Proceedings of the 1st International Conference on Natural Resources Engineering & Technology 2006
24-25th July 2006; Putrajaya, Malaysia, 104-111

Biodegradation Of Linear Alkyl Benzene Sulfonate By Bacterial Consortium

Praswasti PDK Wulan, Misri Gozan, Anondho W, Dianursanti, Mahmud S

Gas dan Petrochemical Engineering University of Indonesia, Depok 16424, Indonesia[1]

Abstract

Surface active agents (surfactants) are chemical compounds which are massively used as raw material in detergent production. Synthetic type surfactants are often used because they perform better and are more economical compared to natural detergents. Linear Alkyl Benzene Sulfonate (LAS) is one of the synthetic surfactants that is widely used. Although LAS is biodegradable, its introduction to the environment in big amounts harms water bodies. Research on biodegradation of LAS with 100 ppm, 400 ppm, 700 ppm, 1000 ppm and 1500 ppm concentrations was conducted by using consortium of bacteria comprising of *Pseudomonas aeroginosa, Bacillus subtilis, Bacillus aglomerans, Bacillus cereus, Bacillus alvae*. Experiments were carried out for twelve days, at 29^{o}C with initial total inoculum of bacteria at 1,59 x 10^{8} CFU/mL. Results showed that this type of bacterial consortium could tolerate 1500 ppm in LAS environment. However, significant growth rate did not occurr, 0.039 – 0.042 hour $^{-1}$ and not too efficiently reduce Chemical Oxygen Demand (COD) for those systems. Surface tension in several variated concentration of LAS: 0 ppm >100 ppm >400 ppm >700 ppm, LAS 700 ppm = 1000 ppm = 1500 ppm.

Keywords: Biodegradation; Linear AlkylBenzene Sulfonate (LAS); Consortium Bactery; Chemical Oxygen Demand (COD)

1.0 Introduction

Until recent years, soap and water with basic ingredients: animal and vegetable fats and oils were the only cleaning agents available. The studies led to the commercial discovery of surfactants which could be made synthetically from petrochemicals, which were readily available. Unlike the traditional soap, the surfactants were more resistant to hard water and therefore improve the efficiency of the cleaning process.

Surfactants are also used in textile industry and mining, either as lubricant, emultion or flocculant. The surfactants used in detergent are in composotion of 10% - 30%. Surfactants are nontoxic material, however, if they enter to environment in big amount, they could make water pollution .

LAS was first commercialized in 1965s as a replacement for the poorly biodegradable AlkylBenzene Sulfonate (ABS) which caused persistent foam in sewage treatment plants, streams and rivers. LAS was the first surfactant introduced to solve an enviromental problem.(HERA Project, 2004).

[1] *Corresponding author: Tel:: 062-021-7863516/78885335; Fax: 062-021-7863515; E-mail : wulan@che.ui.edu, mgozan@che.ui.edu*

LAS is comprised of linear alkyl carbon chains (C_{10}-C_{13}), SO_3^- and Na^+. LAS is made from kerosene and benzene through Linear AlkylBenzene (LAB) with further sulpho-nation and neutralization.

Efectivity and biodegradebility of LAS as synthetic surfactant are the factors which make LAS favorable until now (HERA Project, 2004). LAS has also been widely used in Indonesia. However, there is missperception in public that detergents performing bulk of foam represent the quality of detergents. This, unfortunately, renders the utilization of LAS abundantly in detergent production.

One of technologies to recover polluted site is bioremediation. The method of bioremediation has been applicated in many oil companies in Indonesia. Development of this technology is hencefort done by many institutes. Pusat Penelitian dan Pengem-bangan Teknologi Minyak dan Gas Bumi (LEMIGAS) has found mixtures of microorganism (bacterial consortium) in sewage plant that degrade oil pollutants. LEMIGAS has been developing bacterial consortium in order to make more effective and eficient degradation of oil and petrochemical pollutant.

Gas and Petrochemical Engineering Department University Indonesia examined a bacterial consortium endurance and effectivity in degrading LAS. Bacterial consortium comprised *Pseudomonas aeroginosa*, *Bacillus subtilis*, *Bacillus aglomerans*, *Bacillus cereus*, *Bacillus alvae*.

2.0 Material And Methods

Bacterial consortium was acclimatized toward LAS in Lockhead and Chase (LC) medium containing LAS as a single carbon source. Medium and LAS were added and refreshed periodically to maintain the log phase.

Concentrations of LAS (100, 400, 700, 1000, 1500 ppm) were varied to examine the toxicity of LAS on bacteria consortium in 250 mL Erlenmeyer in room temperature and was put on shaker with constant speed, 22 rpm for 264 hours or 12 days.

Total Plate Count (TPC) methode was used for counting bacteria inoculum. Measuring LAS concentration was done by Standard Test Method for Methylene Blue Active Substances (ASTM D 2330). COD test were done by using

2.0 Results And Discussion

3.1. Cultivation and enrichment

Pure cultivation step was done in nutrient broth medium to increase the number of bacterial consortium in laboratorium. Bacterial consortium contains *Pseudomonas aeroginosa*, *Bacillus subtilis*, *Bacillus aglomerans*, *Bacillus cereus*, *Bacillus alvae*. Usually, *bacillus* in great quantity lives in environment, surrounding *pseudomonas* bacterium. Those of bacteria live in environmental muttualy support others metabolism with less competition of carbon source.

Table 1. Growth of Bacteria Consortium in Nutrien Broth Medium

Hour	Growth (hour^{-1})
0	0,000
24	0,039
120	0,008
144	0,002
168	0,022
192	0,008
264	-0,032
288	-0,011
312	-0,028
336	-0,003
360	-0,004

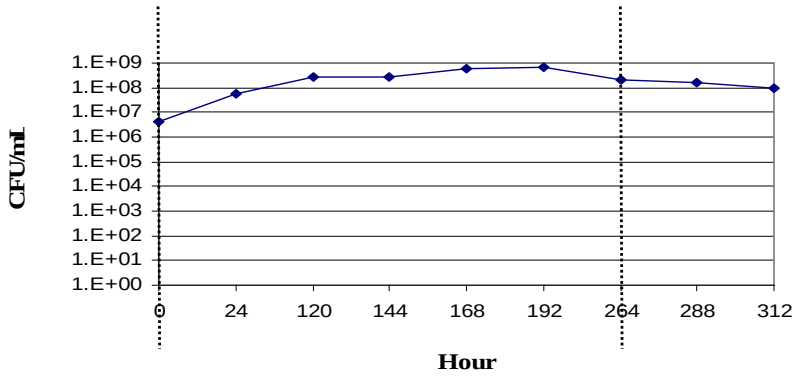

Figure 1. Amount of Bacteria Inoculum versus time in Nutrient Broth Medium

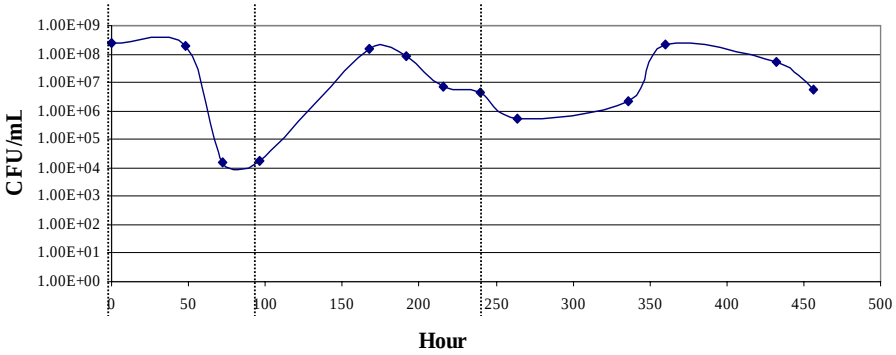

Figure 2. Growth of Bacteria Consortium in Enrichment Step

Table 1 and Figure 1 shows normally the growth of bacterial consortium, with nutrient broth medium. It also can be seen that negative growth starts on the 264th hour (12th day). It means the amount of bacteria started to decrease and research was ceased at 264 hours (12 days).

It can be seen in Figure 2 that the amount of bacteria relatively decreased in first enrichment (300 ppm). It was because of their adaptating phase in new environment which contains of LAS. Sudden contact of bacteria consortium with relatively high LAS could disturb their living. In order to ensure that the consortium could acclimatize with higher concentration of LAS also to definite there was no lack of carbon source, the addition of 100 LAS was done

periodically. On this addition, amount of the consortium could increase again. It means that the consortium could aclimatize with LAS.

3.2. Biodegradation of LAS

Figure 3 shows the amount of bacteria consortium versus time in various concentration of LAS. Generally, it can be seen that this kind of consortium could live in environment which contained LAS. Figure 3 shows negative growth in the beginning of implementation because some bacteria that could not hold out with LAS were killled in that time. Later on, when they had already aclimatized with this environment, number of bacteria (CFU/mL) relatively increased until the maximum level. The consortium bacteria in control vial relatively need less time than others to reach maximum amount of population. It can be seen in Figure 3 that the consortium also reached the stationer phase. This indicate that there was not much difference environment for those aclimatized bacteria consortium so that in control vial they relatively did not need much time to aclimatize.

The use of LC medium which contains adequate nutrition made the consortium could live and grow. After reaching maximum amount of population, and there was lack of nutrition, then the consortium started to reach death phase.

Several factors that may affect grow of bacteria in this research are:

- Agreement of bacterial consortium with LC medium.This factor needs to be tested furthermore.
- Growing time period which did not same for each bacteria in this consortium. This factor needs to be tested furthermore.
- Lower surface tension could make higher risk to the consortium's death

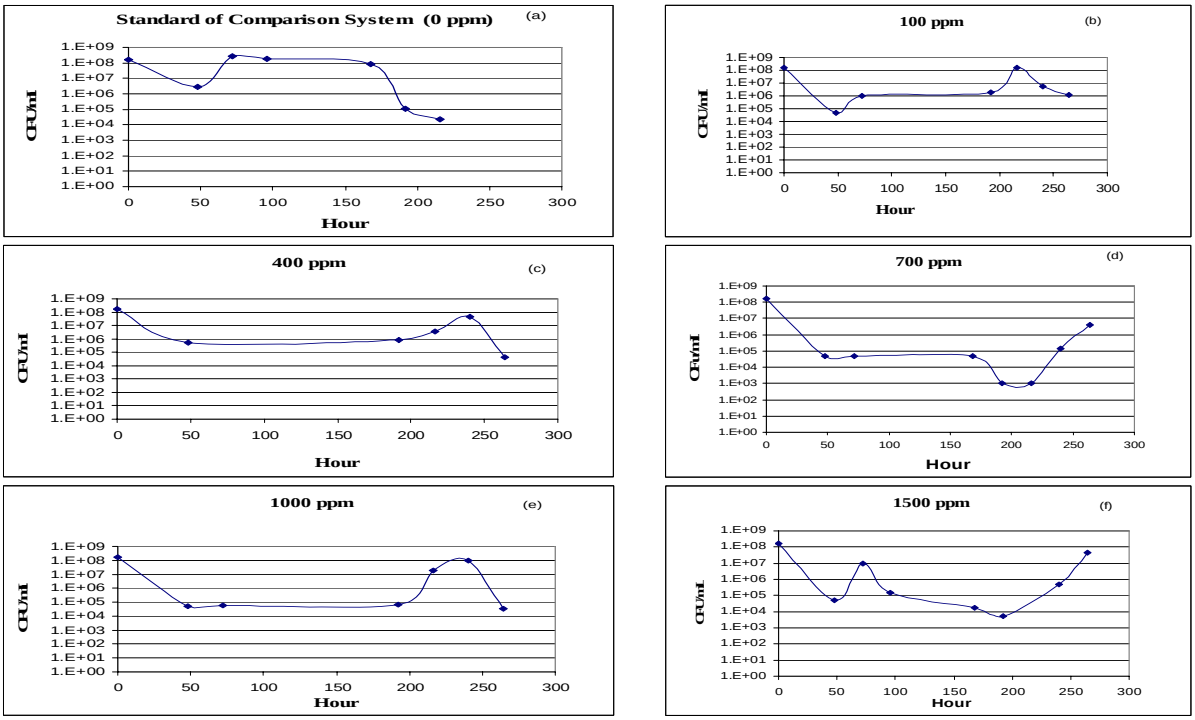

Figure 3. Amount of Bacteria Consortium (CFU/mL) versus time in various LAS concentration (ppm)

Note. Y axis on each curve was made with logarithmic scale.; (a) Control vial (0 ppm); (b)100 ppm; (c) 400 ppm;(d)700 ppm; (e) 1000 ppm; (f) 1500 ppm.

Table 2 . Optimum Growth of Bacteria Consortium in Varying Concentration of LAS

LAS concentration (ppm)	**Optimum Growth (hour^{-1})**
0	0.041
100	0.042
400	0.039
700	0.041
1000	0.042
1500	0.041

Result from parallel research had shown that the *Critical Micelle Concentration* (CMC) for LAS was 513.98 ppm. Instead of that, at concentration below 513.98 ppm, higher LAS concentration, lower surface tension will be happened. However for concentration above 513.98 ppm, surface tension has already reached minimum number and start to be steady. In other words, surface tension in several variated concentration of LAS:
0 ppm >100 ppm >400 ppm >700 ppm, LAS 700 ppm = 1000 ppm = 1500 ppm.

As was happened in 100 ppm, 400ppm and 700 ppm when concentration of LAS became higher and made surface tension became lower, bacteria relatively needed much time to aclimatize and reach their maximum population.

Addition of LAS with larger concentration influenced bacteria's living environment. In this situation, they had to do the adaptation hardly. This could affect the amount of LAS biodegradated by the consortium in 12 days,which was become less.

When surface tension relatively stable in the lowest value, as happened in 700 ppm,1000 ppm, 1500 ppm, the other factors that might affect adaptation time of bacteria to reach their maximum population were their ability to do metabolism by explored LAS as their carbon source and agreement with their living medium which might toxic to them.

Table 2 shows that optimum growth of bacteria in variated concentration of LAS relatively undifferent, even if we compared with optimum growing time in pure cultivation step and enrichment step. Figure 3 shows that the pattern of bacteria growing phase become explicit when LAS in higher concentration was applicated. Indeed, the maximum number of bacteria population only could reach their intial number of population.

3.3. Measuring LAS concentration with Methylene Blue Active Substance Standard Method

Generally, this method may measure LAS concentration because of the reaction between methylene blue toward anionic surfactant (included LAS). Blue colour has changed as the result of this reaction.Then extraction of those blue colour is done with chloroform. Later on, the turbidity was observed with spectrofotometer (650 nm). Calibration curve absorbation versus LAS concentration has to be made before.

This method had been done until calibration step. Instead of limited equipment in laboratpry, this method could not be continued.

3.4. COD in LAS Biodegradation Process

COD symbolizes oxygen which is needed for degradating organic chemical in water. COD value is generally used to detect suitability of water. COD classification are: 1000 ppm for hard, 500 ppm for medium, and 250 ppm for minor pollution (Tchobanoglous, 2004).

LAS was the only organic chemistry in this system, so in this research, COD value could indicated whether biodegradation of LAS has happened or not. The desire was to know whether bacteria in those LAS contaminated systems could decrease COD value until minor pollution condition. COD value was measured at initial, middle and the end of in this research. Decreasing pattern of COD can be seen in Figure 4.

Figure 4 shows that there were decreasing COD values in all systems. These indicated that decreasing concentration of LAS or in other words, the biodegradation, was trully happened.

Generally, bacteria consortium in this research could live in medium which was contaminated by LAS. The consortium could explored LAS as their carbon source, in other words, could biodegradate LAS. The carbon source then has been used to do metabolism so they can multiply their population.

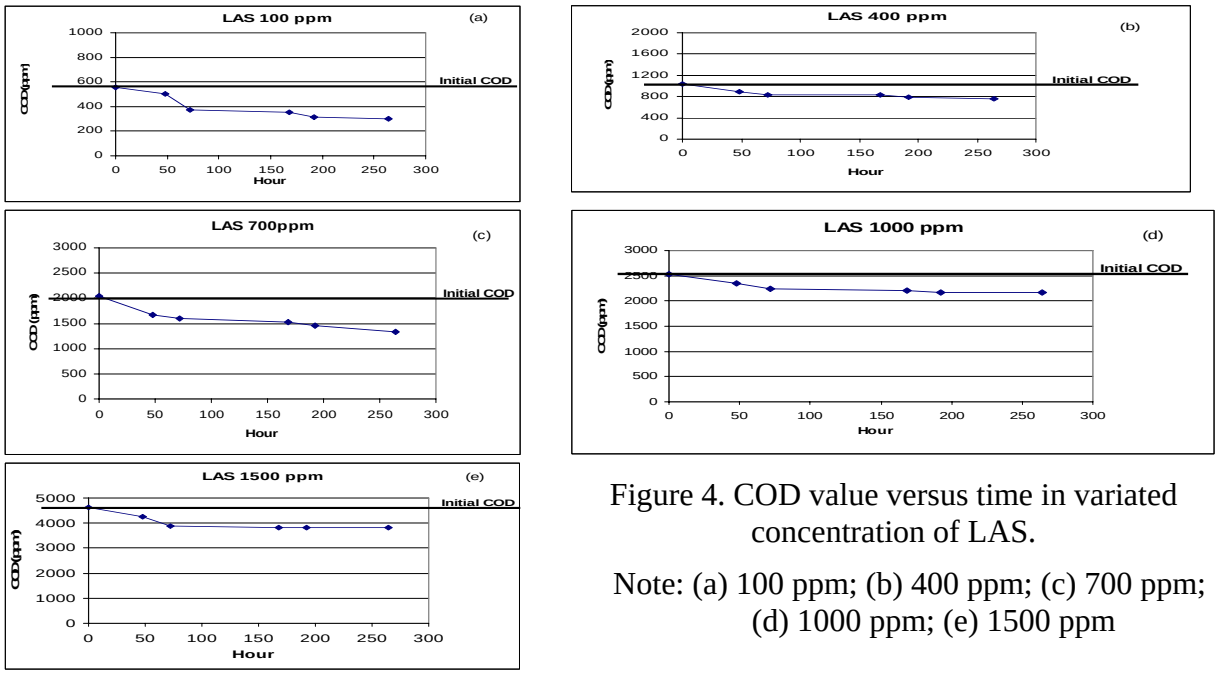

Figure 4. COD value versus time in variated concentration of LAS.

Note: (a) 100 ppm; (b) 400 ppm; (c) 700 ppm; (d) 1000 ppm; (e) 1500 ppm

The obstacle was as LAS concentration increases, growing phase pattern of bacteria consortium relatively more unclear. After they had reached maximum population, they shortly entered death phase. The stationery phase relatively unclear, compare with stationery phase which has happened in nutrien broth medium.

This condition might happened because of competition with each bacteria or because of LAS's toxicity toward some bacteria in this consortium. If the first possibility happened, then LAS

concentration would be reduced to zero and COD value also would be greatly reduced. However it can be seen from datas of this research that:

- Higher LAS concentration could make slower growing time of bacteria; it means that there was signifficant effect made by the addition of LAS.
- COD value was not greatly reduced; it means that biodegradation had happened but there still lot of carbon source available whether in form of LAS or intermediate form. All this organic carbons was counted as COD value.
- Some bacteria which had been used in this research were: *Pseudomonas aeroginosa* → gram-negatif; *Bacillus subtilis*, *Bacillus aglomerans*, *Bacillus cereus*, *Bacillus alvae* → gram- positif

Anionic detergents are effective against gram-positive organism but are relatively ineffective against gram-negative forms which have near their cell surface a phospholipid that forms salts with anionic agents (Frobisher, 8th.ed.).

So the most suitable possibility was LAS could be toxic to some bacteria. Decreasing amount of consortium population was because the decreasing population number of *bacillus* bacteria. It could happened directly or slowly. To make sure of it, come new researches should be done.

The advantage use of bacteria consortium in this research was: when there were lot of bacteria died because of toxic material, there still another bacteria could hold out and do the degradation.

4.0 Conclusion

The consorsium of bacteria (*Pseudomonas aeroginosa*, *Bacillus subtilis*, *Bacillus aglomerans*, *Bacillus cereus*, *Bacillus alvae*) could survive until 1500 ppm of LAS. While at higher LAS concentration the stationery phase was relatively unclear.

LAS concentration above 513.98 ppm, surface tension has already reached minimum number and start to be steady. surface tension in several variated concentration of LAS:
0 ppm >100 ppm >400 ppm >700 ppm, LAS 700 ppm = 1000 ppm = 1500 ppm.

Bacteria consortium that was used in this research could degradate LAS but less efficient to reducing COD value in those systems.

References

[1] Atlas, C. 1995. Bioremediation of Petroleum Pollutants. *Bioscience* Vol 45 No 5.
[2] Atlas, Ronald M. 1984. *Microbiology: Fundamentals and Applications*. New York: Macmillan Publishing Company.
[3] Azar, T.P. dan Nahei M.R. 2 Desember 2005. *Using Bacteria for Degrading of Linear AlkylBenzene Sulfonates in Wastewaters of Detergent Producing Factories*. Lahijan: Department of Microbiology Lahijan Islamic Azad University.
[4] Baker, K.H. dan D.S.Herson.1994. *Bioremediation*. McGraw-Hill, Inc.
[5] Collins, C.H., et. al. 1995..*Microbiological Methods*. Butterworth Heinemann.
[6] Conant, S. dan Overman. *Microbiology, 13th ed*.
[7] Effendi, H. 2003. *Telaah Kualitas Air Bagi Pengelolaan Sumber Daya dan Lingkungan Perairan*. Penerbit Kanisius.

[8] Fitria, R. R. 2005. Skripsi: *Studi Awal Proses Biodegradasi Benzena oleh Bakteri Pseudomonas aeruginosa*. Depok: Departemen Teknik Gas dan Petrokimia Universitas Indonesia.
[9] Frobisher, M. *Fundamentals of Microbiology, 8th ed*. W.B. Saunders Company.
[10] Hart, H., et. al. 2003. *Organic Chemistry*. Houghton Mifflin Company.
[11] HERA Project .Human and Environmental Risk Assessment (HERA) on Ingredients of European Household Cleaning Product.
[12] Rosen, M.J. 1989. *Surfactants and Interfacial Phenomena, second edition*. John Wiley & Sons, Inc.
[13] Sawyer, C.N., et.al. 2003. *Chemistry for Environmental Engineering and Science*. McGraw Hill.
[14] Schkeheck, D. 2003. *Biodegradation of Synthetic Surfactants: Linear AlkylBenzene Sulfonates (LAS) and Related Compunds*. Germany: Department of Biological Sciences University of Konstanz.
[15] Tchobanoglous, G.,et.al. 2004. *Wastewater Engineering Treatment and Reuse*. McGraw-Hill.
[16] Udiharto, M. 1992. Bioremediasi Minyak Bumi. *Prosiding Pelatihan dan Lokakarya Peranan Bioremediasi Dalam Pengelolaan Lingkungan.*