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Effect of Selected Bacteria as Bioremediation on the Degradation of Fats Oils and Greases in Wastewater from Cafeteria Grease Traps

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Abstract
Fats, oils and greases (FOG) are used in the food preparation and cooking. However, FOG are pouring an accumulative load on drainage systems as they can cause blockages. They also can pollute public sewer systems and can deplete oxygen levels in waterways causing aquatic life may be killed. The objectives of this research were to study the activity of immobilized enzyme lipase derived from the selected bacteria; Bacillus subtilis, Staphylococcus epidermidis, and Pseudomonas aeruginosa on FOG degradation and wastewater treatment in cafeteria grease traps. The experiment data collected after bacterial cultivation in the wastewater for 5 days. The comparison between batch and continuous methods was investigated. The results show that activities of enzyme lipase in oily wastewater using continuous method showed the high efficacy more than batch method. The most activity of enzyme lipase was found in P. aeruginosa to be 819.92 unit/ ml. significantly ($p < 0.001$), following by B. subtilis and S. epidermidis to be 579.95 and 559.95 unit/ ml, respectively. Moreover, the most thickness reduction of the fat layer was found in P. aeruginosa to be 61.22 percent significantly ($p < 0.05$), following by B. subtilis and S. epidermidis to be 57.14 and 53.06 percent, respectively. Nevertheless, the most BOD5 treatment efficiency was found in B. subtilis to be 64.51 percent removal, following by S. epidermidis and P. aeruginosa to be 63.58 and 60.72 percent, respectively. In conclusions, enzyme lipase from P. aeruginosa show that the most thickness reduction of the fat layer using continuous method. However, B. subtilis reduced the BOD5 to a minimum by continuous method. This bioremediation method would increase efficiency of oily wastewater and FOG degradation, moreover, this method could minimize area disturbance compared with physical and chemical method.

Keywords: Bioremediation, immobilized enzyme lipase, degradation of FOG in Wastewater, reduced the thickness of the fat layer

1. Introduction

Fats, oils and greases (FOG) are primarily generated from food establishments. They have been discharged from food industries, eatery, abattoir, and household (Bhumibhamon, o., *et al*, 2002). However, FOG are pouring an accumulative load on a grease trap, a plumbing device designed to intercept most greases before they enter a wastewater disposal system, and drainage systems as they can cause blockages of sewerage pipes. Impacts from residential areas particularly multi-family housing can merge as a major contributor to wastewater infrastructure blockages that cause sanitary sewer overflows, errors of pumping stations, and odors. They also can pollute in wastewater often causes major problem in biological wastewater treatment processes. Because they will form layer on water surface and deplete oxygen levels in water causing aquatic life may be killed (Becker, P., *et al*, 1999).

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Generally, treatment of small amount of lipid contaminated wastewater can be achieved by physical operations such as skimming and adsorbing using fabrics (Bhumibhamon, O., & Phattayakorn, K., 2003). Moreover, chemical method commonly used hazardous toxic substances such as sodium hydroxide (caustic soda) and sulfuric acid. However, these chemical operations are costly and harmful to microorganism in wastewater treatment processes. Therefore, biological wastewater treatment is interested by lipase-producing microorganisms because FOG residues are converted to carbon dioxide, water and biomass (Lefebvre, E., *et al*, 1998). The bioremediation is effective treatment in terms of efficacy, safety on long terms use, cost and simplicity of administration (Jain. K.P., *et al*, 2011).

Lipase-producing microorganisms such as yeasts, fungi, and bacteria were interesting. Yeasts produced lipase are *Candida deformans* (Muderwa, J. M., & Ratamahenina, R., 1985), *C. rugosa* (Rao, P. V., *et al*, 1993), *C. cyindracea* (Rungruang, O., & Pattanapipitpaisal, P. 2011), *C. maltose*, *C. tropicalis* (Nonthirach, I., & Pungrasmi, W., 2011), *C. catenulata* (Joo, H.S., *et al*, 2008) and *Yarrowia lipolytica* (Nonthirach, I., & Pungrasmi, W., 2011; Chansirirattana, J., *et al*, 2015). Whereas bacteria produced lipase are *Pseudomonas aeruginosa* EF2 (Gilbert, A., *et al*, 1991; Martínez, A. & Soberón, G., 2001), *P. fragi* CRDA 323 (Pabai, F., *et al*, 1995), *Bacillus* sp. (Nascimento, W.C.A., & Martins, M.L.L. (2004), *B. thermaleovorans* (Markossian, S., *et al*, 2000), *B. subtilis* (Thongpitak, T., *et al*, 2012), and *Staphylococcus epidermidis* (Thongpitak, T., *et al*, 2012).

The lipase producing microorganisms from variety sources have been isolated and studied on the degradable efficiency as both single culture and mixed culture formula (Bhumibhamon, O., *et al*, 2002). For example, Wakelin, G., & Forster, C. (1997) found a single culture and mixed culture which were isolated from grease trap degraded over 90% of fat content in activated sludge system. Moreover, Rungruang, O., & Pattanapipitpaisal, P. (2011) found immobilized-PTL38 lipase on egg shell show the higher BOD_5 treatment efficiency and percent of oil degradation in wastewater than immobilized-PTL38 bacterial cell. The present study was aimed to study the activity of immobilized enzyme lipase on eggshells derived from the selected bacteria; *Bacillus subtilis*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* on FOG degradation and wastewater treatment in cafeteria grease traps.

2. Materials and Methods

2.1 Bacterial strains

The bacteria, *Bacillus subtilis* (TISTR1248), *Staphylococcus epidermidis* (TISTR518), and *Pseudomonas aeruginosa* (TISTR1287), were obtained from TISTR Culture Collection (Thailand Institute of Scientific and Technological Research). Strains were transferred and preserved on nutrient agar (NA) slants at 4°C.

2.2 Lipase production

Each selected bacteria was cultivated in 100 ml Erlenmeyer flasks containing 10 ml of enrichment medium A and incubated on rotary shaker at 150 rpm, 55°C for 18 hours, then the solution was diluted and adjusted to 5% (v/v) in 50 ml of enrichment medium B. The medium was incubated on rotary shaker at 150 rpm, 55°C for 48 hours,

then the medium was centrifuged at 19,000 rpm, 4°C for 10 minutes. The supernatant, extracellular lipase of the each selected bacteria, was isolated from each selected bacteria and filtered by Millipore™ membrane filtration (Rungruang, O., & Pattanapipitpaisal, P., 2011).

2.3 Enzyme lipase immobilization on chicken eggshell

The chicken eggshell, after being crushed into small pieces, was placed in a boiling-water bath for 10 minutes. The pieces were washed with acetone and dried in an oven at 60°C. The pieces were then ground and passed through sieves of 60, 80, and 100 mesh. Crushed eggshell (50 g of 100 mesh) was added to 12.5 ml of each enzyme lipase solution and stirred for 15 minutes. Then, glutaraldehyde (2.0%) was added slowly with stirring, the mixture was allowed to dry in the air for 6 hours (Makkar, H., & Sharma, O., 1983; Rungruang, O., & Pattanapipitpaisal, P. 2011).

2.4 Degradation of FOG in wastewater with enzyme lipase immobilization

2.4.1 Wastewater treatment using batch method

The oily wastewater was obtained from the grease traps of a cafeteria. Each 3% of enzyme lipase immobilization from selected bacteria inoculum was incubated in 5,000 ml Erlenmeyer flasks containing 2,500 ml of oily wastewater, then shaken at 200 rpm, 30°C. One hundred ml of water sample were carried out every 24 hours for 5 days. BOD and SS were analyzed with standard method (APHA, 1998). The lipase activity was determined according to a modification of Horani, K. (1996) and presented as percent degradation.

2.4.2 Wastewater treatment using continuous method

Each 3% of enzyme lipase immobilization from selected bacteria inoculum was incubated in a 2.54 cm. diameter column, 1 m. long. Continuous up flow of oily wastewater with flow rate of 2 ml /minute were used (Matsumiya, Y., *et al*, 2007; Rungruang, O., & Pattanapipitpaisal, P., 2011). One hundred ml of water sample were carried out every 24 hours for 5 days. BOD and SS were analyzed with standard method (APHA, 1998). The lipase activity was determined according to a modification of Horani, K. (1996) and presented as percent degradation.

3. Results and Discussion

3.1 Preliminary characterization of wastewater samples

General properties of the wastewater sample, taken from cafeteria grease traps, were evaluated. The pH was 3.98 – 4.50, the BOD value was 118 - 260 mg/l, the SS values was 260 – 300 mg/l. The thickness of the fat layer was 110 mm, and the value for the enzyme lipase content was 55.56 unit/ml.

3.2 Wastewater treatment with enzyme lipase immobilization by batch method

The most activity of enzyme lipase was found in *P. aeruginosa* to be 419.96 unit/ ml, following by *B. subtilis* and *S. epidermidis* to be 379.96 and 179.98 unit/ ml, respectively (Figure 1). Moreover, the most thickness reduction of the fat layer was found in *P.*

aeruginosa to be 48.98 percent significantly ($p < 0.05$), following by *B. subtilis* and *S. epidermidis* to be 32.65 and 26.53 percent, respectively. Nevertheless, the most BOD_5 treatment efficiency was found in *B. subtilis* to be 62.66 percent removal, following by *S. epidermidis* and *P. aeruginosa* to be 52.31 and 42.05 percent, respectively. Moreover, the most SS reduction was found in *B. subtilis* to be 17.69 percent removal, following by *S. epidermidis* and *P. aeruginosa* to be 16.15 and 9.23 percent, respectively (table 1; Figure 2).

3.3 Wastewater treatment with enzyme lipase immobilization by continuous method

The most activity of enzyme lipase was found in *P. aeruginosa* to be 819.92 unit/ ml. significantly ($p < 0.001$), following by *B. subtilis* and *S. epidermidis* to be 579.95 and 559.95 unit/ ml, respectively (figure 1). Moreover, the most thickness reduction of the fat layer was found in *P. aeruginosa* to be 61.22 percent significantly ($p < 0.05$), following by *B. subtilis* and *S. epidermidis* to be 57.14 and 53.06 percent, respectively. Nevertheless, the most BOD_5 treatment efficiency was found in *B. subtilis* to be 64.51 percent removal, following by *S. epidermidis* and *P. aeruginosa* to be 63.58 and 60.72 percent, respectively. Moreover, the most SS reduction was found in *B. subtilis* to be 40.00 percent removal, following by *S. epidermidis* and *P. aeruginosa* to be 14.61 and 5.77 percent, respectively (table 1; Figure 2).

Table 1: Efficacy percent [1,2] of wastewater treatment and FOG degradation by enzyme lipase immobilization from selected bacteria

enzyme lipase immobilization	pH		activity of enzyme lipase (unit/ ml)		Thickness of fat layer (mm)		Thickness of sludge layer (mm)		SS (mg/ l)		BOD_5 (mg/ l)	
	B	C	B	C	B	C	B	C	B	C	B	C
Control	-0.12^a	-1.04^a	-3.54^a	-1.79^a	+10.67^a	+4.56^a	+42.53^a	+2.53^a	+23.20^a	+10.15^a	+6.67^a	+4.63^a
B. subtilis	-11.31^b	-12.31^b	+683.87^b	+1007.82^b	-32.65^b	-57.14^b	-12.73^b	-31.82^b	-17.69^b	-40.00^b	-62.66^b	-64.51^b
S. epidermidis	-10.05^b	-12.56^b	+323.94^c	+1043.82^c	-26.53^c	-53.06^b	-10.91^b	-30.00^b	-16.15^b	-14.61^c	-52.31^c	-63.58^b
P. aeruginosa	-13.57^b	-14.57^b	+755.86^d	+1475.73^d	-48.98^d	-61.22^c	-10.00^b	-13.64^c	-9.23^c	-5.77^d	-42.05^d	-60.72^c

[1] Means with the different letters (in same column) are significantly different at $p < 0.05$
[2] + refer to increased percentage, - refer to reduced percentage
B refer to batch method, C refer to continuous method

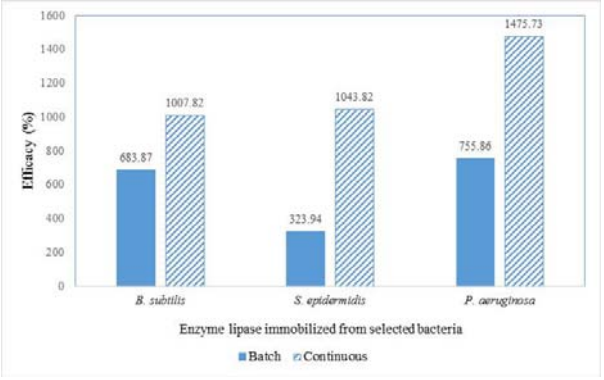

Figure 1: Efficacy of activity of enzyme lipase from selected bacteria in oily wastewater using batch and continuous methods

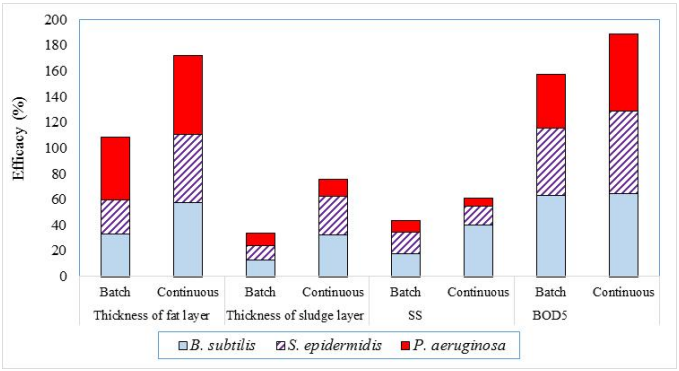

Figure 2: Efficacy of oily wastewater treatment and FOG degradation by enzyme lipase immobilization from selected bacteria using batch and continuous methods

The results of the activity of enzyme lipase from selected bacteria in oily wastewater using between batch and continuous methods indicated that lipase activity in immobilization system in the continuous method was higher than batch method. Microorganisms, on the eggshell and in wastewater, could grow and release the extracellular enzyme. However, in batch method, the eggshell could sink at the bottom of container and FOG in wastewater has been used for eatery. In contrast, the eggshell could intermix with new oily wastewater in continuous method, thus, the enzyme lipase from selected bacteria could still activate.

The most activity of enzyme lipase was found in *P. aeruginosa* to be 819.92 unit/ ml. significantly ($p < 0.001$), following by *B. subtilis* and *S. epidermidis* to be 579.95 and 559.95 unit/ ml, respectively. Moreover, the most thickness reduction of the fat layer was found in *P. aeruginosa* to be 61.22 percent significantly ($p < 0.05$), following by *B. subtilis* and *S. epidermidis* to be 57.14 and 53.06 percent, respectively. Enzyme lipase produced by different organisms might have different reaction such as *Pseudomonas* spp. were dominated hydrolytic reaction of lipase (Dunhaupt, A., *et al*, 1992; Bhumibhamon, O., & Phattayakorn, K., 2003). However, FOG degradation with microorganisms still activities of better than without using microorganisms.

4. Conclusion

Experimental results indicated that activities of enzyme lipase in oily wastewater using continuous method showed the high efficacy more than batch method. The most activity of enzyme lipase was found in *P. aeruginosa* significantly ($p < 0.001$), following by *B. subtilis* and *S. epidermidis*, respectively. Moreover, the most thickness reduction of the fat layer was found in *P. aeruginosa* significantly ($p < 0.05$), following by *B. subtilis* and *S. epidermidis*, respectively. Nevertheless, the most BOD_5 treatment efficiency was found in *B. subtilis*, following by *S. epidermidis* and *P. aeruginosa*, respectively. Therefore, the immobilization of microorganism cells on eggshell has been used for increase efficiency for oily wastewater treatment.

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