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Microorganisms Involved in the Bioremediation of Pentachlorophenol and Lignin Discharged by the Pulp and Paper Industry

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ABSTRACT

Pulp and paper industry are one of the fastest-growing industries due to increased demand in paper products which are proved to affect our environment negatively. Global consumption of paper has increased by 400% in the last four decades and this suggests that more research is required to assess the impact of industrial effluents on our environment and public health. Paper products are generally biodegradable, however, the processes involved in its production which involve the use of mainly bleaching agents and other non-biodegradable substances pose a serious problem to the environment. There are more than 250 chemicals released in paper mill waste and some are xenobiotics. Different methods such as physical and chemical methods can be adopted for the remediation of the effluents but are proved to be costly and not safe to the environment. On the other hand, the biological method is shown to be less costly and environmentally friendly. Microorganisms and their enzymes have shown a promising future for bioremediation of effluents related to the paper mill. Pentachlorophenol is extremely hazardous to living cells and therefore need to be removed from the environment. Microorganisms including bacteria and fungi have the potential to degrade phenolic compounds e.g. *Bacillus stearothermiphilus*, *Pseudomonas putida, Coricus versicolor, Sphingomonas chlorophenol, Fusarium sp, Bacillus subtilis* and *P. aeroginosa*.. Enzymes used for the degradation include phenol hydrooxylase, polyphenoloxylase, laccase, peroxidase among others. Lignin is another important pollutant and is resistant to microbial degradation, but it has been proved that certain bacteria and fungi like can degrade it. This review focused on use of microorganism to reduce or eradicate pollutants released from the paper industry. It can serve as a review for further research to be conducted especially in the field of biotechnology.

INTRODUCTION

The use of microorganisms or their metabolic products in the biodegradation of organic compounds especially environmental pollutants has advanced significantly during the past three decades because of advancement in biotechnology. It has been found that large numbers of microbes coexist and undergo different forms of relationships in almost allnatural environments particularly in soils. Many natural and synthetic organic chemicals are readily biodegradable in the natural environment by organisms that have required enzymes and metabolic pathways. Such organisms are usually isolated from different habitats by researchers. Biodegradation of materials involves initial proximity, allowing absorption or physical access to the substrate, secretion of extracellular enzymes to degrade the substrate or uptake via transport system followed by intracellular metabolism.

The efficiency of biodegradation of organic compounds is influenced by the type of organic pollutants, the nature of the organism, the enzymes involved, the mechanism of degradation and the nature of the influencing factors. Organic pollutants comprise a potential group of chemicals which can be dreadfully hazardous to human health and many of these resistant to degradation. As they persist in the environment as recalcitrant, they are capable of long-range transportation, bioaccumulation in human and animal tissue and disruption of the food chain [1].

Biodegradation is used to describe the complete mineralization of the starting compound to simpler ones like CO_2, H_2O, NO_3 and other organic compounds [2]. The term has been proposed for describing the ultimate degradation and recycling of an organic molecule to its mineral constituents. No natural organic compound is resistant to biodegradation provided that the environmental conditions are favourable [3]. This is known as the principle of microbial infallibility. However, Microbiologists have hardly dipped below the surface of the natural pool of microbial diversity. When new microorganisms have been isolated with biodegradation efficiency, their biochemical versatility has been found to the immense. [4].

Biodegradation of phenolic compounds

Phenolic compounds are hazards pollutants that are toxic at relatively low concentration. Accumulation of phenols creates toxicity both for flora and fauna. Since phenol is toxic and causes pollution, it must be removed from the environment. Phenol and its higher homology are aromatic molecules containing hydroxyl groups linked to the benzene ring structure. The origin of phenol in the environment is both industrial and natural. Phenol pollution is associated with pulp mills and various chemical industries as well as their wastewaters. [5]. The presence of phenol in water imparts carbolic odour to receiving water bodies and can cause toxic effects on aquatic flora and fauna [6]. Phenols are toxic to human beings and affect several biochemical functions [7].

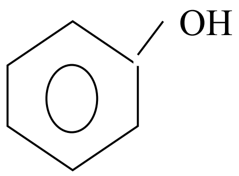

Fig. 1. Chemical structure of phenol (C_6H_6O)

Acute exposure to phenol causes central nervous system disorders. It leads to collapse and coma. Muscular convulsions are also noted. A reduction in body temperature has resulted and this is known as *hypothermia.* Phenol and its derivatives are toxic and classified as hazardous materials [8]. These phenolics compounds possess a various degree of toxicity and their resultant impact on the environment is therefore important [9]. In recent years a great deal of research work has been directed toward the development processes in which enzymes are used to remove phenolic contaminants [10].

Microorganisms involved in phenol biodegradation

Degradation of phenol occurs as a result of an activity of a large number of microorganisms including bacteria, fungi and actinomycetes. Bacterial species including *Bacillus sp*, *Pseudomonas sp*, *Acinetobacter sp*, *Achromobacter sp.*, *Fusarium sp*, *Phanerocheate chrysosporium*, *Coriolus versicolor*, *Ralstonia sp*, *Streptomyces sp* etc. are also proved to be efficient fungal groups in phenol biodegradation. However, these microorganisms suffer from substrate inhibition at higher concentration of phenol, by which the growth is inhibited [11-13]. Many studies on the biodegradation of phenol come from bacteria. The genus *Pseudomonas* is widely applied for the degradation of phenolic compounds. These bacteria are known for their immense ability to grow and utilized various organic compounds. Phenol degradation studies with the bacterial species have resulted in bringing out the possible mechanism and also the enzyme involved in the process. The efficiency of phenol degradation could further be enhanced by the process of cell immobilization [14].

Phenol and other phenolic compounds are common constituents of many industrial effluents. Once a suitable microorganism-based process is developed for the effective degradation of phenol these phenolic effluents can be safely treated and disposed of [15]. The rate of biodegradation of phenol by *Klebsiella oxytoca* strain was studied. It was found that *K. oxytoca* degrade phenol at an elevated concentration where 75% of initial phenol concentration at 100ppm was degraded within 72 h [16]. Phenol was degraded by *Actinobacillus* species. They found that pH7, the incubation temperature of 35 to 37^oC and the agitation rate of 150rpm were the optimal conditions for achieving a higher percentage of phenol degradation. Succinic acid and glycine as respective carbon and nitrogen source were found to be the most efficient co-substrates for the removal of phenol. Immobilized *Alcalegenes* sp was successfully used for the effective treatment of phenolic paper factory effluent [17].

Table 1. Microorganism in biodegradation of phenolic compounds.

Type of phenol	Microorganism
Phenol	*Baccilus staerothermophilus, Pseudomonas putida, Agaricus bisporus, Ochromonas danica, Pseudomonas flurorescens Coriolus versicolor, Acine tobacter sp, Fusarium sp, Bacillus brevis, Streptomyces setonii, Alcaligenes sp e.t.c.*
Pentachlorophenol	*Lentinula edodes, Lentinus bisporous, Sphingomonas chlorophenol*
2- chlorophenol	*Pseudomonas putida*
Nitrophenol	*Nicardioides*
2,4 chlorophenol	*Mixed culture*
Bisphenol-A.	*Coprinus cinereus*
Chlorophenol	*P. pudita, Achromobacter sp*
Dichlorophenol	*P. putida*
Nonylphenol	*Clavariopsis aquatica*

Table 2. Enzymes involved in the biodegradation of phenolic compounds.

Type of phenol	Enzyme
phenol	phenol hydroxylase, polyphenol oxidase phenol oxidase, catechol 2,3 dioxygenase, laccase, peroxidase, catechol1, 2 oxygenase, tyrosinase.
methoxyphenol	laccase
bisphenol	peroxidase
lignophenols	peroxidase

Biodegradation of pentachlorophenol (PCP)

Generally, aromatic compounds are broken down by natural bacteria, however; polycyclic aromatic compounds are more recalcitrant. Derivatisation of aromatic nuclei with various substituents particularly with halogens makes them more recalcitrant. There are reports on many microorganisms capable of degrading phenol through the action of a variety of enzymes. These enzymes include; oxygenases, hydroxylases, peroxidases, tyrosinase and oxidases. Oxygenases include mono oxygenases and dioxygenases. [18-22].

Pulp and paper mills are utility huge amount of lignocellulose components of plants and chemicals during the manufacturing process and are generally regarded as polluting industries owing to the discharge of huge amount of waste materials which enter into the environment [23]. The pulp paper industry has been associated with a variety of potential environmental problems. Regardless of the manufacturing process used pulp mill effluents are complicated mixtures consisting of several hundred compounds among which chlorinated phenolics are an important class of toxicant [24]. The major problems due to the pulp and paper industry are the presence of high colour and chlorinated compounds. Chlorophenols from the pulp bleaching processes are found both in free and bound forms in dissolved organic matter and particles [25].

Complex reactions between chlorine and lignin in wood pulp lead to the production of high and low molecular chlorinated compounds [26]. The environment toxicity due to chlorinated compounds has not yet solved. A multitude of the organo-chlorinated compounds has been chemically characterized as *lipophilic* [27,28], meaning they are easily able to penetrate the cell membrane and frequently possess high bioaccumulation factors. Toxic effects have been recorded acting at the major planktonic and benthic trophic level [29-30], reducing the diversity and abundance of phytoplankton, zooplankton and zoobenthos [31,32] and disrupting benthic algae and invertebrate communities and increasing pathogenic bacteria population in aquatic resources [33-35]. Additionally, a significant share of the chlorinated organic compounds especially those of molecular weights (>1 000), is very refractory to microbial degradation [36]. However, some of this rather refractory substance like lignin, quantitatively significant waste products of the pulping process are known to be biodegraded not only by fungi and other eukaryotes but also a number of bacteria [37-39].

Several methods have been attempted for the removal of colour and toxicity from pulp and paper mill effluents these can be classified as physical, chemical and biological methods. Physical and chemical process is quite expensive and remove high molecular weight chlorinated lignin, colour, toxicant, suspended solids and chemical oxygen demand. But BOD and low molecular weight compound are not removed efficiently. The biological removal process is particularly attractive since in addition to colour and COD it also reduces BOD and low molecular weight chlorolignin [40]. Among the chlorophenols pentachlorophenol (PCP) is expected to be recalcitrant to aerobic biodegradation due to its high chlorinated ring structure, generally, an aromatic compound with higher amount of chlorine are more resistant to biodegradation [41]. The environmental protection agency has registered PCP in the list of priority pollutants. They have now restricted the sale and use of pesticides products containing PCP [42]. The safe permissible limit of PCP in water is $0.30ugl^{-1}$ [43]. However, paper mill effluents contain far above the permissible limit of PCP even after the treatment at industrial scale [44].

Microorganism in pcp biodegradation

Several microorganisms that has the ability to metabolize various industry pollutants including PCP have been isolated from the environment these microorganisms include *Bacillus* sp, B. *subtilis* IS13 [45,46] *pseudomonas sp*, P. veronii, p. fluorescens [47,48] and P. aeroginosa [49], Flavobacterium [50], arthrobacter [51], *Sphingomonas chlorophenol* ATCC 39723, *Desulfomonile tie jei* DCB-1 [52], Arthrobacter chlorophinolicus A6 [53]. Two aerobic bacterial strains namely *Bacillus cereus* (DQ 002384) and *Serratia marcescens* (AY927692) have been able to degrade PCP in the mineral salt medium in presence of glucose and peptone [54]. Other bacterial species known to degrade PCP include *Corynebacterium* sp, *Mycobacterium chlorophenols*, *Mycobacterium fortuitum* and *Rhodococcus* sp [55,56].

Biodegradation of lignin

Lignin is a major non-carbohydrate, polyphenolic structural constituent of wood and other plant material that encrusts the cell walls and cements the cell together, a highly polymeric substance, with a complex, cross-linked, highly aromatic structures of molecular weight of about 10,000 derived principally from coniferyl alcohol (C_{10} H_{12} 0_3) by extensive condensation polymerization [57]. The aromatic polymer lignin is well known for resistance to microbial degradation because of its high molecular weight and presence of various biologically stable carbon-carbon and other linkages. Microorganisms that degrade plant lignin via oxidative process are fungi, Actinomycetes and to a lesser extent bacterium [58-60].

Study shows that bacterial strains degrade the low molecular weight portion of lignin but a probably unable depolymerize the high molecular weight back bone of the polymer because unlike fungi which secrete extracellular enzymes called *ligases,* the bacterial cells do not secrete lignin- depolymerizing enzymes. However, bacterial lignin degradation systems consist of many unique and specific enzymes with the ability to catalized the production of various use full compounds [61]. Mechanical and chemical method have been frequently using for the treatment of lignin. However, these methods are either costly or have hazardous impact on the ambient environment. In contrast, biological treatment is a novel trend, which is not only more efficient in improving quality through degradation of lignin and undigestable fibre content but also of less cost and environmentally friendly. If lignin is not removed from pulp and paper industry wastewater, it presents a serious pollution and toxicity problem in aquatic ecosystems, owing to its low biodegradability and high range of colour [62].

There are many types of lignin the properties and composition of which depend upon the source and method of isolation. Among them, kraft lignin (KL) a waste by-product from alkaline sulfide treatment of lignocelluloses in the pulp and paper industry is the main contributor to the colour toxicity of the plant effluents. It differs from natural lignin as it undergoes a variety of reaction including aryl alkyl cleavages, strong modifications side chains and ill-defined condensation reaction causing the polymer to fragment into smaller water / alkali-soluble fragments. Kraft lignin though not identical to natural lignin has been widely used as experimental lignin for microbial degradation studies [63].

Microorganisms capable of degrading lignin

A wide variety of microorganisms including fungi, actinomycetes and bacteria have been implicated in lignin biodegradation and discolouration of pulp effluent [64,65]. Among them, white-rot fungi have received extensive attention due to their powerful lignin degradation enzymatic systems [66] for example *Fomes lividus* and *Trametes vesicolor* [67]. However, fungi are not stable in practical treatment under extreme environmental and substrate conditions such as higher temperature, oxygen limitations, high extractive and lignin concentration [68].

Bacteria in particular deserve to be studied for ligninolytic potential because of their immense environmental adaptability and biochemical versatility [54]. Several bacterial strains were found to degrade and assimilate lignin e.g. *streptomyces viridosporus* and *Streptomyces* spp [69-72]. Due to their productivity, bacterial enzymes systems are expected to serve as tools for the conversion of lignin into intermediate metabolites. Lignin induced peroxidases; both extracellular and cell-associated were identified and characterized in *Streptomyces* spp [53]. Bacteria isolated from compost soil, viz. *azotobacter*, *Bacillus megatarium* and *Serratia marcescens* were capable of decolorizing or solubilizing lignin *S.marcescens* produce laccase and its activity correlated positively with lignin mineralization and solubilization [73]. The contribution of bacteria has also been reported to the utilization of low-molecular-weight lignin oligomers as the sole sources of carbon and energy that produce enzymes catalyzing the cleavage of intermonomeric linkages 74].

CONCLUSION

Civilization will most probably continue to be accompanied by the production of hazardous waste materials despite new advancement in science and technology, it is, however, necessary to develop efficient strategies for waste management. Biotechnology of hazardous waste management involves the development of biological systems that catalyze the detoxification, degradation or decontamination of environmental pollutants. In future technologies, microbial systems might be the potential tools to deal with the environment pollutants. New technologies must be designed to degrade those chemicals that pose the greatest threat to human health especially those released from pulp and paper industry.

REFERENCES

1. Jayachandra. K., Indu, C.N. and Shashidhar S., Biodegradation of phenol, Afr J Biotechnol. 2008;7 (25): 4951-4958
2. Atlas, M.; Bartha, R. Microbial evolution and biodiversity. In: Atlas, M.; Bartha, R. Microbial ecology. Menlo Park: Benjamim/Cummings Science, 1998. p.27-57.
3. Alexander, M Biodegradation: problems of molecular recalcitrance and microbial infallibility. Adv Appl Microb. 1965 7:35-50
4. Wackett, L. P. and Hershberger, D.C. Biocatalysis and biodegradation, microbial transformations of organic compounds. ASM Press, Am Soc Microbiol. 2001. Washington DC.
5. Paula, M. and Young, L. Y.. Isolation and characterisation phenol degrading denitrifying bacteria. Appl Environ Microbial. 1998; 64(7): 2432-2438
6. Ghandi, SC. and Sangodikar, U.M. Potentials of Pseudomonas cepacia PAA in bioremediation of aquatic wastes containing phenol Proceedings of National symposium Frontier Appl Environ Microbiol. 1995 11-13Dec., Cochin.
7. Nuhoglu, A. and Yakin, B Modeling of phenol removal in a batch reactor. Proc Biochem. 2005;40:233-239
8. Zumriye, A. and Gultac, B. Determination of the effective coefficient of phenol in calcium alginate immobilized Pseudomonas putida. Enzyme Microbial Technol. 1999;25:344-348
9. Bollag, J.M, Shuttle, W.K.N. and Anderson, D.H Larccase medicated detoxication of phenolic contaminants. Appl Environ Microbial. 1951;54 (12): 3086
10. Ghiureliotis, M. and Nicell, J. Assessment of soluble products of peroxidase catalyzed polymerization of aqueous phenol. Enz Microbial Technol. 1999;25:185-193.
11. Prieto, M.B., Hidalgo, A., Rodriquez, F.C., Serra, J.L. and Llama, M.J. Biodegradation of phenol in synthetic and industrial waste by Rhodococus erythropiticus, UPV-1 immobilized in air-stirred reactor with clarifier. Appl Microbial Biotechnol. 2002;58.583-859
12. Aisami A, Yasida NA, Joharic WL, Ahmada SA, Shukora MY. Growth rate abolishment on phenol as a substrate by Pseudomonas sp. AQ5-04 best modelled using the Luong substrate inhibition kinetics. Desalin Water Treat. 2019;;152:214-20.
13. Aisami A. Phenol biodegradation and optimisation of cultural conditions physical by the free cells of newly isolated phenol degrading *Alcaligenes* sp. AQ5-02. Res J Adv Sci. 2020;4;1(1).
14. Annadurai, G., Balan, M.S. and Marugesan, T. Design of experiments in the biodegradation of phenol using immobilized *Pseudomonas dictorium* (NICM-2077) on activated carbon. Bioproc Eng. 2000;22:101-107.
15. Borghei, S.M. and Hosseini, S.H. The treatment of phenolic wastewater using a moving bed biofilm reactor. Proc Biochem. 2004;39: 1177-1181.
16. Shawabkeh, R. and Khaleifat, M., K. Rate of biodegradation of phenol by *Klebsiella oxytoca* in minimal medium and nutrient both conditions. Bioremediation 2007;11:13-19.
17. Nair, I.C., Jayachandra, K. and Shankar, S Treatment of paper factory effluent using a phenol degradation *Alkaligenes* sp. under free and immobilized condition. Bioresour Technol 2007;98:714-716.
18. Ali, S. and Robert F. Meta pathway degradation of phenolics by thermophilic bacillus. Enz Microbial Technol. 1998;23:462-468.
19. Sakurai, A. Removal of bisphenol a. by polymerization and precipitation method using *Coprinis cinereus* peroxidase. Biotechnol Lett. 2001;23:995-978.
20. Xin, L. and Bohuslavek, J. Characterization of 2,6 dichloro-p-hydroquinone 1,2 dioxygenase (PCPA) of *Sphingomonas chlorophenolic* ATTCC 39723. Biochem Phys Res Comm. 1999 ;266: 322-325.
21. Shashirekha S., Uma, L., and Subramanian, G.. Phenol degradation by marine cyanobacterium Phormidium valderianum BDU 30501. J. Ind. Microbial. Biotechnol. 1997;19:130-133.
22. Xia, Z., Yoshida, T. and Fonuoku, M. Enzymatic degradation of highly phenolic lignin based polymers (lignophenols). Eur Polymer J. 2003;39:909-914.
23. Pachrel, D. and Vigaraphavan, T. Treatment of pulp and paper mill waste water. Rev Sci Total Environ. 2004;333: 37-58.
24. Schnell, A., Stell, P., and Meter H. Enhanced biological treatment of bleaching kraft mill effluents and removal of chlorinated organic compound and toxicity. Water Res. 2000;34:493-500.
25. Palm, H., Paasivirta, J. and Lammi, R. Behavior of chlorinated phenolic compounds in bleach-plant treatment system and Archipelago area. Chemosphere. 1995;31:2839-2852.
26. Suntio, L.R., Shiu, W.Y. A and Mackay, D. Review of the nature and properties of chemicals present in pulp mill effluents. Chemosphere 1988;17:1249-1290.
27. Fahreus van- Ree, G.E. and Payne, J.F. Enzymes cytochemical responses of mussels (*Mytilus edulis*) to resin acid constitutes of pulp mill effluents. Bullf Environ Contam Toxicol. 1999;63(4): 430-437.
28. Koistinen, J., Lethonen, M. and Tukia, K. Identification of lipophilic pollutants discharge from a Finnish pulp and paper mill. Chemosphere. 1998 37: 219-235.
29. Gaete, H., Larrain, A., Smith E, Bacza, J. and Rodriguez, J. Ecotoxicological assessment of two pulp mill effluents Rio river basin, Chile. Bull Environ Contam Toxicol. 2000;65:183-189.
30. Kovacs, T.G. and Voss, R.H. Biological and chemical characterization of newsprint / especially mill effluents. Water Res. 1992;26: 771-780.
31. Sibley, P.K., Legler, J., Doxin, D.G. and Barton, D.R. Environment health assessment of benthic habitat adjacent to the pulp mill discharge: acute and chronic toxicity of sediments to benthic macroinvertebrates. Arch Environ Contam Toxicol. 1997;32:274-284.
32. Sobral, O., Ibeiro, R, Concalves, F. and Soares A.M.V.M. Ecotoxicity of pulp and paper mill in Vietnam on receiving water body. Resour Conserv Recyc. 1998;18: 1661-173.
33. Yen, N.T., Dahn N.T.K., Reutergardh I.B, Wise, D.L., and Lan N,T.T. An intergrated waste survey and environment effects of COGIDO, a bleached pulp and paper mill in Vietnam on receiving water body. Resour Converv Recyc. 1996;18: 161-173.
34. Larson, D.G.J. and Forlin, L. Male based sex ratios of fish employs near a pulp mill, temporary recovery after a short-term shutdown. Environ Health Perspect. 2002;110:739-742.
33. Rheinheimer, G. Pollution in the Baltic Sea. Nature 2002. 1998;85:318-329.
35. Chandra, R., Ghosh, A., Jain, R.K. and Singh, S. Isolation and characterization of two potential pentachlorophenol degrading aerobic bacteria from pulp and sludge. J Gen Appl Microbiol. 2006;52: 125-130.
36. Alafonso MD, Victor G,Maria G, Maria JR, Pinho Nm Nanofiltration removal of chlorinated organic compounds from

alkaline bleaching effluents in a pulp and paper plant. Water Res. 1992;22(12):1639-1643

37. Gonzalez, J.M., Hodson R.E. and Morari, M.A. Bacterial populations in a replicate marine enrichment culture, assessing variability in abundance using RNA-based probes. Hydrobiology 1999;401: 69-75.
38. Zimmermann, W.. Degradation of lignin by bacteria. J Biotechnol.1990;13: 119-130.
39. Chandra, R.I., Purolit, H.J., Raj, A. and Kapley, ACharacterization and optimization of three potential aerobic bacterial strains for kraft lignin degradation for pulp paper waste. Chemosphere. 2007;67: 839-846.
40. Nagarathnamma, R., Rajpai, P. and Bajpai, P.K. Study on decolorization, degradation and detoxification of chlorinated lignin compounds in kraft bleaching effluents by *Ceriporioris subvermispora*. Process Biochem. 1999;34: 939-948.
41. Anandaraja, K., Kiefer, P.M, Donohoe, B.S. and Copley, S.D. Recruitment of a double bond isomerase to serve as a reductive dehalogenase during biodegradation of pentachlorophenol. Biochemical. 2000;39: 5302-5311.
42. EPA. Final determination and indent to cancel and deny application for registration of pesticides products containing pentachlorophenol (including but not limited to its salts and esters) for non-wood uses US environmental protection agency. Federal Register 1987;52:2282-2293.
43. EPA. Integration risk information system (IRIS) on pentachlorophenol national centre for environment assessment office of research development. 1999. Washington DC.
44. Raj, A., Chandra, R. and Patel D.KPhysico-chemical characterization of pulp and paper mill effluent. Toxicology. 2005 12:109-188.
45. Niaza, J.H., Prasad, D.T. and Karegoudar, T.B. Initial degradation of dimethylphthalate by esterases from Bacillus species. FEMS Microbiology Letters 2001;196(2): 201-205.
46. Andretta, C.W.S, Rosa, R.M. and Tondo, E.C. Identification and molecular characterization of a Bacillus subtilis 1513 strain involved in biodegradation of 4,5,6 trichloroguianol. Chemosphere 2004;55: 631-639.
47. Thakur, I.S., Verma, P.K. and Upadhaya, C.K. Molecular cloning and characterization of pentachloriphenol degradation monooxygenese of *Pseudomonas* sp. Biophys Res Comm. 2002;290: 770-774.
48. Nam, I.T., Chang, Y.S, Hong, H.B. and Lee, Y.E. A novel catabolic activity of *Pseudomonas veronii* in biotransformation of pentachlorophenol. Appl Microbial Biotechnol . 2003;62: 284-290.
49. Shah S, and Thakur, I.S. Enzymatic dehalogenesis of pentachlorophenol by *Pseudomonas fluorescens* of microbial community from tannery effluents. Current Microbial. 2003;47:65-70.
50. Saber, D.L. and Crawford, R.L, isolated and characterization of flavobacterium strains that degrade pentachlorophenol. Appl Environ Microbiol. 1985;50: 1512-1518.
51. Edgehill, R.U. and Finn, R.K. Microbial treatment of soil to remove pentachlorophenol. Appl Environ Microbial. 1983;35:1122-2.
52. Mohn, W.W. and Kennedy, K.J. Reductive dehalogenation of chlorophenol by *Desulfomonite tiejei* DBC-1. Appl Environ Microbiol.1992;58: 1367-1370
53. Agneta, B., Maraha, N. and Janson J.K Impact of temperature of the physiological status of potential bioremediation inoculants *Arthrobacter chlorophenolicus* A6. Appl Environ Microbial. 2004 5: 2952-58.
54. Ramachandra, M, Crawford, D.L. and Pometto A.L Extracellular enzymes activities during lignin cellulose degradation by *Streptomyces* spp. A comparative study of wild type genetically manipulated strains. Appl Environmental Microbial. 1987; 53:2754-2760.
55. Chandra, R., Raj, A. and Sangaeta, Y. Reduction of pollutants in pulp paper mill effluents treated by PCP degrading bacterial strains. Environ Monit Assess 2008 DO1 10, 1007/s 10661-008-1413-4.
56. Hagglom, M.M., Nohynek, L.J. and Salkonoja-salonen, M.S.. Degradation and O-methylation of phenolic compound by Rhodococcus and mycobacterium of Minnesota strains Appl. Environ Microbial. 1988;54:3043-3052
57. Apajalahti, J.H.A. and Salkinojr-salonen M.S Degradation of polychlorinated phenols by *Rhodococcus chlorophenolicdus*. Appl Microbial Biotechnol 1986;25: 62-67.
58. Hassan, E.A. and El- hanaty, A.A. Lignin biodegradation by ligninolytic bacterial strains and comparison of Bacillis subtilis and Bacillus sp isolated from Egyptian soil. American-Eurasian J. Agric and Environ Sci. 2009;5(1): 39-44.
59. Tien, M. and Kirk, T.K. Lignin degrading enzymes from *Himenomycete chrysosponium* birds. Sci. 1983;221:661-663.
60. Hernandez, M., Hernandez-coronado M.J., Montiel, M.D., Rodriguez, J. and Arias, M.E.. Analysis of alkali-in a paper mill effluent decolorized with two Streptomyces strains by gas chromatography – mass spectrometry after cupric oxide degradation. J Chromat. 2001;919:389-394
61. Trojanowski, J., Haider, K. and Sundman, V. Decomposition of 141 labeled lignin and phenols by a Nocardia sp. Archive of Microbiology 1977;114:149-153.
62. Raj, A., Chandra, R., Hermant, J.P., Mohan M.K. and Kapley, A. Biodegradation of kraft lignin by a newly isolated bacterial strain, *Aneurinibacillis aneurinilyticus* from the sludge of a pulp paper mill World J Microbial Biotechnol 2007;23: 793-799.
63. Berryman D., Houde, F., Deblois V. and Oshe'a M. Non phenolic compounds in drinking and surface waters down stream of treated textile and pulp paper effluents: a survey and preliminary assessment of their potential effects on public health and aquatic life. Chemosphere. 2004;56:247-255.
64. Raj, A., Reddy, M.M.K, Chandra, R., Prohit H.J., and Kaploy., ABiodegradation of kraft lignin by Bacillus sp isolated from sludge of pulp and paper mill. Biodegradation. 2007 18:783-792
65. Perestello, F., Rodriquez A. and Perez, R. Isolation of bacterium capable of limited degradation of industrial and labeled natural and synthetic lignins. World J Microbial Biotechnol. 1996;12:111-112.
66. Odi, D.R., Chandra, H. and Garg, S.K.. Decolorization of biogas based paper mill effluent by the white rot fungus *Trametes versicolor*. Bioresour Technol .1998;66:79-81
67. Hatakka, A. Lignin-modifying enzyme from selected white-rot fungi: Production and role in lignin degradation. FEMS Microbial. Rev. 1994;13:125-135.
68. Selvam, K., Swaminathan, K., Hoon song, M., and Chan, K. PCP biodegradation. World J Microbial Biotechnol 2002;18(6): 523-526
69. Daniel, G. and Nilsson, T. Developments in the study of soft rot and bacterial decays forest products. Biotechnology 1998;32:37-62
70. Ball, A.S., Bells, W.B. and Mccarthy, A.G Degradation of lignin-related compounds by actinomycetes. Appl. Environ. Microbial. 1989;55:1642-1646.
71. Odier, E., Janin, G. and Monties, B. Popular lignin decomposition by gram-negative bacteria. Appl Environ Microbial 1981;41:337-341
72. Pometto, A.L. and Crawford, D.L Effect of pH on lignin and cellulose degradation by *Streptomyces viridosporus*. Appl Environ Microbial . 1986;52: 246-250.
73. Nishnura, M.O and Davies, J. Isolated and characterization of *Streptomyces* sp NL 15-2K capable of degradation lignin related aromatic compounds. J Biosci Bioeng. 2006. 102:124-127.
74. Perostelo, F., Fallon, M.A., Carnicero, A. and Rodriquez, A.. Limited degradation of industrial synthetic and natural lignin by Serratia marcescens. Biotechnol Lett. 1994 16:299-302.
75. Vicuna, R. B. and Gonzalez, D. Ability of natural bacterial isolated to metabolize high and low molecular weight lignin derived molecules. J Biotechnol. 1993. 30: 9-13.