RESEARCH PLAN PROPOSAL

STUDY OF BACTERIAL DIVERSITY IN COMMON EFFLUENT TREATMENT PLANT OF TEXTILE INDUSTRIES WITH SPECIAL REFERENCE TO BIODEGRADATION OF DYES.

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1. Introduction

While the earth's biodiversity has been studied in detail, to date, microbes have been completely omitted from meta-analytical studies of biodiversity data sets (Balmford &Bond,2005; Díaz et al., 2006). In fact, biodiversity data sets are far from being considered a comprehensive global resource (Collen et al., 2008). Since the origin of prokaryotes on Earth over 3.5 billion years ago, the extent of evolutionary diversification within this group has been truly immense (DeLong & Pace, 2001; Payne et al., 2009). Microbial communities play important biological roles, such as the global cyclical change of materials in various environments (Díaz et al., 2006). As a result, existing biodiversity includes a vast and largely undiscovered diversity of microbes, which are probably very important for the sustainability of ecosystems (Swift et al., 2004). Hence, detailed investigation to characterize the global biodiversity of microbes is a very important task.

Microbes have always formed a major component of global biodiversity, either as producers (e.g., phototrophic blue-green algae) or decomposers (e.g., heterotrophic bacteria) (Naeem et al., 2000). Furthermore, in the future, they may serve as producers of useful alternative energy sources (Ohnishi et al., 2010). Microbes are also used for bioremediation, which is the cleanup of pollution caused by human activities (Jørgensen et al., 2000). Culture-dependent approach was used to provide important information about unknown microbial diversity and potential available resources in a fieldscale model of a sewage sludge composter that was set up in Sapporo, Japan (Ohnishi et al., 2011).

Microbes are believed to be the common ancestors of all organisms. Microbes not only grow virtually everywhere but also are present in abundance. This is evident from major studies done exclusively on microbial diversity and its potential.Microbial diversity is fundamental for the maintenance and conservation of global genetic resources. As extreme environments are explored, the richness of microbial diversity is increasingly evident. Measures were taken to estimate, record, and conserve microbial diversity, not only to sustain human health but also to enrich the human condition globally through wise use and conservation of genetic resources of the microbial world. It has always been a very interesting and challenging area to explore. (Alexander P, 1993)

Traditionally, microbiologists have used culture-dependent approaches for the detection and isolation of environmental microbes, and the methods currently in use are based on those developed in the late 19th century (Okabe et al., 2009). These culture-dependent approaches present one of the most serious limitations to studying microbes, as they are essentially very effort intensive and slow down data assimilation (Moter & G bel, 2000). Our perspective on microbial diversity has improved enormously over the past few decades. Estimating the diversity of life is a persistent challenge in biology (Schloss and Handelsman, 2005). There is growing interest in research and development to develop novel tools to study, detect, and characterize microbes and their communities in industrial environments. Bacterial detection, identification, and typing from industrial samples remains an important task. The wealth of new techniques applicable to environmental microbiology is starting to uncover the variety of sequences relevant in biodegradation and biotransformation, as well as conditions in which these are active in their natural niches. Approaches aiming at characterizing microbial communities in the environment, be it by large-scale sequencing of metagenomes or by looking at biodegradative potential by mRNA-type techniques, are likely to go hand in hand with pure culture systems for detailed functional studies of biodegradative functions, to yield information about microbial enzymatic specificity and processivity. In view of the dearth of new enzymes discovered since the late 1970s, the ultimate goal of the ongoing efforts to explore the biodegradation and/or biotransformations gene pool is to open new avenues to our sustainability as industrialized societies (Galvao et al., 2005).

The highly complex microbial communities in ETPs are barely understood,despite their immense technical significance. ETPs are facilities as ideal model systems for fundamental research in microbial ecology. Focus on wastewater treatment is responsible for process performance and stability, which will help in studying detailed microbial diversity in current and future treatment plants. Wastewater microbiology is one area that has benefited greatly from the post genomic revolution. However, progress in characterizing the microorganisms that are important in ETPs is slow compared with/other ecosystems (Daims et al., 2006). The focus on microorganisms relevant to wastewater treatment is increasing but, to date, there are only a handful of genomes being analyzed from these organisms catalyzing key processes (Field and Kyrpides, 2007). An improved understanding of the genomics of wastewater-relevant organisms will demand both traditional genomics and cultivation-independent genomics approaches. Information regarding the genomic variability within a group of functionally similar organisms can be gleaned from such approaches. Biological treatment of wastewaters from industrial sources depends upon the maintenance of a microbial population in the ETP. ETPs at times start functioning in an inefficient manner and may even go dead. Reviving such malfunctioning ETPs becomes a difficult task. However, knowledge of the microbial community of an efficiently functioning ETP can surely help in reviving these ETPs.

The waste waters from industries vary so greatly in both flow and pollutional strength. In general, industrial waste waters may contain suspended, colloidal or dissolved (mineral and organic) solids. These wastes may contain inert, organic or toxic materials and possibly pathogenic bacteria. It may therefore be necessary to pretreat the wastes prior their release into municipal systems or it is necessary to fully treat when the wastes are discharged directly into ground waters or surface waters. The operation of wastewater treatment technologies relies on a combination of physical, chemical and biological factors. For many decades, these systems have been based mainly on information about chemical and physical parameters, having been quite successful in using the advantages of the amazing metabolic potential of microbial communities without detailed available knowledge about the organisms involved (Gilbride et al. 2006). The development of more stringent disposal limits to protect the environment demands an in-depth acquaintance with the parameters involved in wastewater treatment systems in order to improve and optimize their performance. Secondary treatment is one of the key components of a wastewater treatment plant. It involves the biological reduction of organic matter, suspended solids and toxicity of industrial wastewaters, together with the production of l low nutrient effluents. These functions are carried out by the resident microbial (mainly bacterial) community, which is considered the foundation of the secondary treatment process. These organisms present complex interactions among themselves and with the environment, which makes this stage a sensitive process that needs to be carefully studied. In recent years, the application of molecular techniques has made it possible to broaden insights into the vast diversity and interactions of microorganisms present in complex environments. The advantage of these methods is that they do not rely on cultivation and they therefore can be extremely sensitive and capable of describing the diversity of the microbial communities in much fuller detail (Gilbride et al. 2006).Amplification of the 16S rRNA gene through the PCR technique using universal primers and its subsequent sequencing has been extensively used to study bacterial diversity, as it allows identification as well as an indication of the phylogenetic relationship of the samples (Kapley et al. 2007). This technique has been applied to different conventional wastewater treatment systems such as anaerobic digesters (Sawayama et al. 2006), activated sludge (Liu et al. 2006; Kapley et al. 2007), or membrane bioreactors (Miura et al. 2007).

Textile industrial effluent accounts for the largest proportion of dye effluent pollution worldwide because the dye delivery process to the fabrics is not efficient with between 10 and 40% of the dye lost in effluent (Pearce et al., 2003). The coloured effluent in water has a number of undesirable effects such as the reduc tion of aesthetic properties, decreased photosynthesis and gas solubility (Asad et al., 2007).

Wastewater from textile industry is known to be strongly colored with large amount of suspended solids, high COD, and pH level. Many physico-chemical methods like coagulation, flocculation, ion exchange, membrane separation, oxidation, etc., are available for the treatment of heavy metals and dyes. Major drawbacks of these methods include high sludge production, handling and disposal problems, high cost, technical constraints, etc. This necessitates cost effective and environmentally sound techniques for treatment of wastewater containing dyes and metals. Adequate treatment of textile effluent requires more than one stage as there is need for both colour removal and degradation of aromatic compounds from the decolourisation process. Physico-chemical treatment methods are the least desirable owing to their high costs and generation of secondary pollutants. Biological treatment methods are more desirable as they are environmentally friendly, do not produce secondary pollutants and have a higher possibility of wider application (Forgacs et al., 2004; Georgiou et al., 2004; Moosvi et al., 2005; Asgher et al., 2007; Togo et al., 2008). However, viable biological treatment using microorganisms requires cheap carbon sources.

Biological processes have received increasing interest owing to their cost, effectiveness, ability to produce loss sludge and environmental benignity . Biological processes have potential to convert or degrade the pollutant into water, carbon dioxide and various salts of inorganic nature. The isolation of potent species and there by degradation is one of the interest in biological aspect of effluents treatment

In, a Textile Effluent Treatment Plant, colour removal has been a major concern. The effluent originates from textile and dye stuff plant with a continuous discharge of great quantity of remaining dyes to the environment. The efficient treatment of the effluent is an eco- friendly method for the treatment of textile effluent. Bacteria offers a cheaper and environment friendlier alternative for colour removal in textile effluents (Olukanni et al., 2006). Biological treatment has been effective in reducing dye house effluents and when used properly has a lower operating cost than other remediation process.

In the present study, attempt would be made to analyse waste water at different stages of ETP and bacterial diversity of some of the selected strains would be studied for dye degrading potential.

2. Review of Literature

2.1 Textile Effluent Treatment Plants

Textile industry is a very diverse sector in terms of raw materials, processes, products and equipment and has very complicated industrial chain. The textile finishing covers the bleaching, dyeing, printing and stiffening of textile products in the various processing stages (fibre, yarn, fabric, knits, finished items). The purpose of finishing is in every instance the improvement of the serviceability and adaptation of the products to meet the ever-changing demands of fashion and function.

The impacts on the environment by textile industry have been recognized for some time, both in terms of the discharge of pollutants and of the consumption of water and energy (Lacasse and Baumann, 2006). Finishing processes can be categorized into purely mechanical and wet processes. The liquid phase for the latter type is primarily water, and - to a lesser extent - solvents and liquefied ammonia gas. Another important medium is steam. To achieve the desired effects, a range of chemicals, dyes and chemical auxiliaries are used. Environmental problems of the textile industry are mainly caused by discharges of wastewater. The textile sector has a high water demand. Its biggest impact on the environment is related to primary water consumption (80–100 m3/ton of finished textile) and waste water discharge (115– 175 kg of COD/ton of finished textile, a large range of organic chemicals, low biodegradability, colour, salinity). Therefore, reuse of the effluents represents an economical and ecological challenge for the overall sector (Li Rosi et al., 2007). The effluents resulting from these processes differ greatly in composition, due to differences in processes, used fabrics and machinery (Bisschops and Spanjers, 2003). Main pollution in textile wastewater came from dyeing and finishing processes. These processes require the input of a wide range of chemicals and dyestuffs, which generally are organic compounds of complex structure. Because all of them are not contained in the final product, became waste and caused disposal problems. Major pollutants in textile wastewaters are high suspended solids, chemical oxygen demand, heat, colour, acidity, and other soluble substances (Venceslau et al., 1999; World Bank, 2007).

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and the dwindling supply has made the treatment and reuse of industrial effluents an attractive option. Textile effluents are of concern because they colour the drains and ultimately the water bodies. They also diminish the water quality. The ability of microorganisms to degrade and metabolize a wide variety of compounds has been recognized and exploited in various biotreatment processes.

The aims of wastewater treatment are: to convert the waste matter present into stable oxidized end-products which can be safely discharged to inland or coastal waters without any adverse ecological effects to protect public health; to ensure the effective disposal of wastewater on a regular and reliable basis without offence; to provide an economical method of disposal and recover valuable components of wastewater (Gray, 1989). Waters and wastewaters have highly complex compositions and adjustments to the composition are usually essential to suit a particular use or avoid degradation of the environment. Therefore a variety of treatment processes are necessary to treat the range of contaminants (Pearson, 1999). Microorganisms have several important functions in pollution control. Natural waters possess the capacity of self-purification due to the microbial content of aquatic systems in which microorganisms respond to organic pollution by increased growth and metabolism. It is necessary to apply this same process in biological treatment systems to treat wastewater. Wastewater also contains microorganisms themselves and by providing a controlled environment for optimum microbial activity in a treatment unit or reactor, nearly all the organic matter present can be degraded (Sanders and Yevjevich, 1996). For almost a century, the microbiological quality of waters was based on culture-dependent methods, which have been continuously optimized to detect and quantify the presence of organisms relevant in terms of quality control, public health or risk assessment studies (e.g. Leclerc 1994; Leclerc and Moreau 2002; Mossel and Struijk 2004).The operation of wastewater treatment technologies relies on a combination of physical, chemical and biological factors. For many decades, these systems have been based mainly on information about chemical and physical parameters, having been quite successful in using the advantages of the amazing metabolic potential of microbial communities without detailed available knowledge about the organisms involved (Gilbride et al. 2006). The development of more stringent disposal limits to protect the environment demands an in-depth acquaintance with the parameters involved in wastewater treatment systems in order to improve and optimize their performance.

The textile industries produce effluents that contain several types of chemicals such as dispersants, leveling agents, acids, alkalis, carriers and various dyes (Cooper,1995.) Several methods are used in the treatment of textile effluents to achieve decolourization. These include physiochemical methods such as filtration, specific coagulation, use of activated carbon and chemical flocculation. Some of these methods are effective but quite expensive (Do et al., 2002; Maier et al., 2004). Biotreatment offers a cheaper and environmentally friendlier alternative for colour removal in textile effluents. The ubiquitous nature of bacteria makes them invaluable tools in effluent biotreatment. The chemical nature of dyes varies, but azo dyes are the most widely used. The oxidative decolourisation of dyes of several classes has been reported and azo dyes were found to be the most recalcitrant compounds. (Maeir et al., 2004). Considerable attention has been given in evaluating the capability of microorganisms in decolourising and degrading the azo dyes. Many studies on the decolourising capability of microorganisms especially fungi and bacteria have been reported and reviewed (Novotný *et al.*, 2001; Kandelbauer & Guebitz, 2005). In general, complete azo dye mineralisation requires both anaerobic and aerobic bacterial processes. Azo bonds are reduced under anaerobic conditions leading to generation of aromatic amines.

Biological treatment has been effective in reducing dye house effluents and when used properly has a lower operating cost than other remediation process. The reactive azo dyes-containing effluents cause serious environmental pollution. Therefore, industrial effluents containing azo dyes must be treated before discharging into the environment to remove the dye toxicity from textile effluents (Hao et al., 2000; Wafaa et al. 2003). However, the azo dyes are generally recalcitrant to biodegradation due to their xenobiotic nature. The microorganisms being highly versatile are expected to develop enzyme systems for the decolourization and mineralization of azo dyes under certain environmental conditions (Pandey et al., 2007). Anoxic degradation of various azo dyes by mixed aerobic and facultative anaerobic microbial consortia was reported (Moosvi et al., 2005).Anaerobic and aerobic reduction of azo dyes to simpler compounds have all demonstrated the ability of microbes and sludges to effectively reduce azo dyes to their intermediate structures, thus destroying the apparent colour (Chinwetkitvanich et al., 2000) and Razo- Flores et al., 1997)

Aerobic treatment of azo dye wastes though effective, is often the typical method of treatment. Recent combination of chemical and biological, physical and biological treatment has also proven to be effective (Seshadri et al., 1994; Horning, 1977).The decolourization of azo dyes has been found to be effective under anaerobic conditions. However, the anaerobic degradation yields aromatic amines which are mutagenic and toxic to humans and cannot be metabolized further under the conditions which generated them (Chung and Stevens, 1993; Do et al., 2002). In activated sludge treatments of dye effluents, reactive azo dyes and aromatic amino derivatives are a non-biodegradable class of compounds which can even inhibit activated sludge organism (Maeir et al., 2004). It is thus important to explore the possibilities of isolating efficient aerobic degraders for use in decolourization and biotreatment of textile effluents. Several microorganism are reported to be able to transform azodyes.. Those microorganisms are *Pseudomonas luteola, Pseudomonas* sp., *Escherichia coli, Bacillus* sp*, Alcaligenes faecalis, Rhodococcus erythropolis,* dan *Enterococcus faecalis* (Chang *et al*., 2000; Kalyani *et al*., 2009; Mutafov *et al*.,2006; Meitiniarti *et al*., 2008).

To date, although research on biodegradation of reactive azo dyes by microbial consortia have been established internationally, limited studies on the decolourising capability of bacterial strains isolated locally have been reported and their potential in wastewater treatment should therefore, be investigated.

3. Objectives of Study

3.1 Screening of bacteria at different stages of CETP

3.2 Physico- Chemical characterization of effluent at different stages of treatment process.

3.3 Isolation and characterization of bacterial strains responsible for degradation of dyes.

3.4 Process optimization for effective degradation.

4. Relevance of Study

The requirements for treated wastewater are becoming increasingly more stringent, and therefore the improved efficiency of biological treatment processes is indispensable at industrial effluent treatment plants (ETPs). Microorganisms such as bacteria play an important role in the natural cycling of materials and particularly in the decomposition of organic wastes. The knowledge of the interactions among these microbial populations needs to be harnessed for optimum evaluation and functioning of effluent treatment plants. Modern molecular techniques have revolutionized the methods of assessing these microbial populations. The combination of the results of these microbial assessments along with the on-site parameters at ETPs would favor an efficient treatment.

An extensive use of dyes in textile and other industries has created problems in terms of acute ecological effects except certain dye stuffs that exhibit non-toxic effect towards microbial population and carcinogenic potential to human being (Agarwal and Pandey, 1994). As dyes are recalcitrant molecules, they pose difficulties in degradation. Biological waste treatment processes are sometimes more efficient and less expensive than physical-chemical waste treatment procedures and hence it would desirable to provide a biological process using microorganisms that degrade xenobiotic azo dyes (Zheng *et al.*, 1999). Microbial degradation seems to be promising compared to other organisms and the method of application are simpler compared to other available methods (Jayarajan *et al.*, 2011) Microbial degradation is considered to be less expensive and more effective environment friendly alternative.

The general approach of bioremediation is to improve the natural degradation capacity of the native organism. Several microorganisms have been reported by a number of investigators which have the capacity to decolorize various textile azo dyes (An *et al.*, 2002; Sarnaik and Kanekar, 1999). The degradation of azo dyes produces aromatic amines which are carcinogenic and mutagenic. Recently, several reports appeared showing that the microorganism has the ability not only to decolorize dyes but also to detoxify it (Adedayo *et al.*, 2004; Kumar *et al.*, 2007; Rajaguru *et al.*, 2000). The process of biodegradation is a well-established and powerful technique for treating domestic and industrial effluents. Microbial populations has an amazing and extensive capacity to degrade a variety of organic compounds (Manogari *et al.*, 2008).

Currently, extensive research is being focused on finding an optimal microbial biomass that would be as cheap as possible for the removal of contaminating dyes from large volume of polluted water (Youssef *et al.*, 2008)

5.

5.1 Methodology

1) Samples would be be collected, transported ,stored and preserved as per the standard procedures on Waste water treatment, (APHA,1995) from CETP,Pali (Rajasthan)
2) Physico-chemical parameters of samples would be conducted as prescribed by APHA,1995.
3) Samples would be plated onto screening medium(Hayase medium,2000)using serial dilution technique and Stab culture(Wise et al, 2006)
4) Decolourisation assay would be studied for all the samples.
5) The bacterial isolates would be identified by culture –dependent approach(Bergey's Manual of Determinative Bacteriology)
6) Bacterial isolates would be biochemically characterized according to Bergey's manual of Determinative Bacteriology.
7) Process optimization of dye degrading potential of some of the selected isolates would be carried out.

5.

5.1Methodology (Flow Chart)

(Grab Sampling, Standard procedures on Waste Water Treatment, APHA,1995)

DO(Winkler Method)	BOD (Dilution Method)	COD (Reflux Method)	Hardness (EDTA Titrimetric Estimation)	Chloride (Silver Nitrate Method)	Nitrate (UV Spectrophometric Method)	Phosphorous (Stannous Chloride Method)	TS,TDS,TSS

6. Plan of Work

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