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Airlift bioreactor containing chitosan-immobilized *Sphingobium* sp. P2 for treatment of lubricants in wastewater

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ABSTRACT

An internal loop airlift bioreactor containing chitosan-immobilized *Sphingobium* sp. P2 was applied for the removal of automotive lubricants from emulsified wastewater. The chitosan-immobilized bacteria had higher lubricant removal efficiency than free and killed-immobilized cells because they were able to sorp and degrade the lubricants simultaneously. In a semi-continuous batch experiment, the immobilized bacteria were able to remove 80–90% of the 200 mg L^{-1} total petroleum hydrocarbons (TPH) from both synthetic and carwash wastewater. The internal loop airlift bioreactor, containing 4 g L^{-1} immobilized bacteria, was later designed and operated at 2.0 h HRT (hydraulic retention time) for over 70 days. At a steady state, the reactor continuously removed 85 ± 5% TPH and 73 ± 11% chemical oxygen demand (COD) from the carwash wastewater with 25–200 mg L^{-1} amended lubricant. The internal loop airlift reactor's simple operation and high stability demonstrate its high potential for use in treating lubricants in emulsified wastewater from carwashes and other industries.

1. Introduction

Lubricants are widely used to reduce friction and overheating in a variety of machinery, engines, gear, and turbines. They can be released into the environment through accidental leaking and cleaning activities. Wastewater lubricants are present not only as free oil but also as emulsified oil formed by the mixing of oil with a washing agent and wastewater. This emulsified oil cannot be removed by conventional treatment methods such as gravitational oil separation or corrugated plate interception [1]. The objective of this study was to develop a bioreactor for the removal of emulsified lubricants from wastewater. The bioreactor contained lubricant-degrading bacteria immobilized on sorbent, which was expected to sorp and degrade the emulsified lubricants simultaneously.

Lubricants can be degraded by several microorganisms, such as *Zoogloea* sp., *Pseudomonas mandelii* sp., *Rhodococcus* sp., *Nocardia simplex* sp., *Gordona terrae* sp., *Commaonas acidovorans*, and *Bacillus* sp. [2–4]. However, the extent of lubricant degradation in wastewater depends on the lubricant type, wherein mineral lubricants are the most biodegradable, followed by semi-synthetic and synthetic lubricants [5]. The extent of degradation between fresh and used lubricants also differs. The presence of polycyclic aromatic hydrocarbons (PAHs) in used lubricant also alters its degradation and contributes to increased toxic effects [6]. Therefore, this study tested four known PAH-degrading bacteria, *Diaphorobacter* sp. KOTLB [7], *Pseudoxanthomonas* sp. RN402 [7], *Sphingobium* sp. P2 [8,9], and *Sphingomonas* sp. SP2 [10], for the ability to degrade hydrocarbon components in various lubricants. A strain with high lubricant degradation activity was selected as an inoculum in the bioreactor.

In addition to biodegradation, lubricants can be removed from wastewater by many sorbents. For example, Khan et al. [11] reported that kapok fiber removed oil from a gas station run-off by a sorption process more effectively than wood chips, rice husks, coconut husks or bagasse. Polyurethane foam (PUF)'s oleophilic and hydrophobic properties make it an attractive sorbent for oil sorption [12]. This study compared the efficiency of kapok fiber, polyurethane foam, and chitosan for lubricant sorption. Chitosan is a natural, nontoxic, biodegradable, and high molecular weight biopolymer that has excellent properties such as biodegradability,

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biocompatibility, adsorption, and flocculating ability [13]. Powder and flake chitosan have been used to sorp mineral oil, vegetable oil, and cutting oil [14,15]. To reduce oil accumulation and increase the oil sorption capacity of the sorbent, the study immobilized lubricant-degrading bacteria on the surface of oil sorbent. Immobilization also has the added benefit of enhancing microbial cell stability, allowing continuous process operation, and avoiding the biomass-liquid separation requirement [16].A variety of bioreactors are based on the utilization of immobilized bacteria, such as a biological aerated filter, a rotating biological contactor, a trickling filter and a membrane-aerated bioreactor. However, several problems are associated with these technologies, such as clogging by sediments, fouling by materials that build up over time, and competition of other microorganisms [17,18]. To avoid these problems, this study used an internal loop airlift bioreactor, which fluidizes the immobilized bacteria by entering the compressed air at the bottom of the reactor. The absence of mechanical stirring systems in an internal loop airlift bioreactor can also reduce the capital and operating costs. Recently, this bioreactor is popular because of its mechanical simplicity, good mixing, low shear rate, and high capacity handling [19]. In this study, the lubricant removal efficiency of the immobilized cells in the internal loop airlift bioreactor was compared using both synthetic and carwash wastewater.

2. Materials and methods

2.1. Lubricants, sorbents and microorganisms

The lubricants used in this study were the products that manufactured for gasoline and diesel engines by PTT Public Company Limited (Thailand). There are six samples to represent three types of lubricant: mineral (PTT V-120 and PERFORMA), semi-synthetic (PERFORMA SEMI-SYNTHETIC and DYNAMIC PREMIER), and synthetic (PERFORMA SYNTHETIC and DYNAMIC SYNTHETIC) oil. Because PTT V-120 is widely used in Thailand, it was selected as a model lubricant for more detailed experiments.

Kapok fiber, polyurethane foam, and chitosan were selected as oil sorbents because of their low cost and availability in many parts of the world. Kapok fiber was purchased from a local market in one batch, and PUF was purchased from Thai Products Foam Co., Ltd. Shrimp shell and squid pen chitosan flakes with >85% deacetylation were supplied by ELAND Corporation, Ltd. The lubricant sorption capacity of each sorbent was determined according to the standard test method for sorbent performance of adsorbents (ASTM F726-06). All of the lubricants and sorbents were sterilized by autoclaving at 121 °C for 15 min before use.

Diaphorobacter sp. KOTLB, *Pseudoxanthomonas* sp. RN402, *Sphingobium* sp. P2, and *Sphingomonas* sp. SP2 are currently deposited at the Microbiological Resources Center, Thailand Institute of Scientific and Technological Research (TISTR) under the accession numbers TISTR 2091, 2059, 2006, and 2005, respectively. They are PAH-degrading bacteria that have been isolated locally. The bacteria were maintained on 0.25× Luria–Bertani (LB) agar and subcultured monthly.

2.2. Wastewater

Wastewater containing emulsified lubricants was labeled either synthetic or carwash wastewater. The synthesized wastewater was prepared from a stock oil/water emulsion following Panpanit and Visvanathan [1] and Kloet et al. [20] with some modifications. The 1000 mg L^{-1} stock oil/water emulsion was made by mixing 1 mL of lubricant, 0.1% Tween 80 (Merck Co., Ltd.) and 1 L of deionized water in a food blender. The admixture was stirred for 10 min to stabilize the emulsion as well as to defoam before diluting with water to a specific oil concentration. The oil/water emulsion remained stable throughout multiple experimental runs. Carwash wastewater was collected from a grease trap at a Bangkok petrol station with car washing activities. To minimize the amount of free oil, the samples were taken from the last compartment of the grease trap. The wastewater was stored at 4 °C.

2.3. Bacterial immobilization process

Lubricant-degrading bacteria were immobilized on the surface of sorbent by an attachment approach. The selected strain was cultured in carbon-free mineral medium (CFMM) [21] containing 200 mg L^{-1} lubricant for 24–48 h (depending on the strain) to induce for lubricant-degrading activity before cell immobilization. The immobilization process was carried out in a 1000 mL flask containing 20 mL of cell culture (1.33×10^7 CFU mL^{-1}), 1.25 g of sterilized sorbent, 500 mL of CFMM and 0.25% (v/v) lubricant. The flask was incubated at room temperature while shaking at 150 rpm for 24 h. The immobilized bacteria were subsequently filtered through sterilized filter paper and air-dried. SEM micrographs of sorbent with and without immobilized bacteria were taken with a JSM-5410LV scanning electron microscope (JEOL). The samples were prepared by fixation with 2.5% (v/v) glutaraldehyde, dehydration by sequential ethanol gradients and desiccation with a critical point dryer prior to applying a gold coating.

2.4. Batch experiment for lubricant removal study

Small-scale batch experiments were performed to determine the lubricant removal efficiency of sorbent materials and bacterial strains (i.e., free and immobilized cells). For sorbent materials, the lubricant removal efficiency was determined by adding 1.0 g L^{-1} sorbent to a 250 mL flask with 50 mL of synthetic wastewater containing 200 mg L^{-1} emulsified lubricant. The flask was shaken at 200 rpm for 1 h to reach the sorption equilibrium. The mixture was allowed to settle for 30 min, and the sorbent was removed before analyzing the amount of remaining lubricant in the wastewater.

For bacterial strains, the lubricant degradation activity was examined in a 250 mL flask that contained 100 mL of wastewater. The semi-continuous lubricant degradation was performed by repeatedly adding stock oil/water emulsion daily to maintain the initial concentration of 200 mg L^{-1}. The initial bacterial concentration was approximately 10^7–10^8 CFU $flask^{-1}$, which was achieved by adding either 10 mL of the cell suspension (1.33×10^7 CFU mL^{-1}) or 0.1–0.25 g of the immobilized bacteria (1.00×10^8 CFU g^{-1} sorbent) to each flask. To distinguish between lubricant adsorption and degradation, an additional experiment was performed in parallel with killed immobilized cells, where the immobilized bacteria were autoclaved twice for 1 h each before the experiment. The control treatment was the synthetic wastewater alone. Flasks were shaken at 150 rpm at room temperature. The amounts of remaining lubricant were measured daily.

2.5. Design and operation of internal loop airlift bioreactor

A bench scale internal loop airlift bioreactor was constructed using the design criteria from Wongsuchoto and Pavasant [22] and packed with the immobilized bacteria. The reactor was made from a transparent, cylindrical glass column (15 cm diameter, 50 cm height) with a concentric cylinder (internal loop of 10 cm in diameter and 22 cm in height) (Fig. 1). This system has a ratio between downcomer to riser cross-sectional areas (A_d/A_r) of 1.4, which Wongsuchoto and Pavasant [22] found that the critical A_d/A_r should be in the range of 0.988–1.54 for high internal liquid circulation. A clearance between the column base and the bottom of the internal loop was fixed at 5 cm. The water level was fixed at 3 cm above

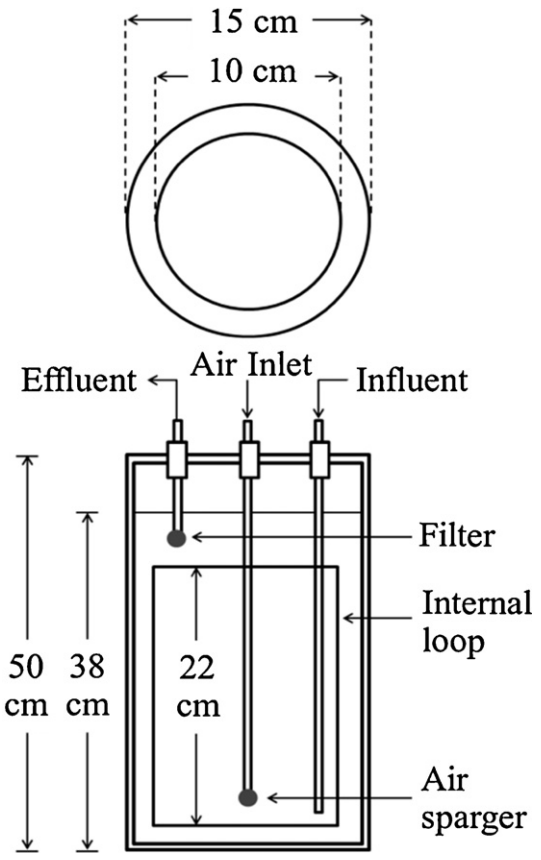


Fig. 1. Schematic of the internal loop airlift bioreactor.

the top of the internal loop. Wastewater and air were entered from the bottom of the reactor by using a peristaltic pump and via a 2.5 cm diameter porous stone air sparger, respectively. DO concentration was maintained between 5 and 6 mg L^{-1} by adjusting the volumetric air flow rate and oxygen transfer rate at 6 L min^{-1} and 10.564 g L^{-1} s^{-1}, respectively. The overall gas hold up was 0.026 which was found to provide a good mixing for 2.5–4.0 g L^{-1} immobilizing sorbent in the bioreactor. To prevent the loss of immobilizing sorbent from the reactor, the effluent tubing was covered by a piece of cheese cloth. The reactor harbored a working volume of 3 L and was operated at various HRT (hydraulic retention time) (0.5–12 h). The efficiency of this bioreactor was determined by feeding synthetic wastewater with various concentrations of lubricant (25–200 mg L^{-1}) or carwash wastewater with and without spiked lubricant (3–200 mg L^{-1}). Influent and effluent were sampled and analyzed for oil and chemical oxygen demand (COD) at various time intervals.

2.6. Analytical methods

The concentration of residual lubricant in the wastewater was analyzed by thin layer chromatography and flame ionization detection (TLC-FID) according to Maruyama et al. [23]. The sample was extracted with chloroform before analyzing for its oil components by Iatroscan ™ MK-6/6S (Mitsubishi Kogaku Iatron, Inc. Japan), where stearyl alcohol (6.25 mg mL^{-1}) served as an internal standard. The tested lubricants contained mainly saturates and aromatics fractions; thus, the peak area of these two fractions was combined to represent the amount of total petroleum hydrocarbons (TPH). The alkane components of these extracted samples were subsequently analyzed by gas chromatography (GC). GC analysis was performed with a Hewlett–Packard 6890 (Agilent Technologies) equipped with an HP-5 fused-silica capillary column and a flame ionization detector. The gas chromatography conditions were as follows: injector temperature of 250 °C, detector temperature of 320 °C, and column temperature of 40–320 °C at a rate of 10 °C min^{-1}.

The COD in the carwash wastewater was determined by a standard closed reflux method. The concentrations of total and lubricant-degrading bacteria were determined by plate count technique on LB and lubricant-CFMM agar, respectively. To quantify the amount of immobilized bacteria, the bacterial cells were extracted from the sorbent before being counted. Briefly, 1 g of the sorbent was rehydrated in 4 ml of CFMM medium for 3 min, sonicated in an ultrasonic bath for 3 min and vigorously vortexed for 1 min.

3. Results and discussion

3.1. Selection of an efficient lubricant sorbent

Among the tested sorbents, kapok fiber exhibited the highest lubricant sorption capacity of 2.90 g oil g^{-1} sorbent followed by PUF (1.71 g oil g^{-1} sorbent), squid pen chitosan (0.24 g oil g^{-1} sorbent) and shrimp shell chitosan (0.19 g oil g^{-1} sorbent) (Table 1). Conversely, kapok fiber removed the lowest amount of emulsified lubricant, with 67% TPH removal efficiency whereas chitosan flakes had the highest TPH removal efficiency of 70–73% (Table 1). The differences between lubricant sorption capacity and removal efficiency were primarily due to the different dispersion behaviors of each sorbent in the tested system. Kapok fiber and PUF floated on the liquid surface; therefore, they easily sorbed the free oil residues. In contrast, chitosan flakes dispersed well in liquid solution, resulting in an increased interaction with the emulsified lubricant. Moreover, chitosan contains a large amount of positive charges, which can bind to the negatively charged oil residues and rapidly remove oil from the water by both agglomeration and sorption mechanisms [14]. The higher oil sorption capacity of squid pen chitosan compared with shrimp shell chitosan was likely due to differences in their molecular structures. Squid pen chitosan is synthesized from beta-chitin, an amine group aligned with the OH and CH_2OH groups, whereas shrimp shell chitosan is synthesized from alpha-chitin, an anti-parallel chain alignment [24]. Because the study focused on the removal of emulsified lubricant, the squid pen chitosan was selected for further study.

3.2. Selection of an efficient lubricant-degrading bacteria

The four PAH-degrading bacteria were able to degrade all of the lubricants in the synthetic wastewater evidenced by the higher lubricant removal efficiency than the abiotic control (Table 2). Other PAH-degrading bacteria, such as *C. acidovorans* Px1, *Bacillus* sp. Px2, and *Pseudomonas* sp. Px3, are also known to degrade lubricant [4]. Comparing to other bacterial strains, *Sphingobium* sp. P2 was the most effective bacterium that degraded approximately 80–90% of 200 mg L^{-1} lubricants within 24 h (Table 2). *Sphingobium* sp. P2 (previously known as *Sphingomonas* sp. P2) can degrade a wide variety of low molecular weight PAHs and co-metabolize high molecular weight PAHs such as fluoranthene and pyrene [9]. The high lubricant-degrading efficiency of this strain might be due to the existence of various oxygenase enzymes involved in hydrocarbon degradation [25].

The lubricant-removal efficiency of each strain was influenced by the types of lubricant. Montagnolli et al. [5] reported that mineral lubricant is the most biodegradable, followed by semi-synthetic and synthetic lubricant; however, the trend was observed only for *Sphingomonas* sp. SP2 and *Diaphorobacter* sp. KOTLB. These bacteria degraded mineral lubricants (i.e., PTT-V-120 and PERFORMA)

Table 1
Characteristics of sorbents used in this study.[a]

Sorbent	Size and form	Lubricant sorption capacity (g oil g^{-1} sorbent)	Lubricant removal efficiency (%)[b]
Squid pen chitosan	<2.50 mm flake	0.24	73
Shrimp shell chitosan	<2.50 mm flake	0.19	70
Polyurethane foam	1 × 1 × 1 cm cube	1.71	71
Kapok fiber	Fiber	2.90	67

[a] The lubricant used during sorbent characterization was PTT-V120.
[b] The lubricant removal efficiency was calculated as percent decrease in TPH concentration after a 24 h incubation relative to the initial concentration in synthetic wastewater. The initial concentrations of sorbent and lubricant were 1 g L^{-1} and 200 mg L^{-1}, respectively.

at a more thoroughly than other lubricants (Table 2). Conversely, *Sphingobium* sp. P2 and *Pseudoxanthomonas* sp. RN402 were able to degrade all of the lubricants at a comparable efficiency. The ability to degrade various lubricants is very useful because carwash wastewater may contain different types of lubricant. In addition to high lubricant-degrading activity, *Sphingobium* sp. P2 grew rather quickly, it was observed by the time needed for cultivation as short as 24 h. Therefore, the bacterium was selected for further study.

3.3. Production of chitosan-immobilized *Sphingobium* sp. P2 and its lubricant removal efficiency in batch experiment

SEM photographs revealed that squid pen chitosan flakes exhibited a rough surface with a few crevices, which makes it potent for *Sphingobium* sp. P2 attachment and colony forming (Fig. 2). The concentration of bacterial cells was approximately 1.00×10^8 CFU g^{-1} chitosan after the immobilization process. The effectiveness of chitosan-immobilized cells was investigated in a semi-continuous treatment system, where the stock oil/water emulsion of PTT-V120 lubricant was added daily to the synthetic wastewater to maintain the initial lubricant concentration of 200 mg L^{-1}. The concentration of TPH in the control treatment increased daily and equaled 996 ± 8 mg L^{-1} at the end of study (Fig. 3a), this value corresponded to the total amount of added lubricant. In contrast, the lubricant was significantly removed from the systems with chitosan-immobilized cells, killed-immobilized cells and free cells, where the amounts of residual TPH were 214 ± 4, 450 ± 2 and 605 ± 4 mg L^{-1} at the end of study, respectively (Fig. 3a). The efficiencies of lubricant sorption by killed-immobilized cells and degradation by free cells were the highest in the first two days. However, the lubricant removal efficiency of both systems rapidly decreased when the lubricant concentrations were increased to 800–1000 mg L^{-1}. These concentrations likely show the maximum sorption capacity of killed-immobilized cells or inhibitory range for *Sphingobium* sp. P2 degradation activity. The maximum lubricant sorption capacity of flake squid pen chitosan was 0.24 g oil g^{-1} chitosan (Table 1); thus, 2.5 g L^{-1} chitosan could sorp the maximum oil concentration of 600 mg L^{-1}. However, the toxicity of mineral-based lubricants on microbial community has been proved [6]. In this study, the number of *Sphingobium* sp. P2 in the wastewater significantly decreased 100-fold (from 1.10×10^7 to 2.03×10^5 CFU/treatment) when the concentration of oil/water emulsion was increased during the treatment.

The limitation of killed-immobilized cells and free cells was overcome by the use of chitosan-immobilized cells. The TPH removal efficiency of chitosan-immobilized cells after each addition of 200 mg L^{-1} lubricant was relatively constant at the range of 80–90% throughout the study (Fig. 3a). A GC chromatogram of the remaining TPH in synthetic wastewater with 200 mg L^{-1} lubricant confirmed that chitosan-immobilized cells were able to remove several hydrocarbon components of the lubricant (Fig. 4). The amount of lubricant on the immobilizing chitosan was analyzed to confirm the degradation of sorbed oil in chitosan which revealed the residual lubricant in chitosan-immobilized cells was closed to zero (data not shown). The high efficiency of chitosan-immobilized cells was likely due to the higher number of bacterial cells both on chitosan flakes and in the wastewater. *Sphingobium* sp. P2 grown well during treatment where the number of *Sphingobium* sp. P2 on chitosan flakes significantly increased 10-fold (1.00×10^8 to 1.67×10^9 CFU g^{-1} chitosan) and the bacterial concentration in wastewater was increased from cell outgrowth to 5.00×10^5 CFU mL^{-1} at the end of treatment. Chitosan flakes have been used to immobilize *Rhodococcus corynebacterioides* QBTo for bioremediation of crude oil polluted seawater [26]. The immobilized bacteria exhibited high oil-degrading activity, which is similar to this study. Gentili et al. [26] suggested that this high oil-degrading activity is due to the protective effect of a biofilm structure on the chitosan surface.

The oil removal efficiency of immobilized cells was further tested with carwash wastewater in a similar semi-continuous treatment system. The initial concentrations of total and lubricant-degrading bacteria in wastewater were 1.90×10^6 and 2.67×10^5 CFU mL^{-1}, respectively. Because the wastewater already harbored indigenous lubricant-degrading bacteria, the amount of immobilized cells was decreased to 1.0 g L^{-1}. The amounts of lubricant in the carwash wastewater without immobilized cells (control) were lower than the total amount of added lubricant after incubation (Fig. 3a and b). The results indicated that the indigenous bacteria were able to degrade lubricant in carwash wastewater. However, the presence of chitosan-immobilized cells significantly

Table 2
Lubricant removal efficiency of the tested bacteria when used as free cells.

Bacterial strain	Cultivation time[a] (h)	Lubricant removal efficiency (%)[b]					
		PTT-V120	Performa	Performa semi-synthetic	Dynamic premier	Performa synthetic	Dynamic synthetic
Sphingobium sp. P2	24	92 ± 1	84 ± 2	85 ± 3	79 ± 4	88 ± 2	89 ± 1
Sphingomonas sp. SP2	48	88 ± 0	79 ± 4	63 ± 4	71 ± 9	49 ± 4	68 ± 4
Pseudoxanthomonas sp. RN402	48	81 ± 9	77 ± 7	76 ± 3	63 ± 9	75 ± 8	89 ± 1
Diaphorobacter sp. KOTLB	48	66 ± 13	60 ± 12	42 ± 4	33 ± 8	19 ± 10	20 ± 7
None (Abiotic control)	–	17 ± 5	11 ± 8	6 ± 8	4 ± 5	14 ± 7	1 ± 7

[a] Cultivation time to achieve the cell concentration of 1.33×10^7 CFU mL^{-1}.
[b] The lubricant removal efficiency was calculated as percent decrease in TPH concentration after a 24 h incubation relative to the initial concentration in synthetic wastewater. The initial lubricant concentration was 200 mg L^{-1}.

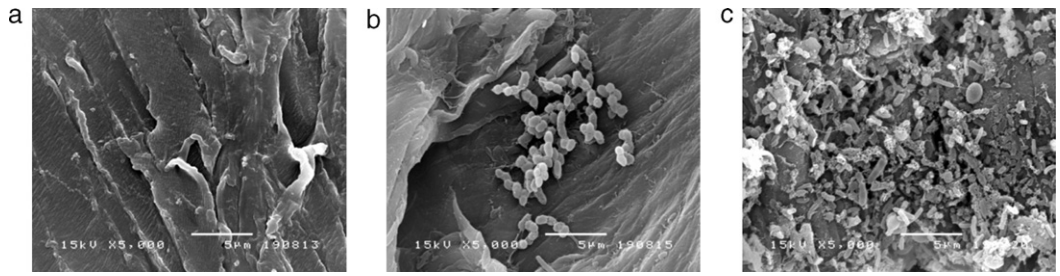


Fig. 2. SEM photographs of flake squid pen chitosan before (a) and after (b) immobilization with *Sphingobium* sp. P2, also after 23 days of use in the internal loop airlift bioreactor containing carwash wastewater (c).

enhanced lubricant degradation, where the amounts of remaining TPH were lower than in control treatment at all-time points (Fig. 3b). Nonetheless, the efficiency of chitosan-immobilized *Sphingobium* sp. P2 in carwash wastewater was lower than that in synthetic wastewater (Fig. 3a and b). This result was likely due to the competition for substrates (such as lubricant and other nutrients) between the indigenous bacteria and *Sphingobium* sp. P2 as well as the toxic effects of other contaminants in the carwash wastewater to the immobilized cells. In addition to lubricant, carwash wastewater may contain hydrofluoric acid, ammonium bifluoride products, paint residues, rubber, phosphates, oil, grease and volatile organic compounds [28]. To maintain the high number of *Sphingobium* sp. P2, the amount of immobilized cells was increased to 2.5 g L^{-1} in the following experiment.

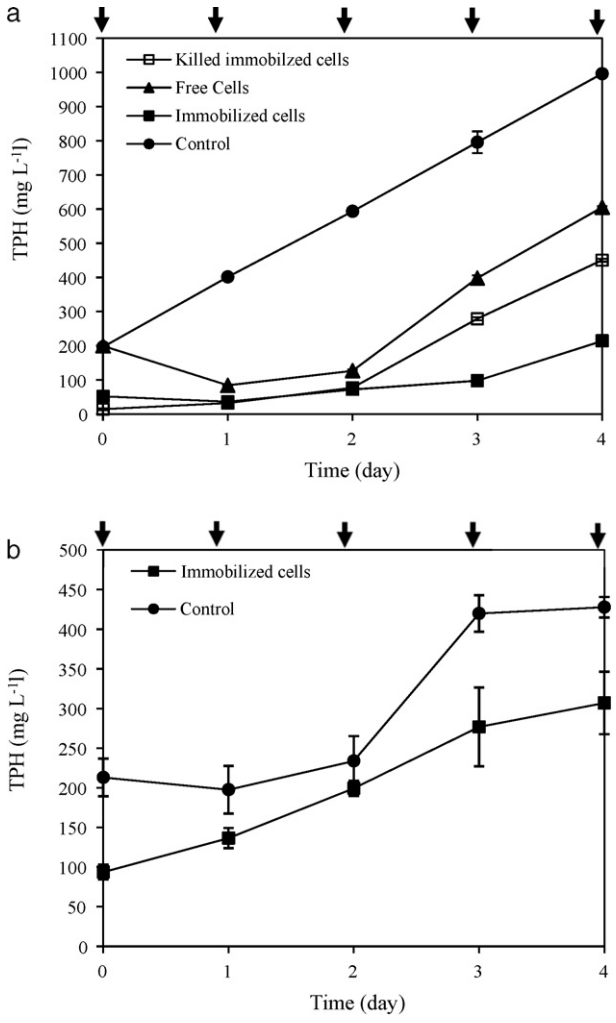


Fig. 3. Removal of PTT-V120 lubricant from synthetic wastewater (a) and carwash wastewater (b) by either free, immobilized, or killed immobilized cells in batch experiments. The amounts of immobilized cells in the experiments with synthetic and carwash wastewater were 2.5 and 1 g L^{-1}, respectively. The stock oil/water emulsion was added daily to maintain the initial lubricant concentration of 200 mg L^{-1}, indicated by the arrows.

3.4. Efficiency of chitosan-immobilized Sphingobium sp. P2 in the internal loop airlift bioreactor for the removal of lubricant in synthetic wastewater

A laboratory-scale internal loop airlift reactor containing chitosan-immobilized *Sphingobium* sp. P2 was constructed and preliminary tested with synthetic wastewater containing various concentrations of PTT-V120 lubricant and at different HRT values. The amount of chitosan-immobilized cells at 2.5 g L^{-1} (1.20×10^8 CFU g^{-1} chitosan) was sufficient for the continuous treatment of wastewater with 25–50 mg L^{-1} lubricant (Fig. 5a). At 25 mg L^{-1} lubricant, TPH removal efficiencies of the reactor at the steady state were 89%, 83%, and 72% at 2.0, 1.0 and 0.5 h HRT, respectively. When the initial lubricant concentrations were increased to 50 mg L^{-1}, the oil removal efficiency decreased (Fig. 5a). However, the chitosan-immobilized cells were able to remove more than 80% of the lubricant at 2.0 h HRT.

The efficiency of the internal loop airlift reactor was further investigated with synthetic wastewater containing 200 mg L^{-1} lubricant. This lubricant concentration was used as a model for hydrocarbon-rich industrial wastewater [27]. The reactor containing 2.5 g L^{-1} chitosan-immobilized cells effectively removed the lubricant at high HRT values. The lubricant removal efficiencies at the steady state were 86%, 82%, 63% and 42% of lubricant at 12.0, 8.0, 6.0, and 4.8 h HRT, respectively (Fig. 5b). To lower the HRT, the chitosan-immobilized cell concentration was increased to 4.0 g L^{-1} (1.00×10^8 CFU g^{-1} chitosan), which was the maximum amount of chitosan-immobilized cells that could disperse well in the reactor. The increased amount of immobilized cells was able to lower the HRT value to 2.0 h and resulted in 82% oil removal (Fig. 5b). Consequently, the airlift loop reactor should contain 4.0 g L^{-1} chitosan-immobilized cells and operate at 2.0 h HRT when treating wastewater containing up to 200 mg L^{-1} lubricant.

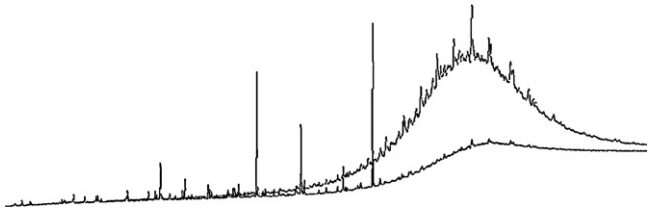


Fig. 4. GC chromatograms show the removal of 200 mg L^{-1} PTT-V120 from synthetic wastewater by chitosan-immobilized cells (lower line) compared to an abiotic control (upper line).

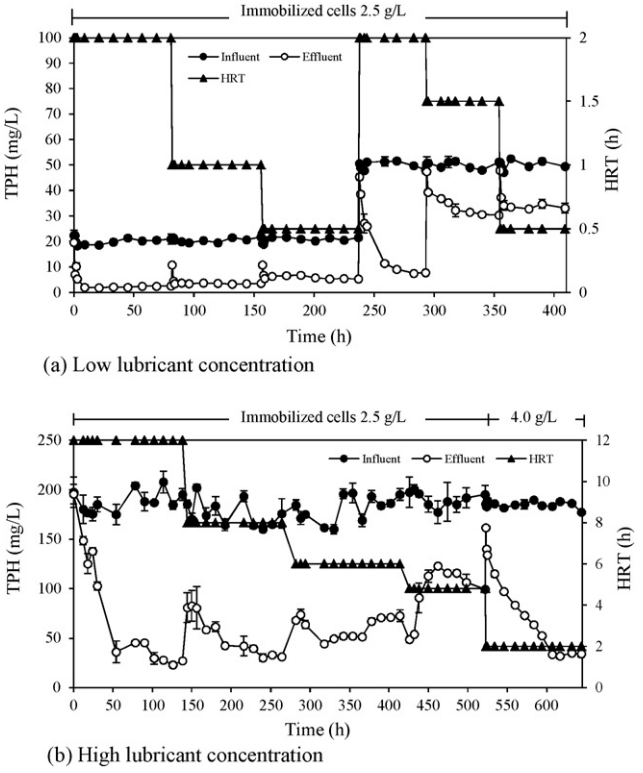


Fig. 5. Removal of PTT-V120 lubricant from synthetic wastewater by the internal loop airlift bioreactor containing chitosan-immobilized *Sphingobium* sp. P2.

This reactor exhibits a high potential in treating large volumes of wastewater because of its low HRT.

3.5. Efficiency of chitosan-immobilized Sphingobium sp. P2 in the internal loop airlift bioreactor for the removal of lubricant and COD in carwash wastewater

To confirm the efficiency of the airlift loop reactor, car wash wastewater was applied and oil/water emulsion was added to the wastewater to obtain the initial PTT-V120 lubricant concentrations of 25–200 mg L^{-1}. The reactor reached a steady state within hours for the lubricant concentrations below 100 mg L^{-1}. A longer acclimatization period was required for a higher lubricant concentration (Fig. 6). For each lubricant concentration, the reactor could remove 85 ± 5% TPH and 73 ± 11% COD from the wastewater at a steady state (Fig. 6a and b). The lower COD removal efficiency could be due to the presence of other contaminants that *Sphingobium* sp. P2 cannot degrade. Nonetheless, the efficiency of chitosan-immobilized *Sphingobium* sp. P2 in the reactor was higher than that in the batch experiment (Figs. 6a and 3b). This result could be due to the increased cell outgrowth overtime as well as the brief toxic exposure period by short HRT operation. Immobilized bacteria removed 200 mg L^{-1} lubricant at similar efficiencies from synthetic and carwash wastewater (Figs. 5b and 6b). The results indicate that the immobilized bacteria had high potential to be applied to real wastewater treatment.

Long-term operation of the reactor was investigated with carwash wastewater for 73 days. The lubricant removal efficiency was low during the first five days where the initial amounts of TPH and COD in wastewater were 4 ± 1 and 60 ± 1 mg L^{-1}, respectively (Fig. 7a and b). The low concentrations of lubricants were probably insufficient to support bacterial activity; therefore, an oil/water emulsion was continuously added to the wastewater to maintain the lubricant concentration of 50 mg L^{-1} after day 15. The

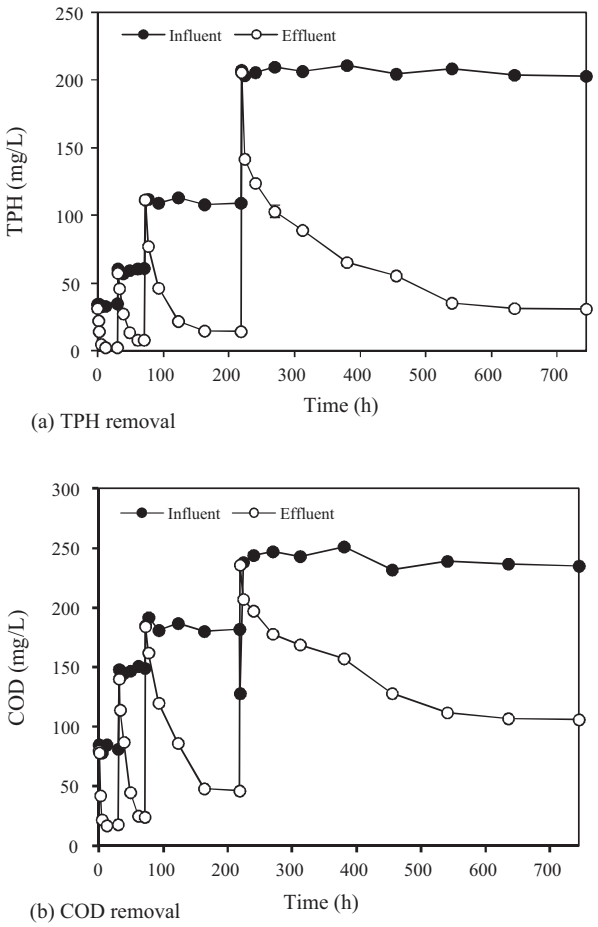
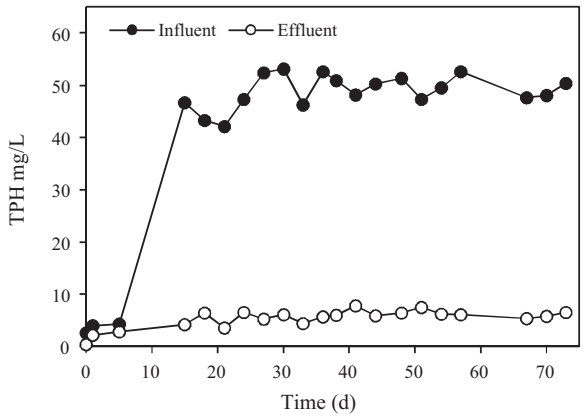


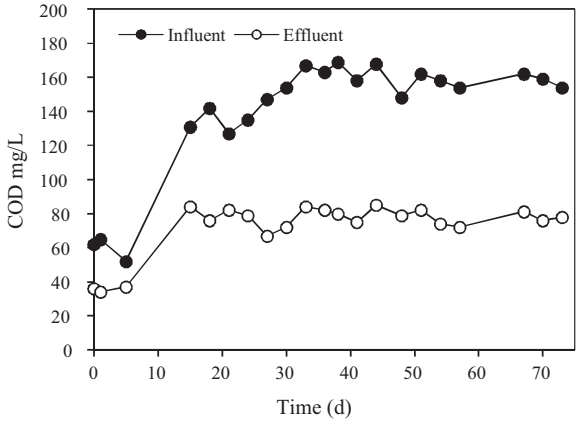
Fig. 6. Effects of initial oil concentrations on (a) TPH and (b) COD removal from carwash wastewater by 4.0 g L^{-1} immobilized cells (1.10×10^8 CFU g^{-1} chitosan) in the internal loop airlift bioreactor with HRT 2.0 h. An oil/water emulsion was added to the wastewater to obtain the initial oil concentration of 25, 50, 100, and 200 mg L^{-1}, respectively.

immobilized cells rapidly and effectively degraded the added lubricant with an average removal efficiency of 88% throughout the study. The amounts of TPH and COD in the effluent were 6 ± 1 and 78 ± 12 mg L^{-1}, respectively, during days 18–73. SEM photographs of the used immobilized cells revealed dense populations of *Sphingobium* sp. P2 and other bacteria on the chitosan surface after a 23-day incubation (Fig. 2c). The results indicated that chitosan was a suitable habitat for bacterial colonization, and the high efficiency of this reactor during long-term operation could be due to the activity of both *Sphingobium* sp. P2 and the indigenous bacteria in the wastewater.

Many types of bioreactors have been developed for oily wastewater treatment. For example, Gargouri et al. [27] successfully used a continuously stirred tank bioreactor to treat petroleum refinery wastewater containing 270–320 mg L^{-1} TPH; however, the reactor operated well after 35 days and required 16 h HRT. Wang et al. [4] investigated the biodegradation of 2000–10,000 mg L^{-1} lubricant in a stainless steel biofilm reactor with 48 h HRT. Although the bacterial biofilm effectively degraded 97% of the lubricant, the formation of the biofilm on stainless steel was slow and may be difficult to scale up. In this study, the preparation of chitosan-immobilized bacteria was simple, and the bacteria could be applied directly to the bioreactor allowing for a brief start-up period and short HRT. The efficiency of this reactor was also comparable to an integrated carwash wastewater treatment by Bhatti et al. [28] that consists of aeration, coagulation by alum, and oxidation by hydrogen peroxide,



(a) TPH removal



(b) COD removal

Fig. 7. Long-term efficiency of (a) TPH and (b) COD removal from carwash wastewater by 4.0 g L^{-1} immobilized cells (1.00×10^8 CFU g^{-1} chitosan) in the internal loop airlift bioreactor with HRT 2.0 h. The experiment was operated for 73 days. An oil/water emulsion was added to the wastewater to maintain the initial oil concentration of 50 mg L^{-1} after 15 days.

which removed 96% of the oil contents. However, the internal loop airlift reactor containing chitosan-immobilized bacteria was much simpler, in which the lubricant was removed by only one process. The system was also stable for over 70 days.

4. Conclusions

The internal loop airlift bioreactor containing chitosan immobilized-*Sphingobium* sp. P2 exhibited a high efficiency in removing automotive lubricants from both synthetic and carwash wastewater. By immobilizing the lubricant-degrading bacteria on chitosan, the lubricant removal mechanism consisted of both sorption and degradation. The immobilization also reduced the toxicity of the lubricant on *Sphingobium* sp. P2, thereby enhancing its degrading activity. The internal loop airlift bioreactor containing 4 g L^{-1} immobilized bacteria continuously removed lubricant and COD from wastewater when operated at 2.0 h HRT. Consequently, it had a high potential for treating lubricants in emulsified wastewater from car washing and other industries.

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