

Bioremediation: A strategic alternative for treatment of textile effluent

¹Patel Feni and ¹Marjadi Darshan

¹Shree Ramkrishna Institute of Computer Education & Applied Science, Surat
Email: fenipatel.5349@gmail.com , darshworld@gmail.com

Abstract— The textile industry, which is one of the largest consumers of water in the world, produces wastewater comprising various recalcitrant agents such as dyes, sizing agents and dyeing aids. Strong colour of the textile waste water is the most serious problem of the textile waste effluent. The disposal of these wastes and their dyes may significantly affect photosynthetic activity in aquatic habitat and damage to the environment. It retards biological activity by reducing the light penetration and also causes metal toxicity to both aquatic and terrestrial life. It also leads to toxicity of fish and mammals. Major problem is that dyes having higher stability under sunlight and resistance to microbial attack. Therefore, care should be taken when releasing these types of wastewater into the environment. For solving these now a novel approach based on bioremediation using bacteria is widely used. In which natural biota and the microbial consortia have been used and found that it is more effective. Bioremediation is the most effective process and their end products are also non-hazardous. For these purposes mostly, bacterial culture was used widely.

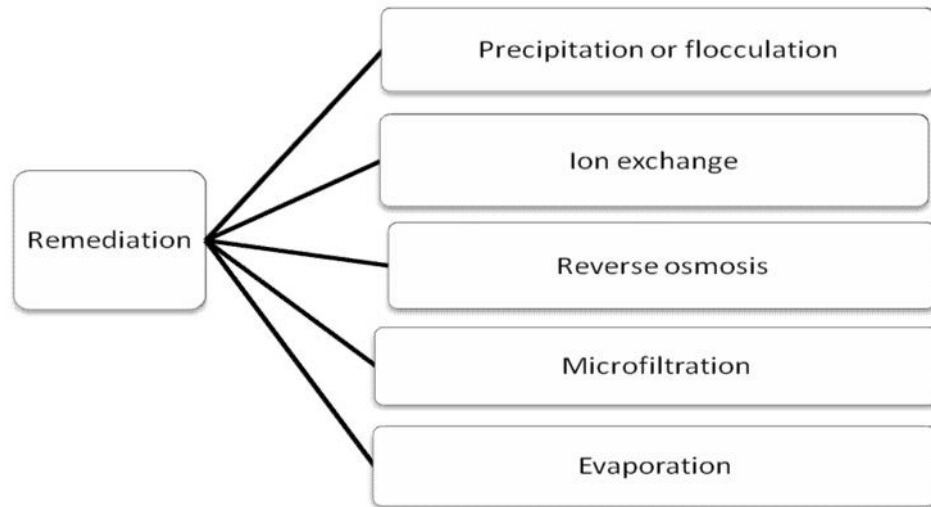
Keywords-Bioremediation; Dyes; Genetically modified organism's; Heavy metals; Pollution

I. INTRODUCTION

Bioremediation constitutes the use of natural biota and their processes for pollution reduction; it is a cost effective process and the end products are non-hazardous [1]. Bioremediation is an integrated management of polluted ecosystem where different microorganisms are employed which catalyze the natural processes in the polluted or in the contaminated aquatic or terrestrial ecosystem [2]. Textile is one of the largest industries and results in water pollution contributed by untreated effluent discharge, which contains high concentrations of consumed metal based dyes, phenol, aromatic amines etc. The discharge of textile effluent alters the Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), suspended solids, salinity, colour, a wide range of pH (5–12) and the recalcitrance of organic compounds, such as azo dyes and gives the rivers intense colouration [3].

Major problem in textile is that dyeing industry effluent alters the color and quality of the water bodies has been proved to be hazardous to aquatic ecosystem and it reduces the sunlight penetration which is essential for photosynthesis, it leads to toxicity of fish and mammals. They also inhibit the activity and growth of microorganisms [4]. Dyes having higher stability under sunlight and resistance to microbial attack and temperature were identified [5]. Many dyes were known as carcinogens, such as benzidine and other aromatic components all of which might be reformed as a result of microbial metabolism. It has been already well documented that azo and nitro-compounds were reduced in the sediment and intestinal environment, resulting in the regeneration of the parent toxic amines this compound was not readily removed by typical microbial based waste treatment processes. The highest rates of toxicity were observed for basic, diazo and direct dyes [6]. Decolorization and degradation of dyes by mixed as well as pure cultures of bacteria and fungi have been studied under aerobic and anaerobic conditions. In most studies, the microbial consortia have been used and found that more effective than pure cultures [7].

Figure 1. Conventional method approaches to reduce heavy metal contamination in water.



Conventional method having some disadvantages that chemical used to remediate heavy metal or dyes which is present in textile raw effluent, that Increased aquatic toxicity, Increased sludge production, Increased filamentous growth, Decreased sludge settle ability, Decreased sludge dewatering characteristics, Increased cost of treatment [8].

Due to the inherent drawbacks of physical, chemical and photochemical approaches to dye and metal removal, the use of biological methods for the treatment of textile wastewaters has received attention as a more cost effective alternative [9].

Bioremediation use the bacteria, fungi and enzymes to degrade environmental pollutants. Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil or water. The residues after the treatment are usually harmless products and include carbon dioxide, water, and cell biomass. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. This eliminates the chance of future liability associated with treatment and disposal of contaminated material. Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible by using bacteria. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation. Bioremediation can prove less expensive than other technologies that are used for clean-up of hazardous waste [10].

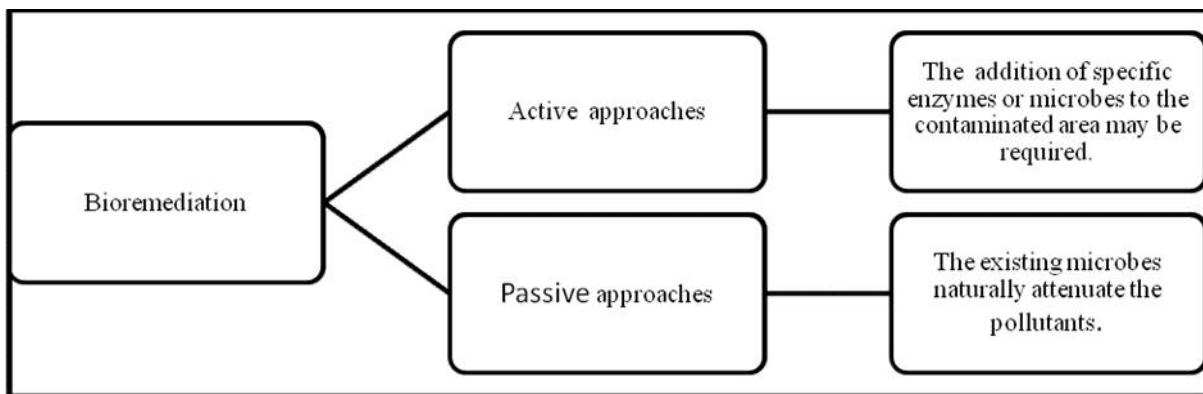
TABLE I. ENVIRONMENTAL CONDITION AFFECTING MICROBIAL ACTIVITY

Parameters	Condition Required For Microbial Activity
Soil moisture	25–28% of water holding capacity
Soil Ph	5.5–8.8
Oxygen content	Aerobic, minimum air-filled pore space of 10%
Nutrient content	N and p for microbial growth
Temperature (°C)	15–45
Heavy metals	Total content 2000 ppm
Contaminants	Not too toxic
Type of soil	Low clay or silt content

II. MECHANISM OF BIOREMEDIATION

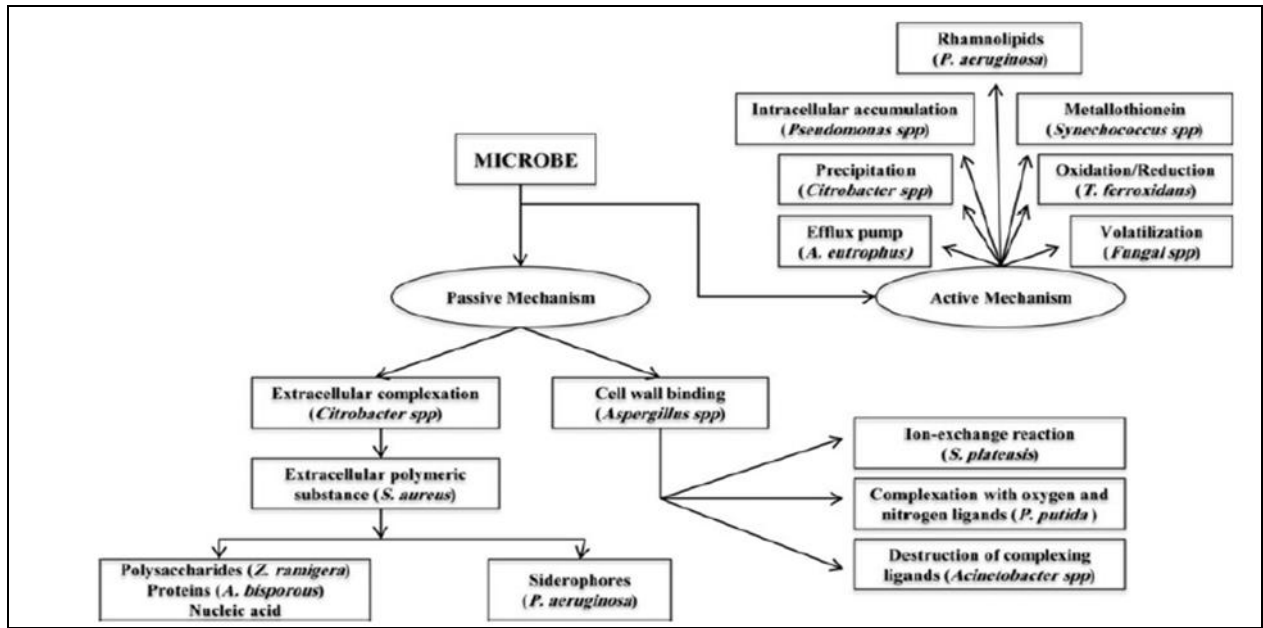
The mechanism by which metal ions bind to the cell surface include, electrostatic interaction, vanderwaals forces, redox interaction, extracellular precipitation, and combination process [11]. The negatively charge groups (carboxyl, hydroxyl, and phosphoryl) of the bacterial cell wall absorb metal cations, which are then retained by mineral nucleation [12]. Bacteria are responsible for the stabilization of wastes coming into a treatment plant. Many of these bacteria form floc particles or clusters of bacteria that break down waste. The floc particles also serve as sites on which waste can be absorbed and broken down later. In addition, filamentous bacteria form trichomes or filaments. These chains of bacteria provide a backbone for the floc particles, allowing the particles to grow in size and withstand the shearing action in the treatment process. When filamentous bacteria are present in excessive numbers or length, they often cause solid/liquid separation or settle ability problems. *B. cereus* performed well because they are nutritionally versatile and carries an efficient enzymatic system for the cleavage of azo bonds, which cause rapid decolourization of different azo dyes and thus they are able to biodegrade many natural and synthetic organic compounds. Azo dyes are the largest group of dyes. More than 3000 different varieties of azo dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries [13]. Azo dyes are characterized by the presence of one or more azo groups – $N = N -$, which are responsible for their colouration and when such a bond is broken the compound loses its colour. They are the largest and most versatile class of dye, but have structural properties that are not easily degradable under natural conditions and are not typically removed from water by conventional waste water system.

Figure 2. Approaches to reduce contamination in water.



Microbial decolouration can occur via biosorption, enzymatic degradation or a combination of both [14]. The uptake or accumulation of chemicals by microbial mass has been termed as biosorption. It was mainly taking place in the cell wall, where as the mechanism includes adsorption and absorption will differ according to the biomass type [15]. Biosorption mechanism for metal removal has been studied extensively using various species of live and inactivated biomass of bacteria, algae, fungi or yeast [16].

Figure 3. General Heavy metal sorption mechanism Operating in various microbes.

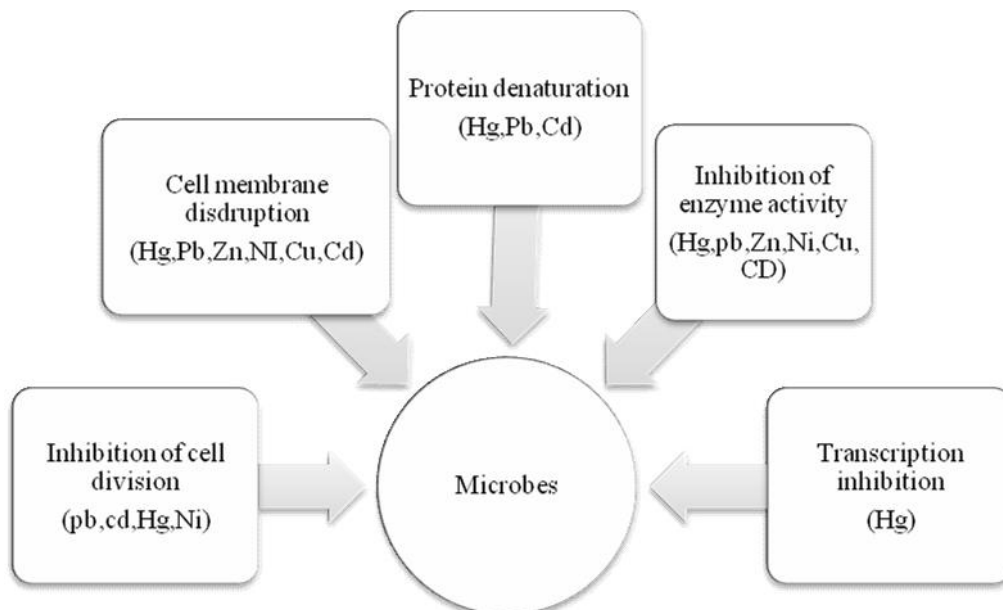


(after ,[8])

III. HEAVY METAL TOXICITY MECHANISM TO MICROBES

Organisms respond to heavy metal stress using different defense systems shown in fig.2 . Such as exclusion, compartmentalization, formation of complexes and synthesis of binding proteins, like phytochelatins (PCs).

Figure 4. Different defence systems of microbes to toxic metal .



IV. GENETIC ENGINEERING FOR METAL REMEDIATION

There are several developments in GEMs which overcome the limits of using wild type microbes. Microbes are confined to aerobic catabolic and co-metabolic pathways and therefore cannot be applied to anaerobic environment. GEMs are developed by inserting genes for oxygenases make it possible to use them in anaerobic environmental conditions. Application of GEMs based remediation of xenobiotics is in the fore front due to eco-friendly and human friendly approaches. Bioremediation using GEO is a relatively cheap, environmentally-friendly and socially acceptable technology which can eliminate waste permanently [17], [18].

Genetic engineering allows introduction of desired traits into cells to metal clean up techniques, and this approach has already been used to construct cells for the bioremediation of mercury. The recombinant *staphylococcus xylosus* and *staphylococcus carnosus*, had gained Ni²⁺ and Cd²⁺ binding capacity and suggested that they could be used in bioremediation of heavy metals [19]. *E. coli* strain that accumulated Ni²⁺ by introducing the nixA gene from *helicobacter pylori* into *E. coli* JM 109 that expressed a glutathione S-transferase pea metallothioneine fusion protein [20]. The recombinant *Escherichia coli* strain accumulated four times more nickel than the wild type. Metal resistant *R. eutropha* isolate, engineered to produce metallothionein accumulated more Cd²⁺ than its wild type and offered tobacco plants some protection from Cd²⁺ when inoculated into contaminated soil [21]. Nickel (Ni) is possibly the most recalcitrant pollutant and can be accumulated by the GE *Escherichia coli* SE5000 strain from an aqueous solution [22]. Arsenic (As) is a very toxic metal which can be found in nature [23]. GM bacteria expressing the ArsR gene can promote the bioaccumulation of as when present in contaminated water [24].

V. BIOREMEDIATION THROUGH BIODEGRADATION

Waste water sample collected from the effluent treatment plant (ETP) of textile industries, from the printing and dyeing mills, or in some case Heavy metal solution prepared in laboratory and used as sample. This raw effluent was treated with different bacteria.

Bacteria were isolated directly from the textile raw effluent or from the soil at nearby areas of textile industry. Mostly bacteria that used for study is *Bacillus cereus*, *Bacillus subtilis* [25], *Escherichia coli* [26], *Pseudomonas aeruginosa* [27]. Dye degrading isolates were identified on the basis of morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology [28].

Dyes and heavy metal: Reactive azo dyes used in some research are, Reactive Orange – M2R (m = 493 nm), Reactive Blue – M58 (m = 572 nm), Reactive Yellow – M4G (m = 413 nm) and Reactive Black - B (m = 574 nm) [26]. In some, Crystal violet (max 523 nm) [25], in some, Cr(VI) bioremediation [27] and in some article, reactive turquoise blue, disperse yellow, reactive orange H3R, cibacron red P4B and cibacron black PSG dyes are used for experiment.

TABLE II. OTHER MICROORGANISM THAT ARE USED FOR BIOREMEDIATION

Microorganism and Source	Process, Conditions and Enzymes	Effluent, % Colour, COD, BOD removal, Toxicity	References
<i>Bacillus cereus</i> isolated from site dye Contaminated	Adsorption and degradation; 30 C, Lac, azoreductase	Real textile effluent, 100% colour	[29]
<i>Escherichia coli</i> and <i>Legionella sp.</i>	Degradation	Textile wastewater, 86% COD, 92% TOC	[30]
<i>Candida tropicalis</i>	Adsorption and degradation; room temperature	Textile wastewater, 100% colour, 70% COD, 76% BOD	[31]
Consortium of <i>Pseudomonas sp.</i> and <i>Aspergillus ochraceus</i>	Adsorption and degradation	Effluent containing reactive and disperse textile dyes, 77% colour	[29]
Consortium of <i>Pseudomonas sp.</i> and <i>Pseudomonas sp.</i>	Degradation; pH 7, 30 C, 48 h; Lac, azoreductase	Effluent with reactive azo dyes, 100% colour, 78% COD, 80% BOD. Reducing toxicity at least 30%	[32]
Sludge from a treatment plant	Adsorption and degradation; 25 C, pH 2–11	Effluents containing Reactive Red 120, 99% colour > 88% COD	[33]
<i>Aspergillus fumigatus</i> isolated from mildewing rice straw	Degradation; pH 3, 150 rpm, 30 C, 72 h	Effluents of a printworks utilizing reactive textile dyes, 100% colour	[34]

VI. CONCLUSION AND DISCUSSION

Several researchers have demonstrated the possibility of utilizing micro-organisms for biotreatment of textile wastewater. Microbiological methods are quite simple to use and the cost of operation is low. South Asian countries are experiencing severe environmental problems due to rapid industrialisation. This phenomenon is very common where the polluting industries such as textile dyeing, leather tanning, paper and pulp processing, and sugar manufacturing thrive as clusters. Among these the textile industries are large industrial consumers of waters as well as producers of wastewater [35].

Various parameter like, the pH of the effluent alters the physico-chemical properties of water which in turn adversely affects the aquatic life, plant and humans. High temperature decreases the solubility of gases in water, which is ultimately expressed as high BOD/ COD. Sediment rate is drastically increased because of the high value of TDS, which reduces light penetration into water, and ultimately decreases photosynthesis. The decrease in photosynthetic rate reduces the dissolved oxygen level of wastewater, which results in decreased purification of wastewater by micro-organisms [36]. High chloride contents are harmful for agricultural crops [37].

The isolation of different micro-organisms from the effluent sample collected from the textile industries indicates natural adaptation of micro-organisms to survive in the presence of toxic chemicals and dyes. A strain of bacterium *Bacillus subtilis* ETL-2211 with strong decolourising ability was isolated from textile effluent to decolourise the textile azo dye Crystal violet (100 mg L⁻¹) within 24 h in aerobic and static conditions. Some study indicated that bacterial species *Bacillus cereus* isolated from the dyewaste effluents have potential to decolourize dyes to varying degrees. Bacterial capable of dye decolourization have been reported.

Like dyes reactive turquoise blue, disperse yellow, reactive orange H3R, cibacron red P4B and cibacron black PSG in which degree of decolorization is obtained 65.33, 88.33, 35, 23, 32% respectively by *Bacillus cereus* [38]. *Pseudomonas sp.* show the capacity to the maximum metal removal by 80.16% at 10 mg/l of Cr (VI) through after 72 hours and remove 78% at 25 mg/l of Cr (VI) [39]. Decolourization of azo dye Reactive Yellow – M4G by *Escherichia coli* was 20%. Decolourization of azo dye Reactive Black - B by *Escherichia coli* was 30%, Decolourization of Reactive Blue - MR by *Escherichia coli* was 60% [26].

A Recombinant DNA and RNA technology is a promising method for the preparation of genetically modified organism able to combat environmental pollutants for successful bioremediation [40]. Genetically engineered microbes are an advanced technology that has attracted public attention when employed in cleaning up toxic waste and heavy metals from contaminated sites [41], [42]. DNA shuffling is a powerful mutagenesis technique that can create new enzymatic activity [43] and Bio-catalysts with higher degradation rates for heavy metals [44].

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