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Biodegradation of automobile service station wastewater

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ABSTRACT

Oil and greases are released from automobile service stations into terrestrial and aquatic environment and they severely damages the surrounding ecosystems. In this research, the initial characteristics of automobile wastewater were analyzed and *Pseudomonas aeruginosa* was isolated and inoculated into wastewater, whereas 50% of oil degradation was observed under the optimized growth conditions at pH 7 of 48 h. Addition of optimized dosage of 0.2 mL glycerol as a carbon source and 0.1 mL surfactant results in oil degradation of 72 and 61%, respectively. A combination of carbon source and surfactant was found to be the most effective treatment, leading to 93% of oil degradation. Further, treated wastewater was subjected to conventional sequential batch reactor, best effluent quality was obtained at 10 h of hydraulic retention and 10 days of solid retention resulting in COD = 100 mg/L and TSS = 85 mg/L, which meets the requirements of the discharge standard.

Keywords: Biodegradation; Automobile service station wastewater; *Pseudomonas aeruginosa*; Carbon source; Surfactant

1. Introduction

Oil released into the environment is a well recognized problem in today's world. Oil contaminated water affect many species of plants and animals in the environment, as well as humans. Automobile workshops are an important component of the service sector industry. The most significant environmental impact associated with the existing workshops is the seepage of used engine oil and washed water into the soil. Contamination of the soil by oil causes it to lose its useful properties, such as fertility, water-holding capacity, permeability, and binding capacity [1].

Wastewater from service stations threatens surface water resources. High amounts of biochemical oxygen demand (BOD) cause dissolved oxygen (DO) depletion in the receiving stream. Oil and grease can coat the fish gills and take to death, toxic hydrocarbons are fatal to the aquatic life and humans, and grease can cause loss of hydraulic capacity of sewers and fouling of wastewater treatment plant. Suspended solids cause turbidity in water, impair photosynthesis, clog the fish gills, and damage their productivity. The removal of oil by conventional treatment methods, such as skimming, filtration, gravity separation, adsorption, coagulation, electro coagulation, advanced oxidation process, etc., have several disadvantages, such as high energy consumption and increasing cost of the process. Physical methods, such as skimming and adsorption just transfer the contaminant from one phase to another and thus lead to the existence of contaminant in the environment itself. Filtration leads to membrane fouling, gravity separation leads to large residence time and

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complicated hydraulic design. Chemical methods such as coagulation, electro coagulation involve the application of a variety of chemicals that enhance the phase separation which may need subsequent processing steps to remove the additives [2]. So far, biodegradation suggests an effective and economic method.

Biodegradation is a natural process by which microbes alter and breakdown oil into other substances. The resulting products can be carbon dioxide, water, and simpler compounds that do not affect the environment. Biodegradation efficiency depends on micro-organisms, capability of producing enzymes and that will degrade the target compounds. Factor such as temperature, pH, and nutrient status are of importance as moderators. Many species of micro-organisms, such as bacteria, yeasts, and fungi obtain both energy and tissue building material from petroleum. Petroleum is a complex mixture of many thousands of compounds. These can be divided into four major groups: the alkanes, the aromatics, the resins, and the asphaltenes. In general, the alkane fraction is the most biodegradable, whereas the polar fraction (i.e. the resins and asphaltenes) is resistant to biological degradation. The aromatic compounds, especially the polycyclic aromatic hydrocarbons are of intermediate biodegradability, but these are of most concern owing to their toxicity and tendency to bioaccumulate [3].

The bioavailability of weakly soluble hydrophobic compounds for microbial conversion is usually low and thus limits their degradation rate in aqueous medium. The use of surfactants has been found to enhance degradation of crude oil, but the degradation rates have depended on the chemical structure [4]. Among various surfactants, rhamnolipids are considered to be the most efficient in degrading hydrocarbons [5]. The biodegradation of long-chain alkanes was stimulated by the addition of rhamnolipid [6]. This facilitated biodegradation is probably due to the increase of cell surface hydrophobicity after extraction of lipopolysaccharides from the cellular envelope by rhamnolipids, which subsequently stimulates uptake via direct contact between cells and hydrocarbon droplets. Thus, the interaction between addition of rhamnolipid and biodegradation of hydrocarbons seems to be highly specific. *Pseudomonas aeruginosa* exhibited more degradative effect than other species, such as *Bacillus subtillus*, *Micrococcus luteus* etc., [7].

P. aeruginosa could degrade most of crude oil with direct or indirect addition of rhamnolipid. *P. aeruginosa* is a typical strain for rhamnolipid production and can utilize vegetable oil or glycerol as the sole carbon source. Rhamnolipid production by glycerol is much higher than that of other substrates including glucose, vegetable oil, babassu oil, n-hexadecane, and liquid paraffin [8,9]. In this research, the oil degradation in automobile service station wastewater was studied using *P. aeruginosa* along with effect of pH, incubation time, carbon source, and surfactant.

2. Materials and methods

2.1. Isolation of oil degrading bacteria

The oil degrading bacteria *P. aeruginosa* was isolated from automobile wastewater, which was collected from the disposal site of an automobile service station, in Tirunelveli, Tamil Nadu, India. The method of serial dilutions of the sample in selective medium cetrimide agar was used for isolation purposes. The plates were incubated at 30°C for 24 h. The well grown colonies were picked up and stored at 4°C. The purified bacterial cultures were identified based on Gram staining and motility [10].

2.2. 16S rRNA sequencing

Isolates are sub cultured on agar plate. A single colony was obtained from a fresh subculture and resuspended in master mixture. A 380-bp fragment of the 16S rRNA (universal primer) used as the target. For 50 µl reaction, 1 µl of 200 mM dNTPs, 5 µl of Reaction buffer (Tris with $MgCl_2$) 10 pM forward primer RW01 (FP) 5´AACTGGAGGAAGGTGGGGAT-3´, 10 pM reverse primer DG74 (RP) 5´AGGAGGTGATCCAACCGCA-3´, 1.2 u/µl of Taq Polymerase were used, amplification was carried out in Thermal Cycler PTC 200 (MJ Research), and a standard three-step protocol was used for all reactions, including 35 cycles for each reaction with an annealing temperature of 55 °C and 10 min extension time. The amplified product purified by Chromous Biotech, Bangalore, India. Cyclic PCR carried out using Big Dye Terminator Ver 3.1. Each purified template sequenced on both strands, reaction done by Genetic Analyzer 3130 (ABI System). The DNA sequences obtained were compared with those in the GEN BANK database using the blast server (Basic local alignment search tool) hosted by national center for biotechnology information (NCBI, Bethesda, MD, USA). The BLAST program is available from the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/).

2.3. Bacterial growth optimization

The bacterial growth was optimized by varying the parameters pH and temperature. The composition of the mineral salt medium (MSM) used in this study was as follows (g/L): $NaNO_3$ 4.0, NaCl 1.0, KCl 1.0,

$CaCl_2{\cdot}2H_2O$ 0.1, KH_2PO_4 3.0, $Na_2HPO_4{\cdot}12H_2O$ 3.0, $MgSO_4$ 0.2, $FeSO_4{\cdot}7H_2O$ 0.001; 2 ml trace element stock solution composed of (g/L): $FeCl_3{\cdot}6H_2O$ 0.08, $ZnSO_4{\cdot}7H_2O$ 0.75, $CoCl_2{\cdot}6H_2O$ 0.08, $CuSO_4{\cdot}5H_2O$ 0.075, $MnSO_4{\cdot}H_2O$ 0.75, H_3BO_3 0.15, $Na_2MoO_4{\cdot}2H_2O$ 0.05. The initial pH was adjusted to 7. The bacterial culture (24 h) was inoculated MSM with 1% (v/v) engine oil as carbon source. The control sample was devoid of inoculums. They were kept in a shaker at 200 rpm at 30°C for 3 days. The growth was monitored through culture densities, measuring absorbance spectrophotometrically at 620 nm wavelength.

2.4. Characteristics of automobile service station wastewater

The untreated and bacterially degraded automobile service station wastewater was sampled and analyzed every 12 h. The bacterial biomass was separated by centrifugation at 7,000 rpm for 20 min prior to analysis for pH, total dissolved and suspended solids, hardness, BOD, chemical oxygen demand (COD), oil, and grease. The analysis for various parameters was done according to standard methods [11].

2.5. Laboratory scale biodegradation studies

Automobile wastewater (100 mL) was sampled in 250 mL conical flasks and then following experimental setups were followed: (1) First four flasks served as a control. (2) 5–8 flasks were treated with 1.0 mL of bacterial inoculums. (3) 9–12 flasks were treated with 0.2 mL glycerol. Glycerol served as a carbon source for the microbial inoculums. (4) 13–16 flasks were treated with bacteria along with 0. 2 mL of glycerol. (5) 17–20 flasks were treated with 0.1 mL of surfactant (Tween 80). (6) 21–24 flasks were treated with surfactant and bacteria. (7) 25–28 flasks were treated with the combination of glycerol (0.2 mL) and surfactant (0.1 mL) (8) 29–32 flasks were amended with both glycerol (0.2 mL) and surfactant (0.1 mL) along with bacteria (1.0 mL). The above flasks were tightly cotton plugged in order to avoid evaporation. The flasks were incubated for 48 h under room temperature. Results were recorded from each flask every 12 h up to 48 h.

2.6. Bench-scale biodegradation studies

The biological treatment for oil degradation was conducted in plexiglass reactor with 21.5 cm × 21.5 cm × 15 cm. Five liters of automobile service station wastewater along with optimized nutrient conditions were applied in the suspended growth batch reactor. An air compressor delivering airflow of 3.2 L/min was used to supply oxygen through porous diffuser stones. The studies were conducted at room temperature (30–35°C).

2.7. Aerobic sequential batch reactor (SBR)

The main biological treatment was conducted in plexiglass reactor with the size of 21.5 cm × 21.5 cm × 15 cm. The reactor was equipped with an air pump and air diffuser to keep dissolved oxygen above 3 mg/L. Two identical reactors with a total working volume of 7 L were operated in parallel with FILL, REACT, SETTLE, and DRAW periods in the ratio of 1:10:0.5:0.5 to constitute a cycle time of 12 h. The SBR was inoculated with aerobic sludge from domestic wastewater treatment plant. Concentration of biomass in the reactor after inoculation was 4,000 mg/L. Mixed Liquor Suspended Solids concentration and COD was analyzed as per standard methods (APHA 2005) [11].

3. Results and discussion

Automobile wastewater was collected from the disposal site of automobile service station, Tirunelveli, Tamil Nadu, India and analyzed for pH, total solids, total dissolved and suspended solids, total hardness, BOD, COD, oil, and greases. The oil degrading bacteria *P. aeruginosa* was isolated from the automobile wastewater and the bacterial growth was determined by optimizing pH, temperature, and incubation time. At optimized conditions, the oil degradation was carried out with this micro-organism. To enhance the oil degradation, carbon source and surfactant were added.

3.1. Characteristics of automobile wastewater

Table 1 shows the initial characterization of automobile wastewater. Oil and grease concentration was about 121 mg/L. The automobile wastewater is reported to have oil and grease concentration from 27 to 140 mg/L [12].

3.2. Isolation and identification of oil degrading bacteria

An oil degrading bacteria was isolated from automobile wastewater. It was observed that the colonies were small, smooth, and yellowish white in color, and not in bluish green color because the soluble blue pigment pyocyanin is produced by many, but not all, strains of *Pseudomonas*. It is a rod shaped, gram negative bacteria observed in gram's staining test. From motility test it was observed that the cells were motile. DNA homology analysis of the 16S rRNA regions of isolated colonies of bacterial culture indicated that

Table 1
Initial characterization of automobile service station wastewater

Parameters	Results
pH	7.4
Total dissolved solids (mg/L)	3,536
Total suspended solids (mg/L)	2,453
BOD (mg/L)	68
COD (mg/L)	752
Oil and grease (mg/L)	121

the bacteria displayed the greatest similarity with *P. aeruginosa* (97%), ATCC 27853, genome accession number AE004091. Crude petroleum oil degradation efficiency of *P. aeruginosa* strain isolated from North East India has been reported by Das and Mukherjee [13]. *P. aeruginosa* has a capacity to produce the glycolipid emulsifier which reduces the surface tension of oil water interface and thus helps in its removal [7]. Cetrimide agar medium was used to isolate *P. aeruginosa* from wastewater collected from oil wells in Northeastern of Brazil [14].

3.3. Bacterial growth optimization

Bacterial growth was determined by optimizing pH and incubation time. The optimization of environmental conditions is very important for the enhancement of bacterial growth and for designing effective biodegradation strategy. Thus, the population of microbial growth was necessary to improve the biodegradation of oil.

3.3.1. Effect of time

The growth of oil degrading bacteria is observed at different time intervals in MSM using oil as a carbon source. It was observed that the minimum growth was occurred at 12 h of incubation due to lag phase of microbial growth. Biomass concentration increases as time increases and reaches the maximum growth at 48 h. The stationary phase was reached after 48 h. After stationary phase, microbe migrates to death phase due to lack of nutrients. Hence, the optimized incubation time was found to be 48 h. The above result was supported by Rosa and his coworkers [15] whose work about *P. aeruginosa* showed that the growth rate decreases after reaching the stationary phase. The pattern of microbial growth differs from organism to organism. The factors that determine this pattern of growth include the incubation period, the nature and composition of the nutrient in which the organism is growing. Stanbury and Whitaker [16] highlighted that different organisms have different incubation periods, which range from minutes to several hours.

3.3.2. Effect of pH

Maintenance of pH is important, since pH strongly affects bacterial growth. A change in pH is largely due to the production and accumulation of waste products. In order to study the effect of pH, the growth of oil degrading bacteria was observed at various pH 5–9. Fig. 1 shows that the optimal growth of bacteria was occurred at pH 7. The requirement of neutral or near neutrality for optimal growth of bacteria on oil is also exhibited by many other oil degrading bacteria [17–20].

3.4. Effect of P. aeruginosa on oil degradation

P. aeruginosa has the capacity to produce the glycolipid emulsifier which reduces the surface tension of oil water interface and thus help in its removal [7]. The biodegradation of oil and grease by *P. aeruginosa* was investigated. The initial concentration of oil and grease is 121 mg/L. The results are shown in Fig. 2. It was observed that the oil and grease concentration was decreased from 121 to 61 mg/L (50% reduction) at 48 h which demonstrated that the most of oil mineralized into CO_2 and H_2O. This result was supported by Bako et al. [21] who reported that 52% of oil was reduced using *P. aeruginosa*.

3.5. Enhancement of biodegradation

The inoculums of *P. aeruginosa* degraded the automobile oil effluent. However, oil is composed only of

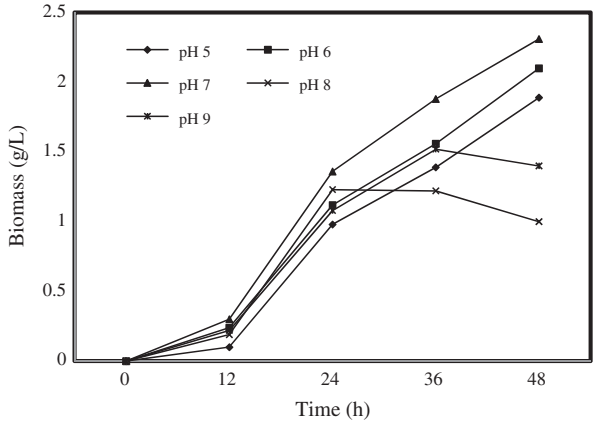

Fig. 1. Effect of pH on bacterial growth.

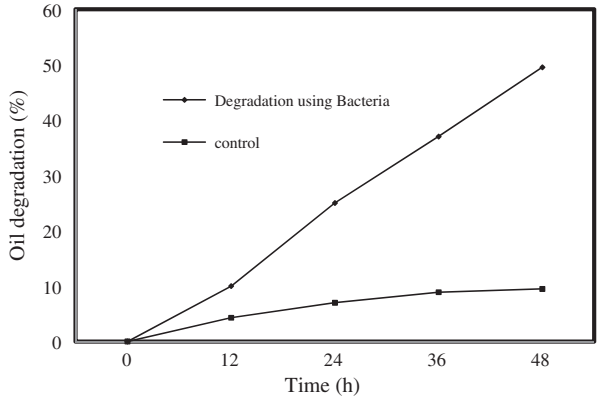

Fig. 2. Effect of *P. aeruginosa* on oil degradation.

hydrogen and carbon, and the bacteria need additional nutrients to grow for fast degradation rate [1]. To enhance the oil degradation, carbon source and surfactants dosage were optimized and added into the automobile wastewater.

3.5.1. Effect of carbon source

Carbon sources have the capability to increase the production of biosurfactant. The use of glycerol as a carbon source to produce biosurfactant seems to be interesting and at a low cost [8]. Glycerol concentration varied between 0.1 and 0.7 mL. From Fig. 3 it is observed that the maximum oil degradation of 80.56% was obtained with the carbon source dosage of 0.2 mL. It was observed that a higher amount of glycerol inhibits the growth of micro-organisms. A minimum oil degradation of 25% was observed with 0.1 mL of glycerol dosage: minimum oil degradation of oil may occur due to a lack of nutrients. It was supported by Santa et al. [14] who reported that the high

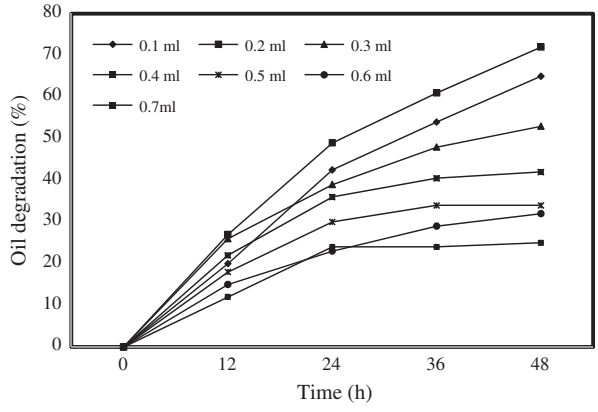

Fig. 3. Effect of glycerol on oil degradation.

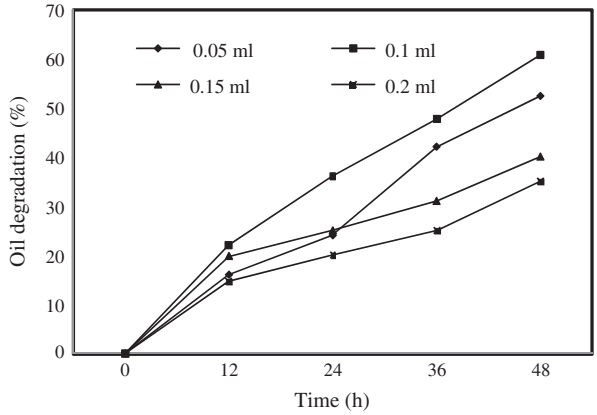

Fig. 4. Effect of surfactant on oil degradation.

amount of glycerol may inhibit the growth of bacteria. The inhibitory effect was ascribed to problems linked to the solubility of glycerol and the difficulty of the bacterium to gain access to the nutrients in the culture medium [8].

Glycerol is needed as a carbon source but it can be toxic to micro-organisms due to the solvent effects of carbon source that could destroy the bacterial cell membrane. Hence, many biodegradation studies on oil are carried out using lesser carbon dosage concentration from 1 to 6% (v/v) [15,18,20,22,23]. Lee et al. [17] and Santa et al. [14] reported that the degradation is generally unfavorable at carbon concentrations higher than 3% (v/v). Several studies have been carried out to define the best carbon source dosage need to obtain high bacterial growth [24].

3.5.2. Effect of surfactant dosage

Micro-organisms growing on petroleum usually produce potent emulsifiers and these surfactants help

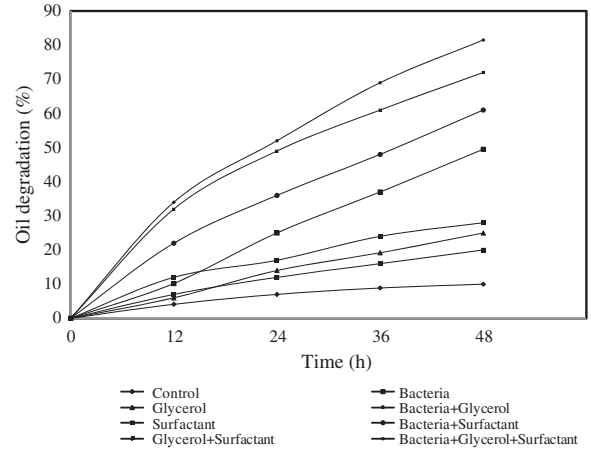

Fig. 5. Effect of carbon source and surfactant on biodegradation of oil.

Table 2
Final characteristics of automobile service station wastewater

Parameters	Biodegradation with *P. aeruginosa*	Conventional SBR	Disposal standard
pH	6.8	7.0	6.5–9.5
Total dissolved solids (mg/L)	2,545	65	100
Total suspended solids (mg/L)	1,692	85	100
BOD (mg/L)	250	10	20
COD (mg/L)	586	100	250
Oil and grease (mg/L)	9	9	10

to degrade petroleum. The surfactants produce the micellization process when they are in contact with water and a non polar solute. The surfactants molecules seek to arrange in minimize the repulsion between hydrophobic groups and water. In this process, the surfactant molecules group around the oil by their nonpolar extremities, while their polar extremities interact with the water. Therefore, the oil becomes more susceptible to biodegradation in facilitating the oil–micro-organisms interaction [25].

The surfactant dose was varied from 0.05 to 2 mL. From Fig. 4 it is observed that the maximum oil degradation was obtained as 0.1 mL. It shows that the oil degradation was increased by the addition of surfactant. Similarly, several researches have reported that the use of surfactants that have enhanced degradation of oil [26,27]. In our study, the degradation at Tween-80 decreased at higher dosage compared to a lower dosage. Some researchers reported that synthetic surfactants were effective on biodegradation of diesel oil and cell growth was inhibited at high concentrations of Tween 80 [18]. These reports have confirmed our results. Finally, these results have shown that the surfactants were able to stimulate the biodegradation of crude oil, but the degradation rates have depended on the chemical structure.

3.6. Biodegradation of oil with the effect of carbon source and surfactant

Microbes which degrade oil and grease produce distinct extra cellular enzymes and bioemulisfiers. Upon the action of the emulsifiers, the lipids are made easily available to the action of the extracellular enzymes [28]. From Fig. 5 it is observed that there is very less amount of oil degradation, 10% in control. 50% degradation was observed in the treatment of automobile service station wastewater with bacteria. Addition of optimized dosage of carbon source and surfactant were found to be of maximum degradation of 72 and 61%, respectively. In the combination of glycerol and surfactant showed the maximum degradation of 82%. Oil degradation was found to be most effective due to the combined effect of carbon source and surfactant. Sathiyamoorthy et al. [1] reported 82% of oil degradation using *Pseudomonas* spp. with the effect of surfactant. The nutrients provide several essential minerals and surfactants also contribute for oil degradation.

3.7. Treatability studies on biodegradation of automobile service station wastewater

The automobile service station wastewater was treated in a bench scale aerobic batch reactor with the addition of glycerol and surfactant along with bacterial inoculums. The biodegradation of oil was found to be 93%. Further treated automobile service station wastewater subjected to biological treatment in a laboratory scale aerobic sequential batch reactor in order to achieve disposal standard. The effects of important process variables, such as hydraulic retention time and solids retention time on COD removal performance of the system were investigated. The effluent COD decreased with increasing hydraulic and solids retention times. The best effluent quality was obtained at 10 h of hydraulic retention and 10 days of solid retention resulting in COD = 100 mg/L and TSS = 85 mg/L, which meets the requirements of the discharge standard. Table 2 shows the final characteristics of treated automobile service station wastewater.

4. Conclusion

The strain *P. aeruginosa* was isolated from a sample of automobile service station wastewater. Maximum growth of the organism was found at pH 7 and 48 h in the considered experimental conditions. *P. aeruginosa* has the capacity to use glycerol as carbon source. The variation in the concentration of glycerol as carbon source from 0.1 to 0.7 mL showed the highest oil and grease degradation with 0.2 mL glycerol dosage, and that when the concentration of glycerol was above 0.2 mL it has an inhibitory effect on microbial

growth. The surfactant concentration was varied from 0.05 to 2.0 mL. It shows the maximum oil degradation with 0.1 mL surfactant. High concentration of surfactant also has an inhibitory effect on the microbial growth. At optimized conditions, 50% of oil degradation was observed in the treatment of automobile wastewater with Bacteria. Addition of optimized dosage of carbon source and surfactant were found to be maximum degradation of 72 and 61%, respectively. The combination of carbon source and surfactant the oil degradation reached 93% and it was found to be the most effective treatment.

Oil degrading micro-organisms have the capability to degrade toxic contaminants for the reclamation of polluted sites. Pollution of water bodies is mainly caused by spillage of used engine oil. Therefore, spillage of oil at the source should be controlled. The workshop owners need to cooperate and maintain strict operating procedures with the contractor collecting the used oil. The effluents from washing activities should not be released into open drains and streams but they should be treated properly before being discharged. The application of biodegradation will be an important aspect of waste management now and in the future.

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