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ISBN 978-81-934349-3-2

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Microbiological Studies

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Manuscript References No. DMBS-0417-001-Khare P.

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Submitted By:

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2017

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INTERNATIONAL ASSOCIATION OF SCIENTISTS & RESEARCHERS

Cite This MLA

Khare, Pankaj. *Isolation And Characterization Of Paper Degrading Bacteria From Paper Press Waste Water Disposal Site*: International Association of Scientists and Researchers, www.xournals.com. April. 2017. Web

"Isolation and Characterization of Paper Degrading Bacteria from Paper Press Waste Water Disposal Site"

A Dissertation Report Submitted In the partial fulfillment for the degree of MASTER OF SCIENCE IN MICROBIOLOGY-2015

Supervised By:

Dr. Madhurima De Roy,

HOD of Microbiology department,

BIMR, Gwalior

Submitted By:

PANKAJ KHARE

MSc Microbiology

B.I.M.R College of Professional Study

Gwalior, Madhya Pradesh

B.I.M.R College of Professional Study Gwalior, Madhya Pradesh INDIA

DECLARATION

I declare that the work entitled, “ISOLATION AND CHARACTERIZATION OF PAPER DEGRADING BACTERIA FROM PAPER PRESS WASTE WATER DISPOSAL SITE” is carried out by me in BIMR college of Professional Studies, Gwalior during the period 10 March 2015 to 10 July 2015.

(Pankaj Khare)

Student of M.Sc. (Microbiology)

Department of Microbiology,

B.I.M.R College of professional study,

Gwalior (M.P.) INDIA

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Dated: 30/07/15

Pankaj Khare

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ACKNOWLEDGEMENT

Success is 1% inspiration & 99% perspiration. This 1% inspiration plays a very important role in every phase of life. I look back in retrospect to all those days of happiness, success accompanied by challenges, difficulties but God bestowed upon me the might to live up to it all confidently, patiently with contentment and pleasure. I am very grateful to the supreme Almighty for showering his divine blessings upon me!!

Every completed task has got many hands to get done it. Mere mention of them will not justify their true contribution. My acknowledgement are, therefore, many than what I am expressing here.

It is my great privilege in availing the golden opportunity to express my deepest sense of gratitude & humble indebtedness towards Dr. A.V. SUBHADRA Principal of BIMR Gwalior for giving me opportunity to accomplish my dissertation in this establishment.

I am highly indebted to my supervisor, Dr. MADHURIMA DE ROY; H.O.D of Microbiology of BIMR Gwalior for her guidance, moral support and her kind & generous encouragement throughout the project.

I specially express my gratitude to Mrs. Reeta sharma, and B. Bhadoriya, lab attenders of microbiology and biotechnology, BIMR, for giving her valuable support to complete my dissertation work and completion of report.

I owe my deep sense of gratitude to Prof. G. Pandya (Retired scientist, Virology, division DRDE) for his help and co-operation during dissertation work.

I am also thankful to my family members and friends for their kind co-operation and encouragement.

I owe my indebtness to my parents Mr. Ramadhin khare and Mrs Uma khare , for keeping immense trust on me and showing their divine and pious blessing upon me.

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Affiliated to Jiwaji University, Gwalior (M.P.)

BIMR/CPS/19/2015 30.07.2015

CERTIFICATE

*This is to certify that **M.Sc. dissertation** entitled **" ISOLATION AND CHARACTERIZATION OF PAPER DEGRADING BACTERIA FROM PAPER PRESS WASTE WATER DISPOSAL SITE"** in partial fulfillment of the requirement for degree of Master of Science in Microbiology from **BIMR COLLEGE OF PROFESSIONAL STUDIES, GWALIOR (M.P.)** as a record of bonafide research/project work is carried out by **Mr.Pankaj Khare** under the supervision and guidance of Dr.Madhurima De Roy, **H.O.D. Microbiology Department.** The thesis has not formed on the basis is of the award of any degree / diploma / associateship / fellowship or other similar title to other candidate of any university.*

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CHAPTER - I
INTRODUCTION

Paper mill effluent is a major source of pollution generating industry discarding huge amount of intensely colored effluent. Some are rich in wood fiber and harbor good carbon source further the primary and de-inking paper mill effluent others carry effluents rich in nitrogen and phosphorus. Lack of infrastructure, technical manpower research and development facilities restrict these mills to recover the chemicals. The chemical oxygen demand of the emanating stream is quite high and floating minuscule of debris. Rapid increase of population and the increased demand for industrial establishments to meet human needs have created problems such as over exploitation of available resources, increased pollution in air and water environment hence there is a growing demand to treat the effluent with the native industrial samples isolates. (Shanthi J1, Krubakaran CTB2 and Balagurunathan R1 1Department of Microbiology, Periyar University, Salem -636 011, India 2012).

Pulp and paper industrial effluent is rich in recalcitrant compounds and causes pollution. For the treatment of such compounds activated sludge process is frequently used in which F/M ratio is kept low. This treatment results in effective biochemical oxygen demand removal but other waste water parameters are not reduced effectively due to lack of dissolve oxygen. In the present study sequential batch reactor was used for the removal of pollutants from the waste water of pulp and paper mill by using bacterial consortium (*Klebsiella sp.*, *Alcaligens sp*. and *Cronobacter sp*.). The aim of present research is to identify the influences of F/M ratio and dissolved oxygen concentration on the microorganism’s growth and pollutant removal. The process of bioremediation was optimized by Taguchi approach.

A significant reduction in colour (55%), adsorbable organic halides (45.4%), total dissolve solids (22%) and total suspended solids (86.7%) was also observed within 14hrs while, the sludge volume index was 52. The wastewater after the treatment process meets the standard given by regulatory agencies and can be discharged into the environment without any risks Kumar V, Dhall P, Naithani S, Kumar A, Kumar R (2014).
In nature, cellulose, lignocellulose and lignin are major sources of plant biomass; therefore, their recycling is indispensable for the carbon cycle. Each polymer is degraded by a variety of microorganisms which produce an enzyme that work synergically. In the near future, processes that use lignocellulolytic enzymes or are based on microorganisms could lead to new, environmentally friendly technologies. In addition, biotechnological innovations based on natural delignification and applied to pulp and paper manufacture are also outlined.

The present study was conceptualized to evaluate the heavy metal absorbing potential of microbes isolated from paper industry effluent. The effluent was used as the source sample, after screening morpho-metrically ten strains were cultured and sub cultured to achieve the pure discrete colonies. he heavy metal tolerant efficiency was determined by analyzing the growth of the bacteria in presence of heavy metal (Cu,Hg,Co and Zn) solution and their optimum tolerance was determined by measuring the optical density at 600nm after 24hr and 48hr of incubation. *Bacillus cereus* and *Corynbacterium xerosis* were both observed as heavy metal tolerant bacteria further these two microbes were subjected to sensitivity testingagainst commercially available antibiotics Satpal Singh Bisht Department of Biotechnology, Roland Institute of Pharmaceutical sciences, Berhampur-760010, Orissa, India.

Pulp and paper industry is considered as one of the most polluter industry in the world (Thompson *et al.*, 2001; Sumathi & Hung, 2006). The production process consists two main steps: pulping and bleaching. Pulping is the initial stage and the source of the most pollutant of this industry. In this process, wood chips as raw material are treated to removelignin and improve fibers for papermaking. Bleaching is the last step of the process, which aims to whiten and brighten the pulp. Whole processes of this industry are very energy and water intensive in terms of the fresh water utilization (Pokhrel & Viraraghavan, 2004). Water consumption changes depending on the production process and it can get as high as 60 m3/ton paper produced in spite of the most modern and best available technologies (Thompson *et al.*, 2001). The wastewaters generated from production processes of this industry include high concentration of chemicals such as sodium hydroxide, sodium carbonate, sodium sulfide,bisulfites, elemental chlorine or chlorine dioxide, calcium oxide, hydrochloric acid, etc (Sumathi & Hung, 2006). The major problems of the wastewaters are high organic content (20-110 kg COD/air dried ton paper), dark brown coloration, adsorbable organic halide (AOX), toxic pollutants, etc. The environmental problems of pulp and paper industry are not limited by the high water consumption

The raw waste water consists of 80-90 mg L (-1) suspended soil and 1,010-1,015 mg L(-1) dissolve solid .however, Ph varied from 5.5 -6.8. The biochemical oxygen demand ranged from 200-210 and 1,120 -1,160 mg L (-1), respectively. Aerobic treatment of row effluent attribute to significant reduction in suspended soil (range between 25 to 30 mg L (-1) and total dissolved solid range between 360 to 390 mg L (-1). However, pH, temperature, and electrical conductivity were found superior after treatment. Copper, cadmium,iron, lead, nickel, and, zinc were found in less quantity in row effluent and were almost completely removed after treatment. The dendrogram of the effluent quality parameters clearly indicate that south India paper Mill does not meet minimal national standard set by central pollution control Board to discharge in agricultural field.

Cellulose is a main component of paper, cellulose is a linear polysaccharide of glucose residues with beta-1, 4-glycosidic linkages. Abundant availability of cellulose makes it an attractive raw material for producing many industrially important commodity products. Sadly, much of the cellulosic waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon. With the help of cellulolytic system, cellulose can be converted to glucose which is a multiutility product, in a much cheaper and biologically favourable process.

Source for cellulose system extraction is the best suitable from microbial system found in the gut of organisms thriving on cellulosic biomasses as their major feed. Insects like termites (Isopteran), bookworm (Lepidoptera), and so forth, are found to have syntrophic symbiotic microflora in their guts responsible for cellulosic feed digestion. Many microorganisms have been reported with cellulosic activities including many bacterial and fungal strains both aerobic and anaerobic.

Cellulose due to its massive applicability has been used in various industrial processes such as biofuels like bioethanol, triphasic biomethanation; agricultural and plant waste management; chiral separation and ligand binding studies. The present work concentrates on the isolation of cellulose-degrading bacteria from invertebrates such as termites, snails, ceterpillars, and bookworms and assessment of their cellulolytic activity. The coculturing of cellulose-degrading bacteria and yeast was also carried out for simultaneous saccharification and fermentation of cellulose into ethanol.
Although the physical and chemical methods are on the track of treatment, they are not on par with biological treatment because of cost ineffectiveness and residual effects.(Richa Sharma, Subhash Chandra, Amrita Singh, Kriti SinghDepartment of Biotechnology and Allied Sciences Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, INDIA july 2014). Pulp and paper mill is a major industrial sector utilizing a huge amount of lignocellulogic materials and water during the manufacturing process, and release chlorinated lignosulphonic acids, chlorinated resin acids, chlorinated phenols and chlorinated hydrocarbon in the effluent. The highly toxic and recalcitrant compounds, dibenzo-p-dioxin and dibenzofuran, are formed unintentionally in the effluent of pulp and paper mill .The untreated effluents from pulp and paper mills discharged into water bodies, damages the water quality. The undiluted effluents are toxic to aquatic organisms and exhibit a strong mutagenic effect. Treatment of pulp and paper mill effluent has not proved successful due to lack of suitable microorganism, loss of genetic potentiality in adverse environmental conditions, formation of recalcitrant compounds of various structural formulation and poor process optimization for treatment at large scale. Although the physical and chemical methods are on the track of treatment, they are not on par with biological treatment because of cost ineffectiveness and residual effects. The biological treatment is known to be effective in reducing the organic load and toxic effects of krafts mill effluents.

The manufacture of papers dates to the ancient Egyptians before 3000 B.C., while the 'modern' method of pulping plant material for paper production was developed by the Chinese in the first century A.D. The utilization of plant fiber for paper production is one of the oldest manufacturing industries and is built upon age-old technologies. It was not until this became mechanized and the scale of production escalated in the early part of lastcentury that many of today's environmental problems associated with the pulp and paper industry emerged. For example, in the industrial manufacture of paper from wood fiber, it was known that natural

Various studies have reported detrimental effects of pulp and paper mill effluent on animals living in water bodies receiving the effluent. The effects are in form of respiratory stress, oxidative stress, liver damage and geno-toxicity [37-39]. A study in 1996 reported health impacts such as diarrhea, vomiting, headaches, nausea, and eye irritation on children and workers due to the pulp and paper mill wasterwater discharge to the environment. The effluent has high chemical diversity of organic chemicals present in it. Many of them are carcinogenic, mutagenic, clastogenic and endocrinic disrupters. A study on B.subtilis reported the mutagenic effects of the sediments contaminated by the effluent of kraft paper mill.
Paper and pulp mill is a large industrial enterprise that generates a significant amount of wastewater containing high concentration of lignin causing brown color and high Chemical Oxygen Demand. There has been consid-erable organic matter in pulp mill effluents on the environment. Some members of this family are known to be toxic, mutagenic, persist ant and bioaccumulating and are thought to cause numerous harmful disturbances in bio-logical systems. There is no industry which does not add wastes into the environment. The introduction of con-taminants through effluent and sludge to different environmental compartments can often overhelm the self-cleansing capacity of recipient ecosystems and thus re-sult in the accumulation of pollutants to problematic or even harmful levels (Radhakrishnan Saraswathi1, Manghatai Kesavan Saseetharan21Coimbatore Institute of Technology, Coimbatore, India june 2010).

Waste water released from various industries is the major concerns for environmentalists now days. Industrial effluents contain various toxic metals, harmful gases, and several organic and inorganic compounds. Both the quality and quantity of effluent result in various impacts on the availability of good quality water as well as on marine environment. Due to the discharge of these toxic effluents, there has been a major loss in the ecological, social and economic perspective. The long-term consequences of exposure also cause fatal diseases like cancer, delayed nervous responses, mutagenic changes, neurological disorders etc [Wahaab, (2000) and Balaji et al., (2005)].

Chromium metal is an example of a heavy metal found in the effluents of industries, such as in metal finishing, petroleum refining, iron and steel industries, textile manufacturing, electroplating, leather tanning metal plating etc [Ramakrishna and Philip, (2005); Horton et al., (2006); Das and Mishra, (2010); Poornima et al., (2010)]. Effluents of these industries contain large quantities of chromium-laden in their wastewater. Hexavalent chromium (Cr (VI)) compounds are considered to be highly toxic, carcinogenic, and mutagenic to living organisms [Poopal and Laxman, (2009) and Ramakrishna and Philip, (2005)]. But trivalent chromium (Cr (III)) is generally toxic only to plants at very high concentrations and is less toxic or non-toxic to human and animals [Shen and Wang, (1994), Megharaj et al., (2003)]. Because of its hazardous nature, toxicity and exposure, Cr(VI) has been designated as a priority pollutant in many countries [Wang and Xiao et al., (1995); Shakoori et al., (2000); Megharaj et al., (2003)]. Phenol is another major pollutant included in the list of EPA (1979). Aqueous phenolic effluents are relatively common industrial wastes, being produced in several industries and operations such as petroleum refineries, gas and coke oven industries, phenolic resins, explosive manufacture, plastic and varnish industries, textiles units using organic dyes, and smelting and related metallurgical operations [Mahadevaswamy et al., (1997); Bandyopadhyay et al., (1998); Jayachandran and Kunhi, (2008); Marrot et al., (2006); Paula and Young, (1998)]. Acute exposure of phenol causes central nervous system disorders. Pulp and paper mills generate varieties of pollutants depending upon the type of the pulping process. Combinations of anaerobic and aerobic treatment processes are found to be efficient in the removal of soluble biodegradable organic pollutants (Swamy et al., 2011). Waste paper based paper mills account for about 35% of Indian paper mill production capacity. Recovery of waste paper has increased from 650,000 tonnes in 1995 to 850,000 tonnes in 2000 but due to alternative uses the recovery rate for paper industry is still about 20 % as against China's 33% & Germany's 71 % (IL&FS Ecosmart limited, 2)

AIM AND OBJECTIVE

1. Isolation of bacteria from water disposal newspaper press site.
2. Identification of bacteria with high potential of degrading newspaper.
3. Study of the biodegradative ability of isolated bacteria.

CHAPTER- II
REVIEW OF LITERATURE

Paper manufacturers produce large quantities of wastewater, which can have deleterious effects on the receiving water; therefore there is a need to find a treatment process which can minimize these effects considerably. A suitable aerobic biological treatment process that can be used with great success involves the use of thermophilic micro-organisms. This technology has many advantages, which include rapid biodegradation rates, low sludge yield, and excellent process stability. Batch studies were conducted on two type of activated sludge (pulp-mill sludge) at 40 degree c,50 degree c, and 60 degree c to determine the feasibility of thermophilc degradation of bleach pulp mill effluent in terms of increasing aeration, biomass concentration and nutrient addition (Prenaven reddy,Visvanathan L pillay,Adinarayana Kunamneni,suren singh, 2005). (Department of Biotechnology and Allied Sciences Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, INDIA 2014).

Chemical composition of paper

The main raw material is recycled paper fiber plant, in addition to raw materials containing cellulose, hemicellulose,and lignin three major components,such as resin, ash, and so on. Each polymer is degraded by a variety of microorganisms which produce abattery of enzymes that work synergically the near future, processes that use lignocellulolytic enzymes or arebased on microorganisms could lead to new, environmentallyfriendly technologies (Akhtar M, Scott GM,Swaney RE,Kirk Tk 1)

a) Cellulose

Cellulose is a linear polysaccharide of glucose residues with beta-1, glycosidic linkages. Abundant availability of cellulose makes it an attractive raw material for producing many industrially important commodity products. Sadly, much of the cellulosic waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon. With the help of cellulolytic system, cellulose can be converted to glucose which is a multiutility product, in a much cheaper and biologically favourable process. Cellulolysis is basically the biological process controlled and processed by the enzymes of cellulose system. Cellulose enzyme system comprises three classes of soluble extracellular enzyme; 1,4 –beta endoglucanase, 1,4-beta exoglucanes (beta-D glucoside glucohydrolase or cellobaise). Endoglucanese is responsible for random cleavage of beta -1, 4- glycosidic bonds along a cellulose chain .exoglucanase is necessary for cleavage of the non-reducing end of a cellulose chain and splitting of the elementary fibrils from crystalline cellulose, and beta-1,4 glucoside hydrolysis cellobiose and water soluble cellodextrine to glucose. Only the synergy of the above three enzymes make the complete cellulose hydrolysis to glucose or a thorough mineralization water and CO_2 Possible.

b) Hemicellulose

Hemicellulose includesxylene, glucuronoxylan, arabinoxylan, glucomanna. These polysaccharides contain many different sugar monomers. In contrast cellulose contains only anhydrous glucose.Hemicelluloses contain most of the D-pentose sugars as well.

c) Lignocellulose

Lignocelluloses in nature derive from wood, grass, agricultural residues, forestry wastes and municipal solidwastes. Several biological methods for lignocelluloserecycling, based on the enzymology of cellulose-, hemicellulose-and lignin degradation, have been suggested.Among them, compostage and their use as raw material for the production of ethanolas an alternative combustibleseem to be the most economically feasible. Moreover, the generaluse of alternative, environmentally friendly technologies that introduce lignocellulose enzymes at different stages of pulp and paper manufacture as a pretreatment to pulping (biopulping), bleaching (biobleaching), or wastewater treatment has allowedconsiderable electrical power savings and a reduction of pollutants in the waste water from these industries. In addition, pretreatment of agricultural wastes with ligninolytic fungi enables their use as raw material for paper manufacturing.

Cellulose biodegradation

Most of the cellulolytic microorganisms belong to eubacteria and fungi, even though some anaerobic protozoa and slime molds able to degrade cellulose have also been described. Cellulolytic microorganisms can establish synergistic relationships with non-cellulolytic species in cellulosic wastes. The interactions between both populations lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic conditions, and carbon dioxide, methane and water under anaerobic conditions. Microorganisms capable of degrading cellulose produce a battery of enzymes with different specificities, working together(Be´guin, P, Auber, JP (1994) the biological degradation of cellulose. FEMS Microbiology)

PULP AND PAPER MILL

The manufacture of papers dates to the ancient Egyptians before 3000 B.C., while the 'modern' method of pulping plant material for paper production was developed by the Chinese in the first century A.D. The utilization of plant fiber for paper production is one of the oldest manufacturing industries and is built upon age-old technologies. It was not until this became mechanized and the scale of production escalated in the early part of last century that many of today's environmental problems associated with the pulp and paper industry emerged. For example, in the industrial manufacture of paper from wood fiber, it was known that natural compounds released during processing caused harm to aquatic population Pulp and paper are manufactured from raw materials containing cellulose fibers, generally wood, recycled paper, and agricultural residues. In developing countries, about

60% of cellulose fibers originate from nonwood raw materials such as bagasse (sugar cane fibers), cereal straw, bamboo, reeds, esparto grass, jute, flax, and sisal. In World Bank studies, pulp and paper manufacturing with unit production capacities greater than 100 metric tons per day. As per the Ministry of Environment and Forest (MoEF), Government of India, the pulp and paper sector is in the "Red Category" list of 17 industries having a high polluting potential. Pulp and paper production is a major industry in India with a total capacity of over 3 million tons per annum.

THE PULP AND PAPER INDUSTRY IN INDIA

The first paper mill in India was set up at Srirampur, West Bengal, in the year 1812. However, large scale mechanized technology of papermaking was introduced in India in early 1905. Since then the raw material for the paper industry underwent a number of changes and over a period of time, besides wood and bamboo, other non-conventional raw materials have been developed for use in the papermaking. The paper industry is categorized as forest based and agro-based and others (waste paper, secondary fibre, bast fibers and market pulp). Currently, the Pulp and Paper industry in India is the 15th largest paper industries in the world. The paper industries in India have been categorized into large-scale and small-scale. Those paper industries, which have capacity above 24,000 tonnes per annum, were designated as large-scale paper industries. Indianpaper industry has been de-licensed under the Industries (Development & Regulation Act, 1951) with effect from 17th July, 1997. Foreign Direct Investment (FDI) up to 100% is allowed on automatic route on all activities except those requiring industrial licenses where prior governmental approval is required. Growth of paper industry in India has been constrained due to high cost of production caused by inadequate availability and high cost of raw materials, power cost and concentration of mills in a particular area. Government has taken several policy measures to remove the bottlenecks of availability of raw materials and infrastructure development. For instance, to overcome short supply of raw materials, duty on pulp and waste paper and wood logs/chips has been reduced. As of 2007-08, the Indian paper industry has a total turnover of more than Rs 10,000 crore and provides direct employment to 200,000 people and indirectly to another 100,000 persons the demand of paper and paper products in India has continuously been increasing over the time. However, per capita paper consumption in India is about 5.5 kg in the year 2003 as against of world average of 50 kg (TERI, 2006). There are about 525 pulp and paper mills with an installed capacity of 6.5 million tonne. The installed capacities of Indian mills vary over a wide range of 5 tpd to 600 tpd. Indian paper mills are categorized into (1) large mills with installed capacity of more than 100 tonne per day, and (2) small mills with capacity less than 100 tonne per day. The small units account for more than 50% production capacity, and characterized by poor energy efficiency. About 80–85% of energy is used for process heating while the share of electricity accounts for 15–20%. More than 80% of electricity used in large wood–based mills is met by cogeneration units (Narayanan et al., 2010).

RECYCLED FIBRE (WASTE PAPER) SOURCES

Waste paper based paper mills account for about 35% of Indian paper mill production capacity. Recovery of waste paper has increased from 650,000 tonnes in 1995 to 850,000 tonnes in 2000 but due to alternative uses the recovery rate for paper industry is still about 20 % as against China‘s 33% & Germany‘s 71 % (IL&FS Ecosmart limited, 2010). To sum up, future fibre supply in India depends very much on the following factors:

a) Increase in availability of domestic wood:

Hardwood pulp production is expected to increase from 0.90 MT in 2000 to1.5 MT by 2015 & 1.8 MT by 2020, depending on further development of plantations. However most of softwood pulp and shortfall in hardwood pulp may still have to be imported the cost of wood to Indian players is $50 per tonne compared to around $30 internationally. Growth in non-wood pulp production which is expected to increase from 1.3 MT in 2000 to 3.2 MT by 2020. Even though the non-wood fibres required are expected to be available in India, the actual utilisation may be lower due to technical/ environmental/logistical reasons.Improvement in waste paper recovery rate in India which is expected to increase to 38% by 2020 (IL&FS Ecosmart limited, 2010).

Energy benchmarking of Indian mills

• Indian forest based mills consume a lot of steam and electricity, but produce usually all from wood material without any major oil or coal usage or purchased power.
• Indian agro based mills consume considerably less energy but have to purchase about half of the electricity consumption. (Jaakko Poyry Consulting, 2002).
• European waste based mills use more energy because deinking process is better and final product cleaner.

CHARACTERSTICS OF PULP AND PAPER INDUSTRY

The pulp and paper industry produces effluents with large BODs and CODs. One of the specific problems that yet not been solved is the strong black brown color of the effluent, which is primarily due to lignin and its derivatives released from the substrate and discharged in the effluents, mainly from pulping, bleaching and chemical recovery stages. The brown color of the effluent may increase water temperature and decrease photosynthesis, both of which may lead to decreased concentration of dissolved oxygen.

The generation of waste water and characteristics of pulp and paper mill effluent depends upon the type of manufacturing process adopted and the extent of reuse of water employed in plant. Effluent depends upon type of manufacturing process adopted and the extent of reuse of water employed in plant. Effluent of kraft pulping is highly polluted, and characterized by parameters unique to these wastes such as colour, adsorbable organic halides (AOX) and related organic compound. The alkaline extraction stage of bleach plant effluent is the major source of colour and is mainly due to lignin and derivatives of lignin. Lignin wastewater is discharged from the pulping, bleaching and chemical recovery sections. Lignin is a heterogeneous, three dimensional polymer, composed of oxyphenylpropanoid units. The high chlorine content of bleached plant reacts with lignin and its derivatives formed into highly toxic and recalcitrant compounds and the responsible for high biological and chemical oxygen demand. Trichlorophenol, trichloroguicol, tetrachloroguicol, dichlorophenol, dichoroguicol and pentachlorophenol are major contaminats formed in the effluent of pulp and paper mill.

TECHNOLOGIES USED FOR THE TREATMENT OF PULP AND PAPER MILL EFFLUENTS

Recent developments in treatment of pulp and paper mill wastewater showed successful application of physical, chemical and biological treatment methods as well as combination of different methods in series. Commonly used physical and chemical treatment methods are electrocoagulation, ultrasound, reverse osmosis photocatalytic systems using titanium dioxide (TiO2) and zinc oxide (ZnO) under UV/solar irradiation], hydrogen peroxide, Fenton’s reagent (H2O2/Fe2+), UV,UV/ H2O2, photo-Fenton (UV/ H2O2/Fe2+), ozonation and peroxon (ozone/ H2O2). Some of these studies have optimized the operating conditionsfor effluent treatment. Biological treatment methods involved the use of fungi, bacteria, algae and enzymes as a single step treatment or in combination with other physical and chemical methods.

The various enzymes involved in the treatment of pulp and paper mill effluent are lignin peroxidase, manganese peroxidase, and laccase. Microorganisms showing good production of these enzymes havethe potency to treat effluent.

ENVIRONMENTAL IMPACT OF PULP AND PAPER MILL: The environmental impact of paper and pulp mills is of particular concern since these units generate 150-200 m3effluent/ton paper with a high pollution loading of 90-240 kg suspended solids /ton paper, 85-370 kg biochemical oxygen demand (BOD)/ton paper and 500-1100 kg chemical oxygen demand (COD)/ton paper. Apart from the pollution, there is a growing water scarcity and deterioration in water quality in many parts of India.

Among the various industries, paper and pulp industry is one of the notorious polluters of the environment.it has been categorized as one of the most polluting industries due to discharge of hugs volumes of highly colored and toxic waste water(effluent) in the environment causing pollution of land (soil), air and water (martin,9198).most of the paper and pulp industries discharge their insufficiently treated waste water into the river or stream which results in serious problems for aquatic life (kesalker et Al, 2012).

The most important problem which the pulp and paper industry is facing today is the disposal of tremendous volumes of waste water. This waste water is rich in dissolved solids such as CHLORIDES and SULPHATES of Na,Ca and varying amounts of suspended organic materials. In addition to these constituents, effluents also contain same trace material like Hg, Pb, and Cr etc. the effluents are generally alkaline in reaction with high chemical and biological oxygen demands. Thu, the effluents discharge into the water unfit for irrigation and potable use and create health hazards (chhonkar et al., 2000). Paper mill effluents often contain high amounts of various organic and inorganic materials, as well as toxic trace elements, which may accumulate in soils in excessive quantities under long term use (Xionge et al, 2001).

Air pollution

Nitrogen dioxide, sulfur dioxide and carbon dioxide are all emitted during paper manufacturing. Nitrogen dioxide and sulfur dioxide are major contributors of acid rain, whereas CO2 is a greenhouse gas responsible for climate change.

Water pollution

Waste water discharge for a pulp and paper mill contains soils, nutrients and dissolved organic matter such as lignin. It also contains alcohols, and chelating agents and inorganic materials like chlorates and transition metal compounds. Nutrients such as nitrogen and phosphorus can cause or exacerbate eutrophication of fresh water bodies such as lakes and rivers.

FATE AND AFFECTS OF PULP AND PAPER MILL EFFLUENTS

Various studies have reported detrimental effects of pulp and paper mill effluent on animals living in water bodies receiving the effluent. The effects are in form of respiratory stress, oxidative stress, liver damage and geno-toxicity. A study in 1996 reported health impacts such as diarrhea, vomiting, headaches, nausea, and eye irritation on children and workers due to the pulp and paper mill wasterwater discharge to the environment. The effluent has high chemical diversity of organic chemicals present in it. Many of them are carcinogenic, mutagenic, clastogenic and endocrinic disrupters. A study on B.subtilis reported the mutagenic effects of the sediments contaminated by the effluent of kraft paper mill.

WASTE CHARACTERIZATION AND SOURCE

Pulp and paper industry is one of the most water and energy consuming industry in the world. This industry uses the fifth largest energy consumer processes; approximately 4% of total energy is used worldwide (Cheremisinoff &Rosenfeld, 2010).

MANUFACTURING TECHNOLOGIES AND PROCESS DESCRIPTION

Pulping process is the first step of the production. The main steps of this part are debarking, wood chipping, chip washing, chip digestion, pulp screening, thickening, and washing. Mechanical and chemical operation processes in pulping are used mechanical processes involve mechanical pressure, disc refiners, heating, and light chemical processes to increase pulping yield; wood chips are cooked in pulping liquors at high temperature and under pressure in the chemical pulping processes. (Sumathi & Hung, 2006). Additionally, mechanical and chemical processes can be combined in some applications. The yield of mechanical processes is higher (90-95%) compared to chemical processes (40-50%). However quality of the pulp obtained from mechanical processes is lower and also the pulp is highly coloured and includes short fibers (Pokhrel & Viraraghavan, 2004). Therefore, chemical pulping carrying out in alkaline or acidic media is mostly preferred. In alkaline media generally referred as Kraft Process, the woodchips are cooked in liquor including sodium hydroxide (NaOH) and sodium sulfide (NaS2).

Wastewater

Different pulping processes utilize different amount of water and all of these processes are water intensive. The quality of wastewater generated from pulping and bleaching is significantly distinctive because of the process and chemical types (Billings and Dehaas, 1971). Approximately 200 m3 water are used for per ton of produced pulp and most of them are highly polluted, especially wastewater generated from chemical pulping process (Cecen etal., 1992). Wood preparation, pulping, pulp washing, screening, washing, bleaching, paper machine and coating operations are the most important pollution sources among various process stages. Wastewaters generated from pulping stage include mostly wood debris, soluble wood materials, and also some chemicals from chemical pulping process. Bleaching process wastewater has a different quality. These wastewaters are not higher strength than pulping process wastewater; however they include toxic components (Holmberg & Gustavsson, 2007).

Solid and hazardous wastes

Wastewater and consequently solid wastes are the main environmental problem of the pulp and paper mills because this industry has a very water intensive production proc(Cabral et al., 1998; Thompson et al., 2001). Solid wastes from pulp and paper industries are mainly treatment sludges, lime mud, lime slaker grits, green liquor dregs, boiler and furnace ash, scrubber sludges, and wood processing residuals. Wastewater treatment sludges have a significant concern for the environment because of including chlorinated compounds (EPA, 2002). The characteristics of all solid waste generated from the pulp and paper mills are organic exception of boiler and furnace ashesses.

Gas emissions

Air pollutants and gas emissions are the other concern about the pulp and paper industry. The most important gas emission is water vapours. Additionally, particulates, nitrogen oxides, volatile organic compounds (VOCs), sulfur oxides and total reduced sulfur compounds (TRS).

CHEMICAL COMPOSITION OF EFFLUENTS

Pollution of water by industrial effluents of process industries is a serious problem in most countries. Industrial waste consists of both organic and inorganic substances. Organic wastes include pesticide residues, various hydrocarbons, solvents, cleaning fluids, dissolved residue from fruit and vegetables, and lignin from pulp and paper etc. Effluents can also contain inorganic wastes such as brine salts and metals. The increased industrial activities have reduced the availability of good quality water by producing a large amount of effluents to the rivers. Industrial effluents often contain various toxic metals, harmful gases, and several organic and inorganic compounds [Balaji et al., (2005)]. Due to discharge of toxic effluents long-term consequence of exposure can cause cancer, delayed nervous damage, malformation in urban children, mutagenic changes, neurological disorders etc. [Govindarajalu, (2003)]. Phenol and chromium are the major contaminant present in the effluent discharged from the various industrial processes such as wood preserving, metal finishing, petroleum refining, leather tanning and finishing, paint and ink formulation, pulp and paper industry, Textile Industry Pharmaceutical industry and manufacturing of automobile parts industries [Song et al., (2009); Tziotzios et al., (2008); Wang and Chirwa, (1998); Yun-guo et al., (2008); Nkhalambayausi-Chirwa and Wang, (2001)].

Phenol

Phenol is a White Crystalline Solid. It contains a six-membered aromatic ring, bonded directly to a hydroxyl group (OH) having chemical formula C6H5OH [Prpich and Daugulis, (2005)]. Phenol is a hygroscopic [Collins and Daugulis, (1997)], slightly acidic by nature. It has a distinctive odour. Its molecular weight is 94.11, density is 1.072 and the boiling point is 181.9°C. Its other names are Carbolic acid, Benzenol, PhenylicAcid, Hydroxybenzene, Phenic acid [Annadurai et al., (2000)].

Uses of phenol

1, Medical Use: Phenol has antiseptic properties and is used for aseptic (germ-free) techniques in surgery and sanitation purposes.

2, Industrial Use: Aqueous phenolic effluents are relatively common industrial wastes being produced in several industries and operations such as herbicides, and synthetic resins industries petroleum refineries, gas and coke oven industries, phenolic resins, explosive manufacture, plastic and varnish industries, textiles units using organic dyes, and smelting and related metallurgical operations etc [Mahadevaswamy et al., (1997); Bandyopadhyay et al., (1998); Marrot et al., (2006); Bodalo et al., (2008); Jayachandran and Kunhi, (2008)].

Laboratory Use: Phenol is used along with chloroform (a commonly-used mixture in molecular biology for DNA & RNA purification from proteins) and also used for cell disruption and lysis purpose Beauty Products: Phenol is also used in the preparation of cosmetics including sunscreens, hair dyes, and skin lightening products.

Toxicity of phenol

Phenol is a major pollutant included in the list of EPA (1979) as reported by Agarry et al., (2008). Acute exposure of phenol causes disorders of central nervous system. Hypothermia , myocardial depression, burning effect on skin , irritation of the eyes, also it causes gastrointestinal disturbance are some of the effects reported by the researchers [Tziotzios et al., (2005) and Chakraborty et al., (2010)]. As per the rules of central pollution control board the minimum permissible level for phenol in environment is 0.1mg/l [Kumaran and Paruchuri, (1996); Nuhoglu and Yalcin, (2005) and Saravanan et al., (2008)].

Chromium

Chromium is a chemical element discovered in 1797 by Louis Nicolas Vauquelin which has the symbol Cr. Its atomic number is 24. It is a hard metal of steely gray colour and also it has a high melting point of 1907°C. It is odourless and tasteless metal. Many of its compounds are intensely coloured. Chromium is important metal due to its high corrosion resistance and hardness. The trivalent chromium (Cr (III)) is required in trace amounts for sugar and lipid metabolism in humans and its deficiency causes disease. Hexavalent chromium (Cr (VI)) is a highly toxic metal pollutant that affects the environment and is abundantly available in the environment. Due to its toxicity and harmful effect on living system its cleanup is highly essential.

Use of chromium

Chromium metal is found in the effluents of industries, such as in metal finishing, petroleum refining, iron and steel industries, textile manufacturing, electroplating, leather tanning metal plating etc [Shen and Wang, (1994); Horton et al., (2006); Das and Mishra, (2010)]. Effluents of these industries contain large quantities of chromium-laden in their wastewater [Poornima et al., (2010)]. Hexavalent chromium Cr (VI) compounds are considered to be highly toxic, carcinogenic, and mutagenic to living organisms [Ramakrishna and Philip, (2005) and Poopal and Laxman, (2009)]. Considering its potential for hazardous toxicity and exposure, Cr (VI) has been designated as a priority pollutant in many countries. [Bae et al., (2000); Cheunga and Gu, (2007); Srivastava et al., (2008)].

Toxicity of chromium

Considering its potential for hazardous toxicity and exposure, Cr (VI) has been designated as a priority pollutant in many countries. Chromium (VI) is toxic and harmful to human health, mainly for the people who are working in industries where Cr (VI) is widely used. Chromium (VI) causes various health problems to human beings as reported by [Dermou et al., (2005)]. It has been reported by various authors that hexavalent chromium causes lung cancer , ulcer, severe damage to the liver and kidneys, perforation of nasal septum, leukocytosis, Skin rashes in humans [Poornima et al., (2010); Pechova, (2007); Kaufman et al., (1970); Li et al., (1987)]. There is sufficient evidence for carcinogenicity of Cr (VI) in animals for the hexavalent chromium compounds like calcium chromate, chromium trioxide, lead chromate, strontium chromate and zinc chromate stated by Poopal and Laxman, (2009). IARC and ACGIH have also classified chromium metal and trivalent chromium compounds as not human carcinogen. According to central pollution control board the minimum permissible limits of Cr (VI) at 0.05-0.1 ppm and 2 ppm for total chromium is sited [Das and Mishra, (2010)].

DIFFERENT PHYSICAL AND CHEMICAL METHODS FOR PHENOL AND CHROMIUM DEGRADATION AND THEIR DRAW BACK

Traditionally, by the use of various expensive chemical and physical processes high concentrations of both Cr (VI) and phenol are reduced from the industrial wastewater. Those methods include ozonization, adsorption, ion exchange, membrane filtration, chemical oxidation etc [Arutchelvan et al., (2006); Aksu and Gonen, (2006); Meghraj et al., (2003); Das and Mishra, (2010)]. But these processes are high energy consuming, non-economic and release effluents and waste waters which require further treatment and thus are alarming for the environment. Also complete removal of the pollutants cannot be possible by the use of physical and chemical processes [Camargo et al., (2003); Shen and Wang, (1994); Wang and Chirwa, (1998)].

Ozonization

Chemical oxidation with ozone can be used to treat organic pollutants or act as disinfectant agents. Ozone is a powerful oxidant that can oxidize a great number of organic and inorganic materials. Ozone based technologies research is also being focused on the catalytic ozonation where the presence of catalyst significantly improved the oxidation rate of organic compounds compared to non-catalytic ozonization [Matheswaran et al., (2007)]. The disadvantages associated with the process are high operating cost. The cost of the equipment is very high and also it requires high voltage and electricity for its operation [Gharbani et al., (2010)] .

Ion exchange

Ion exchange means the removal of an ion from an aqueous solution by replacing another ionic species. There are natural and synthetic materials available which are specially designed to enable ion exchange operations at high levels. So ion exchangers are used to perform this ion exchange for removal of organic and inorganic pollutants along with other heavy metals for purification and decontamination of industrial effluent. Synthetic and industrially produced ion exchange resins are mainly made up of polystyrene and polyacrylate are in the form of small and porous beads. [Liotta et al., (2009) and Sapari et al., (1996)].

Adsorption

Adsorption is a widely used method for the treatment of industrial wastewater containing colour, heavy metals and other inorganic and organic impurities stated by Al-Rekabi et al., (2007) and Patel and Vashi., (2010). Adsorbent materials are basically derived from low-cost agricultural wastes; activated carbon prepared from various raw materials such as sawdust, nut shells, coconut shells etc Zawani et al., (2009). These adsorbents are basically used for the effective removal and recovery of organic and metal pollutants from wastewater streams [Basso et al., (2002) and Park et al., (2006)]. It is a complex process affected by several factors [Gardea-Torresdey et al., (2004)]. This method suffers from low adsorption capacity and in some cases complete removal is not possible and high cost of the adsorbent. After use the disposal of adsorbants creates problems

Membrane filtration

Membrane filtration technique has received a significant attention for the wastewater treatment. It considers the application of hydraulic pressure to bring about the desired separation through the semi permeable membrane [Chen et al in 2004]. Membranes are of different pore size and it is necessary to select membranes of appropriate pore size for specific purpose so that effluent and wastewater could be purified and permeate could be recycled a number of times. Mainly three types of membrane filtration is there. They are Ultra-filtration, Nanofiltration and Reverse osmosis reported by Chauhan and Rekha, (200and Al-Rekabi et al., (2007).

Chemical oxidation

In this process the waste materials from the industrial waste water are removed by the help of chemical oxidation by the use of various chemicals mainly hydrogen peroxide is widely used for this purpose as reported [Dias-Machado et al., (2006) and Ksibi, (2006)]. There are many disadvantages associated with this process like the high cost of the chemicals, emission of various harmful by products, it creates hazardous constituent like secondary effluent problem along with the production of harmful gases.

Biodegradation

Biodegradation is the process of decaying or reduction of different organic materials and toxic metals to their nontoxic formwith the help of microorganisms. In this process complete mineralization of the starting compound to simpler ones like CO2, H2O, NO3 and other inorganic compounds takes place [Atlas and Bartha, (1998)].In the mixed culture of microorganisms phenol degrading organisms utilizes phenol as sole source of carbon and produce energy, metabolites, electron donor which is used by the chromium degrading organisms to reduce chromium [Yun-guo et al., (2008); Tziotzio et al., (2008) and Song et al., (2009)]. Biodegradation is a microbial process in which nutrients and physical conditions plays important role. Temperature and pH are the important physical variables and carbon, nitrogen, oxygen, phosphorus, sulfur, calcium, magnesium, and several metals are the micronutrients that also shows a significant impact on degradation behavior is reported [Khazi et al., (2007)].

Advantages of biodegradation

There are various advantages associated with biodegradation such as the process is a simple process. It is an eco-friendly and cost effective process that requires low capital and operating cost. Being environmentally friendly process it produces no harmful end products.

MICROORGANISMS INVOLVE IN BIODEGRADATION OF PHENOL AND CHROMIUM

Degradation of phenol occurs as a result of the activity of a large number of microorganisms including bacteria and fungi. Bacterial species include Bacillus sp, Pseudomonas sp, Acinetobacter sp, Achromobacter sp etc. Fusarium sp, Phanerocheate chrysosporium, Corious versicolor, Ralstonia sp, Streptomyces sp etc are also proved to be efficient fungal groups that evidenced phenol biodegradation [Chitra et al., (1995);Nair et al., (2008); Basha et al., (2010); Kumar et al., (2005); Mordocco et al., (1999); Chung et al., (2003); Santos et al., (2006); Chen et al., (2003)]. Similarly chromium degradation can be possible with the help of variety of bacterialand fungal groups like Arthrobacter sp. and Bacillus sp., Megharaj et al., (2003), E. coli ATCC 33456 [Bae et al 2000 and Shen et al 1994], Pseudomonas aeruginosa [Aguilera et al., (2004)], Brevebacterium casei [Das and Mishra, (2010)], Acinetobacter sp [Srivastava et al., (2007)] some unidentified species like Pseudomonas fluorescens, Pseudomonas synxantha [Gopalan and Veeramani, (1994), McLean et al., (2000)] Alcaligenes eutrophus Vaneechoutte et al., (2004) A number of bacterias Bacillus spp., Shewanella alga BrY-MT and a few unidentified strains have also been shown to reduce Cr 6+ [Shen and Wang, (1994), Wang and Xiao, (1995); Shakoori et al., (2000); Guha et al., (2001); Camargo et al., (2003)].

Present Scenario (2000-2015) of Indian Pulp and paper industry:

The Indian paper and paperboards industry is on the growth path. The Indian paper and paperboards industry grew by nearly 7.8 percent during the period 2000-2006. This is substantially higher than the Asian average of 5.1 percent. India‘s paper manufacturing capacity is expected to grow at a Compounded Annual Growth Rate (CAGR) of 7.4 percent from 8.4 million MT per annum to 11.2 million MT per annum between 2008 and 2010. The Indian per capita paper consumption is among the lowest at 7.0 kg, while Asian and global averages are 11.0 kg and 49.0 kg respectively. But the demand for paper is increasing given the rising disposable incomes particularly of the expanding middle income group.

The literacy level in India which is on the increase is further set to improve the demand for paper in the future. The Government of India‘s increased budget allocation for education sector is expected to further improve the literacy rates in both urban and rural areas, resulting in increased demand for writing paper. The Indian Pulp and paper industry is expected to grow at 7.4 % CAGR over the period 2008 – 10. With Indian economy in one of its best ever growth mode, the Indian paper industry continues to be a major beneficiary. There are more than 600 paper mills in India in 2010, and installed capacity is about 9 million tonnes / annum. **2010**: In the present scenario, apart from capacity augmentation, there is an immense need to improve the Energy Efficiency of the individual units. Many of the Indian Paper mills are also working actively in the areas of water and environmental management not only to better the statutory norms but also in a proactively move closer to cleaner production. With the liberalization of the Indian economy leading to global competition as well as the growing emphasis on the environment, it is imperative for the Indian Pulp and Paper industry to become World class in operations, energy consumption and environmental impact. (http://www.slideshare.net/Harijan29/indian-paper-industry19902010)

Pulp and paper (P&P) industry is considered a large user and producer of biomass based energy and materials (Svensson and Berntsson, 2014). To maintain their profitability and overcome the declining (Machani et al., 2014) and competitive (Karikallio et al., 2011) markets, P&P mills are no longer limited to production of pulp and/or paper; rather, they may adopt additional measures including waste heat delivery to district heating systems (e.g., Ericsson et al., 2011; Klugman et al., 2009) and production of the electricity, wood pellets, and dried bark as well as valuable chemicals such as ethanol (e.g., Fornell et al., 2012; Phillips et al., 2013) and materials like carbon fiber (see e.g., Maradur et al., 2012), biofuels, etc. (Jönsson et al., 2011). However, the pulp and paper (P&P) industry is now facing challenges to comply with stringent environmental regulations (Pellegrin et al., 1999) and formation of scum, as well as toxicity to the exposed communities, thermal impacts, color problems, and aesthetical issues (Pokhrel and Viraraghavan, 2004), in case of incomplete treatment (Karrasch et al., 2006). Such substrates may include non-biodegradable organic materials, adsorbable organic halogens (AOX), color, phenolic compounds, etc. (Buyukkamaci and Koken, 2010), depending upon the applied pulping process, additive chemicals, and the amount of water consumed. Accordingly, in both traditional and emerging P&P producers (e.g., Chen et al., 2012) such as United States (Schneider, 2011), China (Zhu et al., 2012), and India (Afroz and Singh, 2014), P&P mills are considered a major source of environmental pollutants.

Pollutants which are released during several parts of P&P production process can be reduced by adopting several internal process improvements , especially in combination with management measures. In this regard, European Commission (2001) has described the best available techniques (BAT) to be adopted by P&P mills. Moreover, several studies have been carried out with the final purpose of reduction in the pollution load during the P&P making process (Martín-Sampedro et al., 2011).

Microorganisms used for degradation of pulp and paper industry effluent

For degradation of pulp and paper industry effluent Paper and pulp mill is a source of major pollution generating industry leaving huge amount of intensely coloured effluent to the receiving end. Rapid increase of population and the increased demand for industrial establishments to meet human needs have created problems such as over exploitation of available resources, increased pollution taking place on land, air and water environment. The intention of this research paper is to identify predominant bacteria and fungi in paper and pulp mill effluent in addition to evaluate the degradation efficiency of individual isolates and combination of isolates. Treatment efficiency of individual isolates and combination of isolates are evaluated by shake flask method. Combination of Pseudomonas Alkaligenes, Bacillus subtilis along with Trichoderma reesei shows higher BOD, COD reduction of 99% and 85% respectively. As individual isolates Pseudomonas Alkaligenes show 92% BOD reduction and 77% COD reduction over other bacterial isolates and Trichoderma reesei removed 99% BOD and 80% COD respectively (Saraswathi et al,.2010).

POLLUTION PROBLM OF PULP AND PAPER INDUSTRIES

Pulp and paper mills are a major source of industrial pollution worldwide. The pulping and bleaching steps generate most of the liquid, solid, and gaseous wastes. Pulping is a process in which the raw material is treated mechanically or chemically to remove lignin in order to facilitate cellulose and hemicelluloses fiber separation and to improve the papermaking properties of fibers. Bleaching is a multistage process to whiten and brighten the pulp through removal of residual lignin. Pulping and bleaching operations are energy intensive and typically consume huge volumes of fresh water and large quantities of chemicals such as sodium hydroxide, sodium carbonate, sodium sulfide, bisulfites, elemental chlorine or chlorine dioxide, calcium oxide, hydrochloric acid, and so on. A partial list of the various types of compounds found in spent liquors generated from pulping and bleaching steps is shown in. The effluents generated by the mills are associated with the following major problems:

•Dark brown colouration of the receiving water bodies result in reduced penetration of light, thereby affecting benthic growth and habitat. The colour responsible for causing aesthetic problems is attributable to lignin and its degradation products.

•High content of organic matter, which contributes to the biological oxygen demand (BOD) and depletion of dissolved oxygen in the receiving ecosystems.

•Presence of persistent, bio-accumulative, and toxic pollutants.

Contribution to absorbable organic halide (AOX) loads in the receiving ecosystems.

Measurable long-distance transport (>100km) of organic halides (such as chloroguaiacols), thereby contaminating remote parts of seas and lakes.

Cross-media pollutant transfer through volatilization of compounds and absorption of chlorinated organics to wastewater particulates and sludge. Volatile organics include carbon disulfide, methanol, methyl ethyl ketone, phenols, terpenes, acetone, alcohols, chloroform, chloromethane, and trichloroethane. The extent of pollution and toxicity depends upon the raw material used, pulping method, and pulp bleaching process adapted by the pulp and paper mills. For example, the pollution load from hardwood is lower than softwood. On the other hand, the spent liquor generated from pulping of non-wood fiber has high silica content. Volumes of wastewater discharged may vary from near zero to 400 m3 per ton of pulp depending on the raw material used, manufacturing process, and size of the mill. Thus, the variability of effluent characteristics and volume from one mill to another emphasizes the requirement for a variety of pollution prevention and treatment technologies, tailored for a specific industry.

CHAPTER- III

MATERIALS AND METHODS

APPENDIX

Nutrient broth and agar (**pH 7.0**)

Peptone 5.0g	
Beef extract	3.og
NaCl	5.0
Distilled water	1000.0ml

For nutrient agar add 15.0 g agar. Used for isolation of bacteria and actinomycetes.

MacConkey's agar (pH 7.1)

Peptone	20.0g
NaCl	5.0g
Bile salt	1.5 g
Lactose	10.0g
Neutral red solution	10.0ml
Crystal violet solution	0.001g
Agar	13.5g
Distilled water	1000.0ml

Mannitol salt agar (pH 7.4)

Mannitol	10.0g
Peptone	10.0g
Sodium chloride	75.0g
Beef extract	1.0g
Phenol red	0.025g
Agar	15.0g
Distilled water	1000.0ml

Acetamide agar (pH 7.0)

Acetamide	10.000g
Sodium chloride	5.000g
Dipotassium hydrogen Phosphate	1.390g
Potassium dihydrogen Phosphate	0.730g
Phenol red	0.012ml
Magnesium sulphate	0.500g
Agar	15.0g

Distilled water	1000.0 ml
Appearance – orange color	

Simmons citrate agar (pH 6.9)

Ammonium dihydogen phosphate	1.0g
Dipotassium hydrogen phosphate	1.0g
Sodium chloride	5.0g
Sodium citrate	2.0g
Magnesium sulphate	0.2 g
Bromo thymol blue	0.08g
Agar	15.0g
Distilled water	1000.0ml

Minimal synthetic Media

K_2HPO4	1.0 g
KH2PO4	0.2g
NaCl,	1.0 g
$CaCl_2$	0.002 g
$(NH_4)\ S_2O$	1.0 g
MgSO2.7H2O	0.5 g
CuSO4.5H2O	0.001 g
ZnSO4.7H2O	0.001 g
MnSO4.H2O	0.001 g
FeSO4.7H2O	0.01 g.
Distilled water	1000.0 ml

Tryptone broth (pH 7.5)

Tryptone	10.0g
Sodium chloride	5.0g
Distilled water	1000.0ml

Lactose broth (pH7.3)

Sodium chloride	15.0g
Lactose	5.0g
Peptone	10.0g
Phenol red	0.018g
Distilled water	1000.0ml

Glucose broth (pH7.3)

Glucose	5.0g
Peptone	10.0g
Sodium chloride	15.0g

Phenol red	0.018g

Sucrose broth (PH 7.3)

Sucrose	5.0g
Peptone	10.0g
Sodium chloride	15.0g
Phenol red	0.018g
Distilled water	1000.0ml

MR-VP broth (PH 6.9)

Peptone	7.0g
Potassium phosphate	5.0g
Dextrose	5.0g
Distilled water	1000.0ml

REAGENTS REQUIRED

Crystal violet

Solution –A

Crystal violet (90%dye content) – 2gm

Ethyl alcohol (95%)	20 ml

Solution – B

Ammonium oxalate	0.8gm
Distilled water	80 ml

Dissolve crystal violet in ethyl alcohol and the ammonium oxalate in distilled water, mix solution A and B.

Gram's iodine

Iodine	1gm
Potassium	2gm
Distilled water	300ml

Dissolved and potassium iodide in distilled water.

Ethyl alcohol (95%)

Ethyl alcohol (100%)	95ml
Distilled water	5ml

Safranine

Safranine (2.5%solution in 95% ethyl alcohol) -10ml

Distilled water 100ml

GLASSWARES USED:-

Borosil test tube, beaker, Conical flask, Measuring cylinder , Pipettes, Petriplates , Dropper buttle for staining reagent ,Glass microscope slide , Glass cover slips , test tube racks , Rubber bulb for pipette.

INSTRUMENTS USED

Laminar air flow, Incubator, Microscope, Hot air oven, Pressure cooker, Weighing machine,Refrigerator,Sprit lamp.

TOOLS

Inoculating loop, Forceps, Scissors, Glass marking pen.

MISCELLANEOUS: Aluminum foil

METHODS

SAMPLE COLLECTION

Effluent sample was collected from Dainik jagran press Jhansi. The effluent sample was collected in sterile glass vials and then stored at 4^0 C for future use to carry out different studies. Dainik jagran paper press is located in Jhansi, UP, India. The colour of the water sample was brown and the PH measured was 7.6.

TECHNIQUE FOR ISOLATION OF BACTERIA

A-SERIAL DILUTION OF SAMPLE: - Serial dilution is a set of dilution in a mathematical sequence, as to obtain a culture plate with a countable number of separate colonies, in serial dilution sample is diluted serially.

For serial dilution 5 tubes were taken and in the first test tubes 9ml of distilled water and 1ml the seconds likewise all the tubes contain 9ml of distilled water and then samples from 1^{st} and 2^{nd} and serially diluted samples several times.

The sample were collected serially diluted up to the label

(B) Then each serially diluted sample was in 0.1 ml amount were spreaded on the Nutrient Agar media (NAM), the composition of this media is given in the appendix.The components of this media are mixed properly in distilled water and sterilized in the autoclave before using it for culturing and then incubated for 24 hours at 37^0C then after incubation separated colonies were obtained.

Different techniques were used for isolation of bacteria after serial dilution

1-SPREAD PLATE METHOD: - If the serially diluted sample is spread on an agar surface. So that every cell (Microorganism) grown into a completely separate colony a macroscopically visible grown of duster of microorganism on a solid medium, each colony represents a pure culture. The spread plate is an easy, direct way of achieving this.

Screening of paper degrading microorganisms

Weighed piece of news Paperwas added in mineral salt medium (composition of MSM media is given in the Appendix) at a fine concentration and the paper was kept in shaker for 1 month. The isolated organisms were inoculated on paper containing agar plates and then incubated at 37°C.

IDENTIFICATION

The identification of bacteria was performed on the basis of macroscopic and microscopic examination and biochemical test according to K.R ANEJA.

2- MORPHOLOGICAL IDENTIFICATION: -pure colonies were taken from streaked plate. This pure colony taken for the Gram's staining. The Gram's staining done as follows.

GRAM'S STAINING:- Gram- positive bacteria have a thick mesh like cell wall made of peptidoglycan (50-90 % of cell envelope), and as a result are stained purple by crystal violet , whereas Gram-negative bacteria have a thinner layer (10% of cell envelope), so do not retain the purple stain and are counter stained pink by the safranin. The steps of Grams staining include:

- Thin smears of bacterial culture were made on glass slide.
-The smear was heated and then air dried.
- Each smear was covered with crystal violet (primary stain) for 30 seconds.
- Each slide was washed with tap water for a few seconds using wash bottle.
- Each smear was covered with gram's iodine solution for 60 seconds.
-Slides were finally stained with counter stain 'Safranin'for 30 seconds and washed with decolorizer and with distilled water. (The gram positive bacteria are not affected while all gram negative bacteria are completely decolorized).
- Stained slides were air dried and examined microscopically.

COLONY MORPHOLOGY

This was done to determine the morphology of selected stains on the basis of shape, size, and colour.

BIOCHEMICAL TESTS

A) IMViC TEST

The IMViC consists of four different tests: (1) indole production; (2) methyl-red; (3) voges-proskaur; and citrate utilization.

1. INDOLE PRODUCTION TEST

The indole test is performed by inoculating a bacterium into tryptone broth (tryptone is an essential amino acid), the indole produced during the reaction is detected by adding kovac's reagent (dimethylaminobenzaldehyde) which produce a cherry-red reagent layer.

Procedure:-Tryptone broth was prepared by dissolving 10 g of peptone in one litter of distilled water. Broth was sterilized using autoclave at 15 psi for15 minute.5 tubes were taken for tryptone broth , bacterial culture was inoculated in tryptone broth and fifth tube was kept as an uninoculated comparative control. Inoculated and uninoculated tubes were incubated at 35^0C for 48 hrs.After 48 hours of incubation, 1ml of kovac's reagent was added to each tube including control. The tubes were shaken gently after repeated intervals of 10-15 minutes and result were compared with the control.

2. Methyl-red and voges-proskauer tests (MR-VP broth)

In the MR test, the methyl red indicator in the in the pH range of 4 will remain red with in indicative of a positive test, while turning of methyl red to yellow in a negative test, while turnig of methyl red to yellow in a negative test. In the VP test development of crimson to ruby pink red colour may be most intense of the surface, in indicative of positive VP test while no change in coloration of negative test.

Procedure- Five tubes were taken. 5ml of MR-VP broth was then poured in each tube and sterilized by autoclaving at 15Ib pressure for 15 minute. FourMR-VP tubes were inoculated with bacterial culture and one of thetubes was kept asan uninoculated comparative control. All five tubes were incubated at35^0C for 48 hours under incubator.5 drops of methyl red indicator was added to each tube.

3.CITRATE UTILIZATION TEST

The citric acid is metabolized, the CO_2 generated combines with sodium and water to form sodium carbonate and alkaline product, which change the colour of the indicator from green to blue and this consititutes a positive test.

Procedure:- simmon's citrate agar was prepared and sterilized by autoclaving at 15 Ib pressure for 15 minute. Culture medium was poured in the petri plates, and simmon's citrate agar plates were incubated afterstreaking at 37^o C for 48 hours.

4.MANNITOL TEST:

This experiment is generally to determine whether the bacteria are capable of fermenting mannitol sugar or not. Whenever organisms ferment mannitol agar, the pH of media becomes acidic due to production of acids. The fermentation of the media from red to yellow which shows positive test result.

5. CATALASE TEST

The enzyme catalase in some microorganisms breaks down hydrogen peroxide to water and oxygen. Catalase test performed by adding H_2O_2 to trypticase soy agar. Release of free oxygen gas bubbles is a positive catalase test.

Procedure: -5 plates were taken for trypticase soy agar and one plate was used as a control. Trypticase soy agar was prepared and poured in culture plates and flasks and sterilized by autoclaving at 15 Ib pressure for 15 minute. Bacterial culture was streaked on the trypticase soy agar plates. Hydrogen peroxide was added to smear of 24 hours old culture on a slide.

6.FERMENTATION OF CARBOHYDRATES

Fermentative degradation of various carbohydrates such as glucose(monosaccharide), sucrose (disaccharide), cellulose (polysaccharide), by microbes, under anaerobic condition is carried out in a fermentation tube. Fermentation broth contains ingredients of nutrient broth, specific carbohydrates (glucose, lactose, maltose, and sucrose) and a pH indicator (phenol red), which is red at a neutral pH (7) and turns yellow at or below a pH of 6.8 due to the production of an organic acid.

Procedure: -15 Fermentation broth tubes were taken (5 tubes of glucose,5 tubes of lactose, 5 tubes of sucrose), and autoclaved at 12 Ib pressure for 15 minutes. Each of specified fermentation tubes of media was labeled.The three types of sugar fermentation broth tubes were inoculated with each bacterium and one uninoculated tube of each fermentation broth was kept as a comparative control. All the 12 inoculated and 3 uninoculated tubes were incubated at 35^0C for 24-48 hours.

CHAPTER IV

RESULT

Isolation and identification of paper degrading bacteria

Paper effluent sample was collected from Jhansi paper industry. It was used as a source for isolating bacteria having the ability to degrade the cellulose.

These bacterial colonies were obtained from effluent sample. The dominant bacterial strains isolated after serial dilution were named as B-1, B-2,B-3,B-4,B-5.

The bacterial strains were identified macroscopically by examining colony morphology, surface staining to study the staining behavior, shape and cell arrangement.

Further characterization was done by performing the following biochemical test such as Simmon's citrate, catalase, fermentation broth etc.

Microbial degradation of paper in laboratory condition

Selected bacterial isolates were further tested in the laboratory conditions to check the ability of degrading newspaper.The isolates were allowed to degrade the paper for period of one month. After the period of incubation,the paper was collected, washed thoroughly using distilled water, shade-dried and then weighed to check final weight. Table no.1 shows analysis of paper weight losses with different bacterial strains under laboratory conditions .From the data collected, weight loss of the paper was calculated.

Table No 1- Result of degradation of paper sample by bacteria after one month

stain no	Initial wt(mg)	Final wt(mg)	Difference	Weight per month (in %)
1.Bacillus sp.	50	37	13	26%
2.streptococcus sp.	50	36	14	28%
3. staphylococcus sp.	50	39	11	22%
4.pseudomonas sp.	50	35	15	30%
Control	50	50	0	0%

Table No 2- Identification of paper degrading bacteria isolates

Characteristics	Strain 1	Strain 2	Strain 3	Strain 4
Colony Characteristics				
Shape	Round	Round	Round	Round
Colour	white	Pale yellow	Yellow	Purple
Surface	Dull granular	Dull granular	Raised	Convex
Margin	Entire	Irregular	Entire	Undulate
Morphology				
Straight rod	+	-	-	+
Cocci	-	+	+	-
Gram stain	+	+	+	-
Cell Arrangement	Short rod, single	Short chain,	Cocci in chain	Short chain , single
Spore	C	C	-	C
Motility	+	+	-	+
Carbohydrate Fermentation				
Glucose	A/ +	A/-	A/-	A/+
Sucrose	A/+	A/+	A/+	A/+
Lactose	A/-	A/-	A/+	A/+
Catalase	+	+	-	+
Indole Production	+	-	+	+
Methayl Red	-	-	+	-
Voges proskauer	-	-	+	-
Citrate utilization	+	-	-	+
Simmon citrate Agar	+	-	-	+
Mannitol salt Agar	-	+	+	-

Acetamide agar	-	-	-	+
Identified Microorganisms	Bacillus Sp.	Streptococcus Sp.	Staphylococcus Sp.	Pseudomonas Sp.

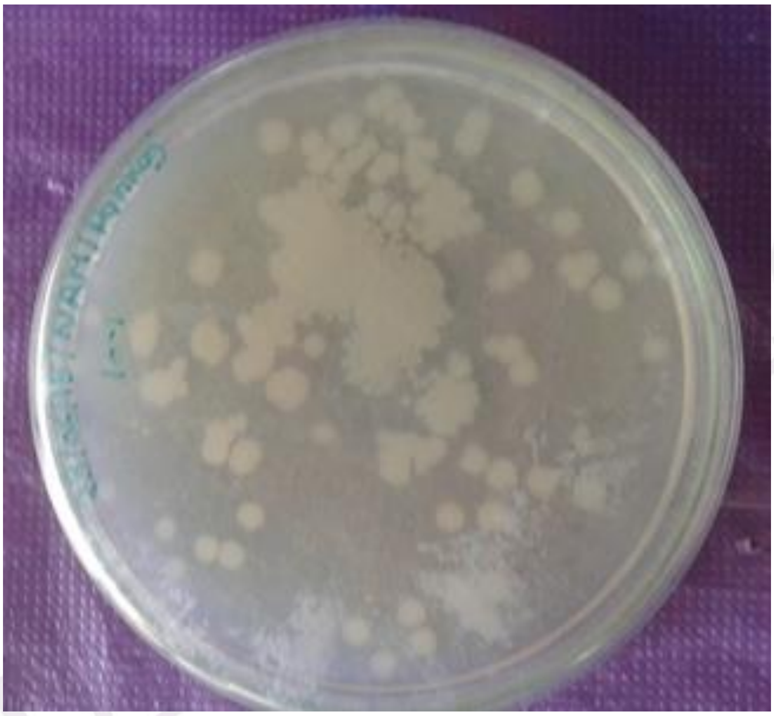

Fig no-1 NAM plate serial dilution 10^{-1}

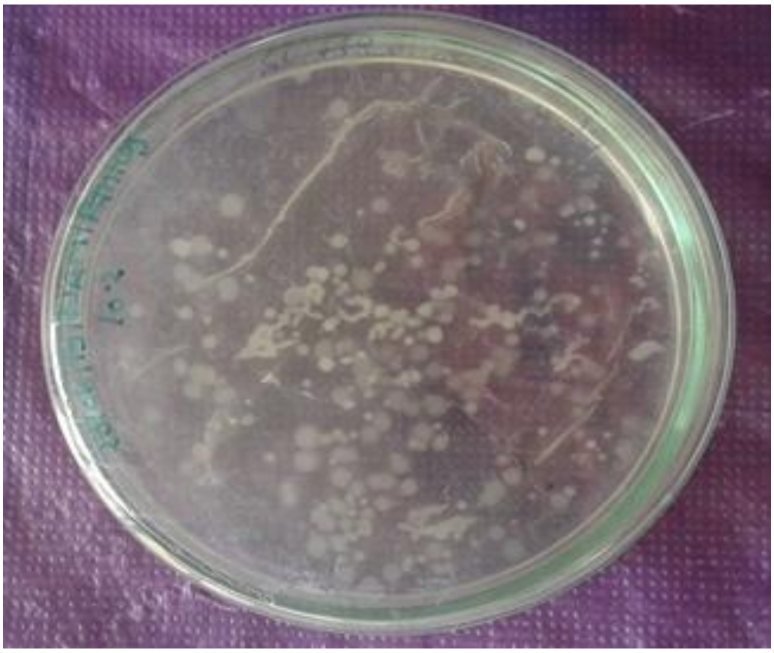

Fig no-2 NAM plate serial dilution 10^{-2}

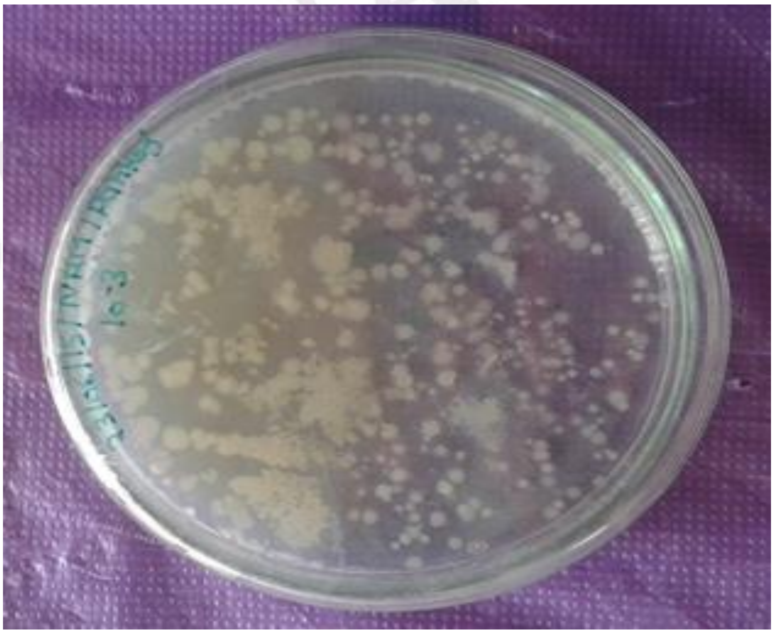

Fig No-3 NAM Serial Dilution Plate 10^{-3}

FIG NO-4 NAM SERIAL DILUTION PLATE 10^{-4}

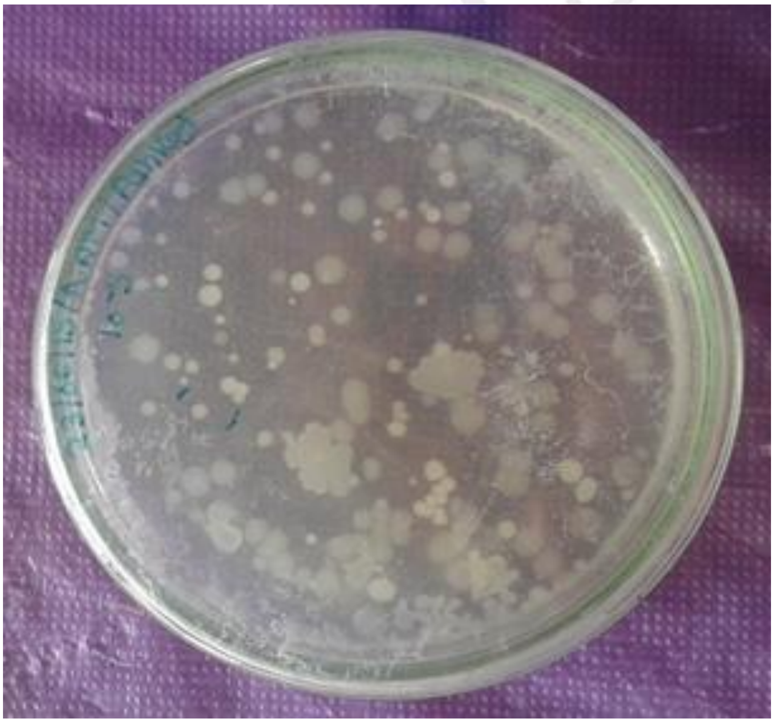

Fig No-5 Nam Serial Dilution Plate 10^{-5}

Fig No-6 NAM Plate Showing Isolated Bacteria After Sub-Culturing

Fig No-7 Mackonky White /Cream Colony Showing Bacteria After Sub-Culturing

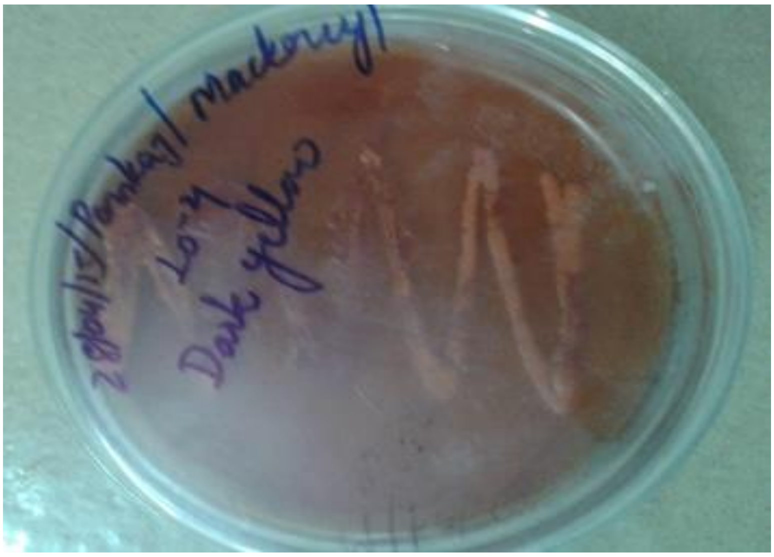

Fig No-8 Mackonky Dark Yellow Showing Bacteria After Sub-Culturing

Fig No 9- Sub-Culturing Of Isolates On Mac'conkey's Agar Plate

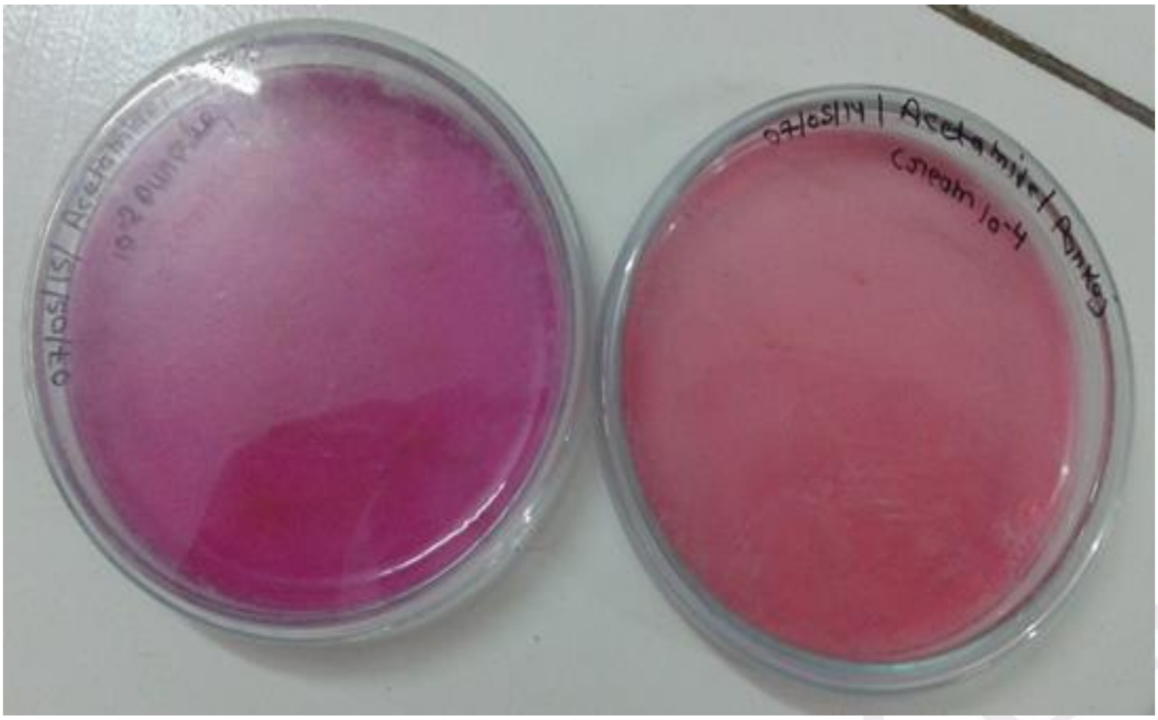

Fig No-10, PurpleAnd White/ Cream Colony Sub-Culturing On Acetamide Agar

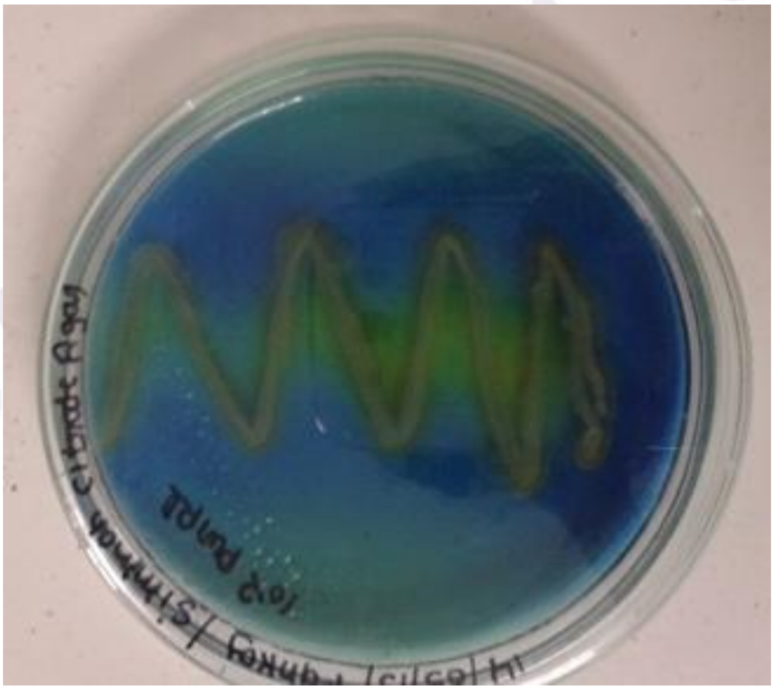

Fig No-11, Purple Colony Showing Isolated Bacteria after Sub-Culturing On Simmons Citrate Agar Plate

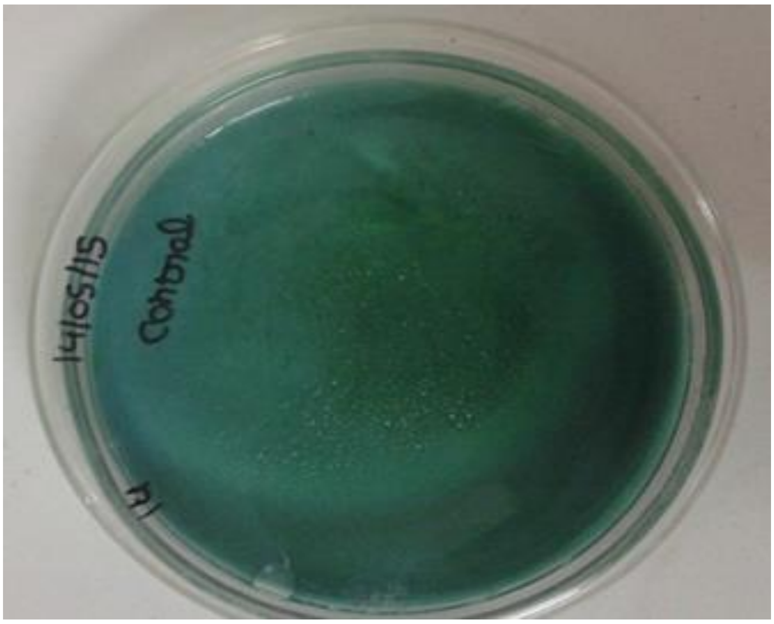

Fig No-12 SimmonCitrate Agar Control Plate

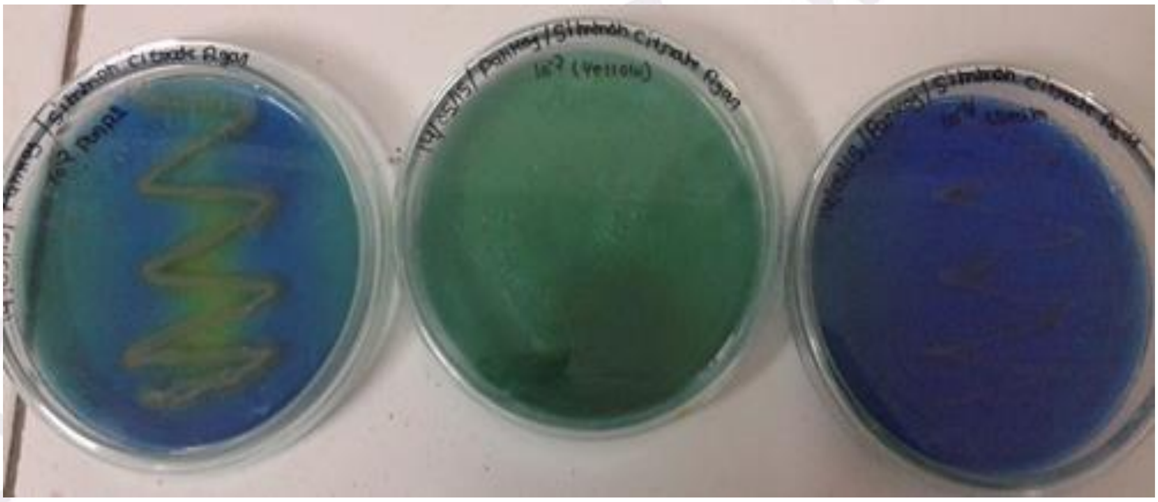

Fig No-13, Purple Colony, White/Cream Colony And Control Plate On Simmon Citrate Agar

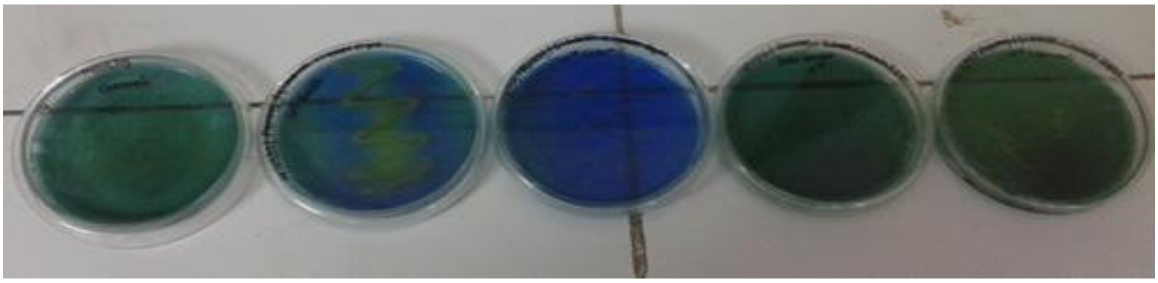

Fig no-14, Purple, white/ cream, yellow, and dark yellow colony showing bacteria after

Fig No- 15 Indole Broth

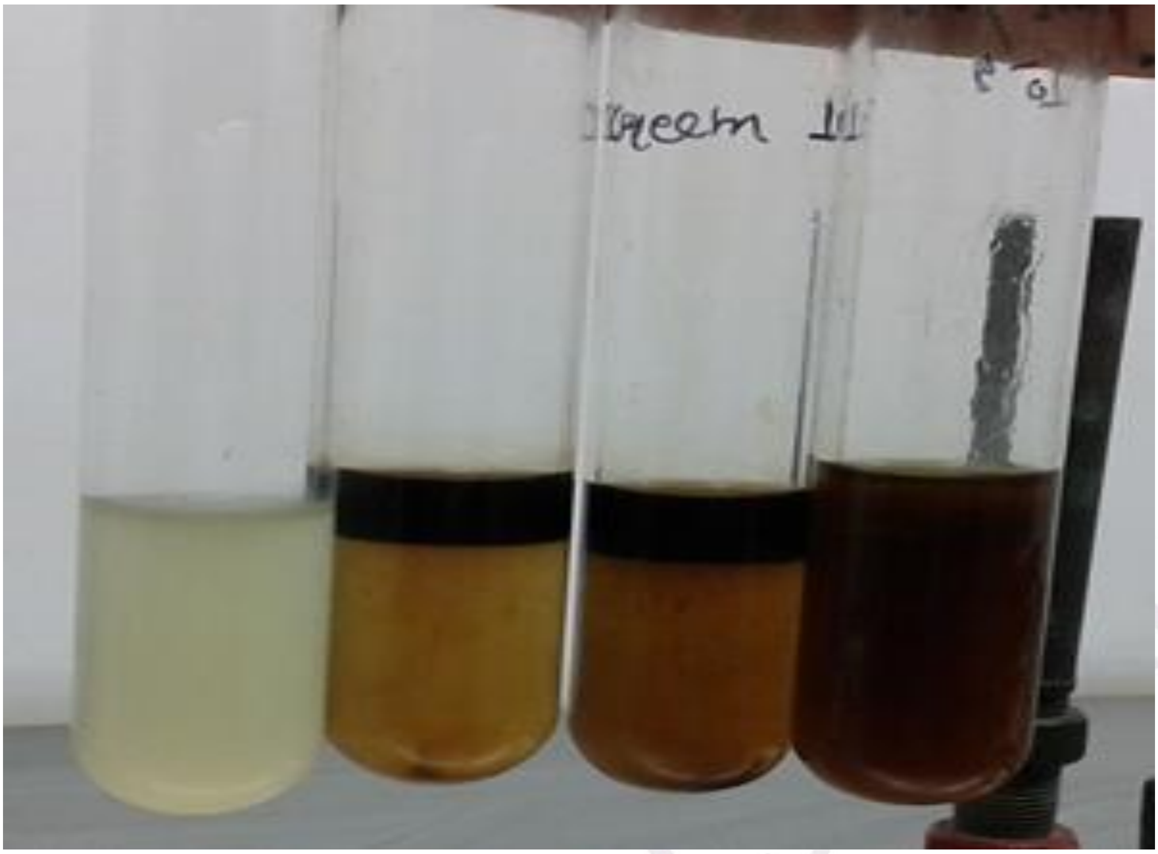

Fig No-16, Indole Test

Fig No-17, Catalase Test Showing Purple Colony

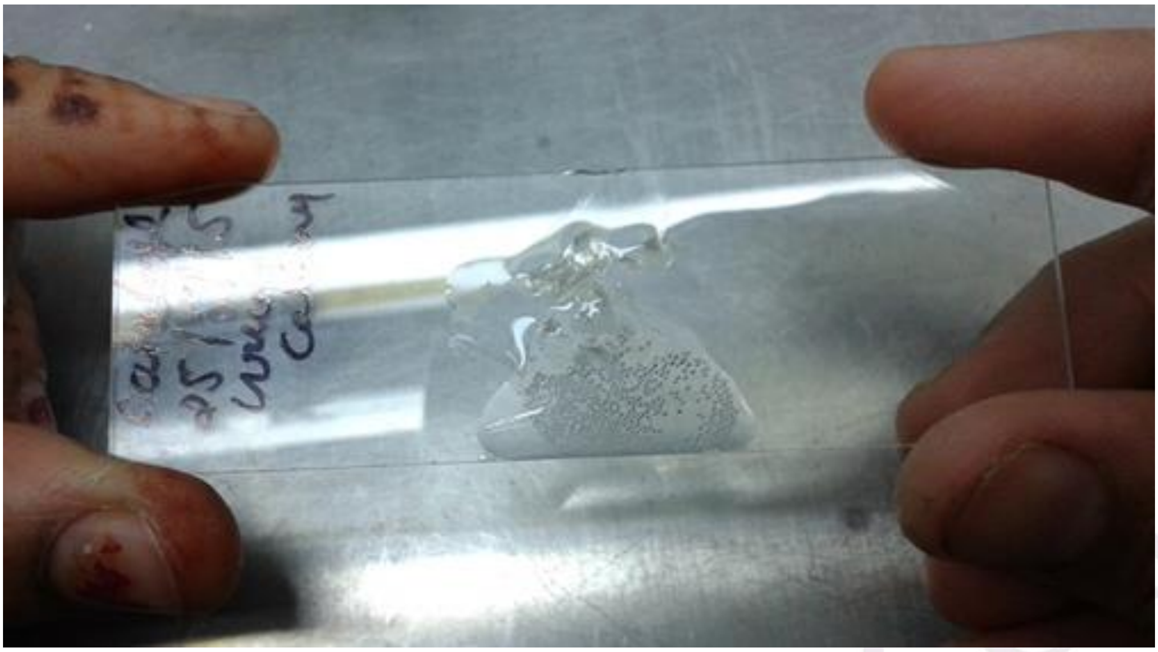

Fig No-18, CatalaseShowing White/Cream Colony

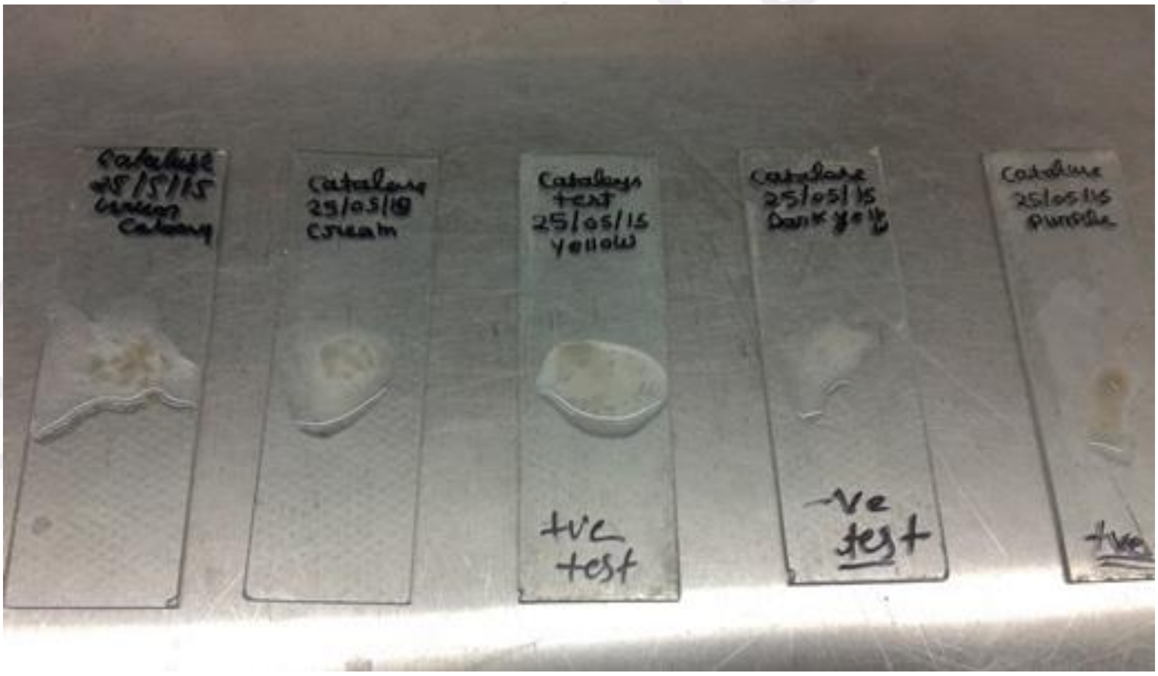

Fig No-19Showing Catalase Test White/ Cream, Purple, Yellow, Dark Yellow Colony

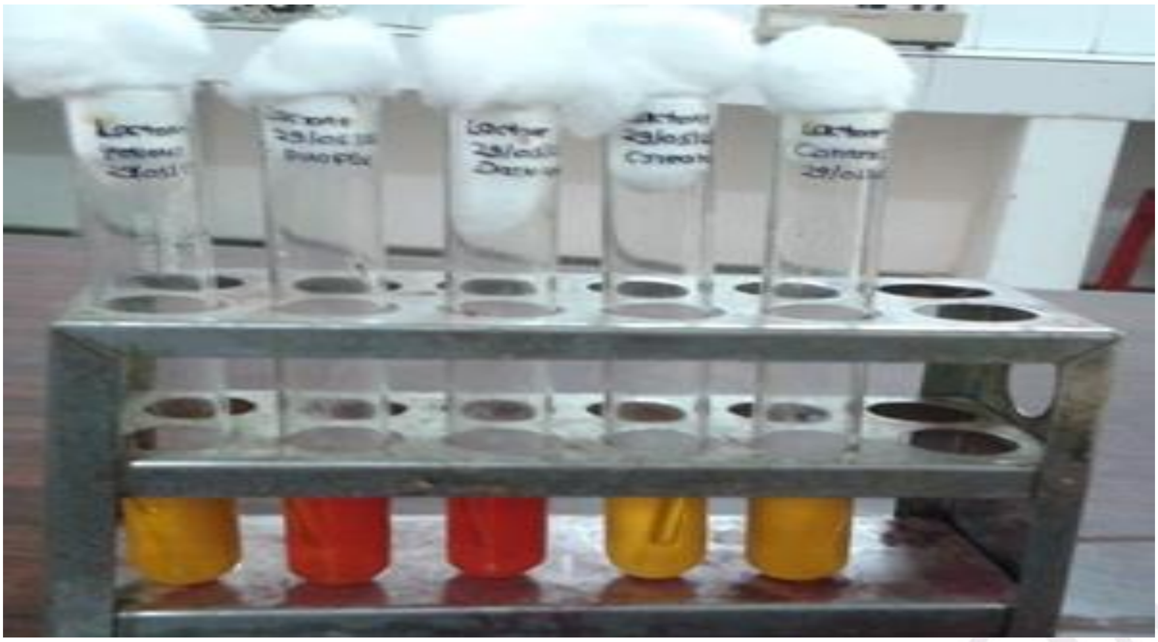

Fig No-20, Showing Fermentation Broth Test (Lactose Broth)

CHAPTER V

DISCUSSION

Pulp paper industry has been considered as one of the biggest consumers of natural resources (wood, water) and energy (fossil fuels and electricity). These industries are also considered as a significant taxpayer of discharge of pollutants to the environment. The high pH and sulphate value of kraft lignin was due to the presence of sodium hydroxide and sodium sulphide which is used in pulping process.

Besides this, the compounds with high molecular weight, such as lignin, chlorophenols and its derivatives do not affect BOD, but would contribute high COD thats why these values were higher in KL compared to ML (Ugurlu et al. 2008; Chandra et al. 2011).

The manufacture of printing inks and the major printing processes have been described in several reviews (National Association of Printing ink Manufacturers, 1988; WilIiams, 1992; Leach & Pierce, 1993; Taggi & Walker, 1996). The development of printing inks followed somewhat different paths, depending upon the printing process. Letterpress and litho printing use high-viscosity inks that are formulated using low-volatility materials and are generically known as 'paste' or 'oil' inks. The flexo and gravure processes use low-viscosity inks formulated with volatile sol vents and are known collectively as 'liquid inks. Screen printing inks generally are of intermediate viscosity.. Pigments determine the colour of the ink, inc1uding its hue (shade) and strength, and also affect physical properties, such as flow characteristics (rheology), opacity (or transparency), fastness and bleed resistance. Vehic1es serve as carriers for the pigment during the printing process and bind the pigment to the substrate. The paper press from which our water samples were taken utilized view ink and the chemical was techno ps chemical.

Microorganisms play a significant role in the degradation of paper and newspaper. The present study deals with the isolation, characterization and degradative ability of bacteria that can degrade newspaper. Water disposed from the press where newspaper is printed was collected from Dainik Jagran press, Jhansi was used in this study. This newspaper was later used to study their biodegradation by bacteria isolated from water disposed of in the press. The pure bacterial isolates were obtained by serial dilution of the water sample and continuous sub-culturing. The characteristic type of growth on solid media like NAM under appropriate cultural condition and colony morphology was used in the presumptive identification of our bacterial isolates. Colony characteristics and Gram staining were recorded for morphological characters whereas biochemical parameters confirmed their identity. The bacterial species found associated with the degrading material were identified as one Gram negative and three Gram positive.The four bacterial isolates that were identified were Pseudomonas, Streptococcus, Staphylococcus and Bacillus.

Many previous reports highlight that microorganisms can degrade paper and describe their association with cellulose and lignin. The microorganism treats the effluents mainly by two process; action of enzymes and biosorption (Malaviya and Rathore, 2007). Microbial enzymes are enabling new technologies for processing pulps and fibers. Xylanases reduce the amount of chemicals required for bleaching; cellulases smooth fibers, enhance drainage, and promote ink removal; lipases reduce pitch; lignin- degrading enzymes remove lignin from pulps. Several of these processes are commercial, and others are beginning to emerge. In the future, enzyme-based processes could lead to cleaner and more efficient pulp and paper processing. Treatment efficiency of individual isolates and combination of isolates are evaluated by shake flask method. Combination of Pseudomonas alkaligenes, Bacillus subtilis along with Trichoderma reesei shows higher BOD, COD reduction of 99% and 85% respectively (Saravanan and Sreekrishnan, 2005). But very few reports have been seen on biological degradation of newspaper which is published, used and dumped at a large scale daily all over the world polluting many water bodies. The discharge of this pulp paper press wastewater may promote skin diseases in human beings who are directly or indirectly contact with this aquatic system (Das et al. 2012).

Thus in our study another area was examined to see whether our bacterial isolates can degrade newspaper by the liquid culture method. It is clear that most recalcitrant polymers can be degraded to some extent in the appropriate environment at the right concentration. The most convenient to determine the degradation is to measure the weight loss. During the degradation time, the systems were maintained as undisturbed with no addition and removal of medium which indicates that the microorganism used the newspaper as carbon source.

When the total biodegradation process of any organic substrate is considered the formation of microbial colony is critical to the initiation of biodegradation. So moving on with our studies, weighed piece of newspaper was inoculated in the liquid culture medium containing our bacterial isolates and kept for 1 month to observe the percentage of weight loss by bacteria. The result shows the degradative ability of the microorganisms after one month of incubation. The 30 percent weight loss of degradation was found by Pseudomonas species. This shows it has the greater potential of degradation than other bacteria. Streptococcus species (28%) and Bacillus species (26%) followed next and least degradation was shown by Staphylococcus species.

CONCLUSION

The bacteria were identified to be Pseudomonas, Bacillus, Streptococcus, and Staphylococcus.

1. Bacillus sp. (strain 1) and Staphylococcus sp. (strain 3) has less capacity to degrade paper as compared to Pseudomonas sp.
2. Streptococcus sp. (strain 2) has less capacity to degrade paper as compared to other bacteria.
3. Pseudomonas sp. (strain 4) degrades paper more than that of other bacteria.
4. The isolated microbes were native to the site of polythene disposal and shown some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media.

5. This gives some suggestion that these microbes can used in both natural and artificial conditions for the purpose of degradation of paper.
6. In our study, further attention and future prospects aims for confirmation of these isolates by 16S rRNA sequencing and application of these isolates in commercial degradation and preparation of eco-friendly papers.

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