Composites Reinforced with Lignocellulose Nanofibers Dried in Melted Ethylene-Butene Copolymer. Materials 7:6919-6929.

Jarboe LR, Grabar TB, Yomano LP, Shanmugan KT, Ingram LO (2007). Development of ethanologenic bacteria. Adv Biochem Eng Biotechnol. 108: 237-261.

Jeoh T, Agblevor FA (2001). Characterization and fermentation of steam exploded cotton gin waste. Biomass and Bioenergy 21(2): 109-120.

Jin F, Zhou Z, Kishita A, Enomoto H (2006). Hydrothermal conversion of biomass into acetic acid. J. Mater. Sci. 41(5): 1495-1500.

John Grasvik J, Winestrand S, Normark M, Jonsson L. J, and Mikkola J (2014). Evaluation of four ionic liquids for pretreatment of lignocellulosic biomass. BMC biotechnology 14:34

Karimi K, Kheradmandinia S, Taherzadeh MJ (2006). Conversion of rice straw to sugars by dilute-acid hydrolysis. Biomass Bioenergy 30(3): 247-253.

Keller FA, Hamilton JE, Nguyen QA (2003). Microbial pretreatment of biomass: Potential for reducing severity of

thermochemical biomass pretreatment. J. Appl. Biochem. Biotechnol. 105(1-3): 27-41.

Kishore KK, Parimala V, Gupta BP, Azad IS, Meng X, Abraham M (2006). Bagasse-assisted bioremediation of ammonia from shrimp farm wastewater. Water Environ. Res. 78(9): 938-950(13).

Krishnani KK, Ayyappan S (2006). Heavy metals remediation of water using plants and lignocellulosic agrowastes. Rev. Environ. Contam. Toxicol. 188: 59-84.

Kumar R, Singh S, Singh OV (2008). Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. J. Ind. Microbiol. Biotechnol. 35(5): 377-391.

Lapuerta M, Hernández JJ, Rodríguez J (2004). Kinetics of devolatilisation of forestry wastes from thermogravimetric analysis. Biomass Bioenergy. 27(4): 385-391

Lim SJ, Kim BJ, Jeong CM, Choi J, Ahn YH, Chang HN (2008). Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor. Bioresour. Technol. 99(16): 7866- 7874.

Lin Y, Tanaka S (2006). Ethanol fermentation from biomass resources: Current state and prospects. J. Appl. Microbiol. Biotechnol. 69 (6): 627-642.

Lissens G, Klinke H, Verstraete W, Ahring B, Thomsen AB (2004). Wet oxidation pre-treatment of woody yard waste: Parameter optimization and enzymatic digestibility for ethanol production. J. Chem. Technol. Biotechnol. 79(8): 889-895.

Liu B, Zhao ZK (2007). Biodiesel production by direct methanolysis of oleaginous microbial biomass. J. Chem. Technol. Biotechnol. 82(8): 775-780.

Lopez MJ, Carmen M, Vargas-García C, Suárez-Estrella F, Moreno J (2006). Biodelignification and humification of horticultural plant residues by fungi. Int. Biodeterioration Biodegradation. 57(1): 24-30.

Lu Y, Mosier NS (2007). Biomimetic catalysis for hemicellulose hydrolysis in corn stover. Biotechnol. Prog., 23(1): 116-123.

Mabrouk MEM, El-Ahwany AMD (2008). Production of _-mannanase by *Bacillus amylolequifaciens* cultured on potato peels. Afr. J. Biotechnol. 7(8): 1123-1128.

Majumdar S, Lukk T, Solbiati JO, Bauer S, Nair SK, Cronan JE, Gerlt JA (2014). Roles of small laccases from Streptomyces in lignin degradation. Biochemistry 53(24):4047-58.

Mani S, Tabil LG, Sokhansanj S (2004). Grinding performance and physical properties of wheat and barley straws, corn stover and switchgrass. Biomass Bioenergy, 27(4): 339-352.

Martín C, Marce M, Thomsen AB (2008). Comparison of wet oxidation and steam explosion as pretreatment methods for bioethanol production from sugarcane bagasse. BioResour. 3(3): 670-683.

Melamane X, Tandlich R, Burgess J (2007). Anaerobic digestion of fungally pre-treated wine distillery wastewater. Afr. J. Biotechnol. 6(17): 1990-1993.

Mohana S, Shah A, Divecha J, Madamwar D (2008). Xylanase production by *Burkholderia* sp. DMAX strain under solid state fermentation using distillery spent wash. Bioreasour. Technol. 99(16): 7553-7564.

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol. 96(6): 673-686.

Mshandete AM, Björnsson L, Kivaisi AK, MST Mattiasson B (2008). Effect of aerobic pre-treatment on production of hydrolases and volatile fatty acids during anaerobic digestion of solid sisal leaf decortications residues. Afric. J. Biochem. Res. 2(5):111-119.

Mtui G (2007). Trends in industrial and environmental biotechnology research in Tanzania. Afr. J. Biotechnol. 6(25): 2860-2567.

Mtui G, Masalu R (2008). Extracellular enzymes from Brown-rot fungus *Laetioporus sulphureus* isolated from mangrove forests of Coastal Tanzania. Sci. Res. Essay 3: 154-161.

Mtui G, Nakamura Y (2005): Bioconversion of lignocellulosic waste from selected dumping sites in Dar es Salaam, Tanzania Biodegradation, 16(6): 493-499

Mtui G, Nakamura Y (2005): Bioconversion of lignocellulosic waste from selected dumping sites in Dar es Salaam, Tanzania Biodegradation, 16(6): 493-499

Mtui G, Nakamura Y (2007). Characterization of lignocellulosic enzymes from white-rot fungus *Phlebia crysocreas* isolated from a marine habitat. J. Eng. Appl. Sci. 2: 1501-1508.

Mtui G, Nakamura Y (2008). Lignocellulosic enzymes from *Flavodon flavus,* a fungus isolated from Western Indian Ocean off the Coast of Dar es Salaam, Tanzania. Afr. J. Biotechnol. 7(17): 3066-3072.

Muruke MHS, Hosea KM, Palangyo A, Heijthuijsen JHFG (2006). Production of lactic acid from waste sisal stems using a *Lactobacillus* isolate. Discovery Innov., 18(1): 1-5.

Muthuvelayudham R, Viruthagiri T (2006). Fermentative production and kinetics of cellulase protein on *Trichoderma reesei* using sugarcane bagasse and rice straw. Afr. J. Biotechnol. 5 (20): 1873-1881.

Namasivayam C, Sureshkumar MV (2006). Anionic dye adsorption characteristics of surfactant-modified coir pith, a waste lignocellulosic polymer. J. Appl. Poly. Sci. 100(2): 1538-1546.

Nazareth SW, Sampy JD (2003). Production and characterisation of lignocellulases of *Panus tigrinus* and their application. Int. Biodeterioration Biodegradation, 52(4): 207-214.

Neves L, Oliveira R, Alves MM (2006). Anaerobic co-digestion of coffee waste and sewage sludge. Waste Manag. 26(2): 176-181.

Öhgren K, Vehmaanperä J, Siika-Aho M, Galbe M, Viikari L,

Zacchi G (2007). High temperature enzymatic prehydrolysis prior to simultaneous saccharification and fermentation of steam pretreated corn stover for ethanol production. Enzyme Microbial. Technol. 40(4):

Ohkouchi Y, Inoue Y (2007). Impact of chemical components of organic wastes on l(+)-lactic acid production. Bioresour. Technol. 98(3): 546- 553.

Ojumu TV, Solomon BO, Betiku E, Layokun SK, Amigun B (2003). Cellulase Production by *Aspergillus flavus* Linn Isolate NSPR 101 fermented in sawdust, bagasse and corncob. Afr. J. Biotechnol. 2(6): 150-152.

Okafoagu CU, Nzelibe, HC (2006). Effect of acid hydrolysis of *Garcinia kola* (bitter kola) pulp waste on the production of CM-cellulase and _- glucosidase using *Aspergillus Niger*. Afr. J. Biotechnol. 5 (10): 819-

Okuda N, Ninomiya K, Takao M, Katakura Y, Shioya S (2007). Microaeration enhances productivity of bioethanol from hydrolysate of waste house wood using ethanologenic *Escherichia coli* KO11. J. Biosci. Bioeng. 103(4): 350-357.

Oliveira LA, Porto ALF, Tambourgi EB (2006). Production of xylanase and protease by *Penicillium janthinellum* CRC 87M-115 from different agricultural wastes. Bioreasour. Technol. 97(6): 862-867

Olofsson K, Bertilsson M, Lidén G (2008). A short review on SSF, an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnol Biofuels 1(7): 1-14.

Orlando US, Baes AU, Nishijima W, Okada M (2002). A new procedure to produce lignocellulosic anion exchangers from agricultural waste materials. Bioreasour. Technol. 83(3): 195-198.

Pérez J, Muñoz-Dorado J, de la Rubia T, Martínez J (2002). Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. J. Int. Microbiol. 5(2): 53-63.

Peterson JD, Ingram LO (2008). Anaerobic respiration in engineered *Escherichia coli* with an internal electron acceptor to produce fuel ethanol. Ann. NY Acad. Sci. 1125: 363-372.

Phan AN, Ryu C, Sharifi VN, Swithenbank J (2008). Characterisation of slow pyrolysis products from segregated wastes for energy production. J. Analyt. Appl. Pyrolysis, 81(1).: 65-71.

Prasad S, Singh A, Joshi H (2007). Ethanol as an alternative fuel from agricultural, industrial and urban residues. Reasourc. Conserv. Recycling, 50(1): 1-39

Qi BC, Aldrich C, Lorenzen L, Wolfaardt GW (2005). Acidogenic fermentation of lignocellulosic substrate with activated sludge. Chem. Eng. Communications, 192(9): 1221-1242.

Rahman SHA, Choudhury JP, Ahmad AL, Kamaruddin AH (2007):Optimization studies on acid hydrolysis of oil palm empty fruit bunch fiber for production of xylose. Bioreasour. Technol. 98(3): 554-559.

Rodríguez-Chonga A, Ramírez JA, Garrote G, Vázquez M (2004). Hydrolysis of sugar cane bagasse using nitric acid: a kinetic assessment. J. Food Eng. 61(2): 143-152.

Roig A, Cayuela ML, Sánchez-Monedero MA (2006). An overview on olive mill wastes and their valorisation methods. Waste Manag. 26(9): 960-969.

Roman HJ, Burgess JE, Pletschke BI (2006). Enzyme treatment to decrease solids and improve digestion of primary sewage sludge. Afr. J. Biotechnol. 5(10): 963-967.

Rosales E, Couto SR, Sanromán MA (2007). Increased laccase production by *Trametes hirsuta* grown on ground orange peelings. Enz. Microbial. Technol. 40(5): 1286-1290.

Rubin EM (2008). Genomics of cellulosic biofuels. Nat. 454(14): 841- 845.

Šiban M, Klašnja M, Škrbi_ B (2008). Adsorption of copper ions from water by modified agricultural by-products. Desalination 229(1-3): 170-180.

Sánchez OJ, Cardona CA (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour. Technol. 99(13): 5270-5295

Schneider UA, McCar BA (2003). Economic potential of biomass based fuels for greenhouse gas emission mitigation. J. Environ. Resour. Economics, 24(4): 291-312.

Sedlak M, Ho NWY (2004). Production of ethanol from cellulosic biomass hydrolysates using genetically engineered *Saccharomyces* yeast capable of cofermenting glucose and xylose. J. Appl. Biochem. Biotechnol. 114(1-3): 403-416.

Shi J, Chinn MS, Sharma-Shivappa RR (2008). Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*. Bioresour. Technol. 99(14).: 6556-6564.

Shide EG, Wuyep PA, Nok A (2004). Studies on the degradation of wood sawdust by *Lentinus squarrosulus* (Mont.) Singer. Afr. J. Biotechnol. 3(8): 395-398.

Silva D, Tokuioshi K, Martins ED, Da Silva R, Gomes E (2005). Production of pectinase by solid-state fermentation with *Penicillium viridicatum*. RFC3 Process Biochem. 40(8): 2885-2889.

Sinegani AAS, Emtiazi G, Hajrasuliha S, Shariatmadari H (2005). Biodegradation of some agricultural residues by fungi in agitated submerged cultures. Afr. J. Biotechnol. (10): 1058-1061.

Singh SP, Asthana RK, Singh (2007). Prospects of sugarcane milling waste utilization for hydrogen production in India. Energy Policy 35(8): 4164-4168.

Sjöde A, Alriksson B, Jönsson LJ, Nilvebrant NO (2007). The potential in bioethanol production from waste fiber sludges in pulp mill-based biorefineries. J. Appl. Biochem. Biotechnol. pp.137-140(1-12): 327- 337.

Songulashvili G, Elisashvili V, Wasser SP, Nevo E, Hadar Y (2007). Basidiomycetes laccase and manganese peroxidase activity in sub merged fermentation of food industry wastes. Enz. Microbial. Technol. 41(1-2): 57-61.

Sun Y, Cheng J (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review. Bioresour. Technol. 83(1): 1-11.

Suthar S (2007). Production of vermifertilizer from guar gum industrial wastes by using composting earthworm *Perionyx sansibaricus* (Perrier). J. Environmentalist 27(3): 329-335.

Swain M. R. Ray R.C. (2007). Statistical optimization for -amylase production by *Bacillus subtilis* CM 3 isolated from cowdung microflora in solid-state fermentation using cassava fibrous residue. Journal of Basic Microbiology. 47(5): 417-128.

Swain M.R. and Ray R.C. (2008). Optimization of culture conditions and their statistical interpretation for production of indole-3-acetic acid by *Bacillus subtilis* CM5 using cassava *(Manihot esculenta Crantz)* fibrous residue. Journal of Scientific Industrial Research. 67: 622-628

Taiwo LB, Oso BA (2004). Influence of composting techniques on microbial succession, temperature and pH in a composting municipal solid waste. Afr. J. Biotechnol. 3(4): 239-243.

Tengerdy RP, Szakacs G (2003). Bioconversion of lignocellulose in solid substrate fermentation. Biochem. Eng. 13(2-3): 169-179.

Thomsen MC, Belinda A (2007). Wet oxidation pretreatment of lignocellulosic residues of sugarcane, rice, cassava and peanuts for ethanol production. J. Chem. Technol. Biotechnol. 82(2): 174-181.

Torre P, Aliakbarian B, Rivas B, Domínguez JM Converti A (2008). Release of ferulic acid from corn cobs by alkaline hydrolysis. Biochem. Enging J. 40(3): 500-506.

Torres ML, Lloréns MCE (2008). Effect of alkaline pretreatment on anaerobic digestion of solid wastes. Waste Manag. 28(11): 2229- 2234.

Vargas-Garc? MC, Suárez-Estrella F, López MJ, Moreno J (2007). Effect of inoculation in composting processes: Modifications in lignocellulosic fraction. Waste Manag. 27(9): 1099-1107.

Wang H, Chen HZ (2007). A novel method of utilizing the biomass resource: Rapid liquefaction of wheat straw and preparation

of biodegradable polyurethane foam (PUF). J. Chinese Inst. Chem. Eng. 38(2): 95-102.

Wen Z, Liao W, Chen S (2005). Production of cellulase by *Trichoderma reesei* from dairy manure. Bioresour. Technol. 96(4): 491-499

Wisselink HW, Toirkens MJ, Berriel MDRF, Winkler AA, van Dijken JP, Pronk JT, van Maris AJA (2007). Engineering of Saccharomyces cerevisiae for efficient anaerobic alcoholic fermentation of Larabinose. Appl. Environ. Microbiol. (15): 4881-4891.

Xiao C, Bolton R, Pan WL (2007). Lignin from rice straw kraft pulping: Effects on soil aggregation and chemical properties. Bioresour. Technol. 98(7): 1482-1488.

Yang C, Shen Z, Yu G, Wang J (2008). Effect and after effect of radiation pretreatment on enzymatic hydrolysis of wheat straw. Bioresour. Technol. 99(14): 6240-6245.

Yue ZB, Yu HQ, Hu ZH, Harada H, Li YY (2008). Surfactant-enhanced anaerobic acidogenesis of *Canna indica* L. by rumen cultures. Bioresour. Technol. 99(9): 3418-3423.

Zhang M, Cui SW, Cheung, PCK, Wang Q (2007). Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. Trends Food Sci. Technol. 18(1): 4-19.

Zhao Y, Wang Y, Zhu JY, Ragauskas A, Deng Y (2007). Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. Biotechnol. Bioeng. 99:(6). 1320-1328.

Zhu S, Wu Y, Yu Z, Zhang X, Li H, Gao M (2006). The effect of microwave irradiation on enzymatic hydrolysis of rice straw. Bioresour. Technol. 97(15): 1964-1968.

❐❐

MICROBIAL BENEFICIATION: AN EFFECTIVE ALTERNATIVE FOR UTILIZATION OF LOW GRADE IRON ORE

M. Mishra[1*], R.C. Mohanty[2], L.B.Sukla[1]

[1]Institute for Applied Environmental Biotechnology Innovation, Bhubaneswar, Odisha

[2]P.G. Dept. of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha-751013

[*]Corresponding Author: mousumi_official@yahoo.com, suklalb@yahoo.com

Abstract

The reserves of high grade ore are getting diminished throughout the world at an alarming rate on the other hand a huge amount of low grade ore, slimes and fines are wasted in form of discards. The basic impurities present in the low grade iron ore are in form of aluminosilicates. The ratio of alumina and silica present in low grade Indian iron ore is about 1.5:3 and in case of fines it is 3:4. The ratio is detrimental for smooth blast furnace operation . To deal with this problem the presently available physico-chemical methods are neither cost nor environmental efficient. Microbial beneficiation can provide eco friendly, promising and revolutionary solution to these problems.

Some heterotrophic fungi like Aspergillus sp. and Penicillium sp. have shown the potential of metal solubilization. Metal dissolution occurs mainly due to the enzymatic reduction of the highly oxidized metal compounds or by the production of organic acids (oxalic, citric, lactic, gluconic acids etc.). Hence the fungal metabolites have dual effect on the process, they lower the pH of the system by production of acids and decrease the concentration of free metal ions by formation of complexes thus allowing more solid to go into the solution. In the present chapter we will discuss the efficiency of fungal strains with emphasis on Aspergillus niger for beneficiation of iron ore, the mechanism behind the process and the future possibilities.

Keywords: Aspergillus niger, alumina , silica, organic acids.

1. Introduction

As a consequence of technological advancement, the depletion rate of valuable mineral is increasing. It is becoming difficult to get minerals in free form. As the high grade ores are getting exhausted at a rapid rate it is high time to think about the extraction of required metals from low grade ores and wastes generated during mining or processing of high grade ores. In the current chapter, we will discuss about the problems with Indian iron ore and how to overcome it with the help of biohydrometallurgical techniques.

In India within last few decades the iron and steel industry has grown in an exponential rate. On the basis of this growth the National Steel Policy has revised the domestic steel production to 180 million tons by the year 2019-20 (Iron and steel vision 2020, Indian bureau of mines). India is very rich in iron ore reserves. Hematite and magnetite are the most prominent ores found in India. Hematite is most used ore due to its high grade and lumpy nature. The total resources of iron ore in the country is estimated to be 285 billion tons (UNFC) out of which hematite accounts for 62.7%and magnetite for 37.3%.

The route of iron production mainly used in India is through blast furnace. The raw materials used in the process are lumpy iron ore, agglomerates, metallurgical coke and fluxes (limestone and dolomite). By using high quality iron ore the productivity of blast furnace increases and energy consumption decreases. Thus higher the iron content in the ore, the lower the amount of slag produced. 1% increase in the iron content of ore improves the productivity by 2% and reduces the coke rate by 1% (Iron and steel vision 2020, Indian bureau of mines). Therefore usually an ore containing more than 60% iron is considered for extraction of pig iron in blast furnace.

Indian iron ore is high in iron content but also have high alumina and silica ratio which affects the grade of ore. The adverse alumina to silica ratio is detrimental for blast furnace and sinter plant productivity. It should be preferably below 1%

for smooth blast furnace operation. High alumina content in the iron ore generates highly viscous slag. It needs higher amount of fluxes and coke. Thereby producing huge volumes of slag hence that productivity of the blast furnace is adversely affected. Increase in content of alumina by 1%, increases the coke rate by 2.2% which eventually decreases the productivity by 4%. It also increases the consumption of flux by 30kg/t of hot metal production (Iron and steel vision 2020, Indian bureau of mines). In Indian iron ore alumina is present in form of kaolinite, gibbsite, laterite and silica in form of quartz. In regard of the above aspects beneficiation of the ore is highly essential.

Now a days there is a greater interest in technologies that use microorganisms .to mobilize or remove unwanted contaminants from valuable minerals. Such technologies, collectively referred to as biohydrometallurgy, are positively acknowledged for their environmental and economic advantages (Jain *et al.,* 2004, Rawlings, 2005) and could be utilised in extraction and purification of different minerals during and after mining operations. (Yusfin *et al.,* 1999; Davis *et al.,* 1978; Elkasabgy, 1984). Several types of autotrophic and heterotrophic bacteria, fungi, yeast and algae are implicated in mineral beneficiation processes. Selective leaching, flotation and flocculation are some of the process involved in mineral bioprocessing (Natrajan and deo, 2001). Microbial metal extraction from non sulphidic minerals has received little attention to date. Non sulphidic ores such as oxides, carbonates and silicates contain no energy source for the chemolithoautotrophs to utilize, so alternatively can be leached or beneficiated by using heterotrophic microbes, which require organic carbon as a source of energy and for their growth.

Almost all knowledge of biohydrometallurgy developed up to now deals with the use of chemolithoautotrophic bacteria for leaching of sulfidic minerals and ores. Leaching mechanisms of nonsulfidic minerals (carbonates, oxides and silicates) using heterotrophs have received less attention from microbiologists (Kiel and Schwartz, 1980). Among the heterotrophic bacteria, members of the genus Bacillus have been found effective in the

leaching and bio beneficiation of bauxite (Karavaiko et al., 1989). Fungi from the genera *Penicillium* and *Aspergillus* have also been used in mineral leaching (Ehrlich and Rossi, 1990). Heterotrophic microorganisms require organic carbon as a source of energy and carbon. They produce metabolic by-products from the organic carbon they consume for energy production that may interact with a mineral surface. In addition to forming several organic acids such as acetic, citric, oxalic, and keto-gluconic acid (Agatzini and Tzeferis, 1997; Castro *et al.,* 2000; Natarajan and Deo, 2001) heterotrophic microorganisms also produce exopolysaccharides (Malinovskaya *et al.,* 1990; Welch *et al.,* 1999), amino acids and proteins that can solubilize the metals via a variety, of mechanisms. However, organic acids occupy a central position in the overall process supplying both protons and a metal complexing organic acid anion (Gadd, 1999).

2. Microbe-metal Interaction

Interaction of microbes with metals and minerals alter their physical and chemical characters and metals also affect the growth and metabolism of the related microbes. The way microbes interact with metals depends in part on whether the organisms are prokaryotic or eukaryotic. Both types of microbes have the ability to bind metal ions present in the external environment at the cell surface or to transport them into the cell for various intracellular functions (Ehrlich, 1997). Metals are directly and/or indirectly involved in all aspects of microbial growth, metabolism and differentiation (Gadd, 1992a). Metals and their compounds interact with microbes in various ways depending on the metal species, organism and environment, while structural components and metabolic activity also influence metal speciation and therefore solubility, mobility, bioavailability and toxicity (Gadd, 1992a, 1993a, 2004, 2005, 2007). Many metals are essential for life, e.g. Na, K, Cu, Zn, Co, Ca, Mg, Mn and Fe, but all can exert toxicity when present above certain threshold concentrations. Other metals, e.g. Cs, Al, Cd, Hg and Pb, have no known essential metabolic functions but all can be accumulated (Gadd, 2010).

3. Fungal Interaction with Minerals

Fungi have significant roles in mineral dissolution and secondary mineral formation (Hughes and Lawley, 2003; Burford et al., 2003a,2003b, 2006; Fomina et al., 2005a, 2005b, Gadd, 2007). There is even some evidence that several fungi can dissolve minerals and mobilize metals at higher pH values, and over a wider redox range, faster and more efficiently than bacteria (Gu et al., 1998; Castro et al., 2000; Burford et al., 2003a, Gadd, 2007). Many activities of fungi influence their interaction with metals (Fig.1). They utilize different metals for the purpose of growth and metabolism and they satisfy their need for trace metals and associated nutrients by interacting with minerals.

Many fungal sps. can dissolute minerals and metals by heterotrphic leaching mode through different processes such as acidolysis, complexolysis, redoxolysis and metal accumulation by biomass (Burgstaller & Schinner, 1993, Gadd, 2007). The primary fungal impact on mineral dissolution appears to result from acidolysis and complexolysis, and occurs as a result of several processes including proton efflux via the plasma membrane Hþ-ATPase and/or maintenance of charge balance during nutrient uptake, the production of siderophores [for iron(III) mobilization], or respiratory CO_2 production (Gadd, 2007). However, in many fungi an important leaching mechanism occurs through the production of organic acids (e.g. oxalic acid, citric acid) (Adams et al. 1992; Francis et al., 1992; Devevre et al., 1996; Sayer et al., 1997). Organic acid excretion by fungi is inter- and intraspecific, and can be strongly influenced by the presence of toxic metal minerals (Sayer et al., 1995; Fomina et al., 2004, 2005c; Sayer & Gadd, 2001). Fungal derived carboxylic acids with strong chelating properties (e.g. oxalic acid, citric acid) can aggressively attack mineral surfaces (Sayer et al., 1997; Gharieb et al. 1998; Gadd, 1999; Gharieb & Gadd, 1999; Fomina et al., 2004). Oxalic acid can leach those metals that form soluble oxalate complexes, including aluminium and Fe (Strasser et al., 1994; Devevre et al., 1996). Moreover, the production of organic acids provides another source of protons. The significance of proton

versus ligand promoted dissolution may depend on the mineral, metabolic activity, and conditions of growth, including nutrient. Insoluble Metal Mineral Phosphate other anions trace organics Metal(s) (Gadd, 2007).

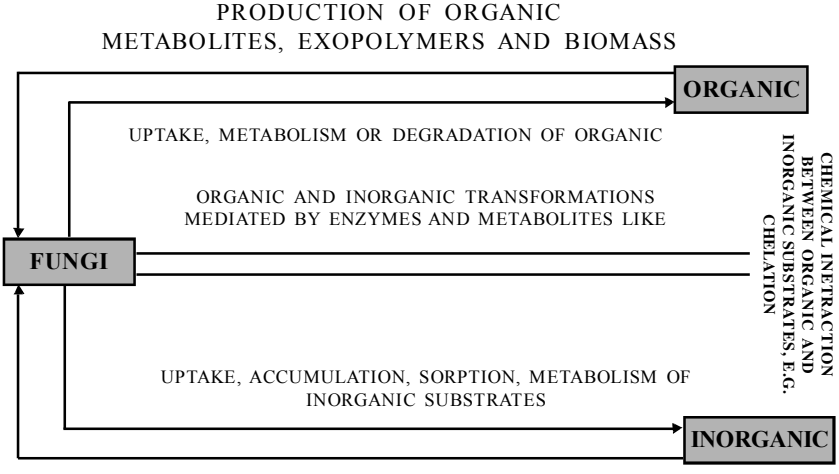


Fig. 1: Model of fungal action on naturally-occurring and/or anthropogenically-derived organic and inorganic substrates (Gadd, 2007).

The metabolites act on the ores in various ways

- **Bioreduction**

Minerals such as limonite, goethite, or hematite can be solubilized by certain microorganisms through reduction (Ehrlich 1986; Ferris et.al., 1989). Production of oxalic acid by a fungus can effect the reduction of Fe (III) to Fe (II) thus increasing iron solubility (Ghiorse, 1988).

Bennet et al.(1997) observed a relation between microbial colonization, iron reduction and silicate weathering. They explained the reductive dissolution of iron oxide minerals and linked the silicate minerals weathering to microbial iron reduction through the production of extra cellular ligands.

- **Acidification**

Welch and Ullman (1993) showed that the lowering of pH

to less than 5 results in an increased dissolution rate of many silicate and aluminium silicate minerals. Acid production is due to the formation of an acidic metabolite or from a preferential utilization of alkaline substrate.

Microbial oxidation of organic compounds may produce non-complexing or weak complexing acids (carbonic, nitric, sulphuric, formic, acetic, butyric, lactic, succinic, gluconic acid etc.). Among the organic acids, 2-ketogluconic acid produced by some bacteria and citric acid and oxalic acid produced by some fungi have been shown to be very active in the dissolution of silicates (Drever and Stillings, 1997; Vandevivere et al., 1994; Welch and Ullman, 1993). They furnish protons that help in breaking Si-O and Al-O bonds, through protonation and catalysis. Table 1 shows the main leaching agents produced by different fungal strains.

Table 1: Organic acids produced as leaching agents by different fungal strains.

FUNGI	MAIN LEACHING AGENT	REFERENCE
Alternaria sp citrate,	Oxalate,Citrate	*KOVALENKO and* MALAKHOVA (1990)
Aspergillus niger	Oxalate, Citrate, Gluconate, Malate,Tartarate, Succinate	DAVE et al. (1981), BOSECKER (1987)
Aspergillus ochraceus	Citrate	OGURTSOVA et al. (1989)
Aspergillus sp.	Citrate, Oxalate	TZEFERIS (1994)
Coriolus versicolor	Oxalate	SAYER et al. (1999)
Fusarium sp.	Oxalate, Malate, Pyruvate, Oxalacetate	BOSECKER (1989)
Mucor racemosus	Citrate , Succinate	MÜLLER and FÖRSTER (1964)
Paecilomyces variotii	Citrate, Oxalate	DAVE et al. (1981)
Penicillium funiculosum	Citrate	BOSECKER (1989)
Penicillium simplicissimum	Citrate, Oxalate, Gluconate	TARASOVA et al. (1993), SILVERMAN and MUNOZ (1971)

- **Ligand Production/ Complexolysis**

Complexolysis is a process that utilizes microbially formed complexing and chelating agents that mobilize mineral constituents (Fe, Al, Cu, Zn, Ni, Mn, Ca, Mg, etc.) (Beveridge, 1989).

Fermentation and degradation of organic macromolecules by microbial results in the production and excretion of organic ligands (Berthelin, 1983; Gadd, 1999; Paris et al., 1996; Tzeferis and Agatzini, 1994; Welch and Ullman, 1999).

These ligands can increase the rates of mineral (Amerhein and Surez, 1988; Wieland et al., 1988). Microbial extra cellular polysaccharides are also produced by microbes, that can enhance mineral dissolution by complexing with ions in solution, or they can inhibit dissolution by irreversible binding to reactive sites on the mineral surface (Welch and Ullman, 1999; Welch and Vandevivere, 1995). Metal solubilzation is also caused by low molecular weight, iron chelating siderophores which specifically solubilize Fe (III) (Liermann et al., 2000). Siderophores produced by bacteria not only mobilize iron for cellular metabolism but also chelate and mobilize other metals (Bennett et al., 1997). As reported, microorganisms are able to mobilize metals by (i) formation of organic acid, (ii) oxidation- reduction reactions, (iii) extraction by complexity agents, (iv) chelate formation. Organic acids however occupy a central position in the overall process and supply both proton and metal complexing organic acid anion. The solubilisation of nickel cobalt, and iron from laterites by means of organic chelating acid of low pH.

- **Alkalinization**

Biosolubilization of silicates is also possible via alkalinization of the media. The silicon-oxygen bond is disrupted under this condition. Avakyan (1985) demonstrated the release of silicon from nepheline, plagioclase or quartz by utilizing the bacteria *Sarcina ureae*, *S. ureae* grows in the presence of urea and produces ammonia resulting in the high alkalization of the medium.

4. Biological Removal of Alumina and Silica

Impurities present in iron ores comprise both metallic and non-metallic components. Usually, siliceous gangue consists of larger proportion of alumina in the form of clay and laterite along with varying amounts of undesirable constituents such as phosphorous, sulphur, titanium, copper and arsenic. Many microorganisms have been reported to solubilise different alumino-silicate compounds found in nature. In silicates, silicon is usually surrounded by four oxygen atoms in tetrahedral fashion (Kretz, 1972) whereas aluminium in aluminosilicates is coordinated with oxygen in tetrahedral or octahedral fashion, depending upon the mineral (Tan, 1986). In minerals, these units are arranged in bi- or tri-layers separated by water layers of variable thickness into which other polar molecules, including some organic molecules can enter. This type of structure makes them susceptible for weathering by microorganisms. Si-O bonds of silloxanes linkages (Si-O-Si) in silicates and aluminosilicates are very strong, whereas Al-O bonds are somewhat weaker. Thus Si-O bonds are relatively resistant to acid hydrolysis (Karavaiko et al., 1985), unlike Al-O bonds. Some bacteria and fungi are known to solubilise silica and silicates. They accomplish this by forming chelators, acids or bases, which react with silicates and exo-polysaccharides, which react with silica and silicates.

In the next section we will discuss some experiments carried out with *Aspergillus niger* to beneficiate guali iron ore.

5. Experimental Studies

5.1 Sample Collection

Iron ore sample was obtained from Guali iron ore mines, Keonjhar, Odisha, India. The sample was analyzed by wet chemical and instrumental techniques (XRF). The sample was collected, ground, dried in a hot air oven (105°C) and analyzed by standard methods.

5.2 Mineralogical Analysis of Ore

The samples were analyzed with X-ray fluorescence (XRF).

The XRF study was carried out against the calibrated samples of similar values (Table 2). The size fraction of the iron ore used in these experiments is 75 to 60 microns.Mineralogical analysis of the original and microbially treated ore was done using high-resolution synchrotronbased X-ray diffractometer (XRD) (X'pert Pro MPD, Model No. 3040/60, P-Analytical, Netherland), which is a Philips model diffractometer with CuK radiation.

5.3 Microorganisms

Aspergillus fumigatus, Aspergillus niger, and *Aspergillus flavus* were used for the beneficiation study. The fungal strains were maintained on potato dextrose agar medium and bacterial strains were maintained on nutrient agar medium.Bromfield medium was used for beneficiation studies which contains (g/L) sucrose-20, Yeast extract-1, K_2HPO_4-0.25, NH_4SO_4-0.27, $MgSO_4$ -0.75, Sodium biphosphate-0.30, pH-6.8.

5.4 Shake Flask Beneficiation Studies

Microbial strains used in the shake flask experiment are, *A. fumigatus, A. niger and, A. flavus.* Experiments were carried out with 90 ml of Bromfield medium and 10% (v/v) inoculum in 250ml Erlenmeyer flask under sterile conditions. Each flask was inoculated with 106 spores/ml of each fungus. The experiments were conducted at 20% pulp density, 35^0C and 150 rpm for 30 days. Solid samples were taken out at regular intervals of time to study the gradual decrease in the alumina and silica concentration in the ore. pH and the carbohydrate concentration of the solution was monitored regularly. At the end of each of the experiment, solid residue was separated by filtration, dried in hot air oven and analyzed for Al, Si and Fe by XRF. The XRF analyses were carried out against standard calibrated samples of similar nature.

6. Results

6.1 Mineralogical Analysis

X ray diffraction pattern of the iron ore sample show that the major minerals present in the sample are hematite, magnetite,

quartz and goethite (Figure.2). Goethite is a very important iron bearing mineral in these samples. It is of two types i.e. vitreous and ochreous. Vitreous goethite is hard and compact whereas ochreous is fine grained and friable. Kaolinite and gibbsite constitute the major volume of aluminous gangue minerals in hematite bearing iron ore sample. These minerals are fine grained and intimately associated with ochreous goethite. The Kaolinite peaks disappear at some places in the XRD results of the microbially treated samples. This shows that the organic acids produced by the microbes selectively attack the alumino silicate bonds of Kaolinite and help in releasing alumina and silica to the solution. The XRD studies also support the removal of quartz from the iron ore. Table 2 shows the initial constituents and their percentage in the guali ore.

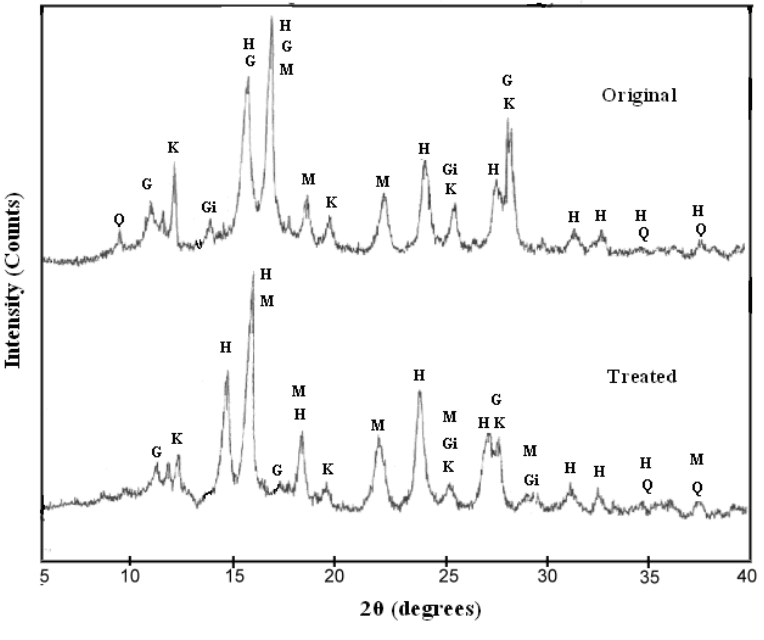


Fig. 2: XRD pattern of original and microbially treated iron ore.

H: Hematite, M: Magnetite, Gi: Gibbsite, Go: Goethite, K: Kaolinite, Q: Quartz

Table 2. XRF analysis of Guali iron ore.

Constituents	*Percentage*
Fe(Total)	53.57
Al	3.18
Si	3.54
Na	0.05
Mg	0.13
K	0.04
Ca	0.06
Mn	0.12
S	0.16
P	0.07
Ti	0.04

6.2 Shake Flask Studies

It is clearly indicated by Figure 3&4 that among the fungal strains, *Aspergillus niger* dominated the beneficiation process by removing 44.2% alumina and 48.7% silica. *Aspergillus fumigatus* and *Aspergillus flavus* removed 40.8%, 28.3% of alumina and 45.9%, 35.9% silica respectively at similar experimental conditions. Fig.5 shows the decrease in pH of the medium during the growth of the organisms. This decrease was due to the production of organic acids via incomplete oxidation of carbohydrate source.

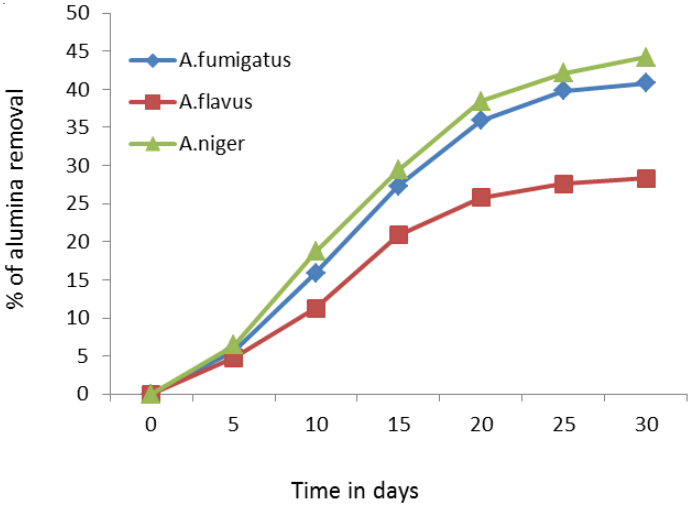


Fig. 3: Alumina removal by different fungal strains.

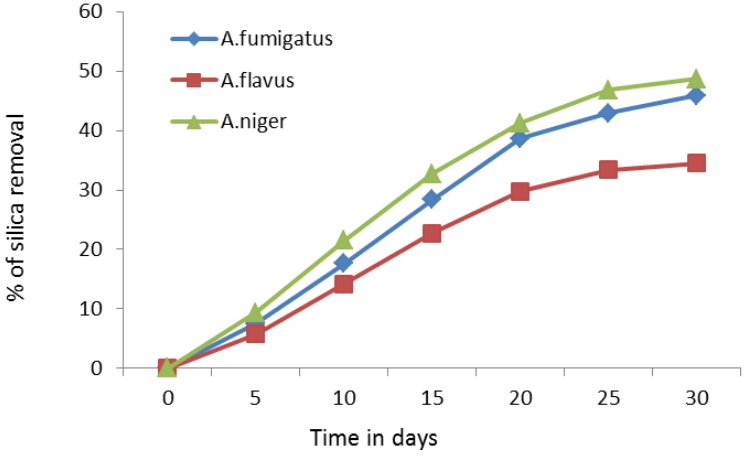


Fig. 4: Silica removal by different fungal strains.

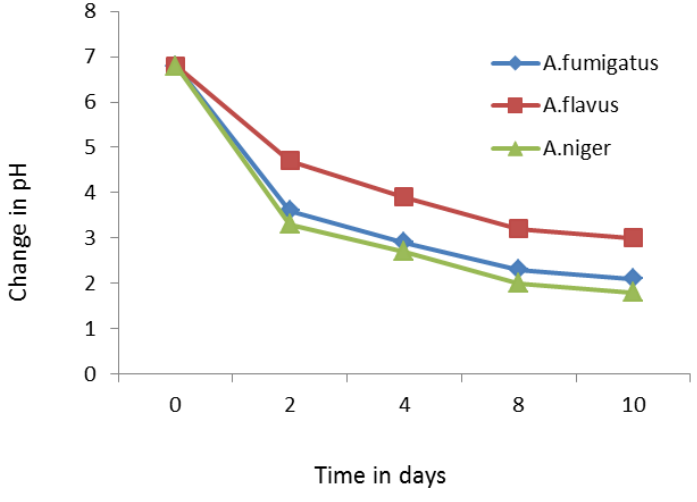


Fig. 5: Change in pH during the beneficiation study.

Conclusions

Results of the above investigation clearly established that fungal strains can effectively remove part of alumina and silica from high alumina and silica containing iron ore. The effectiveness of the procedure is greatly influenced by utilization of sugar and production of organic acids. The mineralogical studies supported the above findings that the Kaolinite structure is getting disrupted due to the attack of organic acid. Among the fungal strains *Aspergillus niger* dominated the beneficiation process by removing 44.2% alumina and 48.7% silica. *Aspergillus fumigatus* and *Aspergillus flavus* removed 40.8%, 28.3% of alumina and 45.9%, 35.9% silica respectively at 150rpm rotation, 10% pulp density and 350C.

Acknowledgments

The authors are genuinely grateful to the Institute of Minerals and Materials Technology (Council of Scientific and Industrial Research) for its constant support.

References

Adams JB, Palmer F, Staley JT, (1992). Rock weathering in deserts mobilization and concentration of ferric iron by microorganisms. Geomicrobiology Journal 10: 99-114.

Agatzini S, Tzeferis P. (1997). Bioleaching of nickel- cobalt oxide ores. Aus IMM Proc, 1: 9-15.

Amerhein C, Surez DL. 1988. The use of a surface complexation model to describe the kinetics of ligand promoted dissolution of anorthite. Geochim Cosmochim Acta 52:2785-2793.

Avakyan ZA. 1985. Microflora of rock and its role in the leaching of silicate minerals. In: Karavaiko GI, Groudev SN, editors. Biogeotechnology of metals. Moscow: Centre of International Projects, GKNT. p 175-194.

Bennett PC, Choi WJ, Roberts JA, Hiebert FK, Ullman WJ. (1997). Microbial metabolism, iron reduction and silicate dissolution: coupled processes in mineral weathering. In: Seventh Annual V.M. Goldschmidt Conference. Lunar and Planetary Institute, Houston, Texas.

Berthelin J. (1983). Microbial weathering processes. In: Krumbein WE, editor. Microb Geochem Oxford: Blackwell. 223-262.

Beveridge TJ. (1989). The structure of bacteria. In: Poindexter JS, Leadbetter ER, editors. Bacteria in nature. New York: Plenum Press. 1-65.

Burford EP, Fomina M, Gadd GM, (2003b). Fungal involvement in bioweathering and biotransformation of rocks and minerals. Mineralogical Magazine 67: 1127-1155.

Burford EP, Hillier S, Gadd GM, (2006). Biomineralization of fungal hyphae with calcite (CaCO3) and calcium oxalate mono- and dihydrate in Carboniferous limestone microcosms. Geomicrobiology Journal 23: 599-611.

Burford EP, Kierans M, Gadd GM, (2003a). Geomycology: fungal growth in mineral substrata. Mycologist 17: 98-107.

Burgstaller W, Schinner F, (1993). Leaching of metals with fungi. Journal of Biotechnology 27: 91-116.

Castro, I.M., Fietto, J.L.R., Vieira, R.X., Tropia, M.J.M., Campos, L.M.M., Paniago, E.B., Brandao, R.L., (2000). Bioleaching of zinc and nickel from silicates using *Aspergillus niger* cultures, Hydrometallurgy, 57, 39-49.

Davies J, Moon JT, Traice FB. (1978) Alkalis in the blast furnace. Ironmaking Steelmaking.; 5(4): 151-161.

Devevre O, Garbaye J, Botton B, (1996). Release of complexing organic acids by rhizosphere fungi as a factor in Norway spruce yellowing in acidic soils. Mycological Research 100.

Drever JI, Stilling LL. 1997. The role of organic acids in mineral weathering. Coll Surf 120:167-181.

Ehrlich HL, Rossi G. (1990). Other bioleaching processes. In: Ehrlich HL, Brierley CL, editors. Microbial Mineral Recovery. New York: McGraw-Hill. 149-170.

Ehrlich HL. (1986). What type of microorganisms are effective in bioleaching, bioaccumulation of metals; ore beneficiation and desulfurization of fossil fuels. Biotechnol Bioengr Symp 16:227-237.

Ehrlich, H. L. (1997). Microbes and metals. Appl Microbiol Biotechnol, 48, 687-692.

Elkasabgy T. (1984), Effect of alkalis on reduction behavior of acid iron ore pellets. Transactions ISIJ.;24:612-621.

Fomina M, Burford EP, Gadd GM, (2005b). Toxic metals and fungal communities. In: Dighton J, White JF, Oudemans P (eds), The Fungal Community: Its Organization and Role in the Ecosystem, 3rd edn. CRC Press, Boca Raton, pp. 733-758.

Fomina M, Hillier S, Charnock JM, Melville K, Alexander IJ, Gadd GM, (2005a). Role of oxalic acid over-excretion in toxic metal mineral transformations by Beauveria caledonica. Applied and Environmental Microbiology 71: 371-381.

Fomina MA, Alexander IJ, Colpaert JV, Gadd GM, (2005c). Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. Soil Biology and Biochemistry 37: 851-866.

Fomina MA, Alexander IJ, Hillier S, Gadd GM, (2004). Zinc phosphate and pyromorphite solubilization by soil plant-symbiotic fungi. Geomicrobiology Journal 21: 351-366.

Francis AJ, Dodge CJ, Gillow JB, (1992). Biodegradation of metal citrate complexes and implications for toxic metal mobility. Nature 356: 140-142.

Gadd GM. (1999). Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. Adv Microb Physiol, 41: 47-92.

Gadd, G. M. (1992a). Metals and microorganisms: a problem of definition. FEMS Microbiol Lett 100, 197-204.

Gadd, G. M. (1993a). Interactions of fungi with toxic metals. New Phytol 124, 25-60.

Gadd, G. M. (1999). Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. Adv Microb Physiol 41, 47-92.

Gadd, G. M. (2004). Microbial influence on metal mobility and application for bioremediation. Geoderma 122, 109-119.

Gadd, G. M. (2005). Microorganisms in toxic metal polluted soils. In Microorganisms in Soils: Roles in Genesis and Functions. 325-356. Edited by F. Buscot & A. Varma. Berlin: Springer-Verlag.

Gadd, G. M. (2007). Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. Mycol Res 111, 3-49.

Gadd, G.M. (2010), Metals, minerals and microbes: geomicrobiology and bioremediation, Microbiology, 156, 609-643.

Gharieb MM, Gadd GM, (1998). Evidence for the involvement of vacuolar activity in metal(loid) tolerance: vacuolar-lacking and -defective mutants of Saccharomyces cerevisiae display higher sensitivity to chromate, tellurite and selenite. BioMetals 11: 101-106.

Gharieb MM, Gadd GM, (1999). Influence of nitrogen source on the solubilization of natural gypsum (CaSO4$2H2O) and the formation of calcium oxalate by different oxalic and citric acidproducing fungi. Mycological Research 103: 473-481.

Ghiorse WC. (1988). Microbial reduction of manganese and iron. In: Zehnder AJB, editor. Biology of anaerobic microorganisms. New York:Wiley.178-210.

Gu JD, Ford TE, Berke NS, Mitchell R, (1998). Biodeterioration of concrete by the fungus Fusarium. International Bioterioration and Biodegradation 41: 101-109.

Hughes KA, Lawley B, (2003). A novel Antarctic microbial endolithic community within gypsum crusts. Environmental Microbiology5: 555-565.

Jain N, Sharma D., (2004) Biohydrometallurgy for nonsulfidic minerals ? a review. Geomicrobiol J.;21:135-144.

Karavaiko G.I., Belkanova, N.P., Eroshchev-Shak, V.A., Avakyan, Z.A., (1985). Role of microorganisms and some *physicochemical* factors of the medium in quartz destruction. Mikrobiologiya., 53, 795-800.

Karavaiko, G. I., Avakyan, Z. A., Ogurtsova, L. V., and Safonnova, O. F., (1989), Microbiological processing of bauxite." In Biohydrometallurgy, (J. Salley, R. G. L. McGready, and L. Wichlacz, Eds.), Ottawa, Canada: CANMET,. 93-103.

Kiel H., W. Schwartz, (1980). Leaching of a silicate and carbonate copper ore with heterotrophic fungi and bacteria producing organic acids, Z. Allg. Mikrobiol, 20:627-636.

Kretz, R., (1972). Silicon: Elements and geochemistry,. In R.W. Fairbridge. The encyclopedia of geochemistry and environmental sciences. Encyclopedia of earth sciences series, Vol.IVA. Van Norstrand Reinhold, New York. 1091-1092.

Liermann LJ, Kalinowski BE, Ferry BJ. 2000. Role of bacterial siderophores in dissolution of hornblende. Geochim Cosmochim Acta 64(4):587-602.

Malinovskaya IM, Kosenko LV, Votseko SK, Podgorskii VS. (1990). Role of *Bacillus mucilaginosus* polysaccharide in degradation of silicate minerals. Mikrobiologiya, 59: 70-78 (Engl. Transl. pp 49-55).Mycological Research 105: 1261-1267.

Natarajan KA, Deo N. (2001). Role of bacterial interaction and bioreagents in iron ore flotation. Int J Miner Process, 62: 143-157.

Paris F, Botton B, Lapeyrie F. (1996). In-vitro weathering of phlogopite by ectomycorrhizal fungi. II. Effect of K+ and Mg2+ deficiency and N sources on accumulation of oxalate and H+. Plant Soil, 179: 141-150.

Rawlings D.(2005) Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. Microb Cell Fact.;4(1):13.

Sayer JA, Gadd GM, (2001). Binding of cobalt and zinc by organic acids and culture filtrates of Aspergillus niger grown in the absence or presence of insoluble cobalt or zinc phosphate. Mycological Research 105: 1261-1267.

Sayer JA, Kierans M, Gadd GM, (1997). Solubilization of some naturally occurring metal-bearing minerals, limescale and lead phosphate by Aspergillus niger. FEMS Microbiology Letters 154:29-35.

Sayer JA, Raggett SL, Gadd GM, (1995). Solubilization of insoluble compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. Mycological Research 99: 987-993.

Strasser H, Burgstaller W, Schinner F, (1994). High yield production of oxalic acid for metal leaching purposes by Aspergillus niger. FEMS Microbiology Letters 119: 365-370.

Tan, K.H., (1986). Degradation of soil minerals by organic acids pp 1-27. In: P.M.Haung and M.Schnitzer, Eds. Interaction

of soil minerals with natural organics and microbes. SSSA Special publication Number 17. Soil Science Society of America Madison, WI.

Tzeferis PG, Agatzini-Leonardou S. (1994). Leaching of nickel and iron from Greek nonsulfide nickeliferous ores by organic acids. Hydrometallurgy 36:345-360.

Vandevivere P, Welch SA, Ullman WJ, Kirchman DL. (1994). Enhanced dissolution of silicate minerals by bacteria at near neutral pH. Microb Ecol 27:241-251.

Welch SA, Ullman WJ. (1993). The effect of organic acids on plagioclase dissolution rates and stoichiometry. Geochim Cosmochim Acta, 57: 2725-2736.

Welch SA, Ullman WJ. (1999). The effect of microbial glucose metabolism on bytownite feldspar dissolution rates between 5 and 350C. Geochim Cosmochim Acta, 63(19/20): 3247-3259.

Welch SA,Vandevivere P. (1995). Effect of microbial and other naturally occurring polymers on mineral dissolution. Geomicrobiol J 12:227-238.

Wieland E, Wehrli B, Stumm W. (1988). The coordination chemistry of weathering, III. A generalization on the dissolution rates of minerals. Geochim Cosmochim Acta 52:1969-1981.

Yusfin Y, Chernousov P, Garten V, Karpov Y, Petelin A,(1999). The role of alkalis and conserving resources in blast-furnace smelting. Metallurgist.;43(2):54-58.

❐❐

MICROBES IN ACIDIC ENVIRONMENTS - POTENTIAL BIOTECHNOLOGICAL TOOLS APPLIED FOR INDUSTRIAL LEACHING OF METALS

Sandeep Panda[1,3*], Srabani Mishra[1,2], Nilotpala Pradhan[1,2], Lala Behari Sukla[3]

[1]Department of Bioresources Engineering, CSIR - Institute of Minerals and Materials Technology (IMMT), Bhubaneswar-751013, Odisha, India

[2]Academy of Council of Scientific and Innovative Research (AcSIR), CSIR - Institute of Minerals and Materials Technology (IMMT), Bhubaneswar-751013, Odisha, India

[3]Institute of Applied Environmental Biotechnology Innovation (IAEBI), Bhubaneswar -751016, Odisha, India

[*]Corresponding Author: panda.sandeep84@gmail.com

Abstract

Acidophilic microorganisms inhabit iron and/or sulphur rich environments. Certain group of specialized acidophilic chemolithotrophic bacteria grow in acidic environments such as those of the mine sites. These microorganisms have been widely used for recovery of metal values from low grade ores, industrial wastes etc. Bacterial assisted leaching of metals has gained a lot of popularity over the years and is now widely accepted as the potential tool for recovery of metal values. This particular review discusses the diversity and features of few bioleaching microorganisms with their inherent property towards leaching of metals.

Keywords: Bioleaching; Acidophilic microorganisms; Chemolithotrophic; Low grade ores; Industrial wastes.

1. Introduction

There exist a specialized group of microorganisms that are especially suitable for leaching or dissolution of metals from low grade ore and other industrial wastes (Schippers, 2007; 2014). Such microbes have a number of features in common that enable

metal dissolution. These microorganisms more appropriately referred to as the chemolithotrophs produce the ferric iron and sulphuric acid which is very much essential for the leaching of metals (Sand et al., 2001). In general, the process of bioleaching involves microorganisms able to oxidize ferrous sulphate ($FeSO_4$) and sulphur (S) to produce ferric sulphate (Fe_3 $(SO4)_2$) and $H2SO_4$. Ferric sulphate is a strong oxidising agent that gives an oxidative attack to the mineral or metal sulphide along with dissolution metals by the sulphuric acid. Another important characteristic feature of leaching microbes is there higher tolerance to heavy metals, which makes them more efficient for leaching process (Panda et al., 2013a,b; 2014). Adaptation to heavy metals is a unique biotechnological property of these microorganisms. More interestingly, such microbes are usually subjected to contamination which makes them especially important in biomining operations (Panda et al., 2012a, b, c, 2015a, b). These microbes grow autotrophically by fixation of atmospheric CO_2 (Das et al., 1999). The microorganism derives their energy by iron and sulphur oxidation with modest nutritional requirement being provided by aeration. Such, chemolithotrophic metabolism as seen in these microbes makes them industrially important. The underlying mechanisms of metal sulphide oxidation by acidphilic microorganisms are now very well known and have been reviewed by many researchers (Rohwerder et al., 2003; Schippers and Sand, 1999; Sand et al., 2001). Therefore, the present mini review will only highlight some of the important microorganisms used in the bioleaching process.

2. Acidophilic Microorganisms and their Bioleaching Capability

Generally, the microorganisms driving the mineral oxidation processes are autotrophic in nature and obtain their carbon for cell/biomass synthesis from atmospheric carbon dioxide. Certain bacteria belonging to the genera such as the *Acidithiobacillus* and *Leptospirillium*, fix CO_2 by Calvin reductive pentose phosphate cycle using the enzyme ribulose 1, 5-biphosphate carboxylase. It is believed that the CO_2 concentration present in

the atmosphere is generally sufficient to avoid carbon limitation where the bacteria such as *Acidithiobacillus ferrooxidans* grow on ferrous iron. The species belonging to the genus *Acidithiobacillus* and *Leptospirillum* are generally considered as the most suitable or industrially important microorganisms and also represent a very versatile group of the chemolithoautotrophic organisms. The diversity of the microorganisms in the bioleaching community is very vast. These microorganisms are generally isolated from acidic mine drainage sites (AMD), soil and water samples inside the pyrite rich mines such as coal deposits etc. The bioleaching community comprises of a vast majority of acidophilies that can be grouped into two major groups based on temperature optimum i.e. the meso-acidophiles and the thermo-acidophilies. The meso-acidophilies can grow up to temperatures of 40^{o}C. The thermo-acidophiles is further grouped into two groups such as the (1) moderate thermo-acidophilies that can grow up to 40^{o}C to 55^{o}C and the (2) extreme thermo-acidophilies that can grow up to 80^{o}C. In addition to the meso and the thermo-acidophilic microorganism certain heterotrophs are also seen to play a significant role in the bioleaching process. The following sections will describe briefly describe certain aspects related to some of the microorganisms capable of metal sulphide oxidation or involved in bioleaching.

2.1.1. *Acidithiobacillus ferrooxidans*

Acidithiobacillus ferrooxidans or *A. ferrooxidans* (earlier known as *Thiobacillus ferrooxidans)* is a rod-shaped bacterium that occurs usually single or in pairs. The microbe is non-spore forming, gram-negative, motile and single-pole flagellated. *A. ferrooxidans* is considered to be obligate autotrophy and can grow only chemolithoautotrophically. The microorganism also poses a very unique property to grow under anoxic conditions, although it has been characterized as being strictly aerobic. Interestingly, *A. ferrooxidans* is endowed with a remarkably broad metabolic capacity. This species lives on the oxidation of reduced sulfur compounds and, in addition, is able to oxidize molecular hydrogen, formic acid, iron (II) ions and other metal ions.

Anaerobic growth of this microbe is possible by oxidation of sulfur compounds or hydrogen coupled with iron (III) ion reduction (Osorio et al., 2013). Under such circumstances, it can grow on elemental sulfur or metal sulfides using ferric iron as electron acceptor. This microbe is seen to oxidize a number of metal sulfides such as arsenopyrite, bornite, chalcocite, chalcopyrite, covellite, enargite, galena, millerite, orpiment, pyrite, marcasite, sphalerite, wurtzite, stibnite, pyrrhotite, tetrahedrite, gallium sulfide and the synthetic metal sulfides such as CdS, CoS, CuS, Cu_2S, FeS, MnS, MoS_2, NiS, PbS, SnS and ZnS (Schippers et al., 2007 and references therein). As a result of metal sulphide oxidation, *Acidithiobacillus* contributes to increased acidification of leaching systems as a result of generation of sulphuric acid. As a result of combined action of sulphuric acid and ferric iron as oxidant, metal sulphide oxidation is possible along with release of metals.

2.1.2. *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum*

Microorganisms belonging to genus *Leptospirillum* are obligately chemolithotrophic acidophilic organism that can grow optimally in inorganic media within pH range of 1.0 to 2.0. These are meso-acidophilic, small, gram negative, vibrio or spiral shaped cells. *Leptospirillum ferrooxidans (L. ferrooxidans)* has quite a different biochemistry than A. *ferrooxidans*. This is because; the microorganism is known to be one of the most metabolically restricted organisms since it uses only ferrous as an electron donor. It oxidizes Fe (II) iron under high redox potentials. This microbe is reported to tolerate metals such as Al, Co, Cu, Mn, Ni and *Zn. Leptospirillum ferriphilum (L. ferriphilum)* can grow up to 45^{o}C and is referred to as a thermotolerant mesophilic Fe (II) oxidizer. The microbe is generally found in tank leaching operations where temperatures of operation are around is 35-50 ^{o}C. It is very interesting to note that, *L. ferriphilum* rapidly oxidizes Fe (II) iron at pH < 1 (Schippers, 2007 and references therein).

2.1.3. *Acidanus brierley*

Acidanus brierley (A. brierley) is an extremely thermophilic

metal sulfide oxidizing bacteria under the phylum Crenarchaeota. The microorganism (type strain DSM 1651) was at first isolated from a sulfatric spring at the Yellow Stone National park in USA to which it was earlier described as *Sulfolobus brierley.* Later on the type stain was reclassified as *A. brierley* by Segerer et al. (1986) and has been described as a facultative anaerobic, chemolithoautotrophic microorgainsm that uses metal sulfides, H_2, organic substances, elemental sulfur as substrates for growth (Schippers, 2007 and references therein).

2.1.4. *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus*

In addition to *A. ferroxidans* and *L. ferrooxidans, Acidithiobacillus thiooxidans (A. thiooxidans)* plays a very important role in metal sulphide oxidation. Unlike, *A. ferrooxidans* that can oxidize Fe (II) iron and sulphur, *A. thiooxidans* can only oxidize sulphur. It is an obligate autotroph that can grow on a number of sulphur compounds such as elemental sulphur, thiosulphate and tetrathionate. This microbe can oxidize covellite, galena, sphalerite, wurtzite but cannot oxidize pyrite. In addition to *A. thiooxidans, A. caldus* can also grow on a variety of sulphur compounds such as the elemental sulphur, thiosulphate and tetrathionate but cannot grow on Fe (II) iron. It is describes as a mixotrophic microorganism that can grow with yeast extract or glucose (Schippers, 2007 and references therein).

2.1.5. *Ferroplasma* sp.

Features of the species belonging to the genus *Ferroplasma* are that they are acidophilic archaea, able to oxidize Fe (II) iron, pyrite and other metal sulfides; their cells lacks cell wall and have irregular pleomorphic cells. They can grow mixotrophically and faculatively under anaerobic conditions via Fe (III) iron reduction. In case of *Ferroplasma acidiphillum (F. acidiphillum)*, growth may be lithotrophically aerobic (Fe (II)+CO_2), organoheterotophic (on yeast extract), or mixotrophic (Fe (II) +

organic carbon source). In case of anaerobic growth for *F. acidiphillum,* yeast extract is used as electron donor. *Ferroplasma acidarmanus (F. acidarmanus)* also grows as in the same way as *F. acidiphillum. F. acidarmanus* possess the capability to grow under As, Cu, Cd metal stress (Schippers, 2007 and references therein).

2.1.6. *Metallosphera* sp.

The metallosphera group belongs to the genera Sulflobales and are capable of metal sulfide oxidation. *Metallosphaera hakonensis (M. hakonensis)* and *M. prunae* are aerobic, facultative chemolithoautotrophic microorganisms that can use metal sulfides, elemental sulfur, tetrathionate, H_2S, and organic compounds as substrates. *M. sedula* is also a aerobic, facultative chemolithoautotrophic microorganism but can oxidise pyrite, chalcopyrite, sphalerite and the synthetic sulfides CdS, SnS, ZnS in addition to elemental sulfur, tetrathionate, H_2S, and organic compounds as substrates (Schippers, 2007 and references therein).

Conclusions and Future Dimensions

The role of acidophilic microorganisms towards leaching of metal values is believed to provide a new dimension to the area of bio-hydrometallurgy. As the diversity of metal leaching microbes is very vast, it is also believed that many new species with better leaching capabilities will be discovered in the near future. A recent web server/tool designed by *Parida et al. (2014),* provides a platform to directly predict the isolated microorganisms from potential mining sites based on the input of their 16s rRNA sequences that further scans and matches the output and the database of the tool to predict acidophilies involved in bioleaching. Many such advanced studies for identification of microbes will tremendously help in improving our existing knowledge. It is thus very pertinent to say that the coming years will see a more advanced mineral biotechnology using acidophilies for recovery of various precious metal values form industrial wastes.

References

Das, T., Subbanna, A., Chaudhury, G.R., 1999. Factors affecting bioleaching kinetics of sulfide ores using acidophilic micro-organisms. Biometals 12, 1-10.

Osorio, H., Mangold, S., Denis, Y., Ñancucheo, I., Esparza, M., Johnson, D.B., Bonnefoy, V., Dopson, M., Holmes, D.S., 2013. Anaerobic Sulfur Metabolism Coupled to Dissimilatory Iron Reduction in the Extremophile *Acidithiobacillus ferrooxidans*. App. Env. Microbiol. 79, 2172-2181.

Panda, S., Biswal, A., Mishra, S., Panda, P.K., Pradhan, N., Mohapatra, U.B., Sukla, L.B., Mishra, B.K., Akcil, A., 2015b. Reductive dissolution by acid processed waste newspaper for enhanced meso-acidophilic bioleaching of copper from low grade chalcopyrite: A new concept of bio-hydrometallurgy. Hydrometallurgy 153, 98 - 105.

Panda, S., Mishra, S., Rao, D.S., Pradhan, N., Mohapatra, U.B., Angadi, S., Mishra, B.K., 2015a. Extraction of copper from copper slag: Mineralogical insights, physical beneficiation and bioleaching studies. Korean J. Chem. Eng. 31, 1-10.

Panda, S., Parhi, P.K., Nayak, B.D., Pradhan, N., Mohapatra, U.B., Sukla, L.B., 2013a. Two step meso-acidophilic bioleaching of chalcopyrite containing ball mill spillage and removal of the surface passivation layer. Bioresour. Technol. 130, 332-338.

Panda, S., Parhi, P.K., Pradhan, N., Mohapatra, U.B., Sukla, L.B., Park, K.H., 2012b. Extraction of copper from bacterial leach liquor of a low grade chalcopyrite test heap using LIX 984N-C. Hydrometallurgy 121-124, 116-119.

Panda, S., Pradhan, N., Mohapatra, U.B., Panda, S.K., Rath, S.S., Nayak, B.D., Sukla, L.B., Mishra, B.K., 2013b. Bioleaching of copper from pre and post thermally activated low grade chalcopyrite contained ball mill spillage. Front. Environ. Sci. Eng. 7, 281-293.

Panda, S., Rout, P.C., Sarangi, C.K., Mishra, S., Pradhan, N., Mohapatra, U.B., Subbaiah, T., Sukla, L.B., Mishra, B.K. 2014. Recovery of copper from a surface altered chalcopyrite contained ball mill spillage through bio-hydrometallurgical route. Korean J. Chem. Eng. 31, 452-460.

Panda, S., Sanjay, K., Sukla, L.B., Pradhan, N., Subbaiah, T., Mishra, B.K., Prasad, M. S. R., Ray, S.K., 2012a. Insights into heap bioleaching of low grade chalcopyrite ores: A pilot scale study. Hydrometallurgy 125-126, 157-165.

Panda, S., Sarangi, C.K., Pradhan, N., Subbaiah, T., Sukla, L.B., Mishra, B. K., Bhatoa, G. L., Prasad, M.S.R., Ray, S.K., 2012c. Bio-hydrometallurgical processing of low grade chalcopyrite for recovery of copper metal. Korean J Chem. Eng. 26, 781-785.

Parida, B.K., Panda, S., Misra, N., Panda, P.K., Mishra, B.K., 2014. BBProF: An Asynchronous Application Server for Identification of Potential Bioleaching Bacteria. Geomicrobiol J. 31, 299-314.

Rohwerder, T., Gehrke, T., Kinzler, K., Sand, W. Bioleaching review part A: Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. App. Microbiol. Biotechnol. 63, 2003: 239-248.

Sand, W., Gehrke, T., Jozsa, P.G., Schippers, A., 2001. Biochemistry of bacterial leaching-direct vs. indirect bioleaching. Hydrometallurgy 59, 159-175.

Schippers, A., 2007. Microorganims involved in bioleaching and nucleic-acid based molecular methods for their identification and quantification. In: Donati, E.R., Sand, W. (Eds.), Microbial Processing of Metal Sulfides. Springer, pp. 3-33.

Schippers, A., Hedrich, S., Vasters, J., Drobe, M, Sand, W., Willscher, S., 2014. Biomining: Metal Recovery from Ores with Microorganisms. Adv Biochem Eng Biotechnol. 141, 1-47.

Schippers, A., Sand W., 1999. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. Appl. Environ. Microbiol. 65: 319-321.

Segerer, A., Neuner, A., Kristjansson, J.K., Stetter, K.O. *Acidianus infernus* gen. nov., sp. nov., and *Acidianus* brierleyi comb. nov.: facultatively aerobic, extremely acidophilic thermophilic sulphur metabolizing archaebacteria. Int J Syst Bacteriol 36, 1986: 559-564.

❐❐

Authors Details

A

A.P. Das

A. Das

A.F. Mulaba Bafubiandi

C

C. Kar

E

E. Priyadarshini

E. Subudhi

G

G. N. Pujari

L

L. B. Sukla

M

M. Mishra

M. Gaur

N

N. Pradhan

P

P. P. Chakrabarti

P. Behera

R

R. R. Nayak

R. K. Sahoo

R. C. Ray

R. Mund

R.C. Mohanty

S

S. Ghosh

S. Mohanty

S. Behera

S. Singh

S. K. Behera

S. K. Panda

S. Sekar

S. Panda

S. Mishra

Applied & Industrial Biotechnology

With rapid industrialization round the corner, the application of novel biotechnological tools has become a priority today. In the present scenario, the field of applied research basically the biotechnological applications have immensely contributed to solve some of the major problems face by the society as a whole. In this context, some of the important aspects that involve biotechnological applications in solving industrial problems through the aid of novel techniques such as bioleaching, bioremediation, use of bio-surfactants etc have been discussed through this book. The experience of some highly qualified researchers working in this applied field of industrial biotechnology is presented. In addition to the above scientific disciplines, the book also presents a set of review articles on biofuels from lignocelluloses biomass, different aspects of environmental biotechnology that are already practiced by mining industries (chemical industries in particular), GIS based mapping and common scientific principles etc. This book is believed to certainly give necessary concise initial information on the applied subject.