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## **Bioremediation – An eco-friendly tool for effluent treatment: A Review**

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### **Abstract**

The use of microorganisms to degrade or reduce the concentration of hazardous wastes on a contaminated site is called bioremediation. Biological treatment systems have various applications, such as cleanup of contaminated sites such as water, soil, sludge, and streams. Rapid industrialization, urbanization, intensive farming and other human activities have resulted in land degradation, environmental pollution and a decline in the crop productivity in all sectors of agriculture. Human activities have increased pressure on the natural resources and has become the source of a myriad of pollutants. Several methods have been designed and developed but more often, these process again produce secondary pollutants, which again are costing the environment. Bioremediation is emerging as an effective and attractive management tool to treat and recover the environment, in an ecofriendly manner. Bioremediation has been used at a number of sites worldwide, with varying degrees of success. Bioremediation, both in situ and ex situ have also enjoyed strong scientific growth, in part due to the increased use of natural attenuation, since most natural attenuation is due to biodegradation. Bioremediation technology, which leads to degradation of pollutants, may be a lucrative as well as environmentally friendly alternative. This article discusses the role of microbes in different waste water treatment methods in different field and puts forward thoughts and scope for further research in the field.

**Keywords:** Bioremediation, Biodegradation, Remedatory

### **Introduction**

Bioremediation is a process in which beneficial microbiological agents, such as yeast, fungi or bacteria are used to clean up contaminated soil and water. It is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the application of biological processes. The first and greatly affected ecosystems in any country are the aquatic ecosystems, affected by either point or non-point source of pollution. Point sources of pollution occur when the polluting substance is emitted directly into the waterway. The common point sources of pollution are municipal and industrial wastewater effluents; run-off and leachate from solid disposal sites; run-off from industrial sites; run-off and drainage from industrial sites; discharge from vessels. The non-point sources includes flow of water from agricultural fields and orchards, urban run-off from unsewered areas, etc. The effects of water pollution are not only devastating to the aquatic organisms but also to the terrestrial animals and birds. More seriously, contaminated water destroys aquatic life and reduces their reproductive ability. Ultimately, the water becomes unfit for human consumption or domestic usage, at severe cases even a hazard to human health. Waste disposal has an environmental cost and a financial cost too, which can be reduced by use of bio remediating agents (Pillay, 1992)<sup>[72]</sup>. To speed up the bioremediation process, seeding of contaminated wastewater with competent microflora that are capable of degrading hazardous waste, is usually practiced in most treatments. The inoculated microorganisms either may be naturally occurring types or prepared in the laboratory to attack the target waste.

### **1. Role of microbes in Bioremediation process**

Microbial bioremediation can cost-effectively and expeditiously destroy or immobilize contaminants in a manner that protect human health and the environment (Heitzer and Saylor, 1993; Gheewala and Annachatre, 1997; Gadd, 2000)<sup>[37, 27, 26]</sup>. Research is underway at a

number of facilities using exogenous, specialized microbes or genetically engineered microbes to optimize bioremediation (Hassan *et al.*, 2003) [35]. A successful, cost effective, microbial bioremediation program is dependent on hydro geologic conditions, the contaminant, microbial ecology and other spatial and temporal factors that vary widely. In any bioremediation process the introduced microorganisms use the contaminants as nutrients or energy sources (Tang *et al.*, 2007) [93]. Bioremediation activity through microbes are stimulated by supplementing nutrients (Nitrogen and phosphorus), electron acceptor (oxygen), and substrates (methane, Phenol and toluene) or by introducing microorganisms with desired catalytic capabilities (Ma *et al.*, 2007, Baldwin *et al.*, 2008) [51, 7]. Some common microorganism used in the process of remediation are *Acromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Cinetobacter*, *Corneybacterium*, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Pseudomonas*, *Vibrio*, *Rhodococcus* and *Sphingomonas* species (Gupta *et al.*, 2001, Kim *et al.*, 2007, Jayashree, 2012) [32, 47, 41]. The main species involved in effective waste water treatment include lactic acid bacteria-*Lactobacillus plantarum*, *L. casei* and *Streptococcus lacti* and Photosynthetic bacteria-*Rhodospseudomonas palustris*, *Rhodobacter spaeroide*, etc. (Narmatha and Kavitha, 2012) [65].

*Arthrobacter sp* was first isolated from natural environment which has the capability to reduce nitrogen by heterotrophic nitrification process (Verstrae and Alexande, 1972) [96]. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform (Derek, 1995) [17]. *Enterobacter cloacae* is capable of growth utilizing trinitrotoluene (TNT) as a nitrogen source. The pentaerythritol tetranitrate reductase an enzyme described to be involved in the degradation of nitrate esters is capable of reducing the aromatic ring of TNT and causing liberation of nitrite (French *et al.*, 1998, 1999) [23, 24]. Endosulfun, a pesticide residual, is extremely toxic to fish and aquatic invertebrates. It can bind to soil particles and persist for a relatively long period, with half-life of 60-800 days. Bacteria such as *Pseudomonas sp.* and *Arthrobacter sp.* can degrade up to 57-90% of  $\alpha$ -endosulfun and 74-94%  $\beta$  - Endosulfun in a period of 7 days (Sutherland *et al.*, 2000). The first patent for a biological remediation agent was registered in 1974 being a strain of *Pseudomonas putida* that was able to degrade petroleum. Several studies reveal, 42 different pollutants including black liquor from a kraft pulp and paper mill effluent, tannery effluent, steel industrial effluent etc., can be biodegraded by using *Pseudomonas sp.* (Prescott *et al.*, 2002) [78]. In the presence of oxygen, the aerobic bacteria such as *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium* are capable of degrading pollutants. These microbes degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy (Boricha and Fulekar, 2009) [9]. Pure culture of *Exiguobacterium aurantiacum* has the capability of phenol degradation and PAHs in batch culture when provided with pure compounds as a source of carbon and energy (Jeswani and Mukherji, 2012) [42]. Other studies show that *Pseudomonas sp.* acts as fuel eating bacteria which can degrade the hydrocarbons (Jayashree *et al.* 2012) [41]. Biological phosphate removal in the activated sludge process was first reported by Levin *et al.* (1972) [49]. *Acinetobacter sp*

was the first bacterium which has been responsible for phosphate removal. (Fuhs and Chen., 1975) [25]. *Micrococcus sp.*, *Lampropedia sp.*, *Tetraphaera sp.*, *Aeromonas sp.*, *vibrio sp.*, and *Pseudomonas sp.* also play an important role in phosphate removal process. Mayer (1991) [54] compare aerobic with anaerobic treatment of waste waters in German brewery and reported that anaerobic treatment achieved 91% COD reduction at loading rates up to 20 g COD/l/day, whereas the aerobic treatment resulted in a 76% reduction at a loading rate of 69 g COD/l/ day. *Pseudomonas sp.* and *Brevibacillus sp.* were nitrate reducing bacterial strains isolated from petroleum contaminated soil (Grishchenkov *et al.*, 2000) [30]. The most common bacteria that carries out ammonia oxidation is *Nitrosomonas* species, while nitrite oxidation is carried out by *Nitrospina*, *Nitrococcus* and *Nitrocystis* (Rittmann and Carty 2001) [80]. Biological treatment by autotrophic nitrogen removal is preferred for concentrated waste water streams with high ammonia concentrations in the range of 100 -5000 mg N/L (Mulder *et al.*, 2003) [61]. The oxidized nitrogenous compounds (Nitrite to nitrate) are reduced to gaseous nitrogen by heterotrophic microorganisms that use nitrite or nitrate instead of oxygen as electron acceptor and organic matter as carbon and energy source. Denitrification is common among the gram negative bacteria such as *Pseudomonas*, *Alcaligenes*, *Paracoccus* and *Thiobacillus*. Denitrification is the slow process particularly for industrial waste waters that contain high concentrations of nitrate. Decrease of ammonia nitrogen concentration from 10 mg/l to below 0.3 mg/l was obtained within 3 days after inoculation of microbial inoculums with aeration in polluted water with use of nitrifiers (Barik *et al.*, 2011) [8]. *Bacillus methylotrophicus* L7, the first reported gram-positive bacterial strain to denitrify nitrite to N<sub>2</sub> and denitrifying nitrite and nitrate to N<sub>2</sub>O under aerobic condition. The strain L7 exhibited efficient heterotrophic nitrifying-aerobic denitrifying ability with maximum NH<sub>4</sub> -N removal rate of 51.58 mg/l/d and maximum NO<sub>2</sub> -N removal rate of 5.81 mg/l/d, besides, more than 90 mg/d ammonia removal efficiency was obtained even in the extremely high ammonia load (>1000 mg/L) (Qing Ling Zhang *et al.*, 2012) [79]. The maximum ammonia removal of 95% was observed in activated sludge of coking waste water was achieved using heterotrophic bacterium *Alcaligenes sp.* in a period of four days (Yongkang *et al.*, 2012) [98].

## 2. Bioremediation in Aquaculture

A successful bioremediation involves: optimizing nitrification rates to keep low ammonia concentration; optimizing denitrification rates to eliminate excess nitrogen from ponds as nitrogen gas; maximizing sulphide oxidation, to reduce accumulation of hydrogen sulphide; maximizing carbon mineralization to carbon dioxide, to minimize sludge accumulation; maximizing primary productivity that stimulates shrimp production and also secondary crops; and maintaining a diverse and stable pond community where undesirable species do not become dominant. (Bratvold *et al.*, 1997) [10]

Ponds pre-inoculated with nitrifying bacteria, were observed to have decreased amounts of ammonia and nitrite in the rearing water and increased shrimp survival (Porubcan, 1991a) [74]. Further the introduction of *Bacillus sp.* in proximity to pond aerators was observed to reduce chemical oxygen demand and increased shrimp harvest (Porubcan, 1991b) [73]. Bioremediation agents serve to modify or manipulate the microbial communities in water and sediment

such that they reduce or eliminate selected pathogenic microbes and generally improve growth and survival of the targeted species. There are approximately 15 species of *Bacillus*, which are the main components of any commercial probiotic (bioremediation) products for pond aquaculture (Jory, 1998) [43]. Bioremediation agents are a significant management tool in shrimp culture practice, but their efficacy depends on understanding the nature of competition between species or strains of bacteria. Bioremediation with certain strains of *Bacillus sp.* have been recorded to also increase shrimp/prawn survival in ponds in addition to their bioremediation efficiency (Morairty, 1998) [60]. Members of the genus *Bacillus*, like *Bacillus subtilis*, *B. licheniformis*, *B. cereus*, *B. coagulans*, and of the genus *Phenibacillus*, like *Polymyxa*, are good examples of bacteria suitable for bioremediation of organic detritus. However, these are not normally present in the required amounts in the water column, their natural habitat being the sediment. When certain *Bacillus* strains are added to the water in sufficient quantities, they can make a significant impact, by competing with the bacterial flora naturally present for the available organic matter, such as excess feed and shrimp faeces (Sharma, 1999) [87]. Two species of *Bacillus* such as *B. subtilis*, and *B. licheniformis* are best candidate species for bioremediation in a prawn grow out system (Singh, 2002) [90]. Beneficial microbes, such as non-pathogenic isolates of *Vibrio alginolyticus*, can be inoculated into shrimp culture systems to suppress the pathogenic *Vibrios* like *Vibrio harveyi*, *Vibrio parahaemolyticus* and *Vibrio splendens* and also reduce the opportunistic invasion of these pathogens in shrimps (Jameson 2003) [40]. The microcosm experiments to assess the efficacy of commercial shrimp farm bioremediators in removing ammonia, revealed that commercial bioremediators failed to remove majority of the total ammoniacal nitrogen when the  $\text{NH}_3$  level was high. Studies on the *in-vitro* antagonistic activity of *Alteromonas*, against several opportunistic crustacean pathogens and observed that these bacteria suppressed the activity of *Vibrio harveyi* and improved the survival of *Penaeus indicus* larvae *in-vivo* (Shubhadeep *et al.*, 2006) [88].

Many studies were conducted on these commercial probiotics which suggest that they improve the water quality parameters in culture ponds (Li *et al.*, 2001; Sharriff *et al.*, 2001) [50, 85]. Many commercial probiotics are used now a days especially in shrimp aquaculture *viz.* Epigreen, Epicin, Environ AC and Super bug (Pradeep *et al.*, 2003; Prabhu *et al.*, 1999) [76, 75]. The application of beneficial bacteria, probiotics and biodegrading microorganisms, to the pond water and soil (bioremediation) is a sustainable approach to minimize the environmental impact of aquaculture. Probiotics are instrumental in maintaining good water quality; maintain higher load of beneficial bacteria and lower load of pathogenic bacteria in aquaculture ponds. They play a major role in maintaining optimum water quality parameters especially dissolved oxygen, ammonia, nitrite, nitrate, phosphates throughout the culture period and can prevent the occurrence of bacterial diseases in fish ponds (Padmavathi *et al.*, 2012). Nitrifying bacteria not only convert ammonia to nitrate but also reduce carbon dioxide to organic matter (carbohydrate), obviously these chemoautotrophic bacteria use energy released by the oxidation of ammonia to nitrate and reduce carbon dioxide to organic carbon (Boyd *et al.*, 1987). Bioremediators consisting of nitrifiers have been used to remove excess ammonia nitrogen from aquaculture system. (Prabhu *et al.*, 1999, Shariff *et al.*, 2001 and Sambasivam *et*

*al.*, 2002) [75, 85, 81]. An improved nitrifying enrichment containing suspended nitrifying cells (ammonia binding inoculum liquid, ABIL) at 5 mg/l decreased the ammonia concentration from 10 mg/l to below the detection limit within 4 days. In this study the factors responsible to decrease the ammonia level are nitrification, quantum of ammonia loss thorough volatilization, Heterotrophic consumption of ammonia and other unknown factors (Grommen *et al.*, 2002) [31]. Bacteriological nitrification is the most practical method for the removal of ammonia from closed aquaculture systems and it is commonly achieved by setting of sand and gravel bio-filter through which water is allowed to circulate. The ammonia oxidisers are placed under five genera, *Nitrosomonas*, *Nitrosovibrio*, *Nitrosococcus*, *Nitrolobus* and *Nitrospira*, and the nitrite oxidisers are placed under three genera, *Nitrobacter*, *Nitrococcus* and *Nitrospira*. There are also some heterotrophic nitrifiers that produce only low levels of nitrite and nitrate and often use organic sources of nitrogen rather than ammonia or nitrite (Ming Yu Li *et al.*, 2011) [58]. Ammonia or nitrate removal process (Nitrification and Denitrification) are essential for the pond water quality and can be carried out by nitrifying or denitrifying bacteria such as *Nitrobacter*, *Thiobacillus*, and *Paracoccus*, with the strain *Paracoccus pantorophus* 768 showing exception capacity to reduce undesirable waste compounds and result in a positive impact on pond soil quality (Mayer *et al.*, 2012) [55]. The ammonia nitrogen degradation rate of the activated *Bacillus amyloliquefaciens* was 93% within 24 h when the initial concentration of ammonia nitrogen was 200 mg/l, with 10% inoculum, temperature 35°C, pH 7.0 and mixing rate of 200 RPM (Cai-Hong Yu *et al.*, 2012) [12].

### 3. Bioremediation of Industrial effluents

Industrial effluents are the most important sources of toxic contaminants in any environment (Mohana *et al.*, 2008) [59]. Globally rapid industrialization and urbanization have enhanced the level of organic contaminants in the environment (Trupti *et al.*, 2009) [95]. The application of cyanobacteria showed immense potential in waste water and industrial effluent treatment, bioremediation of aquatic and terrestrial habitats, detoxification of effluents of chemical, bio fertilizer, food, feed and fuel industries. *Oscillatoria sp.*, *Synechococcus sp.*, *Nodularia sp.*, and *cyanothece sp.* are a group of cyanobacterial species having high biodegradation and biosorption capacity of industrial effluents (Dubey, 2011) [19].

The Composition of the activated sludge treating municipal sewage comprises predominantly of microbes such as *Pseudomonas*, *Flavobacterium*, *Alcaligenes*, *Acinetobacter*, and *Zooglea sp.* (Admase *et al.* 1984) [1]. The bacterial composition may however vary as shown by other studies wherein *Pseudomonas*, *Acinetobacter*, and *Enterobacteriaceae* have been observed to be the dominant bacteria present in municipal sewage water. These group of bacteria are chiefly involved in bioremediation of municipal sewage water (Kappesser *et al.*, 1989) [46]. The presence of organic matter in the aerobic system, promotes the growth of heterotrophs while inhibiting ammonia oxidation. Heterotrophic micro-organisms compete favorably for the oxygen due to the lower growth rate and yield of nitrifying micro-organisms (Hanaki *et al.*, 1990, Wiesmann, 1994) [33, 97]. *Alteromonas sp.*, is osmophilic and alkali tolerant and can be used for bioremediation applications in paper and pulp mill effluents (Murugesan, 2003) [63]. *Pseudomonas*

*pseudoalcaligenes*, when introduced in contaminated sites could degrade higher concentration of tributyl phosphate, utilizing it as a sole source of carbon. Earlier studies show that *P. pseudoalcaligenes* utilizes nitrobenzene as the sole of nitrogen, carbon and energy (He and Spain, 1998) [36] and it also has the ability to utilize 2-4 flurobiphenyl as sole of carbon and energy source (Murphy *et al.*, 2008) [62]. A strain of *Pseudomonas pseudoalcaligenes* having high potential for degradation of tributyl phosphate has also been isolate (Trupti *et al.*, 2009) [95]. *Pseudomonas aeruginosa* and *Brevibacillus choshinensis* isolated from textile effluent, was observed to decolorize the effluent in the presence of 10% glucose very efficiently within 7 days (Annika *et al.*, 2012) [41]. *Bacillus subtilis*, *B.cereus*, *B. mycoides*, *Pseudomonas sp.* and *Micrococcus sp.* have shown to be among the best microbes for bioremediation of textile effluents (Mahmood *et al.*, 2013) [52]. Bacterial species *Pseudomonas putida* and *Bacillus licheniformes* have potential application for the bioremediation of heavy metals from domestic and other industrial waste waters with moderate concentrations of heavy metals (Kamika and Mumba, 2012) [45]. *Pseudomonas* is important in aerobic decomposition and biodegradation, and hence they play a key role in the carbon cycle. *Pseudomonas* species are renowned for their abilities to degrade compounds, which are highly refractory to other organisms, including aliphatic and aromatic hydrocarbons, fatty acids, insecticides and other environmental pollutants. The highest load of *pseudomonas sp.* ( $5.4 \times 10^7$  cfu/ml) in tannery waste water samples was found in effluents during dry season and minimum  $9.6 \times 10^4$  cfu/ml was found in effluents during rainy season.

Studies on the inhibition of chlorination on the biodegradation reveal, low chloride concentrations (<5g/l), did not affect the COD removal and the nitrification process (Hanel, 1986) [34]. However, increased chloride concentration was observed to increase specific nitrogen or COD removal rates during nitrification process of fish meal plant effluent waters (Eilersen *et al.*, 1994 and Gomez., 1995) [21, 28]. Experiments on the degradation of black liquor from a kraft pulp and paper mill in a continuous reactor, with *Pseudomonas putida* and *Acinetobacter calcoaceticus*, showed that 70-80% of COD and lignin could be removed, while around 85% the colour could be removed in 8 days (Jain *et al.*, 1996). *Pseudomonas sp* are responsible for efficient removal of COD in activated sludge (Pala and Sponza., 1996) [70]. During nitrification process the COD removal ranged from 95% to 60 % and the nitrification ranged from 65 to 25% (Juan *et al.*, 1997). *Pseudomonas putida*, *Citrobacter sp* and *Enterobacter sp* have been observed to not only decolorized effluent upto 97% but could also efficiently reduce BOD and COD upto 96.63 and 96.80 % respectively within 24 h. Further these bacteria could also remove heavy metals upto 82 - 99.8% (Chandra, 2001) [15]. The highest COD reduction was obtained by *Coccus* and *Bacillus* 70.7% and 69.5% respectively. The initial COD concentration of 3000 mg/l, could be reduced to 880 and 920 mg/l (Maghsoodi vida *et al.*, 2007) [53]. Sangita *et al.* (2012) studied biodegradation of tannery effluent, using *Pseudomonas sp.* isolated from the effluent and reported that these isolates were efficient enough in degrading the tannic components and render the effluent nontoxic and safe for disposal. Naturally occurring consortia of microorganisms like *Bacillus*, *Pseudomonas*, *Arthrobacter*, and *Micrococcus* species could reduce 95% of COD and BOD in steel industrial effluents (Krishnaveni *et al.*, 2013) [48]. Sugar mill effluents

where reported to have *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumonia*, *Enterobacter aeruginosa*, and *Escherichia coli* as the major bacterial groups with maximum degrading potential observed with *Staphylococcus aureus*. *Staphylococcus aureus* was able to reduce BOD and COD by 64.58% and 63.33% respectively in sugar mill effluents (Buvanewari *et al.*, 2013) [11]. BOD is the most common index used to indicate the strength of any effluent, and is commonly reported for a 5-day incubation period. Initially a rapid increase in BOD for the experimental set up (ES) was observed, which was attributed to the high microbial population present in the waste, consuming higher oxygen levels during the biodegradation of the sewage. *Bacillus sp.*, *Lactobacillus sp.*, and *Klebsiella sp.* have been recognized to be the main detergent and sewage degrading microorganisms. (Nwambo and Kehinde, 2013) [67].

Nutrients present in the dye contaminated solution, play a vital role in dye decolorization process, higher amount of nutrients significantly influences the growth of microorganism and enhances the degradation of dyes in the aqueous solution. *Pseudomonas sp* isolated from azo dye contaminated soil was capable to decolorize azo dye black-E by utilizing it as nitrogen source and utilizing nearly 300 ppm in 36 h (Sudhakar *et al.*, 2012) [91].

#### 4. Bioremediation in Seafood processing plant effluent

In a seafood processing plant, the effluent mostly consists of undefined mixtures of organic substances. It is difficult to generalize the extent of the problem created by this wastewater as it depends on the effluent strength, wastewater discharge rate and the absorbing capacity of the receiving water body (Gonzalez, 1996) [29]. During fish evisceration and cooking high content of COD, nutrient, oil and fat are generated in fish processing wastewater (Aguar and Sant, 1988; Mendez *et al.*, 1992) [2, 57]. Fish canning industries can have a high concentration of organic polluting substances in the range of 10,000–50,000 mg/L (Mendez *et al.*, 1995) [56]. The BOD<sub>5</sub> of tuna waste range from 500–1500 mg/L, and is usually only about 40% of the COD, which ranged from 1300–3250 mg/L (Carawan *et al.*, 1979) [14]. Fish meal blood water contributed to the highest COD value of 93,000 mg/L, among all other processes (Del Valle and Aguilera, 1990) [16]. The level of total soluble and suspended COD vary largely between processing industry and type of fish or raw material processing. In a fish processing industry, the effluent COD is usually higher than BOD<sub>5</sub>. The ratio of process water to product is one of the major factors influencing the organic content of the fish-processing effluent. Effluent BOD<sub>5</sub>: COD ratios varied widely within and among processing plants ranging from 1.1:1 to 3:1. Most of the BOD<sub>5</sub> usually comes from hold water and from the butchering process (Technical Report Series FREMP, 1994) [94]. *Rhodocyclus gelatinosus* removed 86% COD in seafood processing plant effluent for the period of 5 days. (Prasertsan *et al.*, 1993) [77]. *Rhodovulum sulfophilum* isolated from sardine waste water could be reduce the COD by 50% in the sardine waste waters (Azad *et al.*, 2001) [6].

The waste water generated during fish meal production is known to bear an extremely high organic load. Similarly the wastewater produced by the washing of surimi has also been known to have fairly high organic load. The characteristics of the wastewater are found to be greatly affected by the raw materials used in the processing plants. The quality of the raw materials to be processed has also been found to vary as a

function of time (Omil *et al.*, 1996) <sup>[68]</sup>. The high-strength wastewaters such as those generated during fish meal production are often diluted with cooling waters from the overall process, prior to disposal (Alfonso and Borquez, 2002) <sup>[3]</sup>. Further, the wastewater may be contaminated with various microbes that may not assist in bioremediation. To suppress the growth of such heterotrophic microbes in wastewater, an inoculum of good quality and sufficient quantity, of fast growing microbes, need to be introduced to suppress the growth of such microbial contaminants (Noparatnaraporn *et al.*, 1987) <sup>[66]</sup>. The high nitrogen levels are likely due to the high protein content (15–20% of wet weight) of fish and marine invertebrates (Sikorski, 1990) <sup>[89]</sup>. Fish being a rich source of protein the effluents of fish processing units are rich in Nitrogen, the concentration however is minimal in most cases (Gonzalez, 1996) <sup>[29]</sup>. Phosphorus partly originates from the fish, but can also be introduced with processing and cleaning agents (Intrasungkha *et al.*, 1999) <sup>[38]</sup>.

High BOD concentrations are generally associated with high ammonia concentrations (Technical Report Series FREMP, 1994) <sup>[94]</sup>. The degree of ammonia toxicity depends primarily on the total ammonia concentration and pH. Salmon processing effluents contain 42 mg/l of ammonia concentration and 20 mg/L average for normal fish processing units. Sometimes high ammonia concentration is observed due to high blood and slime content in wastewaters. The ammonia concentration of fish processing plant effluent ranged from 0.7 mg/L to 69.7 mg/L, reported by Technical report series (1994). On the other hand, the total ammonia content in the fish condensate, can be as high as 2000 mg/l (Technical report series, FREMP 1994) <sup>[94]</sup>. Studies indicate *Bacillus sp* can reduce ammonical nitrogen from the fish processing waste water from 500 mg/l to  $\leq 10$  mg/l within 5 days of incubation at ambient temperature ( $28 \pm 2$  °C). *Bacillus* species can utilize ammonia via both heterotrophic and chemotropic pathways (Edwards, 2011) <sup>[20]</sup>. This may be the reason for enhanced removal of ammonical nitrogen from waste water only after addition of the culture of *Bacillus sp*. (Anitha *et al.*, 2012 & Sharma *et al.*, 2014) <sup>[5, 86]</sup>. Maximum ammonia degradation was observed for mixed culture of *Nitrosomonas* and *Nitrobacter*, with maximum ammonia degradation of 135 mg/L was observed after 21 days (Selvi *et al.*, 2014) <sup>[84]</sup>. Biological process like anaerobic treatment followed by aerobic treatment involving bioaugmentation using the culture of *Bacillus sp* seems to be a good and economic solution for treating the fish processing industry waste water with high ammonical nitrogen (Sarnaik *et al.*, 2015) <sup>[83]</sup>. *Bacillus cereus* and *Aeromonas veronii* have been identified to efficiently degrade fish processing unit effluent, reducing BOD from 647.74 to 44.67 mg/l, in five days. These microbes performed better in a mixed consortium than individual isolates, and a consortium of 50% each of *Bacillus* and *Aeromonas sp.* could efficiently reduce ammonia and improve on nitrate values indicating biodegradation (Divya, 2015) <sup>[18]</sup>.

## 5. Conclusion

Bioremediation provides a technique for cleaning up pollution by enhancing the natural biodegradation processes. Thus by developing an understanding of microbial communities and their response to the natural environment and pollutants, expanding the knowledge of the genetics of the microbes it is possible to improve on our capabilities to biodegrade pollutants. Identifying the right microbe from the waste to be

biodegraded and conducting field trials with these identified microbes for bioremediation techniques would definitely provide cost effective technologies and offer potential for significant advances in the field. Every microbe have different growth requirements (temperature, pH and nutrients), hence there arises the need to isolate those forms, which can be cultured easily in the lab, with minimal requirement and can be utilized in treating variety of pollutants. Use of genetically engineered microorganisms is not necessary as in most cases there is a wide diversity of naturally occurring microbial strains. As natural resources are major assets to humans, bioremediation of contaminated sites and ecosystems will be the best ecofriendly approach to preserve the scarce resource and also ensure efficient recycling of wastes. Bioremediation is an emerging field, with many players already in the field producing a myriad of products to overcome nutrient deficiency in fish farms, improve nutrient release in agricultural land, improving in composting of industrial waste, removal of toxic chemicals, and the list is growing. Still there is scope for developing new products that could assist in our goal of cleaning up our environment.

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