

Selection of *Pseudomonas* sp. for Lipid and Detergent Degradation

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Abstract

The objective of the research was to identify which *Pseudomonas* species has the ability to alter lipids and surfactants simultaneously. This research was conducted by using three *Pseudomonas* species, vegetable oil, and commercial detergent which contains Linear Alkyl Sulfonate LAS. The result shows that *Pseudomonas aeruginosa* is able to reduce lipids and surfactant 80.53% and 61.22% respectively.

Keywords: Degradation, *Pseudomonas*, surfactants, vegetable oil

1. INTRODUCTION

Lipids from restaurant could be derived from plant, animal and fish on fish waste, for instance *lemuru* fish whose wastes were its head, its digestion parts and liver. These wastes consisted of 5.67 % lipid from head, 5.08% for its digestion parts, and 5.80% for its liver (Khoddami, *et. al.* 2009). Folke and Lander (1999) found that 97.5 % of LAS was distributed in water, 2 % in sediment, suspended solids, biota and small part in water and air. Thus, it emerged that most of LAS still found in the water. The absorption of LAS inside the solid particle was a process which cannot be restored (Petrovic and Fernandez-Alba, 2002). In the waste processing unit, certain amount of LAS will be absorbed by the solids and disposed together with the solids.

Pseudomonas mendocina is able to hydrolyze lipids on the emulsion of substrate - lipids without Tween shows a lower result than lipids with Tween. The increasing of Tween will improve the hydrolysis ability of lipids. This result shows that the existence of surfactants can improve the activity of lipase from *Pseudomonas* (Bendikienè, *et.al.* 2007). Lipase from *Pseudomonas aeruginosa* MB 5001 has an optimum condition on pH 8,0 and temperature 55°C. This condition was different from lipase from any other *Pseudomonas* (Chartrain, *et.al.* 1993).

Bacteria *Escherichia coli*, *Serratia marcescens*, *Proteus vulgaris* and

Pseudomonas fluorescens are able to degrade surfactants lauryl sulfate (Huddleston and Allerd, 1963 *cit* Goodnow and Harrison, 1972). *Pseudomonas* sp and *Klebsiella* sp also succeed to be isolated from contaminated shampoo (Flawn, *et al.* 1973). Degrading bacteria of synthetic surfactants LAS that can be isolated from liquid waste were *Brevibacterium* sp, *Enterobacter agglomerans*, *E. liquefaciens*, *E. majodoratus*, *Escherichia coli*, *Klebsiella aerogenes*, *K. liquefaciens*, *K. oxytara*, *Proteus* sp, *Pseudomonas aeruginosa* and *Staphylococcus albus* (Ojo and Oso, 2008).

2. MATERIAL AND METHODS

Isolates bacteria used were: *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Pseudomonas fluorescens*. These bacteria were tested on inorganic medium that is composed of (per liter of aquades): KH₂PO₄ 0,48 g (Merck), K₂HPO₄ 1,12 g (Merck), and (NH₄)₂SO₄ 2,0 g (Merck). Separately, it was added by MgSO₄·7H₂O (0,1 g/L) in the amount of 1mL (Shabtai, 1991). The source of carbon used was commercial detergent 1 g/L and cooking oil 5 mL/L.

This research was conducted in random design group consisted of 3 factors:

- Factor P: species from *Pseudomonas*, consisted of:

P1: *Pseudomonas aeruginosa*

P2: *Pseudomonas putida*

- P3: *Pseudomonas fluorescens*
- Factor D: concentration of detergent
 - D1: detergent 1 g/L
 - D2: detergent 2 g/L
- Factor M: concentration of cooking oil
 - M1: cooking oil 5 mL/L
 - M2: cooking oil 10 mL/L

The analysis was performed toward the detergent and oil content after 5 days of incubation with 120 rpm of shaking on room temperature. Isolates with the highest degradation ability will be chosen for the next experiment.

3. RESULTS AND DISCUSSION

3.1 The Percentage of Surfactants Decrease

This research found that the decreasing of detergent concentration was influenced by *Pseudomonas* sp, the initial detergent content and the oil percentage in the waste. However, there was no correlation between two factors but there was an interaction from these three factors. This research found the differentiation of *Pseudomonas* in altering surfactants LAS (Linear alkylsulfonates), which was the active material from detergent used, showed a difference. *Pseudomonas aeruginosa* has a better ability to alter LAS than *Pseudomonas putida* and *P. fluorescens* (Table 1).

Table 1. The Percentage of Linear Alkyl Sulfonate (LAS) Degradation by Isolates *Pseudomonas* sp.

Isolates Bacteria	LAS Degradation (%)
<i>P. aeruginosa</i>	80.53
<i>P. fluorescens</i>	67.51
<i>P. putida</i>	74.09

The result of the research conducted by Goodnow and Harrison (1972) showed that *Pseudomonas aeruginosa* 60L-4 is able to decrease LAS 97% with concentration of 10 mg/L in 24 hours. For LAS with concentration of 20 mg/L, the time needed to degrade 90% on the river flow is 4 days (Perales, et.al. 1999). The higher concentrations used in this research are 100 mg/L and 200 mg/L (surfactants content in detergent that is being analyzed is 10-12%). *Pseudomonas aeruginosa* can only decrease LAS 80.53% in 5 days. This low effect of decreasing was caused by the existence of another substrate

(lipids) in substrate. The same result was also shown by *Pseudomonas fluorescens*. Mostly, *Pseudomonas putida* can be found in waste that contains detergent but it is not well-reported for its ability besides its existence. The highlight of *P. putida* is focused more on its ability to metabolize lipids because it has a strong extracellular lipase.

3.2. The Percentage of Lipids Degradation

The statistic analysis result shows that there is a difference *Pseudomonas* species in the decrease of lipids content. *Pseudomonas* sp belongs to true lipase because almost all *Pseudomonas* can produce lipase (Arpigny and Jaeger, 1999). Lipids metabolism on substrate which consisted of two carbon sources shows that metabolism process of lipids is easier than surfactants. This appears on the percentage of lipids decrease which is higher than surfactants. Table 2 explained that the highest decreasing came from *P. Aeruginosa*, *P. Putida* and *P. Fluorescens* respectively.

Table 2. The Percentage of Lipids Decrease by Isolates *Pseudomonas* sp.

Bacteria	Lipid Degradation (%)
<i>P. aeruginosa</i>	61.22
<i>P. fluorescens</i>	50.83
<i>P. putida</i>	49.93
<i>p</i> = 0.001	

Table 2 shows that the ability of *P. aeruginosa* is better than *P. fluorescens* and *P. putida*. Although LAS is an emulsifier for plant lipids in the water, the existence of LAS can inhibit the activity of lipase. The activity of lipase from *Pseudomonas fluorescens* will be obstructed by LAS concentration above 450 µM LAS (Helisto and Korpela, 1998). This causes the low ability of lipid degradation by *P. fluorescens*.

3.3. The Total Number of Bacteria

The statistic analysis result shows that the total number of bacteria during the degradation process of surfactants and lipids decreases from the initial inoculation, but still has the ability to degrade. The measurement of the total number of bacteria was conducted on the fifth day so that the bacteria had possibility to reach degradation phase on its growth curve.

This research found that the bacterial total influenced by lipids concentration (Table 3). This result conducted that lipid in low concentration can be metabolisms by *Pseudomonas* sp but not in high concentration.

Table 3. The Total Number of Bacteria During the Alter Process of Lipids and Detergent by Isolates *Pseudomonas* sp.

Initial Concentration of Lipids (mL/L)	The Total Number of Bacteria (Log)
1	4.46
2	3.72
$p = 0.007$	

The highest decrease of bacteria amount is on *P. Fluorescens*, while the most stable is on *P. putida* (Table 4). The amount of bacteria that shows the highest amount during the research period is *P. Aeruginosa*. This shows that *P. aeruginosa* is the most durable isolates toward the lipids used.

Table 4. The Amount of Bacteria during the alter process of lipids and detergent by Isolates *Pseudomonas* sp.

Bacteria	Initial Concentration of Lipids (mL/L)	The Total of Bacteria (Log)
<i>P. aeruginosa</i>	1	4.63
	2	4.38
<i>P. fluorescens</i>	1	4.30
	2	3.17
<i>P. putida</i>	1	3.96
	2	4.09
$p = 0.001$		

CONCLUSSIONS

From the analysis result, the decreasing of percentage of surfactants, lipids, and the amount of bacteria showed that *Pseudomonas aeruginosa* was the best isolates in the degradation process of surfactants and lipids simultaneously.

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