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Comparative study on biodegradation of lipid-rich wastewater using lipase producing bacterial species

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Studies on bioremediation of high fat and oil wastewater by selected lipase producing bacteria like *Bacillus subtilis*, *B licheniformis*, *B amyloliquefaciens*, *Serratia marsescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were carried out in wastewaters emanating from palm oil mill, dairy, slaughter house, soap industry and domestic wastewater with both individual and mixed culture (consortia). BOD and lipase degradation was analyzed for 12 d. After 12 d of bioremediation, least BOD and lipid content was observed in consortia. Among the six isolates, *P. aeruginosa* showed least BOD (112 mg/L) in palm oil effluent, (82 mg/L) dairy effluent, (145 mg/L) soap and (9 mg/L) domestic water effluent, whereas *S. aureus* showed least BOD (11 mg/L) in slaughter house wastewater. Lipid content was also reduced most by consortia after 12 d of bioremediation. *P. aeruginosa* resulted in very good lipid degradation in palm oil effluent (325 mg/L), soap effluent (300 mg/L) and domestic wastewater (17 mg/L), whereas *S. marsescens* showed good lipid degradation in dairy effluents (280 mg/L) and *S. aureus* in slaughter house wastewater (320 mg/L).

Keywords: Bioremediation, consortia, *Bacillus subtilis, Serratia marsescens, Pseudomonas aeruginosa and Staphylococcus aureus*

Introduction

Lipids (fats, oils and greases) are major organic matters in municipal and some industrial wastewater and can cause severe environmental pollution. Wastewater produced from edible oil refinery, slaughter house, wool scouring and dairy products industry contains a high (> 100 mg/L) concentration of lipids[1,2]. High concentration of these compounds in wastewater often causes major problems in biological wastewater treatment processes. Because of their nature they form a layer on water surfaces and decrease oxygen transfer rate into the aerobic process[3]. Bioremediation of lipid-rich wastes, either aerobically or anaerobically, have been investigated[4,5]. Industrial scale extraction of lipases is carried out in bacteria, fungi, actinomycetes and cultures of plant and animal cells. Among them, microbial lipases are metabolically versatile and hence have advantage in many industrial processes[6,7]. Enzymatic treatment technique has gained more attention because of stringent environmental regulations and clean and friendly applications of enzymes. Lipases are serine hydrolases of considerable physiological significance and industrial potential that can catalyze numerous reactions such as hydrolysis, interesterification, esterification, alcoholysis and aminolysis[8,9]. *Pseudomonas aeruginosa* LP_{602} (a lipase-producing strain isolated from restaurant wastewater) showed good potential for use in treatment of wastewater from a catering unit containing high lipid content[10]. The purpose of the present study was to evaluate the potency of individual bacteria and mixed culture to reduce the biological oxygen demand (BOD) and lipid content from five different lipid-rich wastewater sources.

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Materials and Methods

Isolation of Lipolytic Microbes

For the present study, fat and oil rich wastewater samples were collected from palm oil mill, dairy industry, slaughter house, soap industry and domestic water in sterile containers. For the isolation of lipolytic microbes, 1.0 g of soil sample collected from different sites like oil mill, domestic dump site and dairy was stirred in 100 mL of double distilled water. The serially diluted (10^{-1} to 10^{-6}) samples were plated on tributyrin agar plates. The formation of clear zone around the colony on the plate was considered as lipolytic microbes.

Identification

Microbes which formed large clear zone around the colony were identified based on morphological, biochemical and physiological characters according to Bergey's manual of determinative bacteriology[11]. These were identified as *Bacillus subtilis, B. licheniformis*, *B. amyloliquefaciens*, *Serratia marsescens, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Pure cultures of these organisms were maintained on nutrient agar slant supplemented with 1% olive oil.

Biodegradation of various effluents by isolated bacterial strains was carried out for 12 d, BOD value and lipid content was analyzed on alternative days.

Wastewater Treatment

The wastewater sample (5 L) taken in a 10 L container was inoculated with a 1% (v/v) bacterial culture (O.D. at 600~2). After vigorous shaking, it was divided into 12 portions of 250 mL each in 500 mL flasks and rest of the samples were divided into two portions of 1 L each in 2 L flasks. All the cultures were incubated at 30°C at 200 rpm. Samples were taken at regular intervals of 48 h from 2 L flasks for BOD analysis and from two 500 mL flasks for the determination of lipid content.

Determination of BOD

For the determination of BOD procedure of Helrich[12] was followed. BOD bottle containing 300 mL diluted sample of sterile distilled water was taken, sterile air was blown for 10 min and incubated in dark at 20°C for 5 d prior to test. 2 mL of $MnSO_4$ and 2 mL of alkaline iodine-sodium azide solution was added to each BOD bottle. Stoppers were placed and air bubbles expelled by inverting bottles several times. Bottles were then left for precipitation. 2 mL of H_2SO_4 was added and mixed by inverting the bottles until iodine was uniformly distributed. Starch indicator (2-3 drops) was added to 2 mL sample and then titrated with 0.025 N $Na_2S_2O_3$ until blue colour disappeared. Volume of $Na_2S_2O_3$ was used to calculate BOD (Fig. 1).

Determination of Lipid Content

Lipid content was determined using partition-gravimetric method of Kirschman and Pomeroy[13]. 250 mL sample was taken and acidified with 1:1 diluted HCl up to *p*H 2.0. Lipid was extracted repeatedly with 30 mL portions of 1, 1, 2-trichloro-trifluoroethane (Freon) until the aqueous phase showed no oil layer and the solvent phase became clear. All the solvent extracts were combined and evaporated at 70°C until dried. The dry weight obtained indicated the amount of oil and grease present in the sample.

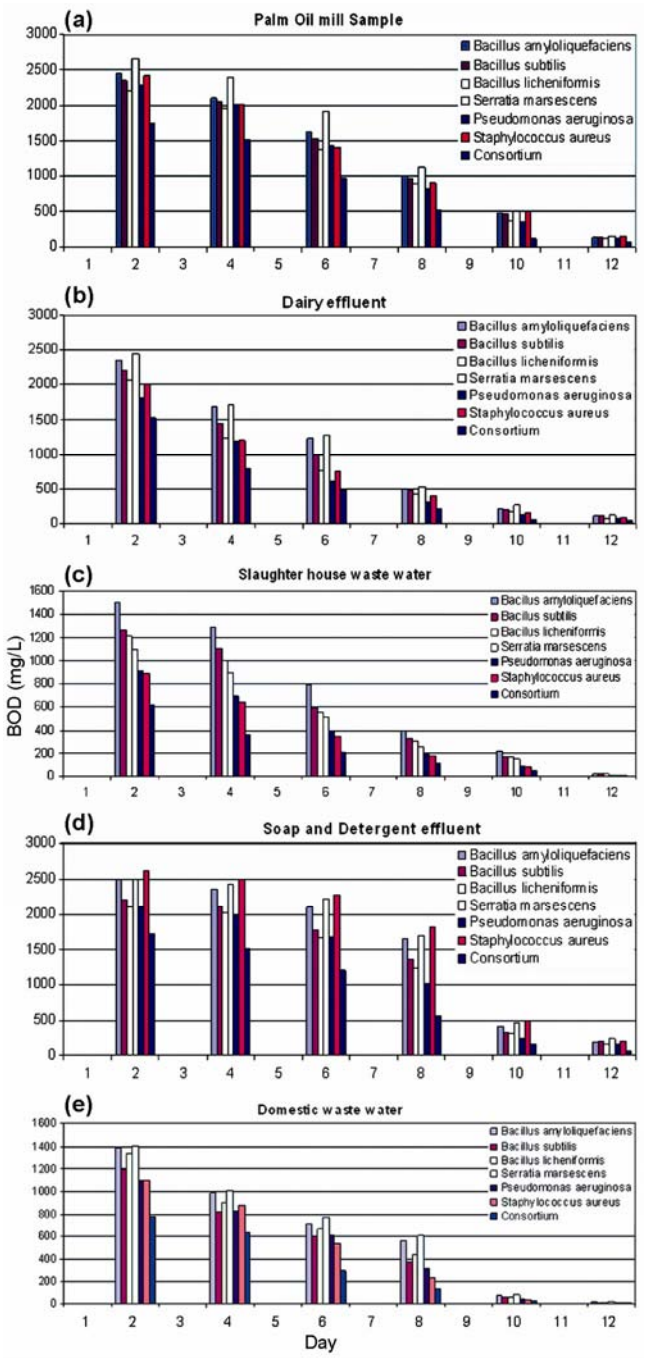

Fig. 1—BOD values for different effluents at different incubation periods: a. palm oil mill effluent; b. dairy effluent; c slaughter house wastewater; d. soap and detergent effluent; & e.domestic wastewater.

Results and Discussion

In the present study, 25 bacterial colonies were isolated which resulted in large clear zones on tributyrin agar media. All the isolates were then cultured individually on tributyrin agar plates and further hydrolysis was analyzed. Eight isolates with sharp clear zones were identified as *B. subtilis, B. licheniformis*, *B. amyloliquefaciens*, *S. marsescens, P. aeruginosa* and *S. aureus*.

Untreated effluents such as palm oil mill effluents, dairy effluent, slaughter house wastewater, soap effluent and domestic water rich in fats and oils were treated with individual organisms and their mixture (consortia). Wastewater samples showed an average *p*H of 5, average BOD value of 3200 mg/L and average lipid content of 25,000 mg/L before the treatment. After treating with individual bacteria and the consortia, the amount of lipid content present in the samples was reduced based on the degrading capacity of the organism (Fig. 2a-e). According to Wiyada and Saovanee[14] similar properties of kitchen wastewater with an average *p*H of 5.2, the reducing sugar content 719 mg/L, average BOD 3600 mg/L and the average lipid content 21,000 mg/L were determined.

Different degradation efficiencies might be due to different reaction systems of lipase from each culture as indicated by the results of fat and oil by mixed culture (consortia) and individual cultures in the two experiments. Lipases present not only catalyzed hydrolysis reaction but also catalyzed interesterification reaction, depending on the source of lipase and reaction condition. Saifuddin and Chua[15] used a combination of microwave irradiation for emulsion breaking and biodegradation of oil by enzymatic method, this methodology was found to be inapplicable to bulk treatment.

The use of the combined culture of *P. aeruginosa* LP_{602} and *B. subtilis* B_{304} could not reduce the BOD and the lipid content in the wastewater to an acceptable level for environmental release. Thus, addition of a third strain of lipase-producing bacteria, *Acinetobacter calcoaceticus* LP_{009} was tried to facilitate the bioremediation process[16].

In this study, *P. aeruginosa* showed very good reduction of BOD value from the day one in palm oil effluent, soap effluent, dairy effluent and domestic water effluent. *S. aureus* resulted in low BOD value in slaughter house wastewater than other organisms. Mixture of all the organisms showed maximum activity with very low BOD value compared to individual bacterial treatment of the wastewater.

A mixed bacterial culture comprising *P. aeruginosa* LP_{602}, *Bacillus* sp. B_{304} and *Acinetobacter calcoaceticus* LP_{009} for use in treatment of lipid-rich wastewater was formulated. In our tests, 11 samples

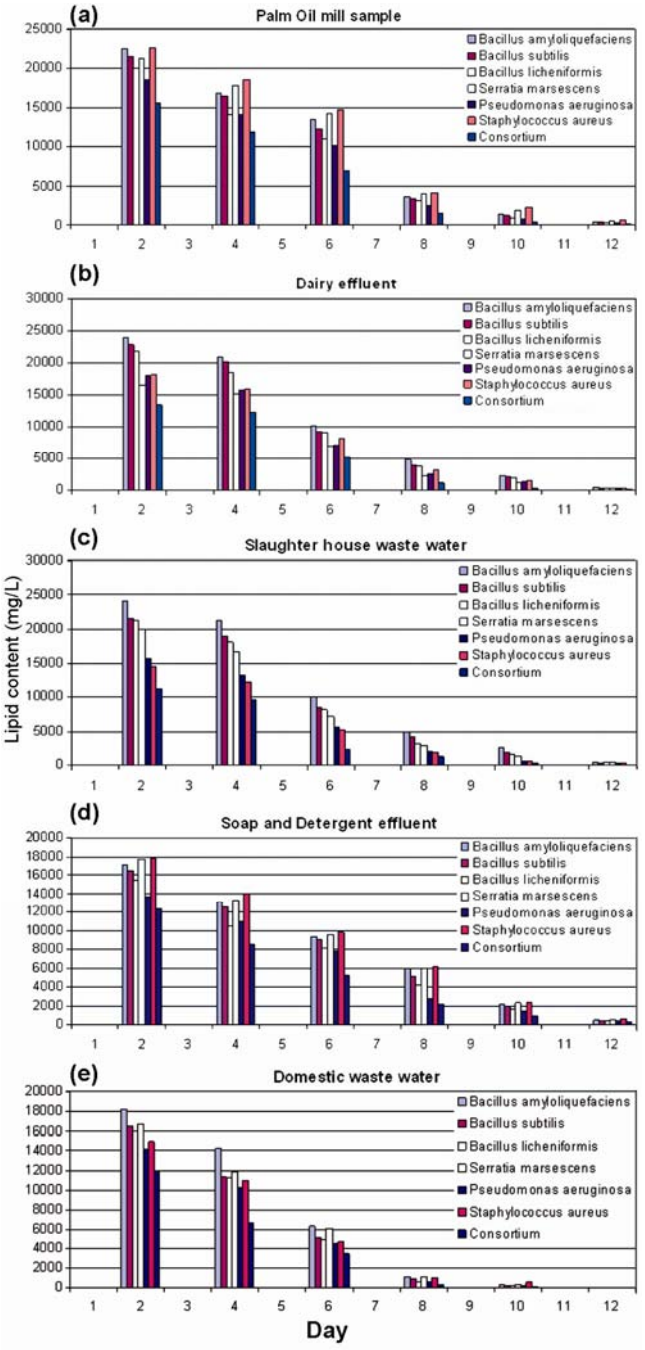

Fig. 2—Determination of lipid content in different effluents: a. palm oil mill effluent; b. dairy effluent; c. slaughter house waste; d. soap and detergent effluent; & e. domestic wastewater.

of wastewater were treated with a mixed inoculum of 6.1×10^8 CFU of LP_{602}, 4.8×10^8 CFU B_{304} and 8.9×10^7 CFU of LP_{009} in 20 ml of basal salt solution. The BOD value and lipid content were reduced from ~3500 and 20, 000 mg/L, respectively, to < 20 mg/L within 12 d under aerobic conditions. *P. aeruginosa* LP_{602}, a lipase-producing bacterium, which was shown to have high potential for use in lipid-rich wastewater treatment, was tested for its ability to treat the kitchen wastewater sample. In this study, *S. marsescens* showed good lipid degradation in dairy effluent, *S. aureus* in slaughter house wastewater and *P. aeruginosa* in palm oil effluent, soap effluent, and domestic water effluent. Mixed culture resulted in better degradation after 12 d of incubation under aerobic conditions.

Wastewater from swine and bovine meat industries with high content of oil and grease was treated in batch anaerobic reactors with and without an enzymatic pretreatment. When the wastewater containing 1,200 mg L^{-1} of oil and grease was pretreated with the lipase preparation obtained by SSF, removal of chemical oxygen demand (COD) increased 22%, compared to a control reactor fed with wastewater without any treatment[17].

Conclusion

In this study, a bacterial consortium comprising *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *S. marsescens, P. aeruginosa* and *S. aureus* for use in treatment of wastewater or industrial effluent rich in lipid content was formulated. The role of lipid degradation capacity for *P. aeruginosa* is high compared to other bacteria. The formulated mixed culture was found to be effective in treatment of lipid-rich wastewater. The average BOD value was reduced from 3200 mg/L to less than 40 mg/L and lipid content was reduced from 25,000 mg/L to ~80 mg/L, respectively within 12 d of incubation.

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