International Journal of Biochemistry and Biotechnology ISSN: 2169-3048 Vol. 2 (2), pp. 268-272, February, 2013. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Optimization for determination of Linear Alkylbenzene Sulfonate(LAS) and AlkylbenzeneSulfonat (ABS) from biodegradation by *Pseudomonas Aeruginosa* bacteria

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International Seminar Biotechnology for Enhancement the Tropical Biodiversity, Padjadjaran University-2010

Received 29 October 2012; Accepted 19 February 2013

LAS and ABS are the detergent active compounds and their presence should always be monitored because they are pollutants. Detergent treatment has been done by way of adsorption, flocculation, coagulation and advanced oxidation, but the process can produces unwanted sludge that need further processing. One alternative technology that is used to treat wastewater is biological treatment using the bacteria *Pseudomonas aeruginosa,* because this method was easier and very economical when compared with other processing methods. Before, biodegradation by *Pseudomonasaeruginosa* is done only for the treatment of LAS but on this study it is also conducted for the treatment of LAS and ABS. So it is necessary to determine optimum conditions. With this method LAS and ABS was degradable to CO_2 and H_2O. In this study, LAS and ABS successfully degraded 99.73%. With the longer time CO_2 formed was increasing, and the biggest CO_2 at pH 7 and the 50th hour, concentration of CO_2was127.6 mg / L.

Key words: Biodegradation, LAS, ABS, *Pseudomonas aeruginosa* bacteria.

INTRODUCTION

LinearAlkylbenzeneSulfonates (LAS) are the most popularly used synthetic anionic surfactants in commercial detergents. Their basic structure consists of a benzene ring connected to a sodium sulphate group (the hydrophilic end) and an alkyl chain containing 10–13 carbon atoms (the hydrophobic end). The commercially available LAS are very complex mixtures of various homologues and phenyl positional isomers. Large amounts of LAS are disposed in municipal wastewaters due to their wide daily use(Constantina et al., 2007).

In the United States and Europe, LinearAlkylbenzene Sulfonates (LAS) has been used since the early 1960s, when the low rate of biodegradation of branched-chain AlkylbenzeneSulfonates (BAS/ABS) was recognized. In some Latin American countries, ABS was currently used in different detergent formulations due to their low costs. Water pollution by ABS is a significant environmental problem in these countries (Jesús Campos-García et al., 1999).

Ciujung River's in Banten-Indonesia attack-polluted industrial and household waste that contains detergent active compounds LAS and ABS because the river is used by the public for bathing, washing and drinking. Consequently, waste treatment as well as routine analysis should be done regularly.

Biodegradation is an important process responsible for the removal of LAS from both raw wastewater and sludge in sewage treatment of plants, while it also enhances the removal of untreated or partially degraded compounds in the environment after their disposal in natural receptors (seas, rivers, lakes etc.), reducing thus their impact on biota. During biodegradation, microorganisms can either utilize LAS as the sole carbon source or co-metabolize LAS by microbial metabolic reactions (Constantina et al.,

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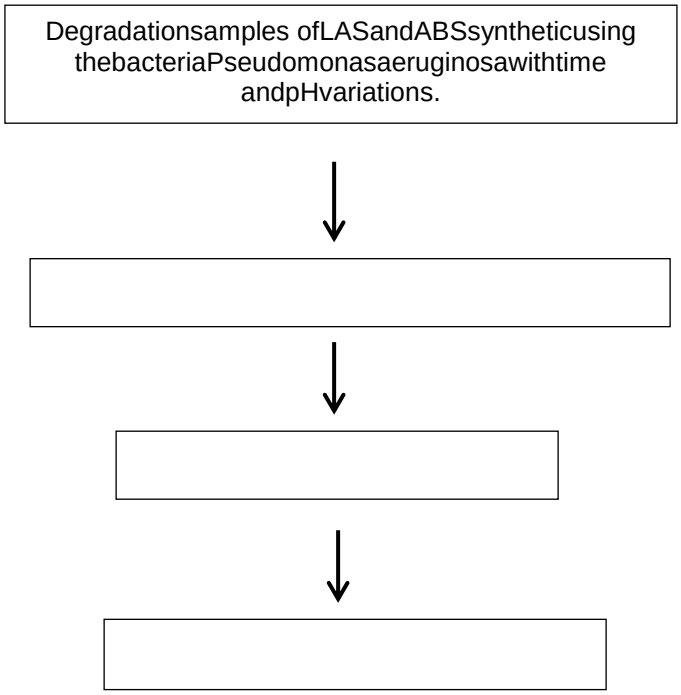

Figure1. Flowchart of Research.

2007;García-L. E et al., 2009; Jean C.,S., and Marie.H.N, 1992;Kristianet al.,2001).

Several studies have been used only for the biodegradation of LAS or ABS (Bielicka et al., 2002; Jurado et al., 2006; Constantina et al., 2007; NourAmirmozafari et al., 2007), the study sample used was a mixture of LAS and ABS so as make it necessary to optimize the operating conditions.

The most important ecological property of any surfactant is the relative ease of their biodegradation. Biodegradation is most often performed by soil or aquatic microorganisms and leads to generation of water and carbon dioxide (CO_2) gas (NourAmirmozafari et al., 2007). In this study, LAS and ABS degraded by *P. aeruginosa* bacteria and the decreasing concentration of LAS and ABS were monitored with CO_2 formed. They were generally analyzed using chromatography (Bielicka et al., 2002; Jurado et al., 2006; Constantina et al., 2007; Kristianet al.,2001; HusyeinKocet al., 2002) or spectrophotometry (NourAmirmozafari et al., 2007). Analysis of CO_2 in this study using the titration method because it was simple, easy but pretty accurate and suitable for routine analysis.

Determination of CO_2 degradation has been done by several methods, for example: the determination of CO_2on decomposition detergent dissolved in water by the method of measuring the value of TOC (Total Organic Carbon) ((ErzsébetSB et al., 2008), (J. Perkowski et al., 2006), Fernández etal., (2004), and (Abu Hassanand SMetcalfe, 2003).Determination of CO_2 in the degradation of anionic surfactant with Turbidimetri method (Otto and RóbertHorváthHuszánk, 2003); Analysis of CO_2 in the degradation of anionicsurfactant4-dodecyl-1, 1-e ksibisbenzenedisulfo-nat (DOBS) by NMR (nuclear magnetic resonance) (Hidaka H. et al., 2004); Determination of CO_2in the degradation of the linear AlkylbenzeneSulfonate non-dispersion infrared spectroscopy (NDIR) (Abu Hassan MA, and IanSMetcalfe, 2003). Analysis of CO_2 based on the value of COD and BOD in the photochemical oxidation of LAS-biodegradable (Merhab et al., 2005).

All of these methods are expensive and require high-tech equipment. Determination of CO_2 that is cheaper and simpler to do with the method of titration is done by determining the flow of carbon dioxide (CO_2) generated in a 100 mL solution of 0.01 M NaOH. Residual unreacted NaOH with CO_2 titrated with 0.01 M HCl using phenolphthalein indicator. At the same flask, dissolved CO_2 is titrated with 0.01 M HCl using methyl orange indicator (Vogel's, 2000).

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Materials and Methods

This study begins with experiments on synthetic samples to obtain the optimum conditions, and then applied to the sample stream Ciujung.

Flow chart of the study can be seen in Figure1.

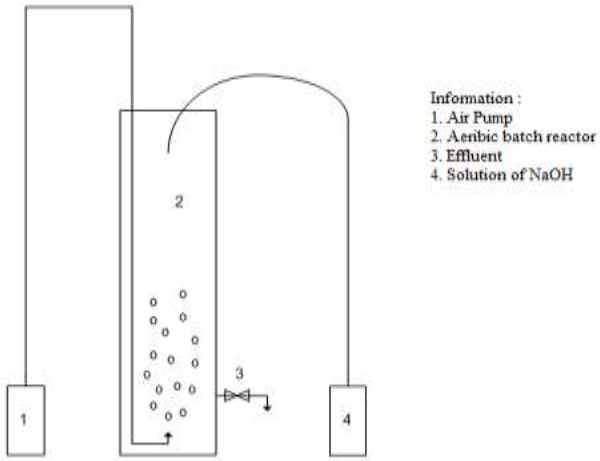

Figure 2. The series of experimental devices.

Chemicals

The material used is a mixture of Linear AlkylbenzeneSulfonate (LAS) or sodium laurel sulfate $C_{12}H_{25}OSO_3Na$ from Merck and ABS. For the analysis used, fenolptalein indicator solution 0.5%, solution of sodium hydroxide 1M from Merck, River water, the bacteria *P. aeruginosa* bacteria, Cation Resin, Anion Resin, Methanol Analysis from, solution of HCl 1Mmerck,. Solvents used were distillate water.

Equipment

Equipment used in research are cation and anion exchange column, a set of aerobic batch reactor, pH meters, glassware, Bunsen, a set of distillation equipment, and stative burrete for titration.

Analitycal methods

In this study, 1000 ml mixture of LAS and ABS 100 mg / L included in aerobic batch reactors. Add Pseudomonas aeruginosa bacteria that have been *acclimation,* as much as 20grams. The variable of time used are: 12, 24, 36, 42, 48, 54, 60 hours and pH: 6, 7, 8, 9. Analysis conducted on the LAS and ABS that has been degraded in aerobic batch bioreactor for a certain time interval analysis method of CO_2, with titration analysis.

Determination of Carbon Dioxide by Titration

Gas stream of carbon dioxide (CO_2) produced into 100 mL solution of NaOH (1M). NaOH that does not react, titrated with solution of HCl (1M). Added solution of phenolphthalein indicator. The amount of HCl that reacts is equivalent to the amount of CO_2 formed.

RESULTS AND DISCUSSION

Effect of pH and time on the amount of CO_2 formed

The enhanced culture exhibited an almost complete LAS elimination after a few days of cultivation (Constantina et al., 2007). A *P. aeruginosa*strain (W51D) was isolated with the ability to mineralize linear AlkylbenzeneSulfonate (LAS) at a significant rate (NourAmirmozafari, 2007) and which is able to mineralize at least 70% of a BAS commercial mixture and completely degrade LAS has been isolated (Jesús Campos-Garcíae t al., 1999).

Upon reviewing the literature concerning the biodegradation of LAS, it becomes apparent that most researchers agree that LAS can be degraded aerobically but hardly anaerobically. On the other hand, information on the ultimate biodegradation of LAS is rather limited. Only scarce studies are available on the kinetics of ultimate biodegradation (mineralization to carbon dioxide, water and sulphate) of the LAS benzene ring. Analysis of the final degradation of LAS and ABS in CO_2 has been done. The C O_2 is trapped in barium or sodium hydroxide and is measured by titration of the residual hydroxide or as inorganic carbon (Constantina et al., 2007).

In this study, a mixture of LAS and ABS degraded by the bacteria P. aeruginosa. Gas stream of carbon dioxide (CO_2) produced into solution of NaOH and is measured by titration with solution of HCl. Figure 3. Show effect of pH on the use of HCl solution.

They were generally analyzed using chromatography or

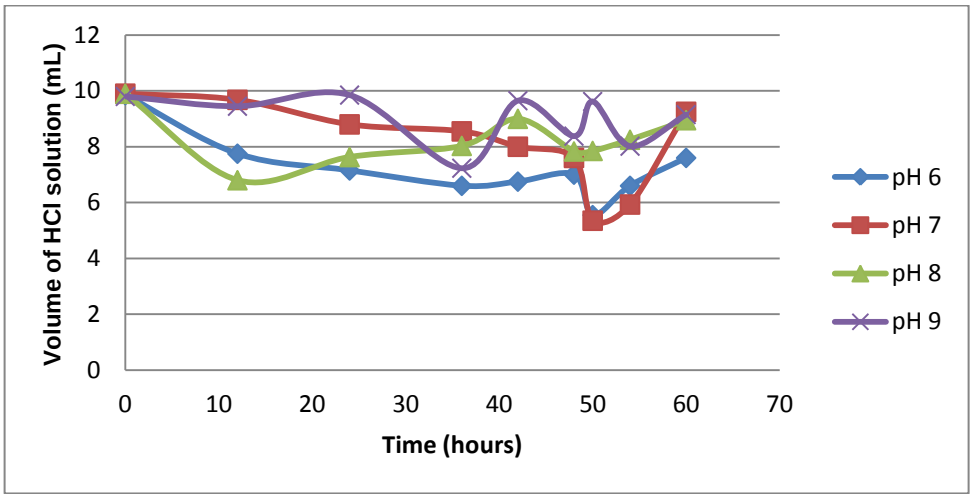

Figure 3. Effect of pH on the use of HCl solution

spectrophotometry, analysis of CO_2 in this study using the titration method because it was simple, easy but pretty accurate and suitable for routine analysis.

Let the volume of standar acid consumed be *v*mL.

$OH^- + H^+ \rightarrow H_2O$

$CO_3^{2-} + H^+ \rightarrow HCO_3^-$

Another titration is performed with methyl orange, methyl orange-indigo carmine or bromophenol blue as indicator.

Let the volume of acid be *V* ml.

$OH^- + H^+ \rightarrow H_2O$

$CO_3^{2+} + 2H^+ \rightarrow H_2CO_3$

$H_2CO_3 \rightarrow H_2O + CO_2$

Then *V*-2(*V*-*v*) corresponds to the hydroxide, 2(*V*-*v*) to the carbonate, and V to the total alkali(Vogel's, 2000).

Effect of pH on the biodegradation of LAS and ABS

The effect of pH on the LAS biodegradability by Burkholderiasp, has been reported that the pH 6.5 is the optimum in which the higher percentage of LAS degradation was obtained and further decreased with further elevation in the pH level. It is possible that the enzyme (s) for LAS degradation affected by the pH condition and their optimum activity at 6.5. (Khleifat et al., 2010). For the combined culture, the greatest LAS biodegradation removal was observed in the nutrient broth of pH 8.5 (90%) with about a 20% decrease at pH 7.5 and 6.5. When the pH was 5.5, a 50% decrease in LAS biodegradation was obtained. Has been reported that, under aerobic degradation conditions, the LAS influences the pH self-regulation capacity, particularly when the LAS concentration is above 20 mg L−1 and thus external neutralization is always required. This is true as long as the cell biomass for pH 7 without LAS had a higher growth biomass than that of pH 8.5 or 5.5–6.5 (Khleifat M. K., 2006). In this study we found that maximum degradation of LAS and ABS solution was at pH 7. It was reported that, the drop in pH was attributed to the production of acidic intermediates as a result of LAS degradation (Khleifat et al., 2010; Khleifat M. K., 2006).

Effect of incubation time at the biodegradation of LAS and ABS

Khleifat., et al., (2010) reported that, to determine the exact time point for achieving complete degradation of LAS, a 2 h time point intervals should be taken for measuring the degradation of LAS below the 300 ppm concentration (100, 200 & 300). It was found that 100 ppm LAS completely degraded within 14 h of incubation compared with previous study, which shows degradation within days. The LAS concentrations of 300 ppm or lower exhibited higher degradation rate than the concentrations above 300 ppm. Under other conditions, the average of degradation rates of LAS was measured by dividing the net amount of transformed LAS for 14 h. Khleifat M. K., (2006) reported that the LAS concentration of 200 ppm resulted in the highest degradation percentage (68%). This percentage of LAS degradation was completed within 96 h. However, the higher LAS concentration utilized resulted in the early termination of degradation activity. This concluded at almost the 48 h time point and at a lower percentage of degradation activity. The reason for the early termination of degradation with the LAS concentration above 200 ppm could be the result of an increase in membrane permeability that causes the dissipation of ion gradients and membrane potential or

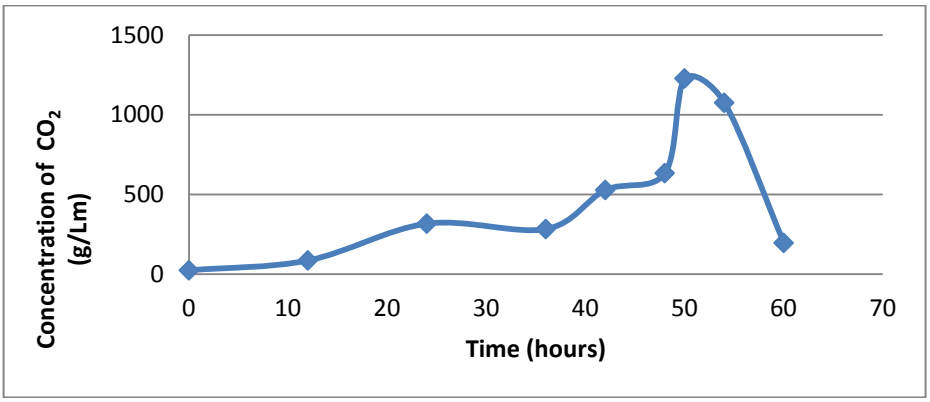

Figure 4. The concentration of CO_2 that was formed at pH 7 and within 50.

$$Removal\ efficiency\ of\ LAS\ and\ ABS$$
$$= \frac{Initial\ concentration - final\ concentration}{Initial\ concentration} \times 100\ \%$$
$$= \frac{100\ mg/L - 0{,}271676\ mg/L}{100\ mg/L} \times 100\% = \ 99{,}73\ \%$$

leakage of essential cell constituents. In this study we found that maximum degradation of LAS and ABS solution (100 ppm) at pH 7 and within 50 hours of incubation.

Concentration of CO_2 that was formed at the optimum condition

The mineralization of LAS during aerobic degradation was also verified through carbon mass balances that were carried out in each experiment. The organic carbon contained in solution (due to LAS or other compounds) and the inorganic carbon (carbonate or gaseous CO_2) produced were summed at each gaseous CO_2 sampling and, as it can be seen, the sum was almost equal to the initial amount of carbon present in the synthetic medium. This means that the carbon balance holds throughout the course of each experiment. Nevertheless, degradation did not reach 100%. Probably it happened because of limited bioavailability due to adsorption on biomass (Constantina et al., 2007).

In this study, a mixture of LAS and ABS degraded by the bacteria Pseudomonas aeruginosa. Gas stream of carbon dioxide (CO_2) produced into solution of NaOH and is measured by titration with solution of HCl. Figure 4. Show that maximum degradation of LAS and ABS solution was 99.73% at pH 7 and within 50 hours.

The LAS biodegradation is influenced by several factors such as the formation of insoluble calcium and magnesium salts surfactants in the presence of metal ions as well as the concentration of dissolving oxygen, pH, additional carbon and nitrogen sources and other growth conditions (Khleifatet al., 2010). In another study, results showed that Enterobactercloacae completely degrade 100 ppm LAS and at the same time could tolerate very much higher concentration (2500 ppm) of LAS (Khleifat et al., 2008). Khleifat M. K., (2006) has been reported that the LAS concentration of 200 ppm resulted in the highest degradation percentage (68%). This percentage of LAS degradation was completed within 96 h. For the combined culture, the greatest LAS biodegradation removal was observed in the nutrient broth of pH 8.5 (90%) with about a 20% decrease at pH 7.5 and 6.5. Biodegradation of LAS by *Burkholderia* sp. has been reported that the pH 6.5 is the optimum in which the higher percentage of LAS degradation was obtained and further decreased with further elevation in the pH level. It is possible that the enzyme (s) for LAS degradation affected by the pH condition and their

optimum activity at 6.5. (Khleifatet al., 2010). In this study, a mixture of LAS and ABS degraded by the bacteria *P. aeruginosa,* the optimum condition of the degradation process at pH 7 and within 50 hours. In this condition the standard solution of LAS and ABS degraded by 99.73% while the LAS and ABS are contained in river water degraded by 94, 22%. The longer time of degradation, the CO_2 that is formed more and more. The biggest CO_2 concentration obtained amounting to 1227.6 mg / L.

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