

Bioremediation for Wastewater Treatment- A Review

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Abstract- *Bioremediation of different waste waters is a relatively new technology that has undergone more intense investigation in recent decades. As rapid industrialisation and urbanisation releases numerous toxic compounds into natural water bodies, polluting both fresh water resources as well as marine water. This process is focused on destroying or immobilizing toxic waste materials present in these water sources.*

Water is undoubtedly the most vital element among the natural resources. In many developing countries, access to clean and safe water is a crucial issue. More than six million people die because of diarrhea. Various methods are used to make water safe and attractive to the consumer. The method employed depends on the character of the raw water. One of the problems with treatment of surface water is the large seasonal variation in turbidity which is caused by polluted water. Commonly used chemicals for various treatment units are synthetic organic and inorganic. In most of the cases, these are expensive since they are required in higher dose and do not show cost effectiveness. Many of the chemicals are also associated with human health and environmental problematic substances.

The term bioremediation has been introduced to describe the process of using biological agents to remove toxic waste from environment. Bioremediation is the most effective management tool to manage the polluted water and recover contaminated wastewater. It is an attractive and successful cleaning technique for polluted environment; it 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 parts of the world due to the increased use of natural attenuation, since most natural attenuation is due to biodegradation. Microbes are very helpful to remediate the contaminated environment. Various microbes including aerobes, anaerobes and fungi are involved in bioremediation process.

Keywords: Wastewater, Microbes, Bioremediation, Environment, Water, Biodegradation, Microorganisms

I. INTRODUCTION

Waste water treatment and bioremediation is a cost effective process due to significant time and planning needed for successful treatment. Now- a-days there are modern wastewater treatment plants, which are highly technical, mechanized and expensive to build and maintain. In most of the under developed countries of the world, optional methods of sewage water and wastewater treatment are required. Sewage water stabilization ponds, lagoons, can provide an appropriate solution for wastewater treatment in developing countries and rural areas.

The world is facing problems with a wide range of pollutants and contaminants from various developmental activities. The population explosion in the world has resulted in an increase in the area of polluted water. The concern on the quantity and quality of waste generated and discharged into natural water bodies has recently indicated the need for different strategies to address water quality challenges in the regions. (Han et al., 2000; Olguin, 2003). Bioremediation technology using microorganisms was reportedly invented by George M. Robinson. He was the assistant county petroleum engineer for Santa Maria, California. During the 1960s, he spent his spare time experimenting with dirty jars and various mixes of microbes. Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste (Vidali, 2001). In order for microorganism to bioremediate the right temperature, nutrients and amount of oxygen must be present in the groundwater, the right combinations of helpful microbes can eat the pollutants until it disappears. Bioremediation works on variety of organic and inorganic compounds. The use of microalgae for removal of nutrients from different wastes has been described by a number of authors (Benemann et al., 1977; Gupta and Rao, 1980; Williams, 1981; Kunikane et al., 1984; Senegar and Sharma, 1987; Tam and Wong, 1989; Gantar et al., 1991; De la Noue, 1992; De-Bashan et al., 2002; Queiroz et al., 2007; Rao et al., 2011).

Wastewater is essentially the water supply of a community after it has been used for a number of applications. It can be defined as a combination of liquid or water carried waste removed from residence, institution and commercial/industrial establishment together with such

ground water, surface water and storm water as may be present (Metcalf, 2003).

Bioremediation:

Bioremediation is the use of microorganisms to destroy or immobilize waste materials.

Bioremediation is a process used to treat contaminated media, including water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants. In many cases, bioremediation is less expensive and more sustainable than other remediation alternatives. Biological treatment is a similar approach used to treat wastes including wastewater, industrial waste and solid waste.

Microorganisms include:

- Bacteria (aerobic and anaerobic)
- Fungi
- Actinomycetes (filamentous bacteria)

By definition, bioremediation is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site (Okonko, et al., 2007)

Microbes for Bioremediation:

- **Aerobic Bacteria :** Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.
- Anaerobic bacteria are not as frequently used as aerobic bacteria. 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.
- Ligninolytic fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corncobs.

- Methylootrophs are aerobic bacteria that grow utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1, 2dichloroethane.
- *Deinococcus radiodurans* bacteria have genetically modified to digest solvents and heavy metals as well as toluene and ionic mercury from highly radioactive nuclear waste.
- *Geobacter sulfurreducens* bacteria can turn uranium dissolved in groundwater into nonsoluble, collectable form.
- *Dehalococcoides ethenogene* bacteria are used to clean up chlorinated solvent that has been linked to cancer. The bacteria are naturally found in both soil and water and are able to digest the solvents much faster than rising traditional cleanup methods.

ADVANTAGES AND DISADVANTAGES OF BIOREMEDIATION:

Advantages-

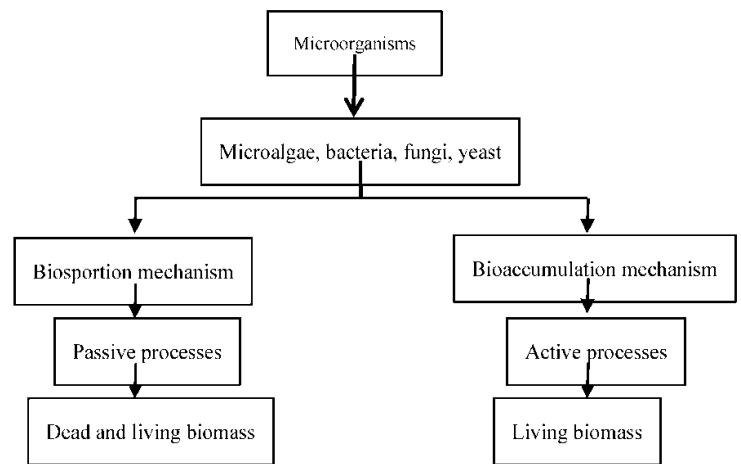
- Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated water. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.
- Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. 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.
- Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. 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.

Disadvantages-

- Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.
- There are some concerns that the products of biodegradation may be more persistent or toxic than the parent compound.
- Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.
- It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations.
- Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids, and gases.
- Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration.

II. PROPOSED METHODOLOGY**Methodology:**

- Physical forces as well as chemical and biological processes drive the treatment of wastewater. Treatment methods that rely on physical forces are called UNIT OPERATIONS. These include screening, sedimentation, filtration, or flotation.
- Treatment methods based on chemical and biological processes are called UNIT PROCESSES. Chemical unit processes include disinfection, adsorption, or precipitation.
- Biological Unit processes involve microbial activity, which is responsible for organic matter degradation and removal of nutrients (Metcalf and Eddy, 1991).

**(Bioremediation of Polluted Waters Using Microorganisms)****Stages Involved In Wastewater Treatment:**

- **Primary Treatment:**

This involves storing the wastewater in settling tanks where metal salts are added to encourage solids to cling together through a process called flocculation, forming sludge at the base of the settling tanks.

- **Secondary Treatment:**

The residual effluent is pumped to aeration tanks, where air is constantly injected and microorganisms are added to break down the remaining solids.

- **Tertiary Treatment:**

This treatment removes the nitrogen through denitrification processing and phosphates usually by chemical precipitation from the effluent.

Microbial Populations for Bioremediation Processes:

- Microorganisms can be isolated from almost any environmental conditions.
- Microbes can adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream.
- The main requirements are energy source and a carbon source (Vidali 2001). Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. Natural organisms, either indigenous or extraneous (introduced),

are the prime agents used for bioremediation (Prescott et al., 2002).

- The organisms that are utilized vary, depending on the chemical nature of the polluting agents, and are to be selected carefully as they only survive within a limited range of chemical contaminants (Prescott et al., 2002; Dubey, 2004).
- Since numerous types of pollutants are to be encountered in contaminated water, diverse types of microorganisms are likely to be required for effective remediation (Watanabe et al., 2001).

Wastewater bioremediation for reuse as drinking water requires the aforementioned processes. Although there are natural microorganisms used in wastewater treatment, the process of bioremediation process requires further addition of various types of microorganisms known as bioremediators.

It is essential to distinguish the types of microorganisms present as well as the organic pollutants to be removed and where they are to be located in the wastewater process. This can be carried out after taking samples and recommendations on the types of microorganism to use along with their best locations in the wastewater treatment processing plant. A typical three phases wastewater treatment plant after the initial screening and coarse filtration will be carried out and major debris removed.

The primary phase involves flocculation and is aided by the addition of metal salts to the wastewater encouraging the solid particles to clump together and settle out in the bottom of settling tanks as sludge containing organic and non-organic matter. The sludge is now subjected to anaerobic degradation. The addition of microorganisms to the natural anaerobic microbes can speed up the process of breaking down the sludge while also producing methane gas used as fuel in on-site power generation.

Flocculation and settling are essential processes used to remove the non-organic matter before the secondary treatment. Since this process relies on microorganisms, these microorganisms will not eradicate non-organic matter.

Addition of microorganisms:

The effluent passes to secondary treatment settling tanks where it is biologically oxidized; air is injected through the effluent and the recommended microorganisms added. This process removes any remaining suspended/dissolved solids including fecal matter; secondary settling following this process removes any remaining particulates. This process also reduces the effluents Biological Oxygen Demand (BOD). The

following conditions are needed for optimal efficiency of the microorganisms and their proliferation in the processing system.

- A constant temperature
- Dissolved oxygen content
- pH
- Nutrient levels

Further Treatment after Bioremediation of Wastewater:

The effluent is now suitable for irrigation purposes, but in order to bring it up to World Health Organization's drinking water standards, a few more processes are required.

- Disinfection – sodium hypochlorite is added
- Filtration – the effluent is passed through filters, with modern ones being polypropylene microfiltration membranes
- Reverse Osmosis – effluent processed through membranes
- Irradiation – irradiated using UV light and with the addition of hydrogen peroxide removes any remaining harmful organic contaminants.

The water is now potable and can be added to existing water supplies, for example reservoirs and dams.

III. RESULTS AND DISCUSSION

BIOREMEDIATION OF MUNICIPAL AND SEWAGE WASTE WATERS:

Municipal wastewater is mainly comprised of water (99.9%) together with relatively small concentrations of suspended and dissolved organic and inorganic solids. Among the organic substances present in sewage are carbohydrates, lignin, fats, soaps, synthetic detergents, proteins and their decomposition products, as well as various natural and synthetic organic chemicals from the process industries. Municipal wastewater also contains a variety of inorganic substances from domestic and industrial sources including a number of potentially toxic elements such as arsenic, cadmium, chromium, copper, lead, mercury, zinc, etc (Endong et al., 2008). Presently, the activated sludge system is the most widely used biological treatment process for both domestic and sewage wastewaters. The system is a biological method that is performed by a mixed community of microbes and uses the metabolic reactions of the microbes to produce high-quality effluent in an aquatic environment. The major microorganisms found in wastewater influents are viruses,

bacteria, fungi, protozoa and nematodes. The constant aeration, agitation and recirculation in an activated sludge system create an ideal environment for the numerous microorganisms present, while inhibiting the growth of larger organisms. Bacteria, fungi, rotifers, viruses, nematodes and protozoa are commonly found in the activated sludge. The species of microorganisms that dominate a system depends on environmental conditions, process design and the mode of plant operation. This will help to ensure science-based decisions with respect to effluent standards and limitations, as set by regulatory bodies and a clearer understanding (Akpoy and Muchie, 2010). The commercial bioremediator, Bioclean® STP is available in dry concentrate bacterial formulations, specially designed to provide improved waste degradation in sewage treatment. Each gram of the product contains up to 4 billion microbes. There are up to 58 different strains of bacteria in each gram of Bioclean® STP product, which can biodegrade very diverse types of molecules. It has been used in compact Bio-engineered Decentralized Sewage Treatment Plants with astounding success. Discharge water of BOD of 6 ppm is achieved in these plants, while the operational cost is half that of conventional plants. In most cases the water is recycled.

Microbial mat in metal and radionuclide removal :

Microbial mats sequester heavy metals, metalloids, radionuclides and oxyanions. The microbial mats removed manganese in ponds receiving drainage from an abandoned coal mine in Alabama, after pH neutralization (Phillips et al., 1995). Other concentration of metals was also removed by mats. Metals such as lead and iron were also reduced by microbial mat in the drainage system (Phillips and Bender, 1998). Microalgae in an Integrated Aquaculture System Portuguese researchers investigated the potential to use microalgae to process fish-farm effluents (primarily inorganic nitrogen and phosphorus) in seawater and use the microalgae as food for the *Tapes decussatus* bivalve clam. The nutrient removal efficiency for ammonium and nitrite-nitrogen was in the range of 80%-100%, for nitrate 41%-100%, and for phosphorus 21- 99%. After treatment, the water is similar in quality to fresh seawater. Researchers note the importance of this process, since fish farm effluent nutrient levels are generally too low for removal using standard bacterial systems. (Borges et al., 2005).

Micro Algae in Municipal Waste Water Treatment:

Municipal wastewater is usually treated to get rid of undesirable substances by subjecting the organic matter to biodegradation by microorganisms such as bacteria. The biodegradation involves the degradation of organic matter to

smaller molecules (CO₂, NH₃, PO₄ etc.), and requires constant supply of oxygen. The process of supplying oxygen is expensive, tedious, and requires a lot of expertise and manpower. These problems are overcome by growing microalgae in the ponds and tanks where wastewater treatment is carried out. The algae release the O₂ while carrying out the photosynthesis which ensures a continuous supply of oxygen for biodegradation. Algae - based municipal wastewater treatment systems are mainly used for nutrient removal (removal of nitrogen and phosphorous). The added benefit is the resulting biomass that can be used as biofuel feedstock. According to (Rose et al., 2002) a pH of 9.2 for 24 hours will provide a 100% kill of *E. coli* and most pathogenic bacteria and viruses. (Pahad and Rao 1962) also found that. *E. coli* could not grow in wastewater with a pH higher than 9.2.

Micro algae in industrial waste water treatment:

Industrial wastewaters are extremely varied and the microorganisms employed to treat these industrial wastewaters also vary accordingly. Biomass immobilization is an efficient mean of retaining biomass during WWT (Nicoletta et al., 2000) and microalgae immobilization in polymeric material such as carrageenan, chitosan, or alginate has been reported by various authors (Chevalier and De la Noue, 1985; Lau et al., 1995; Robinson et al., 1998).

IV. CONCLUSION

It is vital to develop more understanding of microbial communities and their response to the natural environment and pollutants, expanding the knowledge of the genetics of the microbes to increase capabilities to degrade pollutants, conducting field studies of new bioremediation techniques which are cost effective, and dedicating sites which are set aside for long term research purpose, these opportunities offer potential for significant advances.

Despite its short-comings, its importance to the world is unquestionable considering present day environmental hazards. Bioremediation provides a technique for cleaning up pollution by enhancing the same biodegradation processes that occur in nature. There is no doubt that bioremediation is in the process of paving a way to greener pastures and national safety in general.

Bioremediation is a low-cost technology that does not require complex infrastructure, and can be used to treat large volumes of wastewater. In developed countries, bioremediation is highly efficient for removing, transforming or degrading different types of contaminants found in

wastewater, particularly using the mechanisms of phytoextraction and rhizofiltration.

REFERENCES

- [1] Ali, E., Muyibi, A. S. A., Salleh, H. M. M. Salleh, R. M. and Alam, M. Z. 2009. Moringa oleifera seeds as natural coagulant for water treatment,” in Proceedings of the 13th International Water Technology Conference IWTC '09), Hurghada, Egypt.
- [2] Aggarwal, C.S. and Pandey, G.S. 1994. J. Environ. Biol. Vol. 15:49.
- [3] Diaz, A., Rincon, N., Escorihuela, A., Fernandez, N., Chacin, E. and Forster, C.F. (1999). A preliminary evaluation of turbidity removal by natural coagulants indigenous to Venezuela. Content. Process Biochem. Vol. 35: 391-395.
- [4] David Krantz and Brad Kifferstein, Water Pollution and Society.
- [5] Dabigengesere, N., Narasiah, K. S. and Talbot, B. G. 1995. Active agents and mechanism of coagulation of turbid waters using Moringa oleifera,” Water Research, Vol. 29 (2): 703–710.
- [6] Ekambaram Sanmuga Priya, Perumal Senthamil Selvan and Subramanian Venkataraman. 2011. Assessment of the in-vivo and in-vitro antioxidant potential of Strychnos potatorum Linn. Seeds in Freund's adjuvant induced arthritic rats; Journal of Medicinal Plants Research Vol. 5 (19): 4780-4787.
- [7] Environmental Conservation Rules (ECR), Department of Environment. Ministry of Environment and Forest. People's Republic of Bangladesh, 1997.
- [8] G. Folkard, J. Sutherland & Reya Al- Khalili, U.K- Natural Coagulants - A sustainable Approach, 21st WEDC Conference, 1995.
- [9] G. Folkard, J. Sutherland & W.D. Grant, Natural Coagulants at pilot scale, Pickford, J. ed. Water, Environment and Management, 1993.
- [10] Ghebremichael, K.A. (2004). Moringa oleifera seed and pumice as natural alternative materials for drinking water treatment, PhD, KTH land and water resources engineering.
- [11] Ghebremichael, K.A., K.R. Gunaratna, H. Henriksson, H. Brumer and G. Dalhammar, (2005). "A simple purification and activity assay of the coagulant protein from Moringa oleifera seed", Water Research. 39(11): 2338-2344.
- [12] G. Ghebremichael, K. R. Gunaratna, H. Henriksson, H. Brumer, and G. Dalhammar, “A simple purification and activity assay of the coagulant protein from Moringa oleifera seed,” Water Research, vol. 39, no. 11, pp. 2338–2344, 2005.
- [13] Hassan, M. A. A, Li, T. P. and Noor, Z. Z. 2009. Coagulation and flocculation treatment of wastewater in textile industry using chitosan, Journal of Chemical and Natural Resources Engineering, Vol. 4 (1): 43–53.
- [14] Hossain, M. A, Begum, T, Fakhruddin, A. N. M. and Khan, S. I. 2006. Bacteriological and physiochemical analyses of the raw and treated water of Saidabad water treatment plant, Dhaka. Bangladesh Journal of Microbiology, Vol. 23 (2): 133 136.
- [15] Saravanan J., Priyadarshini D., Soundammal A., Sudha G., Suriyakala K. “Wastewater Treatment using Natural Coagulants”, SSRG International Journal of Civil Engineering (SSRG-IJCE) – volume 4 Issue 3 – March 2017.
- [16] Vicky Kumar and Syazwani Asharuddin, “Applications of Natural Coagulants to Treat Wastewater – A Review”, MATEC Web of Conferences 103, 2017.
- [17] Md. Asrafuzzaman, A. N. M. Fakhruddin, and Md. Alamgir Hossain “Reduction of Turbidity of Water Using Locally Available Natural Coagulants” International Scholarly Research Network ISRN Microbiology 20 September 2011.
- [18] Ajayi, T.O., Ogunbayo, A.O. (2012): Achieving environmental sustainability in wastewater treatment by phytoremediation with water hyacinth (Eichhornia crassipes). – Journal Sustainable Development 5(7): 80–90.
- [19] Cañizares-Villanueva, R. (2000): Biosorción de metales pesados mediante el uso de biomasa microbiana, Departamento de Biotecnología y Bioingeniería. – Revista Latinoamericana de Microbiología 42: 131-143.
- [20] Crittenden, J. C., Trussel, R., Hand D., Howe K., Tchobanoglous G. (2005): Water treatment: Principles and design, 2th edition. – John Wiley and Sons, New Jersey, USA, 1948
- [21] Flores-Magdaleno, H., Mancilla-Villa, O. R., Mejía-Saenz, E., Olmedo-Bolaños, Ma. del, C. Bautista-Olivas, A. L. (2011): Heavy metals In agricultural soils and Irrigation wastewater of Mixquiahuala, Hidalgo, Mexico. – African Journal of Agricultural Research 6(24): 5505-5511.
- [22] Hernández-Acosta, E., Quiñones-Aguilar, E. E., Cristóbal-Acevedo, D., RubiñosPanta, J. E. (2014): Calidad biológica de aguas residuales utilizadas para riego de cultivos forrajeros en Tulancingo, Hidalgo, México. – Revista Chapingo, Serie Ciencias Forestales y del Ambiente 20(1): 89-99.
- [23] Pandey, R.S. and Upadhyay. B.V., (2010). Pseudomonas fluorescens can be used for bioremediation of textile effluent Direct Orange-102. Tropical Ecology 51(2): 397-403.

- [24] Ruenglerpanyakul. W., Attasat S. and Wanichpongpan .P., (2004). Nutrient removal from shrimp farm effluent by aquatic plants. *Water Science and Technology*, Vol 50 No 6, pp 321 -330.IWA publishing.
- [25] Venkateswara rao.A., (2008). Bioremediation – an advanced strategy to restore the health of aquaculture pond ecosystem, *Technical Articles – Aquaculture*.
- [26] Boricha, H. and Fulekar M.H. , (2009). *Pseudomonas plecoglossicida* as a novel organism for the bioremediation of cypermethrin. *Biology and Medicine*, 4: 1-10.
- [27] Loredana Stabili, Roberto schirosi, Margharita licciano, Emanuela mola and Adriana gianyrande, (2010). Bioremediation of bacteria in aquaculture waste using the polychaete *Sabella spallanzanii*, *New Biotechnology*, Volume00, Number00, July
- [28] Okonko, I. O. and Shittu, O. B., (2007). Bioremediation of wastewater and municipal water treatment using latex exudate from *Calotropis procera* (sodom apple), *Electronic journal of Environmental, Agricultural and Food Chemistry*, 6 (3):1890-1904.
- [29] Borges, M. T., Silva, P., Moreira, L., and Soares, R.(2005). Integration of consumer-targeted microalgal production with marine fish effluent biofiltration—a strategy for mariculture sustainability. *Journal of Applied Phycology*, 17(3): 187-197.
- [30] Akpor O. B. and Muchie M., (2010). Bioremediation of polluted wastewater influent: Phosphorus and nitrogen removal. *Scientific Research and Essays Volume. 5(21)*, pp. 3222-3230.
- [31] Anjaneyulu Y., Sreedhara Chary,N., Samuel Suman Raj, D., (2005). Decolourization of industrial effluents – available methods and emerging technologies – a review, *Reviews in Environmental Science and Bio/Technology*, 4:245–273.
- [32] Antony and R. Philip .S.P., (2006). Bioremediation in Shrimp Culture Systems, *NAGA, World Fish Center Quarterly*, Volume. 29 (3 & 4).
- [33] Asamudo, N. U., Dava, A.S. and Ezeronye, O.U.,(2005). Bioremediation of textile effluent using *Phanerochaete chrysosporium*, *African Journal of Biotechnology* Volume. 4 (13), pp. 1548-1553.