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Application of rhamnolipid and surfactin for enhanced diesel biodegradation—Effects of pH and ammonium addition

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ABSTRACT

This study investigated the effects of pH and ammonium concentrations on the potential application of two biosurfactants, surfactin (SF) and rhamnolipid (RL), for enhanced diesel biodegradation with a series of bench-scale experiments. In general, compared to the experiments without biosurfactant addition, adding RL or SF to diesel–water systems at concentrations above their critical micelle concentration (CMC) values benefited diesel emulsification, and therefore enhanced diesel biodegradation. The effects of pH on RL or SF-enhanced biodegradation of diesel were in good agreement with the trends of emulsion index values for RL or SF addition, respectively, under different pH conditions, suggesting that enhanced diesel emulsification by RL or SF addition promoted biodegradation of diesel. In diesel–water systems with 50 mg/L of RL addition, an optimum pH condition for microbial growth and diesel biodegradation was found to be at a pH 7.2, while decreasing pH to 5.2 or increasing it to 8.4 reduced those parameters considerably. For the cases where 40 mg/L of SF was added, the enhancing ability shared a general trend with that observed for adding 50 mg/L of RL as the pH increased from 5.2 to 7.2. Further increase of pH to 8.4, however, did not seem to negatively influence biodegradation and biomass growth. With respect to the effects of ammonium concentration on diesel biodegradation in diesel–water systems with 50 mg/L of RL addition, an optimum ammonium addition for microbial growth and diesel biodegradation was found between 200 and 300 mg-N/L, but a dramatic decrease in growth and biodegradation occurred at ammonium addition up to 450 mg-N/L. For the cases where 40 mg/L of SF was added, an increase of ammonium addition from 50 to 200 mg-N/L substantially increased microbial growth and biodegradation of diesel. Further increase of ammonium concentration to 450 mg-N/L, however, did not further improve diesel biodegradation.

1. Introduction

Soil and groundwater contamination with petroleum hydrocarbon compounds receives great concern with respect to environmental and health issues, resulting in an increase in developing innovative and sound remediation technologies. Although bioremediation of petroleum hydrocarbons has been suggested as an effective remediation technology [1,2], bioavailability of hydrophobic organic compounds (HOCs) to microorganisms, however, could be a limiting step during the biodegradation process [3,4]. Addition of surfactants to contaminated soil and water, at concentrations above their critical micelle concentration (CMC) values, can be a potential means of reducing the interfacial tension, increasing the solubility and bioavailability of HOCs, and, thus, facilitating their biodegradation [4–6].

Many studies have demonstrated that surfactants, including synthetic [7–13] and microbial surfactants [11,14–20], are capable in increasing the apparent solubility of sparingly soluble hydrocarbons, but both inhibitory [7,8,12,17,18,21] and enhanced [9,14,16–20] effects of surfactant addition on the biodegradation of insoluble hydrocarbon compounds have been reported. This discrepancy may be attributed to the different physicochemical properties of surfactants and pollutants, and the biological compatibility of surfactants which appears to be system-specific [4]. Potential advantages of biosurfactants for applications on enhancing hydrocarbon bioremediation include their unusual structural diversity that may lead to unique properties and their biodegradability [4,6,15,22,23].

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In a previous study, Whang et al. [24] presented experimental evidence of the capability of two biosurfactants, rhamnolipid (RL) and surfactin (SF), in lowering surface tension, increasing diesel solubility, and their ability on enhancing biodegradation of diesel in diesel/water systems. In addition to their ability in enhancing diesel biodegradation, environmental factors such as pH [25,26] and nitrogen source [27], that may affect interactions between contaminants, biosurfactants, and microorganisms should be taken into account when biosurfactants are applied for facilitating biodegradation. In this current study, the main objective was to investigate optimal conditions of pH and nitrogen addition for diesel biodegradation in the presence of RL and SF. This article specifically presented experimental results that evaluated effects of pH and ammonium addition on the diesel emulsion capability of RL and SF and their ability on enhancing biodegradation of diesel in diesel/water systems.

2. Materials and methods

2.1. Production of biosurfactants

The RL used in this study was produced with *Pseudomonas aeruginosa* J4, an indigenous bacteria isolated from petrochemical wastewater, and the details of RL production can be found in Wei et al. [28]. The SF applied in this study was produced using *Bacillus subtilis* ATCC 21332 grown on an iron-enriched mineral salt medium and the details of SF production can be found in Yeh et al. [29].

2.2. Biosurfactant-enhanced diesel emulsification experiment

In an incubator controlled at 26 °C, a mixture of 3 mL diesel and 2 mL supernatant containing biosurfactants examined was vortexed for 2 min and the height of emulsion layer was measured after 24 h to determine the emulsion index [30]. The equation for determining the emulsion index E_{24} (%) is as follows:

$$E_{24}\ (\%) = \frac{\text{The height of emulsion layer}}{\text{The height of total solution}} \times 100\% \quad (1)$$

2.3. Enrichment of diesel-degrading consortia

In order to enrich diesel-degrading consortia, a 50 g soil sample collected from 3 m deep sandy loam soil (84% of sand) of a diesel-contaminated site was inoculated into a 2 L conical flask containing 1.5 L of growth medium. The Bushnëll and Haas medium (BH medium) composition used to enumerate diesel-degrading consortium followed those in literature [31] with addition of diesel as the main carbon source. The mixed liquor in the flask was well mixed with a magnetic stir machine and the temperature was maintained at 26 °C using an incubator. Air was supplied through diffusers to provide sufficient oxygen and additional mixing during microbial growth. Every other day, 15 g of commercial diesel was added to 1.5 L of mixed liquor to provide a carbon source at an initial concentration of 1%. Every other week, 500 mL of the mixed liquor was transferred to 1 L of fresh BH medium to provide sufficient nutrient for microbial growth. The enriched diesel-degrading consortium was used in batch diesel–water experiments to evaluate the effects of pH and ammonium concentrations on the potential application of SF and RL for enhanced diesel biodegradation.

2.4. Biosurfactant-enhanced biodegradation in batch diesel/water systems

Diesel/water batch experiments were conducted using enriched diesel-degrading consortia to evaluate the effects of pH and ammonium addition on diesel biodegradation. From the flask for enrichment of diesel-degrading consortia, 30 mL of mixed liquor was removed and centrifuged at 10,000 × *g* (gravity) for 10 min. The supernatant was discarded and the solids were resuspended in a 1 L flask batch reactor containing 300 mL of a modified Bushnëll and Haas medium with an addition of diesel (1%) and biosurfactants (40 mg/L for SF and 50 mg/L for RL based on the results in Whang et al. [24]). The modified BH medium was prepared using BH medium with modification on concentrations of K_2HPO_4 and KH_2PO_4 as shown in Table 1 [32] in order to maintain a fixed pH condition in diesel/water systems during the experiments. In addition, the nitrogen source concentrations in batch experiments were varied by adding 0–450 mg-N/L of ammonium chloride. The batch reactor was vigorously shaken at 150 rpm using a reciprocating shaker to keep the dissolved oxygen (DO) concentration above 4 mg/L and the shaker was placed in an incubator maintained at 26 °C. Samples were taken for the determination of biomass (represented by mixed liquor volatile suspended solids, MLVSS), pH, DO, ammonium, and TPH_d throughout the batch experiment for a period of about 200 h. MLVSS and ammonium in batch diesel/water experiments were measured according to standard methods 2540-E and 4500-B, respectively [33].

Table 1
Concentrations of K_2HPO_4 and KH_2PO_4 applied in the modified BH medium to maintain a fixed pH condition in diesel/water batch experiments.

pH	K_2HPO_4 (g/L)	KH_2PO_4 (g/L)
5.2	0.37	13.31
6.3	4	10.47
7.2	12.48	3.85
8.4	16.6	0.62

2.5. Determination of total petroleum hydrocarbon-diesel (TPH_d)

TPH_d in diesel/water was extracted with methylene chloride as extraction solvent, following the procedure recommended in U.S.EPA Test Methods 3510C [34]. Following the procedure recommended in U.S.EPA Test Methods 8015B [34], the quantity of TPH_d in extract was determined using a gas chromatograph with a flame ionization detector (GC-FID, Varian CP-3800, Palo Alto, CA, USA) equipped with a 30 m capillary column (Supelco SPB^{TM}-5, 0.53 mm I.D., 1.5 μm film thickness). The temperature conditions of GC-FID were operated at 250 °C for injection port, 300 °C for detector, and an oven temperature program of 45 °C (held for 3 min) to 300 °C (held for 10 min) at a rate of 12 °C/min. Nitrogen was used as the carrier gas at a flow rate of 5 mL/min.

2.6. Quantification of growth and biodegradation kinetics

In this study, a rate expression as shown in Eq. (2) [1], depending on the concentrations of hydrocarbon (TPH_d) and biomass (X_t), with a rate constant was applied to describe the hydrocarbon consumption rate, mainly due to its superb predictive capability against rate data collected in batch diesel/water experiments. In addition, a yield coefficient Y (mg of dry cells produced/mg TPH_d consumed) can be estimated simultaneously based on the proportional relationship between hydrocarbon consumption and biomass growth. Parameter estimation for k_{bio} and Y were performed using the AQUASIM software package [35].

$$-\frac{d[TPH_d]}{dt} = k_{bio}[TPH_d]X_t \quad (2)$$

where $[TPH_d]$ is the hydrocarbon concentration (mg/L), X_t the biomass concentration (mgVSS/L), and k_{bio} represents the hydrocarbon consumption rate constant (L/mg/h).

Table 2
Diesel emulsion index E_{24} (%) at different pH and RL concentrations.

pH	RL concentrations (mg/L)					
	8	16	32	48	64	160
5.2	0	0	0	1	1	5
6.3	5	10	13	21	60	63
7.2	18	33	60	63	65	68
8.4	0	1	5	10	20	60

Table 3
Diesel emulsion index E_{24} (%) at different pH and SF concentrations.

pH	SF concentrations (mg/L)					
	10	20	40	60	120	200
5.2	1	2	2	5	5	10
6.3	5	10	15	20	30	45
7.2	10	20	30	45	55	65
8.4	30	40	60	65	70	75

3. Results and discussion

3.1. Effects of pH on biosurfactant-enhanced emulsion of diesel

The dependence of emulsion index on RL concentrations and pH studied are shown in Table 2. In Table 2, at a neutral pH of 7.2, the level of diesel emulsion, evaluated based on emulsion index E_{24}, increased rapidly from 18 to 60% with increases in the RL concentration up to 32 mg/L. Further increases in the RL concentration only slowly increased the E_{24} value from 60 to 68%, even though the RL concentration was added up to 160 mg/L. The trend of E_{24} dependence on the RL concentrations observed at pH of 7.2 was in good agreement with the dependence of surface tension reduction on the RL concentrations observed in literature [16,24,28]. At a condition of pH 6.3, the trend of E_{24} dependence was similar to that at pH of 7.2, but the E_{24} value did not increase to 60% until RL addition up to 64 mg/L. At an even more acidic condition (pH of 5.2), the diesel emulsion by RL addition reduced substantially (<10%) regardless that the RL addition was up to 160 mg/L. The trend of E_{24} dependence observed at a pH of 8.4 was similar to that at pH of 6.3, but the E_{24} value did not increase to 60% until RL addition up to 160 mg/L.

The physical properties of RL solutions including morphology and surface tension reduction were sensitive to pH [16,25,36–38]. RL, with an acid–base ionization/dissociation constant (pK_a) of 5.6, has an anionic character at a pH of 6.8, whereas at pH of 5 they are almost totally protonated and exhibit nonionic behavior in the solution [36]. In a study using fluorescent microscopy, Ishigami et al. [36] reported that RL form liposome-like vesicles at pH $\leq$ 6. Between pH values of 6 and 6.6, the RL formed either lamella-like structures or lipid aggregates, and above pH of 6.8 micelles were formed when the rhamnosyl moiety is negatively charged. Using a transmission electron microscopy, Champion et al. [37] observed the RL structure decreasing in size as the pH increased from 5.5 to 8.0, due to repulsion between the more negatively charged head groups effectively creating a larger head diameter, and then causing changes in the morphology from lamellar to vesicles to micelles. Examining the rhamnolipids produced by *P. aeruginosa* ATCC 9027, Zhang and Miller [16] found that the surface tension reduction of the RL was highest between pH 7.0 and 7.5. As the pH value increased to 7.5, 8, 9, 10 and even 11, there was only a slight increase in surface tension from 30 to 32 dyn/cm. As the pH decreased from 7.0 to 5.0, surface tension, however, increased from 30 to >40 dyn/cm. Moreover, a maximum enhancement of aqueous octadecane dispersion occurred at approximately pH 7, with dispersion decreasing with either an increase or decrease in pH. Based on our results, coupled with the results of Ishigami et al. [36], Zhang and Miller [16], and Champion et al. [37], it is speculated that the RL emulsifying capacity for diesel increases as the morphology changes as the pH is increased from 5.2 to 7.2.

The dependence of E_{24} values on SF concentrations and pH studied are shown in Table 3. The general trends of E_{24} dependence on the SF concentrations observed at different pH conditions were similar to those shown in Table 2 for RL addition, except that the emulsifying capacity of SF addition was not reduced as the pH value was increased to 8.4.

During SF production using *B. subtilis* ATCC 21332 grown on an iron-enriched medium, an induced acidification was found to be highly correlated with SF production [39]. The produced SF, however, disappeared rapidly in the medium broth once the sharp decrease in pH fell below 5. In a later study, Wei et al. [40] identified that the disappearance of SF was due to its precipitation triggered by the accumulation of extracellular acidic metabolites. Schaller et al. [41] characterized properties of SF produced by *B. subtilis* ATCC 21332 and found that reducing the pH < 6 would significantly decrease surface tension reduction ability of SF, presumably due to its precipitation under acidic conditions. Moreover, resuspension of the precipitates in nanopure water would regain its ability in surface tension reduction. In Table 3, it is, therefore, speculated that the SF emulsifying capacity for diesel reduces as the precipitate forms as the pH is decreased to <6.3. Many studies demonstrate that biosurfactants have potential for use in enhancing biodegradation rates in the remediation of sites where the mass transfer rate is limited. However, there are several potential challenges associated with the use of biosurfactant as a result of the complex interactions between the contaminant, surfactant, microorganisms, and the target environment. One important environmental factor affecting these interactions is the pH. The results presented above indicate that control of the pH needs to be considered in field applications for improved performance of RL and SF systems.

3.2. Effects of pH on biosurfactants-enhanced biodegradation in batch diesel–water systems

The microbial growth and residual TPH_d percentage profiles in batch tests with 50 mg/L of RL addition at pH conditions of 5.2 and 7.2 are presented in Fig. 1. The enriched diesel-degrading consortia were able to grow, with diesel as the sole carbon source, from 277 mg/L of MLVSS to 987 and 2433 mg/L under the pH conditions of 5.2 and 7.2, respectively. In association with microbial

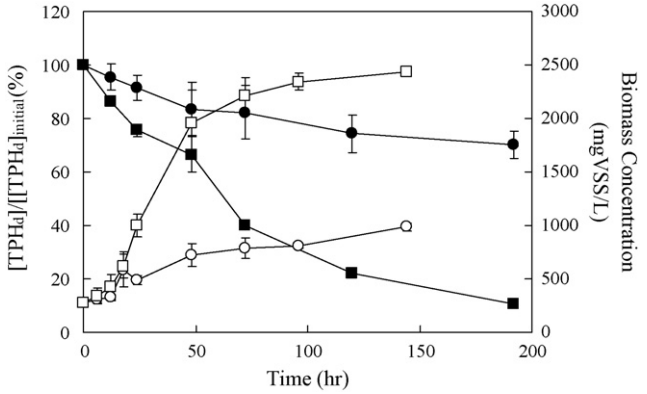

Fig. 1. The microbial growth (open symbols) and residual TPH_d percentage (solid symbols) profiles in batch diesel/water tests with 50 mg/L of RL addition at pH of 5.2 (circle) and 7.2 (square).

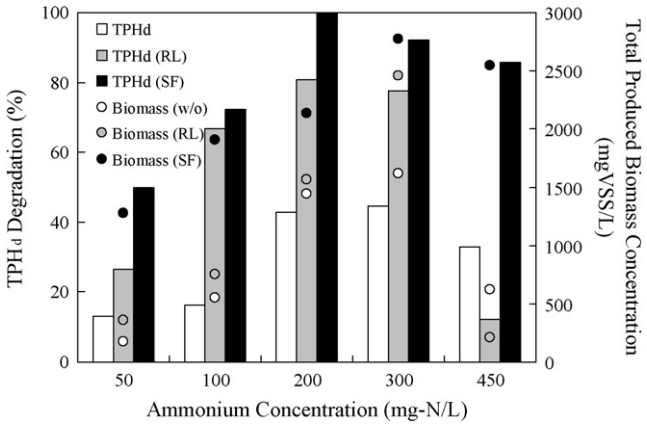

Fig. 2. Total produced biomass concentrations (circle) and degraded diesel percentages (bar) in batch tests without (w/o) and with biosurfactant addition (50 mg/L of RL or 40 mg/L of SF) under different pH conditions.

growth, the residual diesel percentage in batch tests, expressed as $[TPH_d]/[TPH_d]_{inital}$, reduced from 100% to 70.2 and 10.5% under the pH conditions of 5.2 and 7.2, respectively.

The patterns of microbial growth and diesel degradation shown in Fig. 1 were found to be similar for batch tests without or with addition of biosurfactants (50 mg/L of RL or 40 mg/L of SF) under all pH conditions studied (5.2, 6.3, 7.2, and 8.4), but with differences in total quantities of produced biomass and degraded diesel. The measured parameters of total produced biomass concentrations and degraded diesel percentages in batch tests under different pH conditions studied are summarized in Fig. 2. In addition to the results summarized in Fig. 2, estimated parameters of specific growth rate (μ) and rate constants (k_{bio}) for batch experiments studied are presented in Fig. 3. There seemed to be an apparent correlation between biomass production, diesel degradation, μ, and k_{bio} acquired from these batch tests under different pH conditions.

In Figs. 2 and 3, without biosurfactant addition, the optimum condition for maximum biomass growth, diesel biodegradation, μ, and k_{bio} was achieved at the pH of 7.2, while the worst resulted from the pH of 5.2, presumably due to an unfavorable growth environment for diesel-degrading bacteria under a relatively acidic condition. Although the optimum pH for microbial growth depends on the specific microorganisms and their respiration pathways, biodegradation of contaminants is typically favored by near natural pH values between 6 and 8 [27,42]. With experiments adjusting soil pH to 5.0, 6.0, 7.0, and 7.8, Dibble and Bartha [43] found

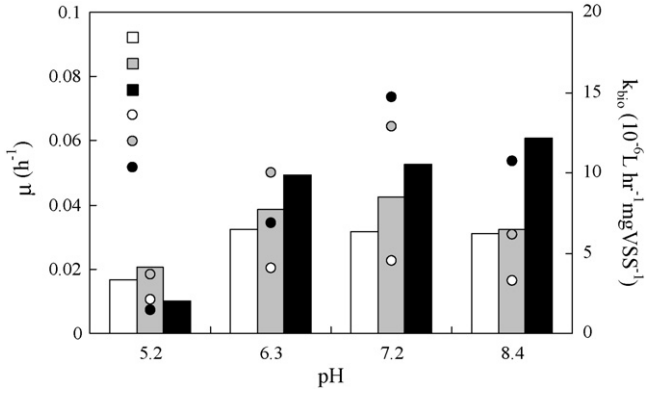

Fig. 3. Specific growth rate (μ, bar) and diesel degradation rate constants (k_{bio}, circle) in batch tests without (w/o) and with biosurfactant addition (50 mg/L of RL or 40 mg/L of SF) under different pH conditions.

a soil pH of 7.8 to be optimal for biodegradation of petroleum hydrocarbons. Similarly, Verstrate et al. [44] observed that adjustment of soil pH from acidic conditions (pH 4.5) to near-neutral conditions (pH 7.4) resulted in a doubling of the rate of biodegradation of gasoline in soil, but a considerable inhibition occurred at pH 8.5. The results presented in Figs. 2 and 3, with respect to the optimum pH condition for maximum biomass growth, diesel biodegradation, μ, and k_{bio}, were in good agreement with previous findings, suggesting the importance of pH during bioremediation of hydrocarbon-contaminated water and soil.

Regarding the mechanisms of insoluble liquid hydrocarbon transport to microbial cells, evidences indicated that surfactants can influence the uptake and consumption of insoluble hydrocarbon by microorganisms [9]. The three mechanisms for the uptake of slightly soluble liquid hydrocarbon by the microorganisms considered include (1) interaction of cells with hydrocarbon dissolved in the aqueous phase; (2) direct contact of cells with hydrocarbon drops considerably larger than the cells; (3) interaction of cells with "solubilized", "pseudosolubilized", "accommodated", "microemulisified", or "submicron" hydrocarbon droplets in entities much smaller than the cells [45]. It is generally agreed that the rate of solubilization of long-chain alkanes in the aqueous phase by physical process of solubilization is so low that the first mechanism cannot support the observed rate of microbial growth [45]. Adding surfactants not only provides micelles for solubilization of the insoluble hydrocarbon, but also facilitates emulsification, with a resulting increase in bioavailability for degradation. In the current study, compared to the results between with and without biosurfactant addition presented in Figs. 2 and 3, it is reasonable to infer that, at pH values between 6 and 8, addition of RL and SF, at a concentration above their CMC values, would be a beneficial measure to enhance biodegradation of diesel.

For the batch experiments with 50 mg/L of RL addition, an increase of pH from 5.2 to 6.3 greatly enhanced measured and estimated parameters presented in Figs. 2 and 3, while only slight increases in those parameters were observed as the pH increased from 6.3 to 7.2. Further increase of pH from 7.2 to 8.4, however, reduced those parameters considerably. For the cases of adding 40 mg/L of SF, the enhancing ability of measured and estimated parameters shared a general trend with that observed for adding 50 mg/L of RL as the pH increased from 5.2 to 7.2. Further increase of pH to 8.4, however, did not seem to negatively affect biodegradation and biomass growth to a large extent, which was different from that observed in batch tests with RL addition. Several studies investigating biodegradation of contaminants in the presence of surfactants have been performed at a pH value close to 7 [11,16,46–49]. Under some conditions, a neutral pH (pH 7) may also correspond to the pH at which greatest contaminant solubilization occurs [16,50]. In a study that evaluated effects of pH and rhamnolipid addition on solubilization and biodegradation of phenanthrene, Shin et al. [26] suggested that the pH selected for optimal substrate dispersion may not be the optimum pH for bioavailability and microbial activity. In our study, the effects of pH on RL- and SF-enhanced biodegradation of diesel shown in Figs. 2 and 3, however, seemed to be in good agreement with the trend of E_{24} values for RL or SF addition under different pH conditions presented in Tables 2 and 3, suggesting that enhanced diesel emulsification by RL or SF addition promotes biodegradation of diesel.

3.3. *Effects of ammonium concentration on biosurfactants-enhanced biodegradation in batch diesel–water systems*

The measured parameters of total produced biomass concentrations and degraded diesel percentages as well as estimated

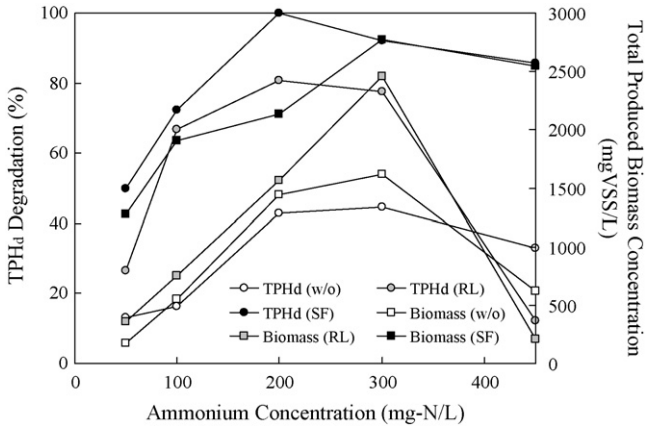

Fig. 4. Total produced biomass concentrations (square) and degraded diesel percentages (circle) in batch tests without (w/o) and with biosurfactant addition (50 mg/L of RL or 40 mg/L of SF) under different ammonium addition conditions.

parameters of μ and k_{bio} in batch tests with or without addition of biosurfactants (50 mg/L of RL or 40 mg/L of SF) at different ammonium addition conditions are summarized in Figs. 4 and 5, respectively. Similar to the results obtained under different pH conditions as shown in Figs. 2 and 3, an apparent correlation was also observed between biomass production, diesel degradation, μ, and k_{bio} acquired from these batch tests under different ammonium addition conditions.

Nitrogen is one of the primary inorganic nutrients necessary for biodegradation [27] and it is required mainly for the synthesis of cellular proteins and cell wall components. In general, the preferred form of nitrogen for growth of soil microorganisms is the reduced form, ammonium (ammonium ions) [51], although both ammonium and low-molecular-weight-organic nitrogen (alanine) can be assimilated concurrently by different populations of soil microorganisms [52]. In Figs. 4 and 5, without biosurfactant addition, an increase in the ammonium addition from 50 to 300 mg-N/L would gradually increase measured and estimated parameters, but a decrease in biomass production and μ occurred once the ammonium addition was up to 450 mg-N/L. Under nitrogen-limited conditions such as experiments with 50 and 100 mg-N/L addition, diesel biodegradation was restricted due to insufficient nitrogen available for biomass growth. For batch experiments with 200 and 300 mg-N/L addition, both measured and estimated parameters seemed to be optimized, presumably due to an adequate nitrogen source for biomass growth in those batches, leading to improved diesel degradation. At a higher ammonium addition of 450 mg-N/L, biomass growth was somewhat inhibited during the first 80 h of experiment (data not shown), resulting in a lower μ value. The inhibition on μ occurring at the ammonium addition of 450 mg-N/L could be attributed to a sudden increase in ammonium concentration for diesel-degrading bacteria, which was acclimated in the BH medium with ammonium addition of 200 mg-N/L.

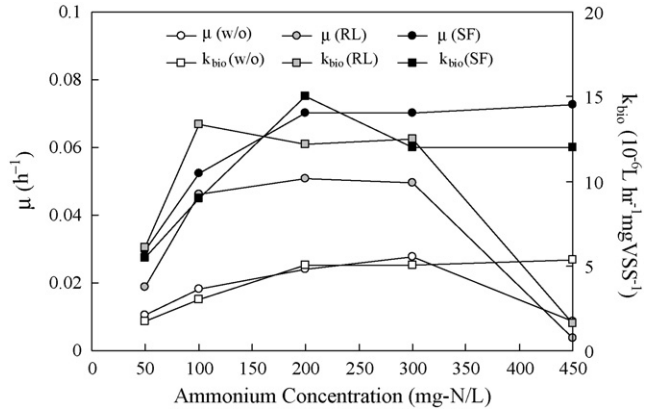

Fig. 5. Specific growth rate (μ, circle) and diesel degradation rate constants (k_{bio}, square) in batch tests without (w/o) and with biosurfactant addition (50 mg/L of RL or 40 mg/L of SF) under different ammonium addition conditions.

For batch experiments with biosurfactant addition presented in Figs. 4 and 5, it was, once again, confirmed that addition of RL and SF, at a concentration above their CMC values, would be a beneficial measure to enhance biodegradation of diesel. With respect to the dependence of ammonium concentration on the measured and estimated parameters, similar trends were also observed for the batch experiments with 50 mg/L of RL addition, but the decrease in those parameters was severe at ammonium addition up to 450 mg-N/L as shown in Figs. 4 and 5. For the batches where 40 mg/L of SF was added, the enhancing ability of measured and estimated parameters shared the general trend with that observed for adding 50 mg/L of RL as the ammonium concentration increased from 50 to 200 mg-N/L. Further increase of ammonium concentration to 450 mg-N/L, however, did not negatively affect biodegradation and biomass growth, which was very different from that observed in batch tests with RL addition. It is assumed that the two acidic residues, asparagine and glycine, of surfactin form a "claw" of sorts which easily stabilizes cations such as ammonium ion, resulting in reduction of inhibitory effect caused by high ammonium concentrations.

4. Conclusions

This study presents experimental results that evaluate effects of pH and ammonium addition on the diesel emulsion capability of rhamnolipid and surfactin and their ability on enhancing biodegradation of diesel in diesel-contaminated water systems. Compared to the experiments without biosurfactant addition, adding RL or SF to diesel–water systems at concentrations above their CMC values, in general, benefits diesel emulsification and, therefore, enhances the extent and rate of diesel degradation. The following conclusions can be drawn from this study.

1. In diesel–water systems with 50 mg/L of RL addition, an optimum pH condition for microbial growth and diesel biodegradation occurs at pH 7.2. Decreasing pH to 5.2 or increasing it to 8.4 reduces those parameters considerably. For the cases of adding 40 mg/L of SF, the enhancing ability shares a general trend with that observed for adding 50 mg/L of RL as the pH increased from 5.2 to 7.2. Further increase of pH to 8.4, however, does not seem to negatively influence biodegradation and biomass growth.
2. The effects of pH on RL or SF-enhanced biodegradation of diesel are in good agreement with the trends of E_{24} values for RL or SF addition, respectively, under different pH conditions, suggesting that enhanced diesel emulsification by RL or SF addition promotes biodegradation of diesel.
3. With respect to the effects of ammonium concentration on diesel biodegradation in diesel–water systems with 50 mg/L of RL addition, an optimum ammonium addition for microbial growth and diesel biodegradation can be found between 200 and 300 mg-N/L, but a dramatic decrease in growth and biodegradation occurs at ammonium addition up to 450 mg-N/L. For the cases of adding 40 mg/L of SF, an increase of ammonium addition from 50 to 200 mg-N/L substantially increases microbial growth and biodegradation of diesel. Further increase of ammonium concentration to 450 mg-N/L, however, does not further improve diesel biodegradation.

Acknowledgments

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