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Biodegradation of PAHs by The Mixed Cultures of Diesel Degradation Bacteria

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Abstract

Polycyclic aromatic hydrocarbons or polyaromatic hydrocarbons (PAHs) is an organic compound in hydrocarbon group. This structure is two or more aromatic rings without heteroatoms. The most abundant groups of aromatic compounds occurring in diesel fuels are naphthalene. Three strains of diesel-degrading bacteria including *Achromobacter insolitus, Candida spp and Xanthobactor polyaromatici yorans* was proved as high capability to degrade diesel. Thus, this study was aims to determine the optimal condition for growth up of diesel-degrading bacteria and diesel degradation efficiency. The experiment was conduct in batch experiment with the varied ratio of synthetic wastewater as nutrient and diesel concentration with surfactants (N:D) at 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100. Then the optimal ratio of N:D was used to determine the effect of initial naphthalene concentration on naphthalene degradation. The different initial concentration of naphthalene was varied in the range of 0 to 100 mg/L. The results showed that the highest percent COD removal (100%) was found at N:D ratio 60:40 and 0:100 followed by N:D ratio 40:60 at 96.7% and N:D ratio 80:20 and 20:80 at 97.4%, respectively. The different of nutrient added was affected the growth of biomass. The highest biomass yield was found in N:D ratio 20:80. The growth of biomass depended not only diesel but also glucose in nutrient. Glucose play as a cometabolism for growth up bacteria. The initial concentration naphthalene was affected the growing of biomass and the efficiency of naphthalene degradation. The highest naphthalene degradation efficiency (99.8%) was found at initial napthalene concentration at 20 mg/L.

Keywords : Biodegradation; Diesel degrading bacteria; Diesel; Naphthalene

Introduction

Sixteen PAHs are regulated by the U.S. Environmental Protection Agency (USEPA) based on their potential human and ecological health effects including Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a] pyrene, Dibenz[a,h]anthracene, Benzo[ghi]perylene, Indeno[1,2,3-cd]pyrene [1]. Wattayakorn (2012) [2] reported that water sample from the estuary of Chaopraya contained 4.71 µg/g of PAHs, the highest types of PAHS was naphthalene. Naphthalene or mothballs are toxic to human and animals include cause of cancer, tumor, acute toxic of nerves system, hematoma system, respiration system and digestion system.

There are many aspects of relation between diesel and naphthalene. PAHs was found in burning of coal, fuel and part of diesel. Diesel is complex and several structure for example n-alkalines, isoand cycloalkanes, PAHs, sulfer and aromatic compound especially naphthalene and alkylnapthalene [3]. Moreover, in the middle distillates such as diesel fuel is identified by a variety of straight, branched, and cyclic alkanes, as well as naphthalene, methylnaphthalenes [4].

From previous study by Singhyakaew (2015) [5], Three strains of diesel-degrading bacteria were collected from the activated sludge process of An-ping wastewater treatment plant in Taiwan. These cultures are high capability to degrade diesel. Moreover, the single culture of microbe is low capacity than mix culture of bacteria because metabolize a limited scope of hydrocarbon substrates [6]. So, this study was utilized Achromobacter insolitus, Candida spp and Xanthobactor polyaromatici yorans to degrade diesel and naphthalene with cometabolism in metabolic process.

The process of bioremediation was influenced by some physical factors as an example temperature, pH, oxygen, nutrient, microorganism number, consortium of microorganism, bioavailability, contaminant characteristics and toxic of end products [7]. The development of limiting factors can lead to more capacity treatment technology. One important factor to limit degradation of diesel is the lack of carbon source. Nutrient was found to stimulate growth of the pollutant degrading microorganisms and enhance their ability to degrade contaminants [8]. Thus, the aim of this work was investigating the optimal nutrient and diesel ratio to enhance diesel degradation and growth up of microorganism. In addition, the effect of initial naphthalene concentration to naphthalene degradation was investigated.

Methodology

Synthetic wastewater

The synthetic wastewater was prepared based on the study of Banu et al., (2009) [9]. About 20,000 ml of synthetic domestic wastewater was prepared by using various chemical as glucose 4.5 g, NH_4Cl 4g, $NaHCO_3$ 8g, KH_2PO_4 0.5 g, microelement solution ($MnCl_2.4H_2O$ 2 ml, $ZnCl_2.2H_2O$ 2 ml, $CuCl_2.2H_2O$ 2 ml, $MgSO_4.7H_2O$ 2 ml, $FeCl_3.6H_2O$ 2 ml, $CaCl_2.2H_2O$ 2 ml). After that added the RO water until the final volume was 20,000 ml. The synthetic wastewater was used as nutrient for all experiment. Synthetic wastewater was kept in refrigerator (4°c) until used and prepared a new one every 2 days.

Diesel solution

Diesel solution was prepared by getting the pure diesel 10 ml and mixed with 1 mL of Ethylene glycol mono-butyl ether (EGBE), ($CH2OHC_{H2}O_{C4}H9$, 99%) and diluted with reverse osmosis water to obtained the final volume at

1,000 ml. It was stirrer for 15 minutes for dissolve the diesel in water. At last, the 180 ml of diesel solution was diluted again with reverse osmosis water to obtained the final volume at 1,000 ml. The concentration of Diesel is 1.8 $x10^{-2}$ (v/v) and surfactant is 1.8 $x10^{-4}$(v/v). The diesel solution was prepared every two days and keep in refrigerator 4°c.

Mixed culture of bacteria

The mixed culture bacteria were collected from previous study of Singhyakaew (2015) [5] and microbial was cultured for 1 month and was stored for 1-2 years. The mixed culture bacteria consist of Achromobacter insolitus, Xanthobactor polyaromatici, and Candida spp. Those microbes were grown-up by feeding diesel with ethylene glycol mono-butyl ether (EGBT).

Experiment for determine the optimum ratio of synthetic solution (N) and diesel with surfactant (D) for diesel degradation

A batch experiment was conducted with varied ratio of N:D in the range of 100:0 to 0:100. The experiment was conducted with 1,000 ml working volume and start-up with 30 ml of mixed culture bacteria. The experiment was done in aeration mode with controlled DO concentration above 2 mg/L at room temperature. Water samples from reactor was collected and analyzed for their total COD (TCOD) and soluble COD (SCOD) every day. In addition, the mixed liquor volatile suspended solids (MLVSS) was investigated to study the accumulation mixed culture of diesel degradable bacteria. The experiment was operated until the COD concentration was increasing from the previous operating day. Furthermore, various parameters including pH, temperature, DO, and MLSS was also measured every day.

Experiment for determine the effect of initial naphthalene concentration on degradation

Two-liter volume beaker was used as reactor by added the optimal ratio of N:D from the previous experiment. About 30 ml of mixed culture bacteria was added as seed sludge. The different initial concentration of naphthalene which varied in the range of 0 to 100 mg/L. The experiment was done in aeration mode with DO concentration above 2 mg/L at room temperature. Various parameters including pH, oxidation-reduction potential (ORP), temperature, MLSS and MLVSS was measured every day. While naphthalene concentration was measured every 2 hours by HPLC technique with fluorescence detection at 254 nm, the mobile phase was a mixture of water and acetroniitrite (70:30 v/v). Separation was carried out with a reverse phase 5 µm c-18 column (250 x 4.6 mm) with flow rate 1 m/m.

Results and Discussions

The optimal ratio of N:D for diesel degradation

The results in **Table 1** showed that the highest percent SCOD removal was found at batch C and F (100%) followed by batch D (96.7%) and batch B, E (97.4%), respectively. The obtained results showed that the mixed culture bacteria used in this study was high efficiency to remove COD from wastewater. Thus, it can be indicated that the ratio of N:D did not affect the degradation of COD by mixed culture bacteria but affect the degradation time. The higher initial SCOD concentration required more degradation time to degrade SCOD.

Table 1 Percent SCOD removal and degradation time

Batch (N:D)	Initial SCOD (mg/L)	Minimum SCOD (mg/L)	% SCOD removal	Time (hr)
A (100:0)	247	10	96.0%	24
B (80:20)	312	8	97.4%	24
C (60:40)	344	0	100%	24
D (40:60)	390	13	96.7%	24
E (20:80)	464	12	97.4%	48
F (0:100)	509	0	100%	72

The results of pH are shown in **Figure 1**. It was found that pH values in all conditions was in the range of 7.0-8.41 except in batch F which lower than 7. The pH of batch F was decreasing along the experiment period because the stationary phase of batch F was reached earlier than the others batch.

MLVSS was measured to identity the increasing of biomass and determine the growth up efficiency. The results are shown in **Figure 2**. Thus, the results showed MLVSS of all batch was increase in same trend. At start of experiment, the initial bacteria 30 ml was added to reactor. The initial MLVSS in all batch was in the range of 30-47.5 mg/L. After 24 hours, the MLVSS of all batch was increasing. The highest increasing of MLVSS was found in batch E (N:D = 20:80). On the other hands, the lowest increasing of MLVSS was found in batch F (N:D = 0:100). As the increasing of MLVSS represented the growth of bacteria. It can be indicated that the bacteria growth of batch F was lowest. The results were the same with batch A (N:D = 100:0) which found the low growth of bacteria. Thus, it can be concluded that only diesel and only synthetic solution has low ability to growth up bacteria. The growth up of bacteria need both diesel and nutrient in synthetic solution. The optimal ratio of N:D was 20:80 as in batch E that provided the highest increasing of bacteria resulted in high growth rate of bacteria.

The biomass yield was calculated as shown in **Table 2**. This study was focus on the volume of the microbial community because it was necessary for startup the system. The highest biomass yield was found in batch E. The result was same trends as MLVSS results. It indicated that the carbon source in batch E was highly rated to degrade and promote growth up of mix culture. It can be indicated that the synthetic solution added to reactor was affected the biomass growth and the ratio 20:80 of synthetic solution: diesel is the optimum for growth up mix culture to degraded diesel. Thus, the growth of biomass depended not only diesel but also glucose in synthetic solution. Glucose play role in a cometabolism or substrate as was found in the study.

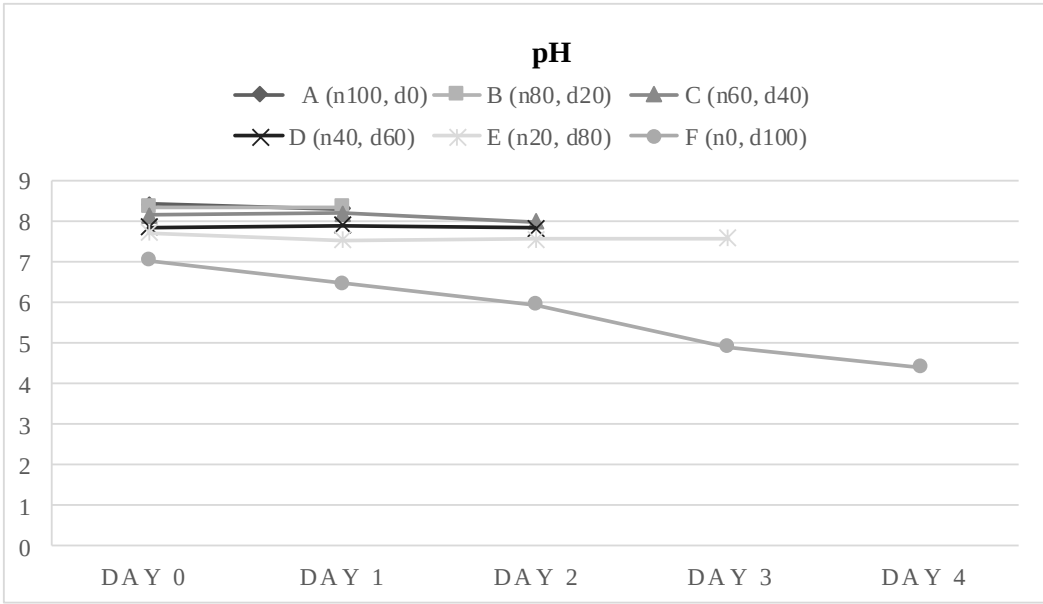

Figure 1 The results of pH in each condition

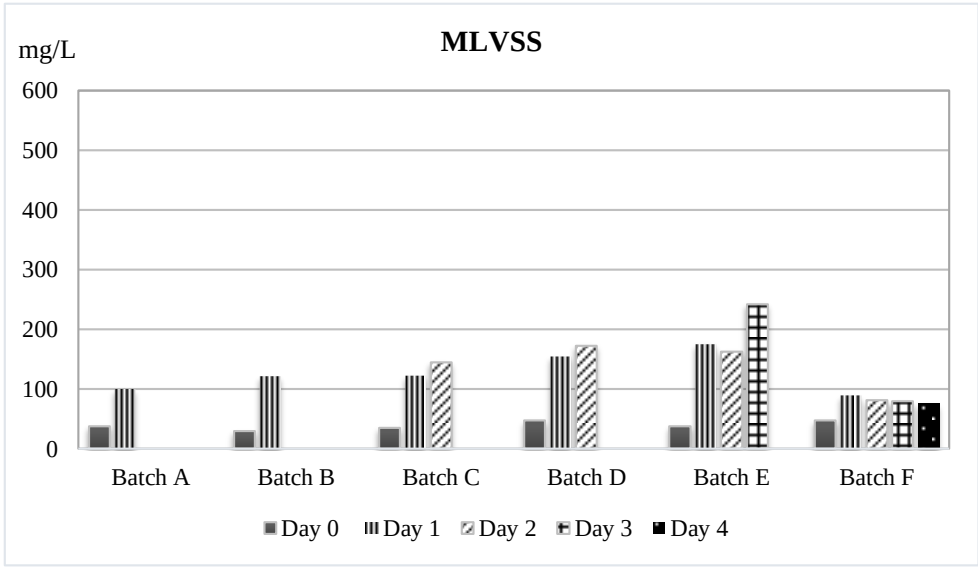

Figure 2 MLVSS in each condition

Table 2 Biomass yield in each batch

	Batch (N:D)					
	A (10:0)	B (80:20)	C (60:40)	D (40:60)	E (20:80)	F (0:10)
Yield (mg of MLVSS/mg of SCOD)	0.2637	0.3026	0.3197	0.3315	0.4535	0.0540

Effect of initial concentration naphthalene on degradation efficiency

The results showed that pH of all batch was found in the range of 6.52-7.99 at all batch conditions**.** The MLVSS in every batch was increase in the same trend. That was indicated varied naphthalene concentration was not affect to growth up the mix culture of bacteria. The biomass yield was calculated to indicate the biomass growth in each batch by normalized the COD concentration in each batch. The results of biomass yield are shown in **Table 3**, it can be indicated that the concentration of naphthalene affects biomass yield. Thus, the optimal initial concentration of naphthalene was 20 mg/L which provided the high growth rate of microbe.

The degradation of naphthalene in each batch was investigated. **Figure 3** showed naphthalene concentration in each batch. The results of degradation of naphthalene was the same trend in all batch. The naphthalene was rapidly degraded in 2 hours and continuous degraded slowly. The highest naphthalene degradation was found in batch A (99.8%) and followed by batch B (87.4%). The naphthalene degradation in batch C to E was the nearly the same (75%). The efficiency and time to degrade naphthalene was affected by the initial naphthalene concentration. The higher decrease naphthalene concentration provided the lower efficiency and required more time to degrade. This resulted can imply that the suitable of initial napthalene concentration was 20 mg/L.

As the government gazette of Thailand, naphthalene does not over 48 mg/L in underground water [10]. Microbe can degrade naphthalene of every batch to lower than 48 mg/L after 2 hours operation periods.

Table 3 Biomass Yield of each batch

	Batch				
	A	B	C	D	E
Initial Naphthalene concentration	20 mg/L	40 mg/L	60 mg/L	80 mg/L	100 mg/L
Yield (mg of MLVSS/mg of SCOD)	1.6	0.5	0.325	0.275	0.195

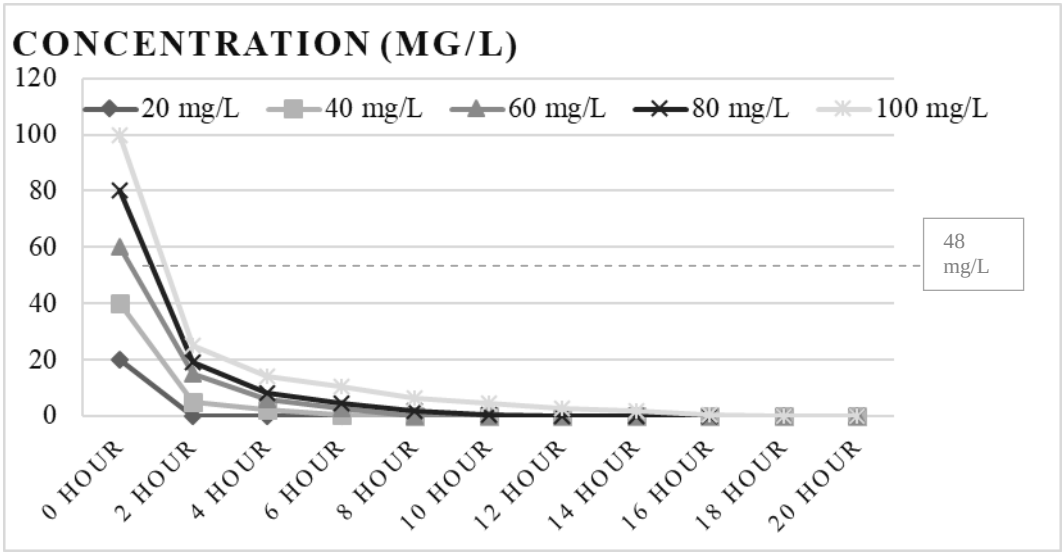

Figure 3 Naphthalene concentration in each batch during operating periods

Conclusion

The mixed culture bacteria was high efficiency to remove organic compound from wastewater. The ratio of N:D did not affect the degradation of COD by mixed culture bacteria but affect the degradation time. The result of MLSS and MLVSS showed that the mix cultured was low degradable when degrade pure diesel or pure synthetic solution. Thus, the ratio of synthetic solution and diesel added was affected the biomass growth. The highest biomass yield (0.4535 mg of MLVSS/mg of SCOD) was found at N:D ratio of 20:80. From the obtained results, the optimal ratio of N:D was 20:80 with the high SCOD removal efficiency and biomass yield.

The mixed cultures of diesel degradation bacteria can degrade naphthalene 100% with varied concentration in varied duration time. The highest efficiency of naphthalene degraded was found in lowest initial concentration of naphthalene (20 mg/L). The suitable initial concentration of naphthalene was 20 mg/L which provided the highest growth rate of microbes and the group of cultures can degrade the others chemical that similar diesel structure as naphthalene.

Acknowledgement

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