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Optimization of pH, Temperature and Agitation Rate on Biodegradation of Lipids and Detergents in Food Wastewater by *Bacillus sp* N-09

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ABSTRACT

The purpose of this research was to determine the optimum conditions of pH, temperature and agitation rate for the biodegradation of lipids and detergent by *Bacillus cereus* N-09. A response surface method was used to analysis the data. The materials used in this research was the waste created by boiling instant noodles as a reference on the packaging and then diluted ten times and added a commercial detergent containing LAS as much as 1%. Three factors namely pH (6.0 to 8.0), temperature (28 - 32^{0}C) and agitation rate (100-140 rpm) were studied. Erlenmeyer flask filled with 100 ml medium and sterilized and then inoculated with Bacillus cereus N-09 age of 2 days. Incubation was carried out for 5 days. The results show that the optimal condition for pH was 6.00, temperature of 30^{0}C and agitation speed of 130rpm. In these conditions the degradation of lipids and detergents achieved respectively were 73.5% and 93.35%.

Key words: biodegradation, detergent, lipid, food waste.

INTRODUCTION

Lipids that are suspended will be easily removed by physical and chemical processes. Lipid who escaped this process will contribute to the value of BOD and COD in the effluent so that the required handling both aerobic and anaerobic biological [1]. Lipolytic activity of Actinomycetes *Amycolatopsis mediterranei* DSM 43304 is at 28 - 35^{0}C. At temperatures below 25^{0}C and above 35^{0}C showed a decrease [2]. Increasing the agitation rate from 0 - 150 rpm will increase the amount of biomass and oil degradation then experienced a stable condition and does not occur degradation at a rate of 200 rpm [3].

High levels of detergent in water are toxic to some aquatic organisms that can cause interference aquatic ecosystems. At a concentration of more than 0.45 ppm, LAS affect gastrulation in sea urchin embryonic skeleton formation (*Paracentrotus lividus*), snail developmental disorder (*Lymnea rubiginosa*) in the formation of blastomeres and the shell [4], improve abnormal embryo and frog tadpoles (*F. cancrivora*) if concentrations above 3.2 mg/l [5].

Degradation of detergents by microbial populations occur best at alkaline pH and room temperature [6]. The growth of surfactant decomposer bacteria strains MH1 and MH2 in alkaline medium were optimum at pH 7.4 and 8.0. Within 5 days, MH1 able to remodel LAS in growth media at 93.6% while the MH2 amounted to 84.6%. LAS degradation highest occurs during the logarithmic growth phase [7]. Not much research on the influence of of temperature on the degradation of detergents, Khleifat *et al,* [8] reported that the optimum temperature degradation of surfactant by *Burkholderia* sp is 37^{0}C. Prats *et al*, [1] also reported the existence of microorganism that is able to removed LAS at low temperatures.

Khleifat *et al,* [8] reported that the optimum surfactant degradation occurred at a rate of agitation was 150 rpm. These results suggest that agitation will make the contact of bacteria with the substrate take place optimally. At a high rate of agitation will spread to the existence of metabolites throughout the medium and not concentrated around the bacteria so that the bacterial activity was not inhibited.

Bacillus cereus N-09 is a bacterium that has the ability biodegradation of lipids and detergents (with active ingredient linear Alkylbenzene sulfonate). These bacteria were isolated from the sewer line restaurants. Biodegradation activity is better than *Burkholderia cepacea, Pseudomonas aeruginosa, P.* and *P. fluorescens putida* [9].

The purpose of this study was to determine the optimum conditions for pH, temperature and agitation rate on the degradation LAS and lipids from *Bacilluc cereus* N-09 on food waste.

MATERIALS AND METHODS

Bacillus cereus N-09 obtained from the Laboratory of Bioindustry of Agricultural Technology Faculty of Brawijaya University. The liquid waste is made from boiled noodle soup instances the advice of the presentation on the packaging are diluted ten times and the commercial detergent plus 1% and then 100 mL of wastewater was entered in 250 mL Erlenmeyer and sterilized at a temperature of 121^{0}C for 15 minutes. Experiment design using Response Surface Methodology. Factors used in this study were pH (6, 7 and 8), temperature

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(28, 30 and 320C) and agitation rate (100.120 and 140 rpm). Liquid waste in the Erlenmeyer was inoculated with Bacillus cereus N-09 was 1 mL and incubated for 5 days. Analysis was performed on levels of lipids and detergents that are stated as LAS (Linear Alkylbenzene sulfonate). Response analysis conducted using design expert software ver 7.1.6. Analysis performed includes LAS and lipid levels.

RESULTS AND DISCUSSION

Biodegradation of Linear Alkylbenzene Sulfonate

LAS degradation is strongly influenced by environmental conditions. LAS is a surfactant that easily degradation and is widely used for domestic purposes in the form of detergent. Results of analysis of variance showed that the model is significant (alpha = 0.0056) with a very significant factor is the rate of agitation that is quadratic (alpha = 0.0018). Regression model obtained is:

$Y_1 = 183{,}62 - 4{,}44X_1 - 10{,}51X_2 + 1{,}33X_3 + 0{,}44X_1X_2 - 0{,}07X_1X_3 + 0{,}06X_2X_3 - 0{,}01X_3^2$

$R^2 = 0{,}76$

Explanation:

Y_1 : Percentage decrease in LAS;

X_1 : pH; X_2 : temperature (^{0}C); X_3 : agitation rate (rpm)

Optimal point optimization results were pH = 6.00, temperature = 30^{0}C and agitation rate = 130rpm which would result in a decrease of 93.35% LAS. Optimal point is different from the use of *Burkholderia* sp is pH = 7.4 temperature of 37^{0}C and agitated rate at 150 rpm [8]. This difference is caused by bacteria that are used are different. *Bacillus cereus* N-09 is a mesophyll bacteria isolated from the region of Yogyakarta which has an average daily temperature of 26 - 32^{0}C. LAS can be used by bacteria as a source of carbon and sulfur. Bacteria would do desulfonation of end alkyl chain. Short alkyl chains more easily degradation than the longer and LAS can be found on the outer surface and in bacteria [10].

Agitation plays an important role in the process of contact between the microbes with the substrate. Good agitation will increase the rate of substrate metabolism of bacteria so that the degradation is going well (Figure 1).

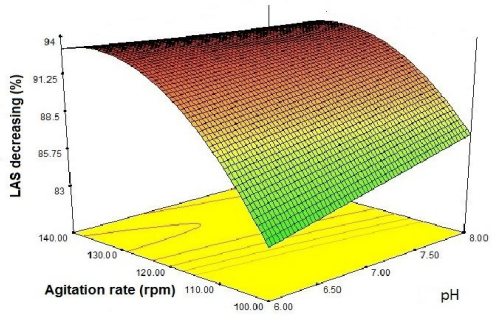

Figure 1. The effect of agitation rate (rpm) and pH on LAS decreasing percentage by Bacillus cereus N-09.

From Figure 1 can be seen that the rate of agitation play a very significant effect on the degradation of LAS in all treatments. The higher rate of agitation, the degradation rate will be higher with optimum agitation at 130 rpm. Optimum agitation rate was lower than that observed Khleifat et al, [8] using Burkholderia sp. This is due to the high speed it is prone to bacterial cell lysis due to collisions and detergent. These results also indicate that the agitation of good bacteria will make contact with the substrate take place optimally. At a high rate of agitation will spread throughout the existence of metabolites of medium and not concentrated around the bacteria so that the bacterial activity was not inhibited.

pH less effect on the rate of high agitation. At a low agitation rate probably less than perfect homogeneity of the process so that the metabolic processes can take place less well. Metabolism increased with increasing pH toward the optimum pH (pH 6.00). pH value is lower than indicated by Veenagayathri and Vasudevan (2010) that a consortium containing Bacillus cereus bacteria can grow and degrade phenolic compounds up to 99% with optimum pH around 7.

In the agitation rate above the optimum value (> 130 rpm), the amounts of degraded LAS tend to start to fall. Optimum point of agitation was lower than that reported by Khleifat et al, [8]. Total LAS is degraded by *Burkholderia* sp did not increase until 15 days of incubation.

1.1. Lipid Biodegradation

The presence of lipids in the wastewater is often a serious problem because it does not dissolve in water. Although lipids can be degraded by microbial lipolytic, but lipids are not water soluble so it is often not available as substrate for microorganisms. Bacillus cereus N-09 is a lipolytic bacterium that is able to degrade LAS.

Based on analysis of responses is known that a significant model (α = 0.0002). pH is linear (α = 0.0232), the temperature is quadratic (α = 0.0001), agitation rate is quadratic (α = 0.0043) and no interaction between pH and temperature (α = 0.0039). Quadratic equation:

$Y_2 = -2307{,}82 - 190{,}56X_1 + 180{,}45X_2 + 5{,}39X_3 + 6{,}12X_1X_2 - 3{,}68X_2^2 - 0.02{*}X_3^2$

$R^2 = 0{,}82$

The results of this model showed that the optimum temperature in the range of temperature treatment. Optimal point optimization results were pH = 6.00 temperature = 30 and agitation = 130 which will result in a decrease of 73.5% lipid. This result was lower when used in waste activated sludge containing radish seed oil 500 mg/l [11]. However, the research Wang, et al [11] there is no LAS.

Lipase enzyme activity is influenced by environmental conditions. Enzymes will work well in optimum condition. Use of *Bacillus cereus* N-09 on the waste with a content of lipid and detergent showed a quadratic pattern (Figure 2).

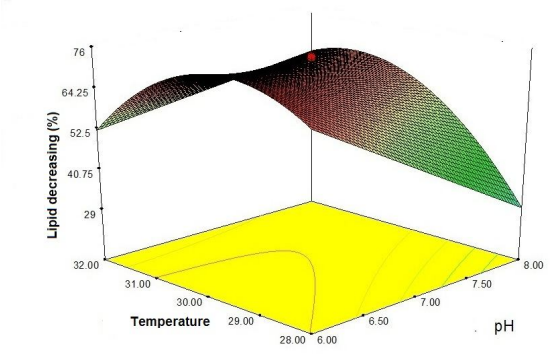

Figure 2. The effect of temperature and pH on LAS decreasing percentage by Bacillus cereus N-09.

Based on Figure 2 can be seen that the effect of temperature on the degradation of lipids is quadratic. Lipid metabolism increases with increasing temperature of incubation and reached a peak at a temperature of about 300C which is then followed by a decrease in the amount of lipid degradation. The optimal temperature is lower than the *Rhodococcus* sp NCIM 5126 on crude oil [3] and *Pseudomonas fluorescens* 27 [12]. In general, enzyme activity will decrease after the optimum point. However, the influence of pH will be different at different temperatures.

Effect of pH on lipid degradation is linear and there is interaction between pH and temperature. At the optimum temperature the percentage of lipid degradation was not influenced by pH. At low temperature, the higher the pH reading, the lipase activity decreased. This is due to the pH optimum of lipid degradation by Bacillus cereus N-09 is 6.00 which is the lowest point of this research. At high temperatures the lipid degradation activity still showed an increase with increasing pH. This is different to that reported by Wang, et. al [11] which indicates that the use of activated sludge in waste containing radish seed oil is relatively stable at alkaline pH and decreased at acidic pH. At pH 10 the substrate biodegradation activity dropped dramatically.

Agitation is a quadratic significant effect on the percentage of lipid degradation (Figure 3).

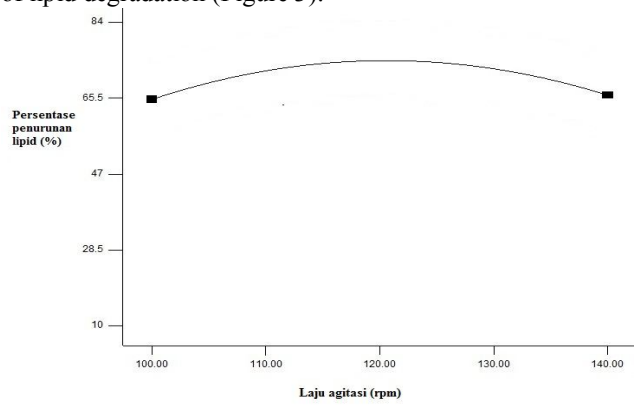

Figure 3. The effect of agitation rate (rpm) on lipid decreasing percentage by Bacillus cereus N-09.

From Figure 3 it can be seen that the higher rate of agitation, the more lipids are degraded. Lipid degradation is highest at 130 rpm agitation rate, followed by decreased degradation of lipids. The optimum value of agitation rate is 130 rpm. These results are lower than the *Rhodococcus* sp [3]. The uses of palm oil wastes require agitation speed of 150 rpm [13]. This shows that the optimum agitation rate depends on the organism used.

Conclusion

Bacillus cereus N-09 can reduce the detergent by 93.35% and 73.50% lipid with the optimum temperature of 30^0C, pH 6.0, and agitation rate of 130rpm.

REFERENCES

1. Prats, D., C. López., D. Vallejo., P. Varó and V.M. León. 2006. The effect of temperature on the biodegradation of linear alkylbenzene sulfonate and alcohol ethoxylate. Journal of Surfactants and Detergents. 9: 69 – 75. (Abstract).
2. Dheeman, D.S., J.M. Frias., and G.T.M. Henehan. 2007. Influence of cultivation conditions on the production of a thermostable extracellular lipase from *Amycolatopsis mediterranei* DSM 43304. J. Ind. Microbiol Biotechnol. 37: 1 – 17.
3. Sharma, S.L and A. Pant. 2001. Crude Oil Degradation by a Marine Actinomycetes *Rhodococcus sp.* Indian. J. Marine. Sci. 30: 146 – 150.
4. Retnaningdyah, C., S. Samino, Suharjono, A. Kurniawati dan Prayitno. 1999. *Pengaruh Surfaktan Deterjen (LAS dan ABS) terhadap Perkembangan Embrio Siput (Lymnaea rubiginosa) dari Kali Mas Surabaya***.** Makalah Seminar Nasional Biologi Menuju Milenium III. UGM. Yogyakarta.
5. Abdulgani, N. 2002. Pengaruh Deterjen Linear Alkylbenzene Sulfonate terhadap Perkembangan Embrio Katak Sawah *(Fejervarya cancrivora).* KAPPA 20: 20 – 27.
6. Ojo, O.A and B.A. Oso. 2008. Isolation and Characterization of Synthetic Detergent-degraders from Wastewater**.** Afr. J.Biotechnol. 7: 3753 – 3760.
7. Amirmozafari,N., F. Malekzadeh., F. Hosseini and N. Ghaemi. 2007. Isolation and identification of Anionic Surfactant degrading bacteria from Activated Sludge**.** Iranian Biomedical Journal 11: 81 – 86.
8. Khleifat, K.M., R.A. Halasah, K.A. Tarawneh., Z. Halasah., R. Shawabkeh. and M.A. Wedyan. 2010. Biodegradation Of Linear Alkylbenzene Sulfonate By *Burkholderia* Sp.: Effect Of Some Growth Conditions. Int. J. Agric. Biol. 12: 17 – 25.
9. Hidayat, N. 2011. Optimasi Proses Produksi Inokulum Bakteri Pendegradasi Lipid dan Surfaktan

pada Limbah Cair. Disertasi. Fakultas Pertanian Universitas Brawijaya

10. Kertesz, M.A., P. Kolbener, H. Stockinger, and S. Beil.1994. Desulfonation of Linear Alkylbenzene sulfonate Surfactants and Related Compounds by Bacteria. Appl. Env. Microbiol.60: 2296 – 2303.
11. Wang, X., Y. Ren and T. Qiang. 2011. Study on the Biodegradability of Oxidized Sulfiting Rapeseed Oil Fatliquor. http://www.oceandocs.org/bitstream/1834/820/1/Nweke,C.O.293-295.pdf Tanggal akses: 5 Februari 2011.
12. Wang, X., Y. Ren and T. Qiang. 2011. Study on the Biodegradability of Oxidized Sulfiting Rapeseed Oil Fatliquor. http://www.oceandocs.org/bitstream/1834/820/1/Nweke,C.O.293-295.pdf Tanggal akses: 5 Februari 2011.
13. Nizam, M.K. 2008. Bioremediation of Oil from Domestic wastewater Using Mixed Culture: Effects of Inoculum concentration and Agitation Speed. Thesis. Faculty of Chemical and Natural Resources Engineering. Universiti Malaysia. Pahang.