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Degradation of oil and grease from high-strength industrial effluents using locally isolated aerobic biosurfactant-producing bacteria

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ABSTRACT

The potential of oil and grease (O&G)-degrading ability of three local bacterial isolates was evaluated using wastewaters obtained from food processing, electrical and electronic and oil palm (POME) industries. These bacteria were chosen based on its high bacterial adherence to hydrocarbon (BATH), culture turbidity and maximum biosurfactant production (BSF) capabilities. From the 16S rRNA analysis, the food-processing isolate was identified and deposited in GenBank as *Serratia marcescens* EU555434, electrical & electronic (*Aeromonas hydrophila* KF049214) and POME (*Bacillus cereus* KJ605415). Prior to evaluation for its O&G degradation ability (effect of contact time, different concentrations of wastewater, pH and initial organic loading rate), *S. marcescens* was adapted in used cooking oil while *B. cereus* in POME. *S. marcescens*, with the highest BSF and BATH values, showed maximum oil and grease degradation ability (91%) at pH 7.0 after 12 days of incubation and initial organic loading rate of 1.46×10^{-1} kg O&G l^{-1} day^{-1}. For *B. cereus*, 100% (v/v) of POME (3012 mg l^{-1} oil and grease) was degraded after 7 days of incubation at 200 rpm, 30 °C and pH 6 while *A. hydrophila* was able to degrade 100% (v/v) of 4.88 mg l^{-1} of O&G from the electronic wastewater, supplemented with tryptone and lactose after only 2 h of incubation at 200 rpm, 30 °C at pH 7.0. The role of tryptone and lactose in complete biodegradation of O&G by *A. hydrophila* is significant as neither the addition of tryptone or lactose only resulted in enhanced O&G degradation, compared to E&E wastewater only. This finding showed the potential of using local aerobic bacterial isolates as an alternative solution to remove the presence of O&G in various industrial wastewaters.

1. Introduction

The discharge of oil and grease (O&G) containing wastewater to the environment increases every year due to rapid urbanization and industrial development. Major industrial sources of oily wastewater include petroleum refineries, metal manufacturing and machining, food processors, electronic and electrical and palm oil mill effluent (POME). Unlike free or floating oil spilled in the sea, most of the industrial wastewaters contain oil-in-water emulsions which can lead to severe problems in the different treatment stages. The presence of O&G in water treatment units will cause fouling in process equipment, complication in water discharge requirements and problems in biological treatment stages (Ahmad et al., 2006).

Amongst existing treatment technologies available include chemical coagulation, gravity separation, parallel-plate coalesces, gas floatation, cyclone separation, granular media filtration, microfiltration and ultrafiltration. However, very few of these technologies provide satisfactory solution to meet the increasing stringent water quality regulations as well as the expensive initial and operating cost. This makes it imperative to develop effective, economical and sustainable O&G treatment technologies that can serve as an alternative to existing treatment systems. One example of current application of alternative treatment system is the ponding system which is also the most common treatment system used by Malaysian palm oil mills to treat POME. One of the most attractive features for the ponding system is the low capital cost which can be attributed to the limited requirement for mechanical mixing, operational control and monitoring (Yacob et al., 2009). This system consists of a series of specifically built ponds including the anaerobic and facultative ponds incorporated with physico-

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chemical and biological treatments, respectively (Tong and Jaafar, 2004; Vijayaraghavan et al., 2007).

Recent study showed that the removal of Chemical Oxygen Demand (COD) as well as O&G by aerobic oxidation was higher in anaerobically digested POME as compared to diluted raw POME at Hydraulic Retention Time (HRT) of 60 h (Vijayaraghavan et al., 2007). Single culture such as *Acinetobacter* sp. (KUL8), *Bacillus* sp. (KUL39) and *Pseudomonas* sp. (KLB1) showed higher O&G removal capacity compared to mixed culture (Bhumibhamon et al., 2002). Lipase producing bacteria such as *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* has also been reported as a potent agent for lipid degradation from O&G-containing wastewaters such as those emanating from palm oil mill, dairy, slaughter house and soap industries (Prasad and Manjunath, 2011). The aim of this study was to investigate the feasibility of using indigenous bacteria to remove O&G in specific industrial wastewater namely POME, Electrical & Electronic, Food Processing Industries wastewater in bench-scale laboratory treatment system.

2. Materials and methods

2.1. Industrial wastewater

The O&G wastewater was obtained from the Wastewater Treatment Plant section of three different industries located in the Johor Bahru district, Johor, Malaysia. Each industry has been identified to incorporate O&G in some stages of their operation namely palm oil mills (mixing pond, POME), electrical and electronic industry, E&E (discharge point) and the food industry (discharge point). The wastewater samples were collected in clean and sterilized glass containers and the pH of the samples were adjusted to pH < 2.0 with either 1 M HCl or 1 M NaOH (to prevent abiotic redox reaction process that might change the chemical speciation in industrial effluents) and transported to the laboratory using refrigerated ice chests in dark condition (APHA, 2005). Each wastewater sample was then immediately stored at 4 °C prior to use. The average O&G contents in the wastewater was determined to be between 1886 and 14,510 mg l^{-1} for POME, 1.45–10 mg l^{-1} (E&E) and 180–79,000 mg l^{-1} (food industry).

2.2. Isolation and screening of O&G degrading bacteria

The O&G degrading bacteria were isolated from POME, E&E and food industry wastewaters using the following procedures: 2.5 ml of each wastewaters were transferred into a series of 250 ml of Erlenmeyer flasks containing 22.5 ml of Nutrient Broth, NB (8 g l^{-1}, Merck) and incubated at 30 °C, 200 rpm for 24 h (Certomat, B. Braun). Then, one loopful of each bacterial culture broth was inoculated onto Nutrient Agar, NA plates (20 g l^{-1}, Merck) followed by overnight incubation at 30 °C (Memmert, USA). The bacterial colony was then sub-cultured onto fresh NA plates using similar procedures until a pure culture was obtained.

For POME, the isolated bacteria were further screened for O&G degradation properties (lipolytic activity) using the Tween 80 peptone agar (Plou et al., 1998). The selected bacterial isolates were evaluated for its cell surface hydrophobicity properties using the bacterial adherence to hydrocarbon (BATH) test (Rosenberg et al., 1980). The BATH tests were carried out using early exponential phase cells which were first harvested by centrifugation at 9500 rpm for 5 min (Allegra™ 25-R Centrifuge, Beckman Coulter) and resuspended in 0.01 M potassium phosphate buffer (pH 7.0) to achieve OD_{600} value of 0.5. Then, 0.5 ml of either palm oil or paraffin oil was added into 5 ml of the cell suspension and vortexed for 30 s (Thermolyne Mixer II) and let to stand for 15 min. The absorbance of the aqueous phase was recorded at 600 nm and the percentage of microbial adhesion to substrate was calculated as follows:

$$1-\frac{OD_{600}\text{of aqueous phase after the addition of substrate}}{OD_{600}\text{of initial phase before the addition of substrate}}\times 100\% \quad (1)$$

All 25 bacterial isolates from the E&E wastewater were profiled for growth, cell concentration (CFU ml^{-1}) and culture turbidity (OD_{600}) using the following methods: one loopful of respective 24 h-old bacterial colony was inoculated into a series of 250 ml Erlenmeyer flasks containing 25 ml of NB. This was followed by 24 h incubation at 200 rpm and 30 °C where bacterial isolates with the shortest time to reach early stationary phase and OD_{600} values of greater than 1.0, were earmarked for subsequent experiments. From this evaluation, four bacterial isolates denoted as isolate A8, B1, B6 and B7 were selected for subsequent studies. Bacterial strain which showed good growth in minimal basal salt medium with highest cell count was chosen as potential bacteria to degrade O&G since it has capability to grow well in low minimal medium. The composition for the minimal basal salt medium is as follows: $(NH_4)_2SO_4$ (3.0 g l^{-1}, Univar), $MgSO_4{\cdot}7H_2O$ (0.5 g l^{-1}, Merck), K_2HPO_4 (0.5 g l^{-1}, BDH), KCl (0.1 g l^{-1}, Merck) and yeast extract (0.1 g l^{-1}, Oxoid). One isolate each from respective wastewaters that shows highest turbidity and fastest growth was identified using the 16S rRNA analysis which was carried out by First BASE Laboratories Sdn. Bhd., Malaysia and Ktrade Enterprise, Malaysia.

For the food industry wastewater, all 10 isolated strains showed good growth with OD_{600} of more than 1.0. One of the isolates, i.e. isolate A was evaluated for its lipid-degrading ability using Tween 80 peptone agar (peptone – 10 g l^{-1}, NaCl – 5 g l^{-1}, $CaCl_2$ – 0.1 g l^{-1}, Tween 80 – 5 ml and agar – 18 g l^{-1}) where formation of opaque zone around microbial colonies indicates lipolytic activity. Isolate A was adapted in 1, 3 and 5% (v/v) of cooking oil at 30 °C and 200 rpm (Certomat, B. Braun) for 24 h while isolates X7 and X10 was adapted in 100% (v/v) of POME in 2 days. Isolates X10, B1 and A were maintained in Luria–Bertani glycerol medium and stored at 4 °C for biodegradation study.

2.3. Biosurfactant production and activity

The biosurfactant production ability of the bacterial isolate was evaluated using isolate A by inoculating 5 ml of a 24 h-old culture broth of isolate A in 500 ml Erlenmeyer flask containing 50 ml mixture of minimal medium (3 g l^{-1} of $(NH_4)_2SO_4$, 0.5 g l^{-1} KH_2PO_4, 0.1 g l^{-1} KCl, 0.5 g l^{-1} $MgSO_4{\cdot}7H_2O$) and 5% v/v of cooking oil. The mixture was then incubated for 12 h, 30 °C and 200 rpm and harvested at 10,000 rpm, 20 min and 4 °C. The resulting supernatant was extracted with chloroform and methanol (2:1 v/v). The extract was concentrated using rotary evaporator and determined for biosurfactant activity using the following procedure (Matsumiya et al., 2007); 30 μl of the concentrated extract was pipetted onto the center of a series of petri dishes containing a mixture of 50 ml of distilled water (DW) and 100 μl of cooking oil. One unit of biosurfactant activity (U) is defined as the diameter of the clearing zone formed after 1 min from the addition of surfactant. The oil displacement test is an indirect measurement of surface activity of a biosurfactant sample tested against oil where a larger diameter represents a higher surface activity of the test solution.

2.4. O&G biodegradation study

The O&G biodegradation study using different industrial wastewaters was carried out as follows: isolate A (10% v/v) was