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Research Article

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Characterization and isolation of paper mill effluent degrading microorganisms

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ABSTRACT

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. The intention of this research paper is to identify the predominant bacteria and fungi in paper mill effluent in addition of evaluating the degradation efficiency of individual isolates and combination of isolates to treat the released effluent. Effective floc formation and degradation was attained in Pseudomonas alkaligenes + Enterobacter spp. combination which enhance clearing and settling process in the treatment plants. 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.

Keywords: Microorganisms, Degradation, Paper mill effluent.

INTRODUCTION

Paper industries are the sixth largest toxic effluent generating industries of the world [1]. Effluents discharged by industries constitute one of the major causes of environmental pollution and a significant public health hazard, particularly in developing countries [2]. These units correspondingly generate large quantities of wastewater, approximately 150–200m3 effluent/ton of paper being produced. The environmental impact of wastewater emanated from paper mill industries is therefore of particular concern. The high chlorine content of bleached plant reacts with lignin and its derivatives and form highly toxic and recalcitrant compounds that are responsible for high biological and chemical oxygen demand. Trichlorophenol, dichlorophenol, dichloroguicol and pentachlorophenol are major contaminants formed in the effluents of paper mills [3]. Waterborne infections are the most common causes of mortality and morbidity in the under developed and developing countries and 80% of the infectious diseases are waterborne in India [4]. Due to the modern trend towards enclosing process water systems, paper machine waters have become richer in nutrients for microbial growth. In addition, suitable temperatures (30 –50^{0}C) and a neutral pH favor the growth bacteria, in the process water [5]. Up to now, very few studies have addressed the characterization of the microflora of paper sludges and its treatment.

Increase in total microbial activity and populations following paper pulp effluent discard to soil has been reported. The presence of N_2-fixing members of the Enterobacteriaceae, including Klebsiella sp. strains, in pulp and paper mill water systems has long been known [6]. White rot fungus isolated from soil samples enriched by continuous paper mill effluent irrigation enabled identification of *Phanerochaete chrysosporium* [7]. Organic and inorganic contents of the effluent also provide ample opportunity to the flourishing of a variety of pathogenic microorganism [8]. Due to high chemical diversity of the organic pollutants in paper mill effluent, a high variety of toxic effects on aquatic communities in the recipient watercourses have been observed. A significant number of these substances have been classified as carcinogenic, mutagenic and clastogenic and endocrinic [9]. The untreated effluents from paper mills discharged into water bodies, damages the water quality and living organisms. The aim of this research study is to isolate the predominant bacteria and fungi present paper mill effluent and evaluate the degradation efficiency of individual and combination of isolates in laboratory scale.

EXPERIMENTAL SECTION

Sample Collection

Highly colored effluent samples from the paper mill was collected from paper mill located in South India. The paper pulp effluent from the inlet and outlet of primary settling tank was used for investigation. The sample was collected using a sterile plastic container and filtered through ordinary filter paper to remove large suspended particles. The filtered effluents were stored at 4°C until use.

Isolation and Identification of Microorganisms

The total microbial load of the effluent samples was determined with the help of the standard plate count method (SPC). The effluent samples were serially diluted 10-folds, and 100 µL of the diluted sample was spreaded over an enrichment medium by adding 5 g of sludge to a conical flask (250 mL) containing 100 mL MSM (minimal salt medium) supplemented with lignin and cellulose 0.2% for enhancing growth. The plates were incubated at 37^0C for 48 hours and the total colony count was determined. Colonies were screened and identification by colony morphology, gram staining, microscopic observation, confirmed with the help of Bergey's manual of systematic bacteriology [10].

Effluent treatment

The mother inoculum was prepared by inoculating one loopful of the four selected strains of individual bacterial isolates separately in 25 mL sterilized nutrient broth. The inoculated broths were incubated in an orbital shaker 200 rpm at 35^0C for 16–24 hours, OD was constantly monitored to reach 1.2. The cultures were centrifuged at 10000 rpm for 10 min at 4°C to collect the pellet. The pellets were mixed in combination *Pseudomonas alkaligenes* + *Enterobacter* spp. and *Citrobacter freundii* + *Bacillus subtilis* in a 250mL of flasks containing 100mL of effluent and incubated in shaker at 200 rpm at 35^0C.

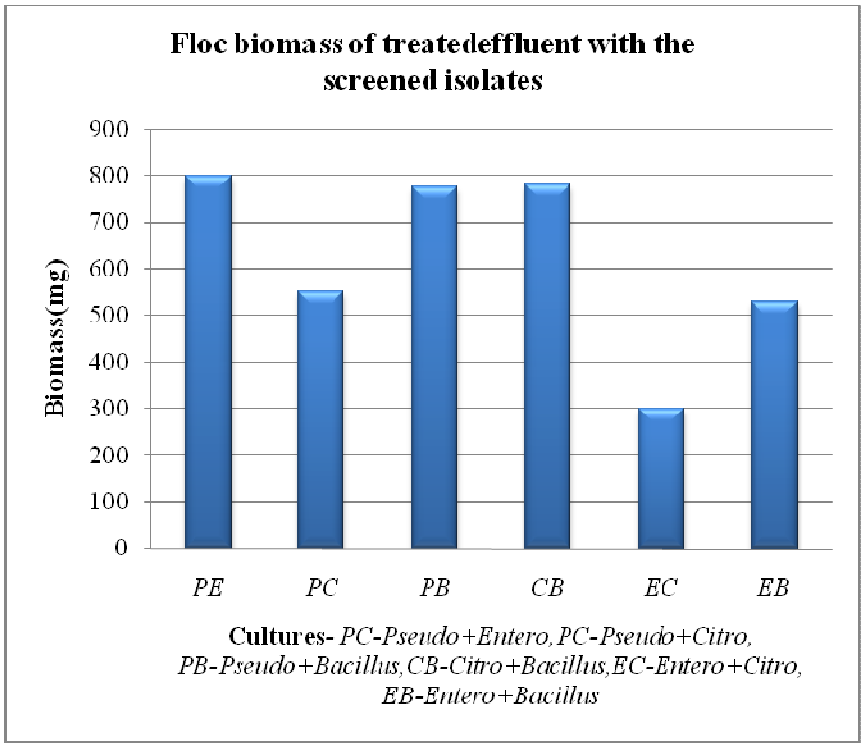
RESULTS AND DISCUSSION

The investigation of the paper mill effluent showed the presence of seven prominent bacterial species *Bacillus, Pseudomonas, Enterobacter* spp., *Bacillus subtilis Citrobacter freundii, Alcaligenes* and *Burkholderia*. The isolated bacterial colonies were diverse in their morphologies ranging from small pinpointed to large sized colonies with fluorescent to pale whitish in color, flat to umbonate, and smooth margined to wrinkle marginal periphery. These isolated bacterial colonies ranged between 0.9 x 10^{-2} to 6.0 X10^{-6} cfu/mL and indicates a dense population of bacteria in the paper mill effluent. The bacterias were selected out of 36 isolates; on the basis of their fast growth on the medium containing carbon source of cellulose and lignin. Among them, *Pseudomonas* spp. with 6.0 X10^{-6} cfu/mL was found to be the maximum percentage present (26%), suggesting its dominance and adaptation to the paper mill environment. Effective floc formation with all four strains the possible permutation and combinations that enhanced settling process was better attained in *Pseudomonas alkaligenes* + *Enterobacter* spp. combination followed by *Citrobacter freundii*+ *Bacillus subtilis*. *Paecilomyces* spp. and *Trichoderma* were the fungal isolates identified from the effluent. On testing all the isolates showed varied resistance and sensitivity property for cephalosporins, quinolone and aminoglycoside antibiotics. Out of 36 isolates, 70.7% were found sensitive against ampicillin, kanamycin, gentamicin, norfloxacin, nalidixic, polymyxin, erythromycin, tetracycline, and ciprofloxacin antibiotics. The screening of bacterial resistance property towards different classes of antibiotics showed maximum sensitivity for aminoglycoside class of antibiotics followed by cephalosporins.

Pseudomonas alkaligenes occupies the maximum percentage among the bacterial species, and it is hypothesized that low concentrations of available organic carbon in water distribution systems favors the growth of bacteria particularly belonging to Pseudomonadaceae family [11]. All the isolates, except few *Citrobacter* spp. were found sensitive against ciprofloxacin; similar findings were also reported [12]. Multiple antibiotic resistances due to pollutants have been previously reported [13] but in this study we have not encountered very high resistance pattern. Drug and a wide range of toxic agents provoke many biochemical changes in cells that allow them to overcome the toxic effects of either the same or other chemical compounds [14]. Plasmid conferring resistance may also play important role in transferring resistance to more than one drug [15]. Cell-wall modification and bio precipitation are other mechanisms employed by the bacterial cells to reduce the toxic effect [16]. Study of [17] revealed that the effect of various stress conditions resulting in the induction of outer membrane protein (OMP) in *P. aeruginosa*. The resistance mechanism of Gram negative bacteria involves the alternation of membrane permeability that results in the adsorption of metals which can attack the lipopolysaccharide layer of outer membrane of these bacteria.

Resistance mechanism among bacteria can be proposed that high level of toxic compounds generally exerts a selective pressure on microorganism that may result in appearing variants possessing resistant property [18]. The study is also supported that not only heavy metals but presence of pollutants either sewage, environmental or industrial may be responsible for multiple drug resistance patterns of bacteria [17]. In addition proteins also play important role in resistance mechanism towards heavy metal or any other stress environment [19]. A number of research studies have discovered that a group of extracellular isoenzymes lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and lactase produced by some microorganisms are capable of degrading lignin present in the paper and pulp mill effluent. The ability of these bacteria's to remove color, pollutants and COD from paper mill effluents was evaluated [20]. Biological methods are of particular interest because they can also reduce chemical and biological demands (COD, BOD), which are also the significant problem in pulp wastewater and reduce holding times in aeration and sedimentation tanks prior to wastewater discharge into the environment [21,22] Since chemical oxidation/precipitation methods are tedious, providing an additional environmental load biological method are often preferred since it has many advantages like rapid biodegradation rates, low sludge yield and excellent process stability. Our attempt in screening the predominant isolate and treating the effluent in pilot scale was successful and needs to be standardized in large scale.

Figure: 1



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