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Biodegradation of Linear Alkylbenzene Sulfonate by *Burkholderia* sp.: Effect of Some Growth Conditions

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ABSTRACT

This study aimed to isolate Linear Alkylbenzene Sulfonate (LAS)-degrading bacteria from soil irrigated with wastewater. These bacteria were identified as *Burkholderia* sp. and could use LAS as sole carbon and energy sources. *Burkholderia* sp. was grown in the presence and absence of LAS and monitored by using OD_{600} measurements in two different medium, nutrient broth (NB) and M9 minimal medium (MM). Growth of *Burkholderia* cells in both media were mathematically modeled by using *Riccati* and *Voltera* models. The results indicate that LAS is not toxic to the *Burkholderia* sp. cells. Growth of *Burkholderia* sp. in M9 minimal media-lacking LAS showed fit to the *Riccati* model, whereas in LAS containing M9 minimal media, the growth fitted both *Riccati* and *Voltera* models. Three different models (*Monod, Andrew & Tessier*) were used to predict the specific growth rates in different LAS concentration. Three models were shown to be fit Andrew is shown to be the best fit. Incubation temperature of 30°C, agitation rate of 150 rev per min and a pH of 6.5 were shown to be optimum for LAS degradation. Addition of different carbon and nitrogen sources enhance LAS degradation of 300 ppm LAS with different degradation rates. Carbon starvation of *Burkholderia* sp. cells for 24 h and their prior adaptation to LAS caused almost the same induction pattern in the degradation percentage of LAS (7-10%).

Key Words: LAS; Biodegradation; *Burkholderia* sp.; Bioremediation

INTRODUCTION

The pollution of soil and water with xenobiotics is widespread in the environment and is creating major health problems. The utilization of microorganisms to clean up organic compounds from a polluted environment represents a potential solution to such environmental problems (Widada *et al*., 2002). 25-30% of a widely applied linear alkylbenzene sulfonate of the world total synthetic surfactants and it is used in domestic as well as industrial detergent formulation, so it is discharged into waste water collection systems worldwide (Larson *et al*., 1993; Khleifat, 2006a). The commercial LAS is a mixture consisting of about 20 different compounds of closely related homologues and isomers, each containing an aromatic ring sulphonated at the para-position and attached to a linear alkyl chain (Olmo *et al*., 2004; Garcia *et al.*, 2005).

Biodegradation is the most important mechanism for the total removal of chemicals from the environment. It is nature's way of getting rid of wastes by breaking down organic matter into nutrients that can be used by other organisms (Rapaport & Eckhoff, 1990; Kloepper-Sams *et al*., 1996). Insufficient biodegradation led to the development of great masses of foam in streams and rivers (Jakobi & Löhr, 1987; Rapaport & Eckhoff, 1990; Jiménez *et al*., 1991; Schleheck, 2003). Thus all efforts must be directed to biodegrade LAS in high concentration.

Biodegradation of a chemical in soil is determined by their bioavailability and the presence and size of degrader populations as well as environmental conditions that affect their activity. The primary biodegradation by the individual cultures and the consortium was established by measuring the disappearance of LAS in the culture and the formation of biomass upon consumption of that substrate (Swisher, 1987; Schleheck, 2003; Khleifat *et al*., 2008). The studies show that the two members or more in a consortium complement each other as long as the degradation ability was much higher than by their individual cell, which indicates a catabolic cooperation between the consortium members (Schleheck, 2003). In another study, results showed that *Enterobacter cloacae* completely degrade 100 ppm LAS and at the same time could tolerate very much higher concentration (2500 ppm) of LAS (Khleifat *et al*., 2008).

The LAS biodegradation is influenced by several factors such as, formation of insoluble calcium and magnesium salts surfactants in the presence of metal ions

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the concentration of dissolving oxygen, pH, additional carbon and nitrogen sources and other growth conditions (Krueger *et al*., 1998; Utsunomiya *et al*., 1998; Scott & Jones, 2000; Khleifat, 2006).

The aim of this study is to isolate LAS-degrading bacteria from soil irrigated with waste water and test its ability to degrade LAS at high concentration (>100 mg L^{-1}) under optimum growth conditions.

MATERIALS AND METHODS

Bacterial strain isolation: A LAS–degrading bacterium was isolated from soil irrigated with wastewater that had been exposed to different concentrations of LAS ranged between 100-3000 ppm. The colonies that were able to grow in 1000 ppm LAS were purified, identified on the basis of morphological, physiological and biochemical identity using the REMEL kit (RapID ONE & RapID NF plus systems) and API 20NE strip (bioMerieux) procedures. The Remel Kit was obtained from Remel Inc., Lanexa, KS, USA. Cells were gram-negatively stained, motile straight rod with single polar flagellum; positive in production of oxidase and catalase; cells were negative in the production of acid from the following starch, sucrose, raffinose, maltose, cellobiose and erythritol. Also negative in the production of H_2S, acetoin and indol. Negative in hydrolysis of starch, cellulose and casein. Cells were positive in nitrate reduction; negative in urease production, positive in acid production from the following; glycerol, L-arabinose, D-glucose, fructose, D-galactose, lactose, inositol, mannose, mannitol, melibiose, rhamnose, sorbitol, ribose, trehalose, sorbitol and xylose. They could use succinate, citrate and malate as carbon source. This bacterium identification results predict it as it belongs to *Burkholderia* sp.

Materials: LAS (linear alkylbenzene sulfonate) was supplied by Jordan Sulphochemicals Co. Ltd., Jordan. It was an aqueous sodium salt solution with a minimum purity of 96.5% and an average molecular weight of 320 g mol^{-1} that was used in the preparation of working standards. Benzethoinium chloride (hyamine) and patent blue (disulfide blue) were supplied by Acros Organics, (Fisher Scientific, UK). Most of the chemicals used were either from Sigma, USA or from Fluka Chemika, Switzerland. Other chemicals were of analytical grade and were obtained from commercial suppliers.

Growth conditions: In all experiments conducted the LAS-containing *Burkholderia*-free media were taken as control. The nutrient broth and agar medium were used for isolation of bacteria and in some degradation tests performed as described previously (Khleifat *et al*., 2006a). Minimal medium (M9) was used to verify the degradability of LAS by bacteria in study. The minimal broth media as described by Miller (1972) consists of the following: 3.0 g Na_2HPO_4, 1.5 g KH_2PO_4, 1.0 g NH_4Cl and 0.5 g NaCl were dissolved in 500 mL distilled water and the pH adjusted to 7.4 with 1 N NaOH. Then the following compounds were added: 0.24 g $MgSO_4$, 0.05 g $CaCl_2.6H_2O$ and 0.05 g $FeCl_3.6H_2O$. After autoclaving, the following were added by predissolving each in 2 mL distilled water and sterilizing by filtration through a 0.45 μm Millipore filter: 0.001 g thiamine-HCl. LAS was used in the concentration of 300 ppm as carbon and energy source.

The initial cell mass (starting inocula) for each experiment on both NB and MM media experiments were grown from single colonies in two consecutive shake flask cultures (overnight followed by 3 h recovery) in nutrient broth. Cells from three hours cultures were harvested by centrifugation and then washed with and resuspended in NB at a concentration of 0.5 A_{600} units. One mL of these bacterial cells was used to inoculate 100 mL of either media in a 250 mL Erlenmyer flask for shake flask experiments. Samples were then taken at time intervals and assayed for cell mass OD_{600}. In all experiments acid-washed glassware was used.

Analytical methods: LAS (linear alkyl benzene sulfonate) was analyzed in culture media using the classic methylene blue method (Longwell & Maniece, 1955), modified by Li and Rosen (1981). The LAS was extracted in an organic solvent (chloroform) and titrated against standardized hyamine (benzethonium) in the presence of patent (anionic dye). The endpoint was detected by the pink to blue oily droplets color development of the organic layer. The disappearance of LAS was qualified by reverse-phase HPLC on a ODS2 column (250 by 4.6 mm; particle size, 5 micron); the motile phase was 0.11M$NaClO_4$ with an acetonitrile gradient (0–100%; vol./vol.) and compounds were detected spectrophotometrically at 255 nm (Kertesz *et al.*, 1994). In order to compare the degradation ability achieved between two different conditions the percentage degradation (%) of the LAS compared to the corresponding control was conducted.

Effect of different growth conditions on LAS biodegradability: Different pH's (6, 6.5, 7, 7.5, 8 & 8.5) of growth media were used to assess the effect of variation in pH on the percentage degradation of LAS by *Burkholderia* sp. The effect of different incubation temperature (20, 25, 30, 35, 37 & 42°C) upon the percentage degradation of LAS by bacterial isolate was conducted. The same growth conditions were used (pH 7.4, agitation rate of 150 rpm). Different shaking rates (50, 100, 150, 200 & 250 rpm) were used to assess the effect of aeration on the LAS degradation rate (ppm/h). The same growth conditions were used except that agitation rate was varied (pH 7.4, 37°C).

Different carbon and nitrogen sources supplied to the M9 minimal medium (MM) to study their effect on the biodegradation of LAS as a function of time. The carbon sources included glucose, fructose, maltose and sucrose as carbon sources, whereas the nitrogen sources being tested included lysine, glysine, urea, yeast extract and trypton. All of the nutrient sources were supplied independently to MM at a concentration of 0.2%.

Growth curves: Two different media, nutrient broth and M9 minimal medium (MM) were used for growth curves. The first was denoted NB and the second were MM, respectively. Each medium was supplemented with 300 mg L^{-1} LAS; and in minimal medium LAS was the only carbon source utilized. Growth curves experiments were performed on the NB and MM media under the same conditions. The same initial cell mass (starter inoculums) for the three cases was taken into consideration as mentioned above. Samples were then taken at time intervals and assayed for cell mass OD_{600}.

Effect of carbon starvation on LAS degradation: Starvation experiments were conducted according to the procedure described previously (Khleifat, 2006b). To make cells starved for carbon, the bacterial cells of *Burkholderia* sp. were grown on nutrient broth (37°C, 150 rpm & pH 7.4) to med-log phase (OD600 ≈ 0.50). Then the cells were centrifuged (5000 rpm, 10 min, 4°C), washed twice with equal volumes of sterile M9 minimal medium and suspended in the same medium to an OD600 of 0.25 (≈ 3 x 10^8 cells/mL). The later cell suspension was considered as the non-starved experimental control to assess LAS degradation by *Burkholderia* sp. cells. A sub-sample of the cell suspension was C-starved in the M9 minimal medium at 37°C with an agitation rate of 150 rpm and left for 24 h. The minimal media that contain carbon starved cells was supplemented with 300-ppm LAS and tested for their LAS degrading ability as usual.

Effect of LAS adaptation on the percentage degradation: *Burkholderia* sp. cells were inoculated into M9 minimal media containing 300 ppm LAS as the sole carbon source. After 24 h of incubation the cells were harvested by centrifugation and washed twice with equal volume of sterilized M9 minimal media. The same procedure was repeated twice and the harvested cells were suspended into 300 ppm LAS-containing M9 minimal media as adapted cells (Brandt *et al*., 2001; Khleifat, 2007). Two controls were used, first the usual experiment (no adapted cells) and the second (Uninoculated M9 minimal media). The degradation percentage was analyzed as a function of time as described above.

RESULTS AND DISCUSSION

Bacterial identification: Based on morphological, physiological and biochemical characteristics results placed the bacterial strain as *Burkholderia* sp. To our knowledge, this is the first study concerning the biodegradation of LAS compound by *Burkholderia* sp. Although the microbial degradation of LAS compounds is discussed widely in the literature, no studies including any of *Burkholderia* sp. have been published.

Effect of substrate concentration on the LAS biodegradation: Various substrate concentrations were used to assess their effect on the percentage degradation of LAS (Fig. 1a). To determine the exact time point for achieving complete degradation of LAS (Fig. 1b), a 2 h time point intervals were taken for measuring the degradation of LAS below the 300 ppm concentration (100, 200 & 300). It was found the 100 ppm LAS completely degraded within 14 h of incubation compared with previous study, which shows degradation within days (Schleheck, 2003; Khleifat, 2006a). The LAS concentrations of 300 ppm or lower exhibited higher degradation rate than the concentrations above 300 ppm. Under other conditions as shown in (Fig. 1a & Table IV), the average of degradation rates of LAS was measured by dividing the net amount of transformed LAS for 14 h, since within this time period there was many having no further degradation, such as in Fig. 1. The reason for calculating the average degradation by this method to avoid any erroneous caused by different lengths of lag phases and the difficulty to ascertain the time required achieving complete degradation or when the degradation had stopped (Khleifat, 2006b). Therefore although the 100 ppm completely degraded still the 300 ppm LAS concentration resulted in the highest rate of LAS degradation (9.6 ppm/h). Therefore further experiments were done using 300 ppm concentration. These results were similar to that obtained by *Enterobacter cloacae* (Khleifat *et al*., 2008). Thus an inverse relationship between the extent of LAS concentration and extent of biodegradation was shown. The decrease in LAS degradation at higher concentration was unlinked to a toxicity effect on the bacterial culture as reported with white rot fungus *Phanerochaete chrysosporium* (Yadav *et al*., 2001).

Growth curves and mathmatical interpretation: Bacterial cells were grown in both nutrient broth (NB) and 300 ppm LAS containing NB media (Fig. 2a & b). In the second culture media M9, the LAS were used as sole carbon and energy source (Fig. 3a & b). When cells were grown in NB and LAS-containing NB, the later had shorter lag phase than in the LAS-free NB media. The extent of bacterial growth in M9-minimal media (Fig. 3b) is a function of exhaustion of LAS since no other carbon source was existed in the M9 minimal media and thus confirming the biodegradation ability of LAS. Other possibility for removal of this anionic surfactant from the culture by surface adsorption or extensive chemical degradation is unlikely. Moreover the lag phase for the cells grown in M9 minimal media was shorter than the same counterpart cells grown in nutrient broth. It was reported that the single bacterial isolates are known to have limited ability for degrading the alkyl chain as well as lacking the capability for cleaving the sulfonated aromatic ring exists in LAS aromatic ring (Schleheck & Cook, 2003). This growth was modeled based on increasing the cells mass with time in the presence and absence of population history (OD_{600} measurements). Two growth models were applied to the experimental data as shown in (Figs 2a & b; 3a & b), these models are *Riccati* and *Voltera* one. *Riccati* model assumes that the inhibition of the cells is directly proportional to the square of population growth according the equation:

$dx/dt = k(x-ßx^2)$ ……..... (1).
$x(0)=xo$.

Where β is constant related to the percentage of inhibition and x is the cell mass. Thus the *Riccati* equation can be integrated to give the logistic curve according the equation:

$$x = \frac{x_o e^{kt}}{1 - \beta x_o (1 - e^{kt})} \text{ ……..…….. (2).}$$

The logistic curve drawn from this equation shows a sigmoidal shape that leads to a stationary population of size $x_s=1/\beta$ On the other hand, *Volterra* model is used in order to predict a phase of decline after the stationary population has exhausted all a viable resources. It can be described as:

$$\frac{dx}{dt} = kx(1 - \beta x) + K_o \left| \int_0^t x(r)dt \right| \text{ ……..….. (3).}$$

$$x(0) = 0 .$$

Where K_o is a constant account for the history or memory of population. The sign of K_o is taken as negative for an inhibitors and positive for a compound which promotes growth.

The population of the cells in NB media free of LAS (Fig. 2a) and the NB media plus LAS (Fig. 2b) is the same, which indicates that LAS has less toxicity on these cells. Both *Riccati* and *Voltera* models fit the experimental data adequately. The corresponding models parameters are shown in Table I. Similar findings were obtained when comparing these results with those in minimal media. However *Riccati* showed best fit to the experimental data compared to *Voltera* model especially for the M9 control (LAS-lacking M9) (Fig. 3a). The experimental data for the growth of the cells in M9 plus 300 ppm LAS showed an adequate fit for both models to these data (Fig. 3b). The corresponding parameters for both models are shown in Table II. It is obvious form these experimental data that cells has little memory effect for the grown, which indicates that cells can resist the toxicity of the LAS.

Toxicity of LAS toward bacterial cells: In order to determine the toxic concentration of LAS toward *Burkholderia* sp. culture or cells, a series of batch experiments were conducted (Fig. 4). In these experiments initial LAS concentrations were varied from 500 to 3000 ppm as previously conducted with the bacterium *Enterobacter clocae* (Khleifat *et al*., 2008). Based on the effect of these concentrations on the cell number, the specific growth rate "μ" can be determined experimentally by having the ratio of the difference in the cells concentration to the differences in time (Nuhoglu & Yalcin, 2004) according the:

$$\mu = \frac{\ln(X_2 / X_{1)}}{t_2 - t_1} \text{ ……..…….. (4).}$$

Table I: *Ricatti* and *Voltera* model parameters for cells grown in NB and NB plus 300 ppm LAS

Parameters\Model Name	k	β	K_o
Riccati	1.5	0.65	-
Voltera	0.55	0.73	0.025

Table II: *Ricatti* and *Voltera* model parameters for cells grown in M9 minimal medium (MM) and MM plus 300 ppm LAS

Parameters\Model Name	k	β	K_o
Riccati	3.3	4.1	-
Voltera	0.9	3.5	0.0001

Table III: Model parameters that fit the experimental data which showed the relationship between substrate concentration and specific growth rate

Parameters\Model Name	μ_{max}	α	K_S	K_i
Andrews model	0.26	2.3	600	1500
Tessier model	0.25	-	0.2	-
Monod model	0.24	-	0.1	-

Table IV: Effect of carbon and nitrogen sources on the LAS biodegradation by *Burkholderia sp*. all carbon and nitrogen sources were added in 0.2% concentration

Carbon and Nitrogen Source	LAS Degradation Rate (ppm/h)	Cell Mass (OD_{600})
Control	5.8	0.27
Glucose	6 ±0.3	0.30±0.3
Fructose	5.9±0.3	0.27±0.4
Maltose	6.2±0.4	0.32±0.2
Sucrose	6±0.5	0.30±0.3
Succinic acid	6.9±0.2	0.35±0.2
Lysine	5.2±0.4	0.27±0.3
Glysine	5.5±0.5	0.26±0.2
Urea	5±0.6	0.25±0.2
Yeast Extract	6.2±0.4	0.29±0.2
Trypton	5.7±0.4	0.35±0.2

Several models such as *Monod*, *Tessier* and *Andrews* models were used to describe the relationship between substrate concentration and specific growth rate assuming the formation of saturation kinetics for cells grown in different concentrations of substrate. The *Monod* model has the form:

$$\mu = \frac{\mu_{max} S}{K_s + S} \text{ ……..…….. (5).}$$

Where μ_{max} the maximum specific growth rate when S>>Ks. Other related forms specific growth rate dependence have been proposed, which in particular instances give better fits to experimental data. For example, *Andrews* and *Tessier* suggest the following models:

Tessier $\mu = \mu_{max}(1 - e^{-S/K_s})$ ……..…….. (6).

Fig. 1: Effect of different substrate concentration on the biodegradation of LAS by *Burkholderia* sp. (a) data collected at 24 h intervals, (b) data collected every 2 h intervals, the figure represents the percentage of remaining LAS expressed as active matter under the same experimental conditions (37°C, pH 7.4 & agitation rate of 150 rpm), all data are average of three trials with error bars indicating STDEVs (σ_{n-1})

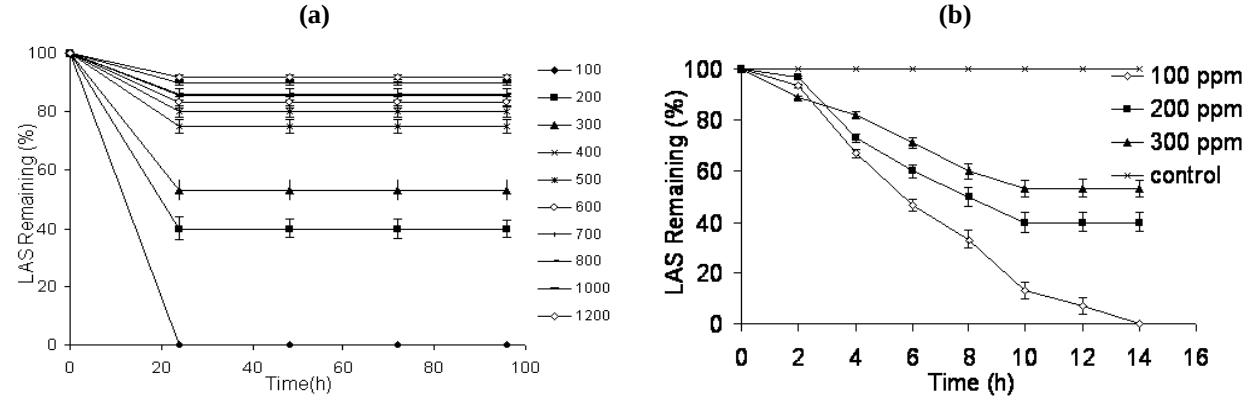


Fig. 2: Growth curve of *Burkholderia* sp. on (a) nutrient broth media (LAS-free NB) and (b) LAS-containing NB medium, the curve represents the absorbance at 600 nm as a function of time, data were fitted by *Riccati* and *Volterra* models this was done under the same experimental conditions (37°C, pH 7.4 & agitation rate of 150 rpm)

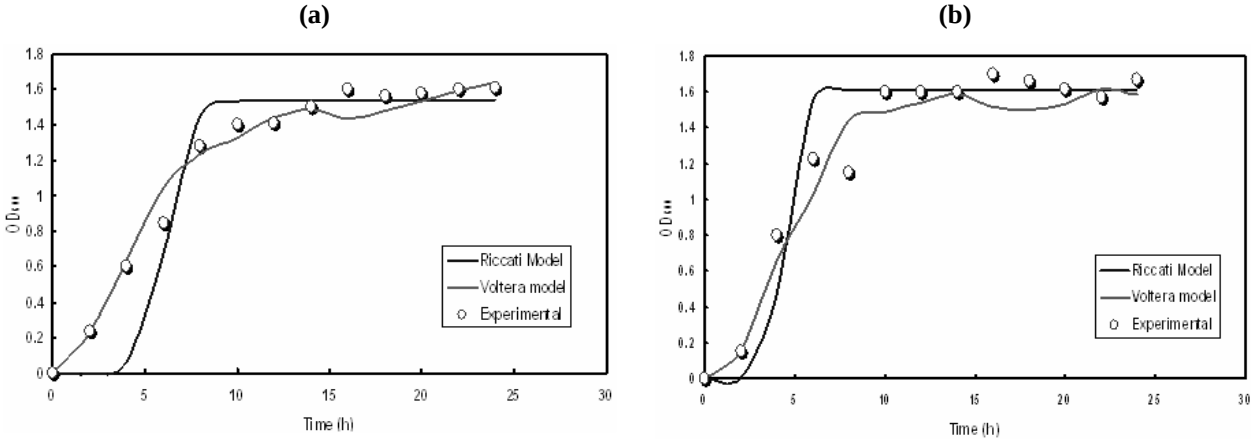
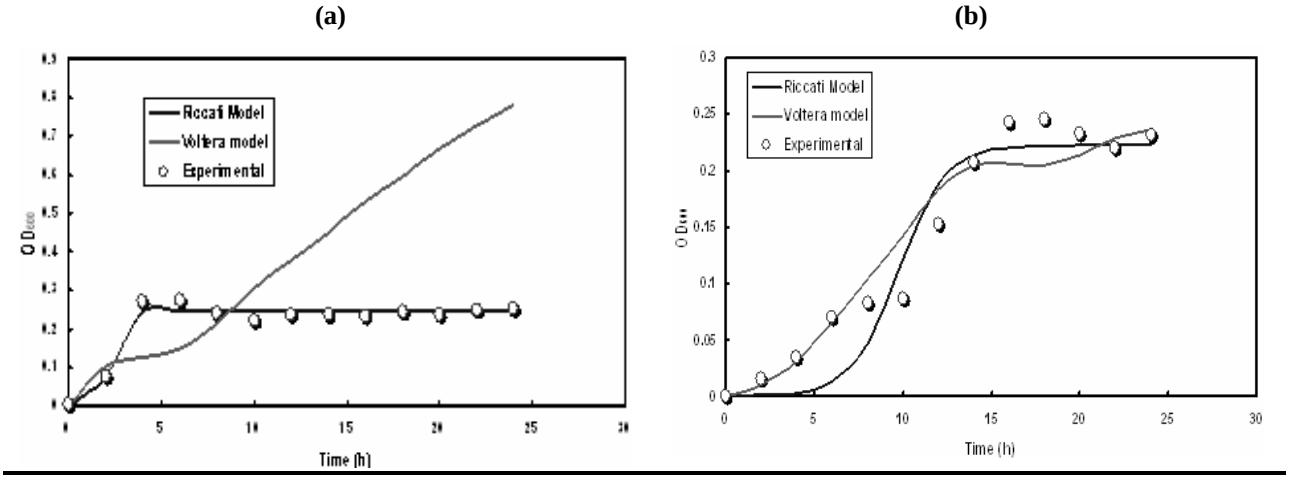


Fig. 3: Growth curve of *Burkholderia* sp. on (a) M9 minimal medium LAS-free MM and (b) LAS-containing minimal medium, the curve represents the absorbance at 600 nm as a function of time. Data were fitted by *Riccati* and *Volterra* models this was done under the same experimental conditions (37°C, pH 7.4 & agitation rate of 150 rpm)



The specific growth rate may be inhibited by medium constituents such as substrate or product. This inhibition effect may be predicted by *Andrews* Model according:

$$\mu = \frac{\mu_{max} S}{K_s + S + S^2 / K_I} \quad \text{..... (7).}$$

By taking into account the last experimental data are error, all the models could fit these data (Fig. 4) and the corresponding parameters are shown in Table III. Previous studies have shown that single bacterial species and specific soil processes can be very sensitive to LAS (Elsgaard *et al*., 2001). Toxic effects of LAS on soil organisms should therefore be evaluated to ensure safe use of sewage sludge as fertilizer (Holmstrup & Krogh, 2000). In very recent study of our Lab, *Enterobacter colacae* have been confirmed to acheive complete degradation of 100 ppm LAS and was able to resist a very high LAS concentration (Khleifat *et al*., 2008). In the present study, almost similar results were achieved with the bacterium *Burkholderia* sp.

Effect of agitation rate on the biodegradation of LAS: It is clear that increasing the agitation rate caused an increase in the percentage degradation of LAS by this organism (Fig. 5). Increasing the agitation rate beyond 150 rpm did not result in any increase indicating that the 150 rpm is the optimum agitation rate for achieving the highest degradation level. When Erlenmeyer flasks was 100% fully filled with LAS-containing growth media, no significant LAS removal was noticed until 15 days. This result presumes the (mono) oxygenation of LAS as previously described by others (Swisher, 1987; Jiménez *et al*., 1991).

Bacteria use essentially two strategies to access the carbons of surfactants. The first strategy involves an initial separation of the hydrophile from the hydrophobe (hydrophile attack), which is then oxidatively degraded. Secondly the hydrophobic is initially oxidized, while still attached to the hydrophile (hydrophobe attack). Both mechanisms lead to immediate loss of amphiphilicity in the molecule, which therefore no longer behaves as a surfactant (Jiménez *et al*., 1991; Abboud *et al*., 2007). Thus the LAS degradation is basically an aerobic process and hence the introduction of air to the solution will favor it and aerobic degradation of LAS is preferred due to the fact that LAS can be anaerobically degradable only if preceded by a period of aerobic exposure (Larson *et al*., 1993), where the oxygenation at the end of the alkyl chain, requires oxygen (Swisher, 1987).

Effect of temperature on the LAS biodegradation: The data on the percentage degradation brought about by this bacterial culture under study at different incubation temperatures are shown in Fig. 6. It is seen from the results that there was a rise in percentage degradation of LAS with rise in temperature between 25 to 30°C followed by early steep fall (within 24 h) with further rise in temperature from 37 to 42°C. The increase in temperature above threshold

Fig. 4: The relationship between substrate concentration and growth rate, this carried out by three different models, all the models could fit the experimental data (*Riccati, Volterra* & *Andrews*) being *Andrews* the best fit, this was done under the same experimental conditions (37°C, pH 7.4 & agitation rate of 150 rpm)

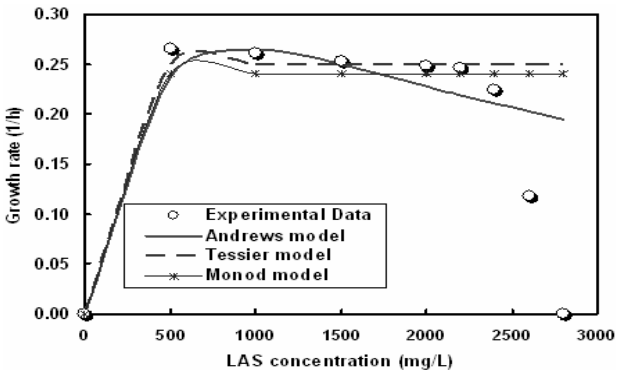


Fig. 5: Effect of agitation rate (rpm) on the biodegradation rate (ppm/h) of 300 ppm LAS, the data represent the LAS degradation expressed as active matter under the same experimental conditions (37°C & pH 7.4), all data are average of three trials with error bars indicating STDEVs (σ_{n-1})

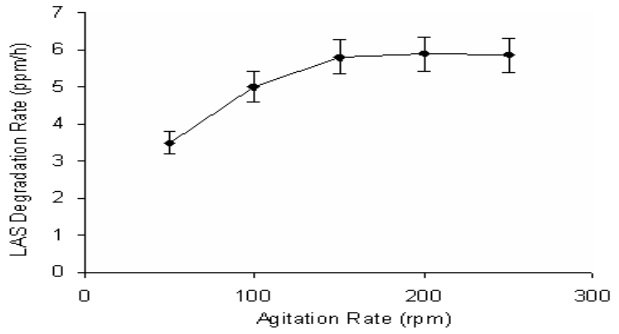
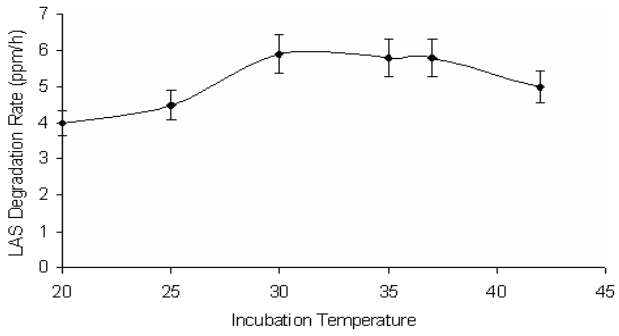


Fig. 6: Effect of different incubation temperature on the biodegradation rate (ppm h^{-1}) of 300 ppm LAS, the data represent LAS degradation expressed as active matter under the same experimental conditions (pH 7.4 & agitation rate of 150 rpm), all data are average of three trials with error bars indicating STDEVs (σ_{n-1})



point (37°C) may increase microbial membrane toxicity as well as the growth itself is affected. The data therefore showed that temperature 30°C would be optimum for better degradation of LAS as confirmed by HPLC (data not shown). Temperature apparently had a strong impact on the fate of the aromatic compounds, as the mesophilic temperature produced the best conditions for their degradation, or this could be solely the consequence of a temperature effect on enzyme activities (Nuhoglu & Yalcin, 2004). It has been reported that the temperature could play an equivalent or larger role than nutrient availability in the degradation of phenol (Margesin & Schinner, 1997).

Generally speaking, temperature affects the rate of biodegradation of xenbiotics by influencing the physical and chemical properties of the LAS compound, microbial metabolism, the specific growth rate of microorganism, the rate of enzymatic activity involved in oxidation process and the composition of microbial community (Gibbos *et al*., 1975; Takamasu *et al*., 1996). The result of the present study showed that biodegradation of LAS by combined culture is temperature-dependent.

Effect of pH on the LAS biodegradation: Fig. 7 shows the effect of pH on the LAS biodegradability by *Burkholderia* sp. It is clear 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. It was reported that, the drop in pH was attributed to the production of acidic intermediates as a result of LAS degradation (Swisher, 1987). The very last observation gives further capacity for belief to the notion that biodegradation rather than sorption was primarily responsible for LAS removal (Brandt *et al*., 2001). All the experiments were made so far, were conducted under 37°C, pH 7.4±0.2 and shaking rate of 150 rpm.

Effect of carbon-starvation on the LAS biodegradation: LAS degradation under starvation conditions was compared with the same activity under normal conditions. Fig. 8 shows that carbon starvation has positively enhanced the percentage of LAS degrading activity (10%). The same results were noticed during the phenol degradation by *Ewingella americana* (Khleifat, 2006). It is possible that carbon starvation, initiates an early expression of the LAS catabolic genes (Matin *et al*., 1999; Reardon *et al*., 2001).

Effect of adaptation on the LAS biodegradation: The LAS pre-cultured (adapted) cells were shown to have slightly higher percentage LAS degradation in all incubation time points (Fig. 9). However the time required to achieve this higher LAS percentage was unchanged. This slight effect of adaptation on the degradation ability, probably, because the bacteria were already isolated from soil contaminated with this compound or its related compounds. In other words, the bacteria were already adapted to presence and absence of this compound. These results are in close agreement with those of Brandt *et al*. (2001).

Fig. 7: Effect of different pH's on the biodegradation rate of 300 ppm LAS, the data represent LAS degradation expressed as active matter under the same experimental conditions (37°C & agitation rate of 150 rpm), all data are average of three trials with error bars indicating STDEVs (σ_{n-1})

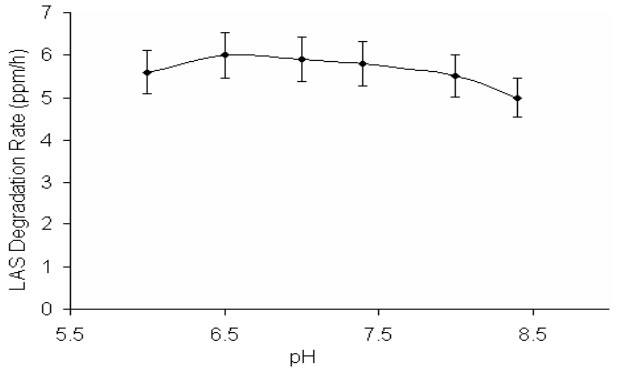


Fig. 8: Effect of carbon-starvation on the 300 ppm LAS degradation percentage, the data represent the percentage of degrading LAS expressed as active matter under the same experimental conditions (37°C & agitation rate of 150 rpm), all data are average of three trials with error bars indicating STDEVs (σ_{n-1})

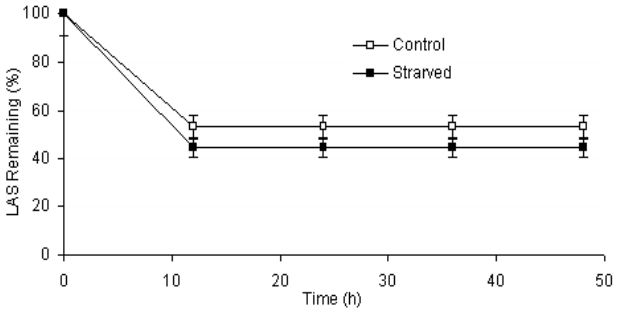
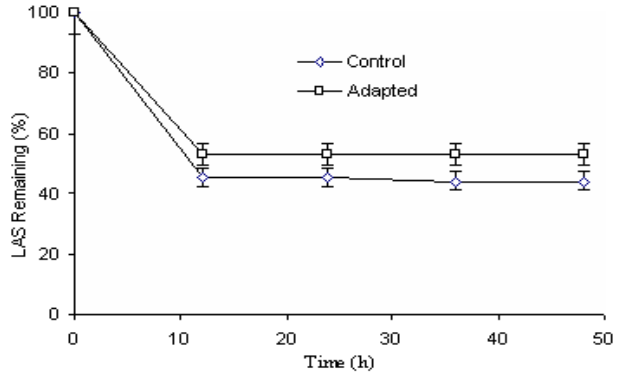


Fig. 9: Effect of LAS adaptation on the 300 ppm LAS degradation percentage, the data represent the percentage of degrading LAS expressed as active matter under the same experimental conditions (37°C & agitation rate of 150 rpm), all data are average of three trials with error bars indicating STDEVs (σ_{n-1})



Effect of carbon and nitrogen source: The degradation of LAS by *Burkholderia* sp. was not repressed by any of the carbon source (Table IV). Succinate was being the highest giving 1.2 fold upon the control. Of the many nitrogen sources tested, only yeast extract caused an enhancement in the LAS degradation by 0.08-fold. Thus lysine, glysine, urea and trypton could be transformed into easily mobilized intracellular carbon, nitrogen and energy sources that could provide a selective advantage to the strain, keeping these substrates at a specific level in order not to totally repress LAS-degrading enzymes and maintaining cellular growth capacity. Generally the induction of the LAS degradation, when adding these carbon and nitrogen sources, probably occurs as a result of increasing cell biomass, using more readily metabolizable carbon and nitrogen sources (Khleifat, 2006) such yeast extract, that cause faster degradation rates of LAS (Table IV) after a short acclimation period (data not shown). It is possible that the simultaneous utilization of conventional nutrients and LAS enables the cells to overcome the inhibition effect of growth caused by LAS (Loh & Wang, 1998). Previous studies have reported that there an optimal amount of yeast extract should be supplemented for the optimal rate of phenol biodegradation (Lob & Tar, 2000). The reason for the enhanced degradation rate of LAS by *Burkholderia* sp. could be attributed to the attenuation of LAS toxicity by available nutrients and consequently the build-up of more cell mass (Loh & Wang, 1998).

The data presented here represent the first report about the capability of LAS degradation by *Burkholderia* sp. isolated from a wastewater plant. This could be a unique organism in the degradation of high concentrations of LAS.

REFERENCES

Abboud, M.M., K.M. Khleifat, M. Batarseh, K.A. Tarawneh, A. AlMustafa and M. Al-Madadhah, 2007. Different optimization conditions required for enhancing the biodegradation of linear alkylbenzosulfonate and sodium dodecyl sulfate surfactants by novel consortium of *Acinetobacter calcoaceticus* and *Pantoea agglomerans*. *Enz. Microbiol. Technol.*, 41: 432–439

Brandt, K.K., M. Hesseloo, P. Roslve, K. Henriksen and J. Sorensen, 2001. Toxic Effects of Linear Alkylbenzene Sulfonate on Metabolic Activity, Growth Rate and Microcolony Formation of *Nitrosomonas* and *Nitrosospira Strain*. *Appl. Environ. Microbiol*., 67: 2489–2498

Eden, P.A., T.M. Schmidt, R.P. Blakemore and N.R. Pace, 1991. Phylogenetic analysis of Aquaspirillum magnetotacticum using polymerase chain reaction-amplified 16SS rRN-specific DNA. *Int. J. Syst. Bacteriol*., 41: 324–325

Elsgaard, L., S.O. Petersen and D. Kasia, 2001. Effect and risk assessment of linear alkylbenzene sulfonate in agriculture soil. *Environ. Toxicol. Chem*., 20: 1656–1663

García, M.T., E. Campos, I. Ribosa, A. Latorre and J. Sánchez-Leal, 2005. Anaerobic digestion of linear alkyl benzene sulfonates: Biodegradation kinetics and metabolite analysis. *Chemospher*., 60: 1636–1643

Gee, J.E., B.K. De, P.N. Levett, A.M. Whitney, R.T. Novak and T. Popovic, 2004. Use of 16S rRNA gen sequencing for rapid confirmatory identification of Brucella isolates. *J. Clin. Microbiol.*, 42: 3649–3654

Gibbos, C.F., K.B. Pugh and A.R. Anderews, 1975. Quantitative studies on marine biodegradation of oil. II. Effect of temperature. *Proc. R. Soc. London B*., 188: 83–94

Holmstrup, M. and P.H. Krogh, 2000. Effect and risk assesment of linear alkylbenzene sulfonates in agriculture soil. *Environ. Toxicol. Chem*., 20: 1673–1679

Jakobi, G. and A. Löhr, 1987. Detergent ingredients. *In*: Gerhartz, W. (ed.), *Ullmann's Encyclopedia of Industrial Chemistry*, 5th edition, pp: 338–372. VCH, Weinheim

Jiménez, L., A. Breen, N. Thomas, T.W. Federle and G.S. Sayler, 1991. Mineralization of Linear Alkylbenzene Sulfonate by a Four-Member Aerobic Bacterial Consortium. *Appl. Environ. Microbiol*., 57: 1566–1569

Kertesz, M.A., P. Kölbener, H. Stockinger, S. Beil, A.M. Cook, 1994. Desulfonation of Linear Alkylbenzenesulfonate Surfactants and Related Compounds by Bacteria. *Appl. Environ. Microbiol.* 60: 2296–2303

Khleifat, K., 2006a. Biodegradation of Phenol by *Ewingella americana*: Effect of Carbon Starvation and Some Growth Conditions. *Proc. Biochem.*, 41: 2010–2016

Khleifat, K.M., 2006b. Biodegradation of linear alkylbenzene sulfonate by a two-member facultative anaerobic bacterial consortium. *Enz. Microbial. Technol.*, 3: 1030–1035

Khleifat, K.M., K.A. Tarawneh, A.A. Al-Tarawneh, K. AlSharafa, M.A. Wedyan, 2008. Growth kinetics and toxicity of *Enterobacter cloacae* grown on linear alkylbenzene sulfonate as sole carbon source. *Curr. Microbiol*., 57: 364–370

Khleifat, K.M., 2007. Effect of substrate adaptation, carbon starvation and cell density on the biodegradation of phenol by *Actinobacillus* sp. *Fresenius Environ. Bull*., 16: 726–730

Kloepper-Sams, P., F. Torfs, T. Feijtel, J. Gooch, 1996. Effects assessments for surfactants in sludge-amended soils: a literature review and perspectives for TRA. *Sci. Tot. Environ.*, 185: 171–185

Knaebel, D.B., T.W. Federle, D.C. McAVOY and J.R. Vestals, 1994. Effect of Mineral and Organic Soil Constituents on Microbial Mineralization of Organic Compounds in a Natural Soilt. *Appl. Environ. Microbiol*., 60: 4500–4508

Krueger, C.J., K.M. Radakovih, T.E. Sawyer, L.B. Barber, R.L. Smith and J.A. Field, 1998. Fate and transport of a linear alkylbenzenesulfonate in a sewage-contaminated aquifer: A comparison of natural-gradient pulsed tracer tests. *Environ. Sci. Technol.,* 32: 3954–3961

Leven, L. and A. Schnürer, 2005. Effect of temperature on biological degradation of phenols, benzoate and phthalates under methanogenic conditions. *Internat. Biodeter. Biodeg*., 55: 153–160

Li, Z. and M. Rosen, 1981. Two-phase mixed indicator titration method for determination of anionic surfactants. *Anal. Chem. J*., 53: 1516–1519

Loh, K.C. and S.J. Wang, 1998. Enhancement of biodegradation of phenol and a nongrowth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. *Biodegradation,* 8: 329–338

Lob, K.C. and P.P. Tar, 2000. Effect of additional carbon sources on biodegradation of phenol. *Bull. Environ. Contamin. Toxicol.,* 64: 756–763

Longwell, J. and W.D. Maniece, 1955. Determination of ionic detergents in sewage, sewage effluents and river waters. *Analyst Lond.,* 80: 167–171

Larson, R.J., T.M. Rothgeb, R.J. Shimp, T.E. Ward and R.M. Ventullo, 1993. Kinetics and significance of biodegradation of alkylbenzene sulfonate in the environment. *J. American Oil Chem. Soc*., 70: 645–657

Margesin, R. and F. Schinner, 1997. Effect of temperature on oil degradation by a psychrotrophic yeast in liquid culture and in soil. *FEMS. Microbiol. Ecol*., 24: 243–249

Matin, A., M. Baetens, S. Pandza, C.H. Park and S. Waggoner, 1999. Survival strategies in the stationary phase. *In*: Roserberg, E. (ed.), *Microbial Ecology and Infectious Disease*, pp: 30–48. ASM Press, Washington, DC

Miller, J.H., 1972. *Experimental in Molecular Genetics*. Cold Springs Harbor laboratories, New York

Nuhoglu, A. and B. Yalcin, 2004. Modeling of phenol removal in a batch reactor. *Proc. Biochem*., 40: 1233–1239

Olmo, M., A. Garballo, M. Nimer, I. Lopez, J. Ferrer and J. Vtichez, 2004. Development of Methodologies for Different Degrees of Resolution of linear alkylbenzene sulfonates in Groundwater and Wastewater by Liquid Cromatograpy Using Sodium Dodecyl Sulfate. *Chromatographia,* 60: 157–164

Widada, J., H. Nojiri and T. Omori, 2002. Recent developments in molecular techniques for identification and monitoring of xenobiotic-degrading bacteria and their catabolic genes in bioremediation. *Appl. Microbiol. Biotechnol*., 60: 45–59

Rapaport, R.A. and W.S. Eckhoff, 1990. Monitoring linear alkyl benzene sulfonate in the environment: 1973-1986. *Environ. Toxicol. Chem*., 9: 1245–1257

Reardon, K.F., D.C. Mosteller, J.B. Rogers, N.M. Du Teau and K.H. Kim, 2002. Biodegradation kinetics of aromatic hydrocarbon mixtures by pure and mixed bacterial cultures. *Environ. Health Perspect. Supplem*., 110: S6

Sacchi, C.T., A.M. Whitney, W.M. Leonard, R. Morey, A. Steigerwalt, B. Arijana, S.W. Robin and T. Popovic, 2002. Sequencing of 16S rRNA gene: a rapid tool for identification of *Bacillus anthracis*. *Emerg. Infect. Dis*., 8: 1117–1123

Schleheck, D., 2003. Biodegradation of synthetics surfactants: linear alkylbenzene sulfonates (LAS) and related compounds. *PhD. Thesis*, University of Konstanz, Germany

Scott, M.J. and M.N. Jones, 2000. The biodegradation of surfactants in the environment. *Biochemica Et Biophysica Acta (BBA) Biomembranes.,* 1508: 235–251

Swisher, R.D., 1987. *Surfactant Biodegradation,* 2nd edition, Vol. 18, pp: 587–614. Maarcel Dekker, New York

Takamasu, Y., O. Nishimura, Y. Inamori, R. Sudo and M. Matsumura, 1996. Effect of temperature on biodegradability of surfactants in aquatic microcosm system. *Water Sci. Technol.,* 34: 61–68

Utsunomiya, A., Y. Mori and K. Hasegawa, 1998. Adsorption of linear alkylbenzenesulfonates and their complexes with cationic surfactants on river sediment and their biodegradation in river water. *Japan J. Toxicol. Environ. Health,* 44: 264–276

Yadav, J.S., D.L. Lawrence, B.A. Nuck, T.W. Federal and C.A. Reddy, 2001. Biotransformation of linear alkylbenzene sulfonated (LAS) by Phanerochaete chrysosporium: oxidation of alkyl side-chain. *Biodegradation,* 12: 443–453

Schleheck, D. and A.M. Cook, 2003. Saccharin as a sole source of carbon and energy for *Sphingomonas xenophaga* SKN. *Arch. Microbiol.,* 179: 191–196

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