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Review Article

Advances in Environmental Biotechnology in India: Status Report

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Sustenance of human beings depends upon the availability of water, food and good environmental conditions. However, the evolutionary changes in our lives have been affected by the industrial developments, which have influenced our ever-increasing demands for more food and energy. To meet our daily needs, we depend heavily upon fossil fuels, which results in environmental pollution. It has obliged us to look for novel energy resources. In addition, to meet the food demand, agricultural practices especially the use of pesticides, antimicrobials, and fertilizers have also added to soil contamination. In this article, we have provided the status of biotechnological advancements being made in India on the following issues: i) Bioremediation, ii) Microbial processes and products, iii) Environment and nanotechnology, and iv) Microbial diversity.

Keywords: Biodegradation; Bioremediation; Bioenergy; Bioproduct; Biopolymer; Nanotechnology

Introduction

The detrimental impact of pollutants on the environment, including global concerns for energy security and food security, has resulted in shifting of the global priority towards finding solutions for environmental protection.

Bioremediation: Oil, Parafin and Petroleum

Among the most important issues to be dealt with is the bioremediation of environmental pollutants. To achieve the goal of sustainability, the Environmental and Industrial Biotechnology Division (EIBD) at TERI is actively engaged with research areas spanning the domain of microbial-based interventions to explore sustainable approaches for protection of the environment, enhanced production of oil from matured oil wells as well as for alternate renewable energy production. Innovative research explorations of Dr. Banwari Lal and group at EIBD, TERI finally paved the way for the development of a couple of microbial-based technologies such as 'Oilzapper' (for bioremediation of oil spill and oily sludge), 'MEOR' (Microbial Enhanced Oil Recovery) and 'PDB' (Paraffin Degrading Bacteria), for prevention of paraffin deposition in oil well tubing. These technologies were eventually implemented successfully in field scale.

Recognizing the need for sustainable mitigation of oil spills, TERI with the aid of financial assistance in 1991 from Department of Biotechnology (DBT), Ministry of Science and Technology, India, developed the technology 'Oilzapper'. It is a sustainable approach for remediation of oil spill and oily sludge. Oilzapper is a consortium of crude oil and oily sludge degrading bacteria, derived from various bacterial cultures existing in the natural environment. It is environment-friendly, cost effective, non-hazardous and cleans up and restores the site to its original condition, leaving behind no harmful residue. Following the successful remediation of oil contaminants by this bacterial consortium in field scale in a cost-effective manner, 'Oilzapper' technology got acknowledged and has been successfully employed since 1998 by several oil industries like: ONGC, IOCL, BPCL, OIL India Limited, Numligarh Refinery, Bharat Petroleum Corporation Ltd, Tata Power, and Reliance Industries.

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For the production of Oilzapper, a Bioreactor facility with 15000 litres capacity was established at TERI Gram, Gurgaon and is mostly supplied to BG Exploration and Production India Ltd., Mangalore Refinery & Petrochemicals Ltd., CanadianNexen Petroleum Yemen, Kuwait Oil Company, Kuwait, and Abu Dhabi National Oil Company. In order to cater the needs of all the oil industries a state of the art Fermentation Technology Research Centre has been set up at TERI, Gual Pahari Gurgaon with the support from Department of Biotechnology and oil industries such as Oil Industry Development Board, Oil and Natural Gas Corporation Lt, Indian Oil Corporation Limited, Hindustan Petroleum Corporation Limited, Bharat Petroleum Corporation Limited (BPCL) and TERI towards construction of a building for bioreactor facility. With these states of the art large scale fermentation facilities ranging from 150 to15,000 litres (at TERI) and broad scale application of 'Oilzapper', present 'Oilzapper' production has increased to 15 tonnes per day. Broad scale application of indigenously developed 'Oilzapper' in mitigating oil spills and Oily sludge in diverse areas across the nation, poised this technology further to get global recognition overseas. This is one of those biotechnologybased processes that not only helped to restore the oil contaminated environment globally, but also restored the livelihood of several farmers and created ample of job opportunity. Till date, more than 1,00,000 hectares of cropland/farmer's land contaminated with crude oil spills has been reclaimed, and more than 4,50,000 m^3 of oily sludge, oil contaminated soil and drill cuttings successfully have been treated with Oilzapper, in India and abroad. Currently,Oilzapper is being used in a major project of Kuwait Oil Company, valued US $40 million, for cleanup of oil spill in 4 sq. Km area in Kuwait (Middle-East).

Also, successful field scale attempts were made for prevention of paraffin deposition in oil well tubings by Dr.Banwari Lal and group. Production of waxy crude oil is a serious global concern for the oil producing companies due to high viscosity, which results in blockage of the oil well tubings. Conventional techniques for removal of paraffin/wax deposition in the oil well tubing/oil pipelines were highly expensive and plagued with other associated problems. With a goal to provide solution to this problem, research studies were extensively carried out at TERI with the financial assistance from IRS (Institute of Reservoir Studies), ONGC, Ahmedabad. Four years of extensive research explorations finally helped to develop a technology 'PDB' (Paraffin Degrading Bacteria) for Prevention of paraffin deposition in well tubings through the use of paraffin degrading bacterial strains 'PDS 10". These strains could degrade crude oil paraffin under both aerobic and anaerobic conditions at temperature 45º to 50-ºC and thus selected for prevention of paraffin deposition in oil well tubings (Lavania *et al*., 2012; Lavania *et al*., 2015; Lavania *et al*., 2011; Sood *et al*., 2008). This technology was initially explored in 2 oil wells of ONGC, and eventually commercialized by applying it in 269 oil wells of ONGC and Oil India Ltd till date, through ONGC TERI Biotech Ltd.

Microbial Products and Processes

Bioenergy

With ever increasing waste contamination and need of environment friendly treatment methods, various microbial process can be employed for the economic treatment of waste material and simultaneously be used for generation of energy. Various research projects have been undertaken in the past to counter the waste generation and meeting the energy crisis. A novel high-rate Bio-Electrochemical Anaerobic Digestion (BEAD) process for renewable energy recovery from distillery waste water was developed. The main goal of this research project was to establish a high-rate bio-electrochemical digestion process specialized in distillery wastewater for organic pollutants stabilization and methane gas recovery, leading to the initial phase of pilot scale development of BEAD process, providing a solution for appropriate management of distillery industrial wastewater. In this process, methane recovery will be realized in a novel BES submersed in an anaerobic reactor. The system performance and potential limitations will be addressed and technical solutions will be developed. The proposed project milestone includes identification of problems associated with distillery waste water. Limitations of current anaerobic digestor system for methanogenesis, inhibitors of methanogenesis, collection and characterization were studied. Reduction of inhibitors like sulfate were studied and it was observed that methane was obtained from biologically treated wastewater followed by chemically treated wastewater. Further the BEAD reactor studies are under progress (Krishnan *et al.,* 2006; Sood *et al.,* 2009; Kaur *et al.,* 2009; Sharma *et al.,* 2013; Sharma *et al.,* 2014; Lavania *et al.,* 2014; Rathi *et al.,* 20115; Jain *et al.,* 2015).

Another integrated approach for biofuel production by coupling with microbial based textile wastewater treatment was developed in a project that deals with research investigation on utilization of textile dye effluent for algal biomass production, that would help in the development of a process for bioremediation of textile waste along with energy generation (biodiesel). It involves characterization of treated and untreated wastewater from reactive textile dye industry, selection of robust algal strains for the algal biomass production and removal of dye colour from untreated reactive textile dye wastewater, and optimization of processes available for enhancement and scale up of algal biomass. Further, separation of algal biomass, extraction and characterization of algal oil is performed. Research findings proved that the textile dye wastewater treatment and biofuel biomass production efficiency of selected algal strains and consortium was maximum in real textile dye wastewater. Further pilot scale production study will be helpful to scale up the present technology close to field level implementation.

To meet the energy needs, a method based on microbial process has been developed for *in-situ* generation/enhancement of methane from underground coal seams. This microbe-based methane production (biogenic methane production) method employs coal as feedstock, and has got the potential for applications in enhancement of methane (which is trapped inside the pores in the coal) recovered from underground coal seam (Coal bed methane). Coal has the potential to store 6 to 7 times more methane than the equivalent volume of another rock. The biogenic methane production process (developed by Microbial Biotechnology at TERI) is now scaled up to next phase and presently being explored in field scale in one of the coal-mine in Jharia, in collaboration with ONGC energy center.

Fast depletion of conventional fossil fuel reserves along with the global concern for energy supply security have drawn global R&D efforts towards alternative energy generation from renewable resources. With a converging desire to combat climate change and to improve energy security, hydrogen has been identified as a futuristic fuel and there is need of bio-hydrogen production (Clean energy Technology). Research activities are being carried out to explore robust microbe(s) for development of process to produce clean energy forms: bio-hydrogen. With the aid of financial supports from HPCL (Hindustan Petroleum Corporation limited), Center for High technology, MoP&NG (Ministry of Petroleum and Natural Gas) and DBT (Department of Biotechnology), TERI researchers developed a bio-based process for fermentative hydrogen production from sugar industry organic waste, in pilot scale (1000 litre working volume) through dark fermentative route with the employment of anaerobic microbial consortium: 'TERI-HP-BH05', as well as purified anaerobe: '*Clostridium butyricum* TM-9A' (Isolated by TERI). Subsequently an integration process for bio-hydrogen production was developed in 100liter scale by combining dark and photo fermentation process. Carrying forward these activities, TERI researchers also developed (with the financial assistance from Ministry of New and Renewable Energy, Got. Of India) a thermophilic bio-hydrogen production process in laboratory scale (10 litre scale bioreactor) by using organic rich food waste as feedstock. Further TERI researchers are now actively engaged with development of bio-hydrogen production process in 100 litre pilot scale by using second generation non- food competitive feed stocks, or biomass sugars. (Jayasinghearachchi *et al.,* 2009; Jayasinghearachchi *et al.,* 2010; Singh *et al.,* 2010; Sanjukta and Lal, 2011; Jungare *et al.,* 2011; Subudhi *et al.,* 2013; Singh *et al.,* 2014; Lavania *et al.,* 2014; Verma *et al.,* 2015; Navaneeth *et al.,* 2015; Subudhi *et al.,* 2016).

Along with the working on alternate fuel generation methods, techniques to enhance oil recovery in present time is highly solicited. Usually, ageing of oil wells is a perpetual problem that the oil industries are facing globally, as oil wells get stripped after recovery of around 20-30% of oil in place. Eventually further oil flow decreases and stops production, leaving behind more than 70% of oil in place. Keeping in view of energy security, microbial enhanced oil recovery technology was developed under a project sponsored jointly by Department of Biotechnology, Government of India and Oil and Natural Gas Corporation Ltd. (ONGC). After six years of research work at TERI, thermophilic, barophilic, anaerobic bacterial consortium was developed by assembling of three bacterial species which could produce CO_2 and methane gas, and metabolic products including volatile fatty acids, alcohols and bio-surfactants. These metabolic products aid in increasing the sweep efficiency of crude oil from pores of oil bearing rocks (Rathi *et al.*, 2015). MEOR technology was initially explored in laboratory scale and then subsequent tests were carried out in 1 well, 10 wells and then 45 wells of ONGC, Ahmedabad and Mehsana oil fields. With the application of MEOR technology, crude oil production from stripper wells increased to 30-200%. Following the success of this technology in field scale, 'MEOR' technology is now being used on a large scale by ONGC and Oil India Ltd. This technology is now commercialized through OTBL (ONGC TERI Biotech Ltd, a joint venture company) and has been applied in 178 oil wells of ONGC, OIL and in oil wells of Georgia, Russia.

Bio-synthesis of Chemicals

Various products like fuel additives, textiles, polymers, synthetic rubbers and plastics require intermediate specialty chemical molecules for their synthesis. Specialty chemical segment in India has been growing at a rapid pace owing to the growing key end use markets. 2,3-Butane Diol (2,3-BD) is a specialty chemical with profound application as a precursor molecule for synthesis of range of important downstream chemicals: 1,3-butadiene; butenes; methyl ethyl ketone (MEK); gamma butyrolactone; diacetyl, esters, and for use in fuel additives, textiles, polymers, synthetic rubbers and plastics. The potential global market for such molecules is more than 30 million tons per annum accounting to > $40 billion in sales revenues. TERI researchers developed a microbial process for 2,3-BD production in pilot scale from commercial grade glucose by employing *Enterobacter cloacae* (non-pathogenic strain, isolated by TERI), which is competitive to employ for industrial scale production of 2,3-BD (Priya *et al.*, 2016). Commercialization of the green 2.3-BD production technology, will play a pivotal role in green chemical industry.

New sustainable technological innovations are being developed for synthesis of platform chemicals and biofuels (ethanol and acetic acid). A method based on bioconversion of synthesis gas to platform chemicals and biofuels (ethanol and acetic acid) through microbial interventions was designed and laboratory scale implementation was done. Syngas bioconversion for bioethanol production has some unique benefits as it can be produced from non-fermentable material, such as lignin (Singla *et al.*, 2014). Thus, bioethanol production from lingo-cellulosic biomass by using synthesis gas fermentation is a relatively new technology. Ethanol is easy to transport, making it easily available at any place wherever required, and use in Flexy Fuel Vehicles. The technology is quite new and fascinating and leads to the elimination of complex pretreatment steps and costly chemical catalysts,and produces an environment friendly and sustainable energy source. After a lot of experimental work from 10 liter bioreactor, an efficient syngas fermenting microbial consortium TERI SA1 was prepared. This microbial consortium efficiently converts Syngas into platform chemicals (ethanol and acetic acid), which are useful for various industrial purposes. Furthermore, it is now aimed at designing an efficient 100 liter prototype for further pilot scale studies in higher volume (100 liter) bioreactor (Patle and Lal, 2008a, 2008b; Sharma *et al.*, 2013; Singla *et al.*, 2014).

Industrial Processes and Enzymes Production

In line of development of microbial processes for production of fuel additives, scale up of microbial based Xanthan gum production was undertaken. Xanthan is a polysaccharide-based biopolymer and has got profound applications in several downstream processes in oil industries, where it is mainly used as an additive for drilling mud chemical in oil industries. Scale up of microbial Xanthan production is in progress at TERI.

Solid State Fermentation (SSF) has been a useful technique for utilization of large volumes of agro-industrial biomass in producing value added enzymes and platform chemicals with simultaneous detoxification of 'toxins or anti-nutrients' present in the biomass. Studies were performed for SSF based detoxification of agro-industrial wastes in case of phorbol esters (in jatropha seed cake), gossypol (in cotton seed meal) and glucosinolates (in rapeseed meal), with simultaneous production of value added products like lipase, xylanase, GABA, lactic acid etc. (Grewal and Khare, 2016; Joshi and Khare, 2013; Joshi and Khare, 2011; Joshi *et al*., 2011; Sadaf and Khare, 2014) The developed SSF based process to completely eliminate phorbol esters from deoiled *J. curcas* seed cake has been patented (Joshi and Khare, 2012). Enzyme systems viz. galactosidases, phytase, and *A. oryzae* cells have been used to obtain soymilk free of anti-nutrients factors and the by products of soymilk/ tofu processing have been utilized by SSF in obtaining useful industrial products viz. citric acid, single cell protein and food enzymes (Gupta *et al*., 2002; Khare *et al*., 1994a; Khare *et al*., 1994b). Similarly, grease waste was bio-remediated by lipase pre-treatment, followed by SSF by *Penicillium chrysogenum* to produce important fatty acids like palmitic acid (Kumari *et al.*, 2017). This addressed the disposal problem of these wastes by channelling them for meaningful utilization.

Another industrially useful enzyme, alkaline protease, has largely been a focus of research groups, as protease enzymes are biocatalysts that are used as alternatives to chemical reactions to improve efficiency and cost-effectiveness of a wide range of industrial systems and bioprocesses. They are currently applied in both basic and applied arenas of research as well as in a wide range of product design and manufacturing processes e.g. those pertaining to the food, detergent, beverage, leather processing, waste water treatment, and in pharmaceutical industries. Till date, the largest share of the enzyme market has been held by alkaline proteases which are active and stable over a wide alkaline pH range. Unfortunately, alkaline protease production from naturally occurring wild type bacterial strains cannot meet the growing market demand due to its low yield and incompatibility with the standard industrial fermentation media formulations as well as fermentative conditions required for optimal growth of the host bacteria. Remarkably, the enzyme production could be enhanced by taking the advantages of modern molecular biology techniques or recombinant DNA technology, whereby, the use of homologous or heterologous overexpression of genes coding for the target enzyme could be achieved for higher productivity in shorter time and that would reduce the costs of production as well. Therefore, studies on construction and application of recombinant strain of *Bacillus subtilis* for industrial scale production of a commercially important enzyme will be undertaken by a research group at IIT Delhi. Further, its improvement and growth optimization for the alkaline protease production using sustainable agro-industrial residues as feedstock carbon source will be performed. The group also intends to investigate the growth and process optimization of the culture during fermentation and other physicochemical parameters up to proto scale production level and its downstream purification to recover the final product. Hence, this study will provide a new perspective for the future bioprocess technology development and commercial success of an industrially important enzyme.

Production of Biopolymers and Bioproducts

Biodegradable plastics, as an alternate to petroleum derived plastics, are in great demand due to the environmental issues posed by non-biodegradable plastics like low density/high density polyethylene (LD/ HDPE), polypropylene (PP), etc. In the absence of proper collection and recycling procedures, plastics, especially in the form of covers and bags, are getting accumulated in the environment in our country. This is leading to clogging of the drains, disrupting seepage of rain water in to earth and creating an unhygienic environment.

Polyhydroxyalkanotes (PHA), natural bioplastic, is produced by several microorganisms as an intracellular storage material, under carbon rich and nitrogen or phosphorous depleting conditions. Properties of PHA are similar to PP and hence, it is gaining attention as an alternate to non-biodegradable plastics. It can be used for packaging applications, medical filed as stents, sutures, wound dressing material, feminine hygiene products, etc. (Angelina and Vijayendra, 2015).

Extensive work on PHBs production has been done by Kalia *et al*., which reported *Bacillus* sp. as a potential producer of PHB from biowaste (Kumar *et al.*, 2009) and later reported *Bacillus subtilis* as a potential producer (Singh *et al*., 2009). Studies were performed using glucose in two stage system for hydrogen and PHB production abilities of *Bacillus* sp. (Patel and Kalia, 2011). In exploring the economic feed for production of bioplastics, group explored the use of pea shells for production of hydrogen and polyhydroxybutyrate using the defined bacterial culture (Patel *et al.,* 2012). The studies later, were thoroughly performed and reported the production of co polymers by *Bacillus thuringiensis* (Singh *et al*., 2013) and reviewed the eco-biotechnological approaches for exploiting the Bacillus for production of copolymer of PHAs (Kumar *et al*., 2014) and highlighted the challenges and opportunities for customizing PHA (Singh *et al.*, 2015). The group also reviewed the integrative approaches for production of biohydrogen and bioplatics, as a mean of economic and sustainable methods for commercial purpose. Mixed waste of biological origin was also explored for this purpose (Patel *et al*., 2016). In attempt to understand how substrate modulation can affect molecule production, studies on bioconversion of crude glycerol under non-limiting nitrogen conditions by *B. thuringiensis* was performed. (Kumar *et al*., 2015). Studies were attempted to understand how the hydrolysis of biowaste regulates the production of co-polymers of PHAs. Recently study has reported how the co-metabolism of substrates affects the copolymer composition by *B. thuringiensis* (Ray and Kalia, 2017a) and the patterns of production and degradation of PHAs in *Bacillus* species. (Ray and Kalia, 2017b).

Research has been carried out for the production of PHA using several bacterial species. A method has been established to identify PHA producing *Bacillus* species using molecular methods, which can save time while screening for PHA producers (Shamala *et al*., 2003). PHA production by a selected *Bacillus* sp. was optimized using response surface methodology with corn steep liquor as a nitrogen source (Vijayendra *et al*., 2007) and an unexploited carbohydrate rich Mahua Flower Fxtract (MFE) (Anil kumar *et al*., 2007). However, the efficiency of utilization of MFE for PHA synthesis varied from one genus to another. The rate of accumulation of PHA co-polymer (poly(hydroxybutyrate-co-hydroxyvalerate)-P(HB-co-HV) - of 90: 10 mol%) in terms of biomass content (%) was highest with *Bacillus* sp. (51%), followed by 31% in *Rhizobium meliloti* and 22% by *Sphingomonas* sp. (Kumar *et al*., 2007). Halami (2008) reported production of PHA by a *Bacilluscereus* using starch as a carbon source.

A simplified Monod's kinetic model and Luedeking-Piret model were developed to describe the biomass and PHA production pattern by *Bacillus flexus* (Divyashree *et al*., 2009a). For easy recovery of PHA from enzymatically lysed cells of *B. flexus*, an aqueous-aqueous two-phase system (ATPS) consisting of polyethylene glycol (12%, w/v) and potassium phosphate (9.7%, pH 8.0) was used (Divyashree *et al*., 2009b). Prior to extraction using ATPS, the bacterial cells were lysed using the culture filtrate of *Microbispora* sp. containing of protease (3 U/mL). *B. flexus* could produce higher quantity of PHA (50%) in an inorganic medium, whereas, substitution of inorganic nitrogen by yeast extract, peptone, or beef extract resulted in reduced PHA yields of 40%, 30% and 44%, respectively (Divyashree and Shamala, 2010). Divyashree and Shamala, (2009) used gamma irradiation (5-40 kGy), a novel technique for PHA recovery, for easy recovery of the PHA produced by *B. flexus* cells grown in sucrose or sucrose with castor oil. Gamma irradiation at 10 kGy found to induce cross-linking and it increased molecular weight of PHA copolymer from 1.5×10^5 to 1.9×10^5, simultaneously.

The effect of various carbon and nitrogen sources, including hydrolysates of rice bran and wheat bran on simultaneous production of PHA and ±-amylase by a *Bacillus* sp. was explored to reduce the production cost of PHA and obtain significantly higher amounts of PHA (524 mg/l) and amylase (73 U/ml) with the supplementing the production medium with wheat bran hydrolysate (50 ml/l) (Srikanth *et al.,* 2012). In another study using the hydrolysates of both rice and wheat bran along with unhydrolysed corn starch, maximum quantity of biomass (10 g L^{-1}) and PHA (5.9 g L^{-1}) from *Bacillus* sp. CFR 67 could be obtained and the PHA was found to be a co-polymer of polyhydroxybutyrate-co-hydroxyvalerate of 95:5 to 90:10 mol% (Shamala *et al.,* 2012). Optimization of the polyhydroxybutyrate (PHB) production by a pigment (•-carotene) producing *Micrococcus* sp. was carried out and among the various sugars tested, sucrose found to support maximum production of PHA~35 and 40% of the dry cell weight when grown at 37°C under aerobic conditions (Vijayendra *et al*., 2008).

In another study, supplementation of the polymer production medium with 20% rice bran hydrolysate (RBH) at 0 h resulted in an increase in simultaneous production of PHA and EPS (2.71g/L and 2.01g/L, respectively) by a fast growing *Sinorhizobium meliloti* MTCC 100. However, addition of RBH after 24 h of fermentation further increased the amount of EPS by 5 folds after 72 h at 30°C. Through this study, a biomass content of 7.45 g/L and PHA of 3.60 g/L could be obtained (Saranya *et al*., 2012). For the first time, biosynthesis of multiple biopolymers simultaneously by *S. meliloti* CFR 14 in high cell density cultures through fed batch fermentation (FBF) was attempted (Shamala *et al*., 2014). With sucrose and ammonium sulphate as feed nutrients, it supported the production of 17.2 g/L PHA, 3.5g/L of EPS and 34.5g/L of biomass along with 0.3g/L each of glycogen, cellular and excreted ^{2}-(1,2)-glucans. Addition of ammonium hydroxide as a nitrogen source as well as for pH adjustment during FBF reduced the yields of biomass and PHA to 29.6 g/L and 9.4 g/L, respectively. However, it increased the amount of EPS, glycogen, cellular and excreted ^{2}-(1,2)-glucan to 9.0,1.1,1.3 and 2.0g/L, respectively (Shamala *et al*., 2014). The potential of film forming microbial biopolymers including both exopolysaccharides and PHA for commercial applications have been reviewed by Vijayendra and Shamala (2014) and later, Angelina and Vijayendra (2015) emphasized on the use of microbes as a cell factory for the production of microbial biopolymers.

Biopolyesters polyhydroxyalkanoates (PHA) have evolved into an industrial value chain ranging from bacterial fermentation, packaging materials, biofuels and fine chemicals to biomedical applications. In particular, the synthesis of PHAs with novel monomer structures holds the potential to pave the way for another interesting research field. PHA-copolymer became of industrial interest to as biodegradable able thermoplastics for applications, such as surgical sutures, long-term carriers for drugs or moulded plastics and films. In another study, for improved synthesis of polyhydroxyalkanoates (PHA)-*co* polymer by high active *pha* synthase and its application in drug delivery primers specific to high activity *pha* synthase (*pha* C gene) harbouring environmental bacterial isolates were designed and PCR conditions were optimized. Also, studies focusing on extracellular enzymes e-PHA depolymerases were performed.

Besides bioplastics, versatile biopolymers like psyllium husk, agar, cellulose, sodium alginate, gaur gum, and bacterial polymer powders may be useful as soil conditioner to increase maximum water holding capacity permanent wilting point. Studies were performed to test water holding power, permanent wilting point and germination and seedling growth of the cotton (*Gossypium herbaceum)* at laboratory scale (Patil *et al*., 2011). It was observed that 1% bacterial polymer and 0.6% psyllium amended soil significantly increased the maximum water holding capacity by 233 and 242%, and also lead to delaying in the permanent wilting point by 108 and 84 h, respectively, at 37 °C, as compared to control soil which was not amended. All biopolymers found to increase permanent wilting point, water holding, organic matter, total nitrogen and microflora, significantly, as compared to control. Comparatively psyllium husk and bacterial EPS showed better enhancement in organic matter, biomass, and microflora. After 288 weeks where it was observed that in cellulose, gaur gum, and sodium alginate water holding capacity gets reduced, but psyllium and agar retained its water holding capacity, i.e., 14.5% and 24%, respectively even after 288 days. These polymers were also found nontoxic against test organism *E. foetida* (earthworm). Toxicity study proved the safe nature of amended polymer and with the results of research work, it was deciphered that biopolymer may be a feature smart agent as soil conditioner to avoid drawbacks of available synthetic polymeric soil conditioner (Patil *et al*., 2011).

Among various biopolymers, microbial cellulose (MC) is a versatile biopolymer produced by microbe with superior properties. The MC sphere obtained under shaking condition is hydrophilic network with translucent, loose and porous nature. There is growing interest in untouched field of applications due to the unique features of biocellulose (BC). Outcome of this investigation describes the novel use as potent and environmentally safe adsorbent for the elimination of pollutant dye, removal of proteinaceous pollutant and toxic metals. Previously we optimized the media and parameters for enhanced BC production for use in safe environment applications that are rarely reported (Mohite *et al*., 2012).

The microbial cellulose produced *G. hansenii*by shaking condition was tested forits potential for removal of dye and proteinaceous pollutant as well as toxic metal (Mohite and Patil, 2014). The microbial cellulose removed dyes like aniline blue, an azo dye upto 400 mg/L with 80% efficiency within 60 min.

The adsorption and elution of protein and heavy metals such as nickel, cadmium and lead were carried out. The study highlighted exclusion ability with reusability of MC. Similarly, it showed potential of BSA adsorption and removal. The effect of temperature and pH on BSA adsorption has also confirmed. The bio-adsorption (82%) and elution ratio (92%) of MC for lead was more than for cadmium and nickel. MC also works as soil conditioner resulted in increased porosity of soil and water-holding capacity. The study revealed the signiûcance of BC as renewable effective and ecofriendly bio-adsorption agent. In summing up this is an endeavour to focus the versatile nature of MC produced by *G. hansenii* in various green environment applications (Mohite and Patil, 2014; Mohite *et al*., 2012).

Further studies were performed to assess production of exopolysaccharides from the biofilm forming bacteria and their applications for metal tolerance. It is well known that heavy metals are essential trace elements, which are at high concentrations turn toxic to all living beings. The search for new polysaccharides will resulted in the development of several new and useful applications with commercial potentials. Two promising isolates from indigenous soil samples showed the tolerance against Cd metal up to 200mg/L. Newly synthesized compound used as a probe has specific attraction towards Cd metal and upon binding with metal the probe emits fluorescence. The completion of this, will pave the way to screen metal tolerant strains from the environment rapidly.

Environment and Nanotechnology

With advancement of material sciences, the recent major challenge to the environment is toxic effects of nanoparticles. Extensive efforts have been made in this direction in deciphering nanotoxicity mechanisms in microbial systems and many research findings have been published on microbial nanotoxicity in esteemed journals in recent times.

Research work on plant system (*Brassica nigra*) by studying nanotoxic effect of CeO_2 nanoparticles on its physiology has led to some understanding in mechanisms of phytotoxicity deciphering the direct impact on environment and human health (Rahul *et al*., 2016) and insights into phytonanotoxicity were also documented (Sinha and Khare, 2015). The mechanism especially cytotoxicity and molecular aspects of interactions of nanoparticles of silver and zinc oxide were investigated on mesophilic and halophilic bacterial cells. *Enterobacter* sp., *Marinobacter* sp., *Bacillus subtilis, Halophilic bacterium* sp. EMB4, was employed as models. The bacterium nanoparticle interactions were probed by electron microscopy and energy dispersive X-ray analysis. Electrostatic interactions between nanoparticles and cell surface were indicated as the primary step towards nanotoxicity. It was followed by cell morphological changes, increase in membrane permeability, accumulation in the cytoplasm and in binding to protein and DNA (Sinha *et al*., 2011b; Sinha and Khare, 2013). The differential interaction of two halophilic protease and one non-halophilic protease with silver and zinc oxide nanoparticles hypothesize that stable halophilic cells could be a versatile and useful systems for their remediation (Sinha and Khare, 2014). Proteomic investigations on interaction of silver nanoparticles with halophilic *Bacillus* sp. EMB9 revealed the differential expression patterns which are indicative of adaptive strategies being employed by the bacteria for functioning of the cellular machinery amidst nano-stress. These multiple investigative approaches at cellular and proteome level provided significant insight into mechanistic interpretations of bacteria-nanoparticle interactions (Rajeshwari *et al*., 2015). It is widely known that the physical and chemical properties of nanoparticles have raised various questions about the environmental safety as well production cost. Thus, more attempts were made for green synthesis of nanoparticles and its application in aromatic dye transformation and mercury detection. As solutions for these problems an eco-friendly method of silver nanoparticles (SNPs) synthesis by using latex of medicinally important plant *Jatropha gossypifolia* at ambient parameters and was characterized by different techniques. Potential of these green synthesized SNPs was investigated in degradation of aromatics dyes like methylene blue and eosin B which are major pollutant of textile and dye industry waste. For this, the latex of *J. gossypifolia* was employed for rapid synthesis of AgNPs. Biochemical studies and FTIR analysis of latex revealed role of protein, flavonoids, terpenoids and polyphenols of latex in nanosynthesis. This novel work on use of latex synthesized SNPs in reduction of methylene blue and eosin B dyes resulted in novel green alternative for nanofabrication with environmental application (Borase *et al*., 2014a).

Another highly toxic metal Mercury which is used in thermometers, fluorescent lamp and blood pressure cuffs has various toxic effects i.e. nephrological, cardiac, immunological and reproductive disorders are well documented. It has long environmental persistence due to its anthropogenic exploitation and natural processes. Method which can detect mercury with higher sensitivity, selectivity, low cost and on field applicability is more appreciable than currently available methods. On this background Latex-synthesized AgNPs which showed potential in selective and sensitive detection of toxic mercury ions (Hg^{2+}) with limit of detection around 100 ppb were designed. Addition of Hg^{2+} showed marked deviation in color and surface plasmon resonance spectra of AgNPs. Toxicity studies on aquatic non-target species *Daphnia magna* showed that latex-synthesized AgNPs (20.66±1.52 % immobilization) were comparatively very less toxic than chemically synthesized AgNPs (51.66±1.52 % immobilization) (Borase *et al*., 2014b).

Microbial Diversity

Microbial diversity of a particular environment provides insights in to the life of microbes existing in those niches. Usually, ruminants rely on the bionetwork of anaerobic rumen fungi (ARF), bacteria, methanogens, protozoa and bacteriophages for conversion of ingested feedstuffs into metabolizable energy and overall well-being of animals. Many of these microbes especially fibre degrading bacteria and fungi are of immense biotechnological potential owing to their efficient lignocellulolytic machinery which comprises of free enzymes and complex cellulosomes. Similarly, the rumen methanogenic archaea are also extremely important as methane production represents a pathway for the disposal of metabolic hydrogen during microbial metabolism. Methane emission in the rumen accounts for the loss of 2-15% of ingested energy and is second most potent greenhouse gas. Therefore, reduction of methane emission is important for ensuring the sustainability of ruminant-based agriculture production. Keeping the importance of methane emissions and animal production in mind, our group worked on molecular diversity of rumen methanogens and strategies for methane mitigation *in vitro*. Our group was first to isolate methanogens from Indian cattle and molecular diversity showed the abundance of *Methano brevibacter* spp. in Indian subcontinent. Besides diversity, three approaches for methane mitigation: dietary shift from low concentrate to high concentrate, rumen microbial manipulation and use of bacteriocin were also employed. (Kumar *et al*., 2011; Kumar *et al*., 2013a; Kumar *et al*., 2013b; Dagar *et al*., 2014).

Anaerobic fungi are regarded as the primary colonizers of plant materials in the herbivorous guts, and most active lignocellulose degraders known in the biological world. These remarkable biomass degraders are also capable of simultaneous saccharification and fermentation of the cellulosic and hemicellulosic fractions, making them ideal for renewable energy applications. In addition, their feeding in the form of microbial feed supplements has been found to increase weight gain, milk production, and total digestibility of poor quality feed components in ruminants, thereby improving overall animal productivity. Therefore, work on different aspects of these fungi including diversity, taxonomy, ecology and biotechnological applications are important. In this direction researchers have isolated novel genera of anaerobic fungi, developing molecular markers to distinguish different species of anaerobic fungi, profiled their enzymatic activities. (Dagar *et al*., 2011; Sirohi *et al*., 2013a; Sirohi *et al*., 2013b; Sirohi *et al*., 2013c; Callaghan *et al*., 2015; Dagar *et al*., 2015).

North-east India especially Meghalaya is endowed with a vast richness of natural caves and the microbial diversity prevalent in the caves has got attracted attention of microbiologists. The research team is working on the microbial composition of the caves of Meghalaya with reference to microbial biofilm and their role in cave biogenesis and endolithic bacteria and sacred groves. (Banerjee and Joshi 2015, 2016; Bhattacharjee and Joshi, 2016). In this region, sacred groves form an intricate part of the culture and traditions of the indigenous tribes. There are pristine and virgin patches of forest which are preserved in their natural conditions by the local community and the indigenous faith and traditions forbid the usage of the forest products and their interferences. This has led to sustained biodiversity in their pristine conditions. Considering the relevance of the traditions and pristine nature of the sacred groves, researchers are working on the microbial diversity in the prevailing environment of the pristine groves and also the epiphytic and endophytic microbes associated with the ethnomedicinal plants traditionally used for curing ailments by the local practitioners. (Lyngwi *et al*., 2016; Lyngwi and Joshi, 2015). Also, in this region, the food habits of the ethnic tribes invariably consist of meat items and traditionally fermented foods which are openly sold in unhygienic conditions in the local markets. These foods also are a source of pathogenic contaminants as the safety parameters are rarely maintained in their preparation and sale. Thus, the occurrence and nature of microbial composition of the traditionally fermented foods and other foods items hold importance in health and transmission of antimicrobial resistant organisms to the consumers of such foods. Hence, the environmental prevalence of antimicrobial resistant bacterial isolate associated with foods and tertiary hospital has generated some data on the prevalence of antibiotic resistance among environmental and food isolates. (Upadhyay *et al*., 2015; Upadhyay and Joshi, 2015; Thokchom and Joshi, 2012). In line of such vast diversity studies, a web-based database on soil microbes of Northeast India has been generated by the group which is being updated with the new isolates characterized from the region. The webpage is available at www.mblabnehu.info/nemid (Bhattacharjee and Joshi 2014). The database provides details on the sample collection site, growth conditions, identification and biochemical parameters of the characterized isolates of microbes along with the research publications generated from the research outcome (Joshi *et al*., 2015).

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List of Patents

1. Microbes for viscosity reduction of heavy oil and process thereof (application no. 3608/DEL/2015)
2. A Process for Enhanced recovery of crude Oil from Oil Wells using Novel Microbial Consortium - US Patent no. 7484560.
3. A process for Enhanced recovery of crude oil from oil wells using novel multimicrobial strain. Indian Patent no. 197554.
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5. Microbial nutrients for biodegradation of oil refinery waste and process for bioremediation of oily sludge and crude oil spill sites. Indian Patent No. 191744.
6. Bioremediation of acidic sludge. Patent No. 385. MUM/2004.
7. A Process for Enhanced Recovery of Crude Oil from Oil Wells using Novel Microbial Consortium - Filing of Canadian National Phase Application based on PCT/IN2004/000206; Application reference no. FPA029
8. A Process for Enhanced Recovery of Crude Oil from Oil Wells using Novel Microbial Consortium - Filing of Russian Patent Application based on PCT/IN2004/000206; Application reference no. FPA032
9. A Method for Preventing Wax Deposition in an Oil Well-bore using microbial means. Series no. CBR 1955, Application number 1437/DEL/2007.
10. Process for sequential bio-hydrogen production through integration of dark fermentation process with photo fermentation process. Indian Patent application number ‘3532/MUM/2013’, dated 8-November 2013.