Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2020.26410

207 (2020) 197–204
December

Determinants of linear alkylbenzene sulfonate destiny in the environment: a study of linear alkylbenzene sulfonate interactions with *Aspergillus niger*

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Received 23 January 2020; Accepted 28 July 2020

ABSTRACT

Like water, linear-alkylbenzene-sulfonate is indispensable for human development. Therefore, this study tests, for the first time, in one experimental setup, the interactions between a wide range of linear-alkylbenzene-sulfonate concentrations and *Aspergillus niger* growth on a daily basis. The tested conditions are linear-alkylbenzene-sulfonate concentrations, 0.15–1,000 mg/L, starving and fresh *Aspergillus niger*, presence and absence of an external carbon source, and during a 7 d period. The results show the interactions between linear-alkylbenzene-sulfonate concentrations and the *Aspergillus niger* growth fluctuate between; no-observed-effect-concentration, inhibition, growth and no-growth. Astonishingly, any of these interactions, even under the same experimental conditions, occurred at more than one linear-alkylbenzene-sulfonate concentration value, and more than one interaction is observed for a single LAS concentration. Therefore, this study recognizes linear-alkylbenzene-sulfonate "interaction" by pattern, rather than by a single concentration value. Linear-alkylbenzene-sulfonate patterns of interaction with *Aspergillus niger* forms increasing-decreasing trends with peak values, which are influenced by test conditions. Actually, the increasing-decreasing growth patterns are a response to multi-interrelated-mutual-and-reciprocal factors that shape the interactions of linear-alkylbenzene-sulfonate with their environment components over time. Thus, increasing decreasing-growth patterns show the combined effect of linear-alkylbenzene-sulfonate toxicity and biodegradability.

Keywords: Linear-alkylbenzene-sulfonate; Biodegradability; Toxicity; *Aspergillus niger*; Circular economy

1. Introduction

Linear alkylbenzene sulfonate (LAS) is like water, at one stage it is considered an essential element for human development, while at a later stage it becomes a waste; xenobiotic compounds, similar to wastewater, both create challenge to the environment. The global production of LAS, is evidence on its importance, that has started since 1960, to replace hardly biodegradable branched alkylbenzene sulfonate [1]; since then the production is in an ever increase, from 1, 2, and 2.4 million tons in 1980, 1990, and 2000, respectively [2] to 3.5 million tons in 2016 [3]. This study recognizes both, used LAS and contaminated water, not as waste but as valuable nonconventional resources that should be exploited; this is in line with the concept of circular economy. To assign value for these nonconventional resources, it is important to understand their behavior. In other words, it is imperative to understand the LAS interactions with its environment. The first and main carrier of "used LAS" is water in the form of "wastewater" and the first destination is wastewater treatment plant (WWTP). In this stage, LAS is partitioned, based on the treatment processes, into three fractions (1) precipitated and adsorbed to sludge (biomass), (2) residual (remaining) in reclaimed

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water, and (3) mineralized released to air in the form of gases. Therefore, the ultimate destination of LAS fractions is the environment compartments; soil, water, and air.

LASs are basically organic compounds that are distinguished by its hydrophobic and hydrophilic characteristics. These characteristics, which promote the use of LAS in many applications, are the same characteristics that create challenges to handling LAS after use. Therefore, LAS xenobiotic compounds have gained increasing attention in the literature. The findings in the literature show that LAS is aerobically biodegradable [4,5] and toxic to many organisms of terrestrial-waters and sea-waters [6]. Moreover, strikingly, most of the biodegradability studies of the LAS were examined at 10 mg/L [7–9], although, this concentration could overlap with the toxic concentrations that were found to be above 0.1 mg/L [6]. Furthermore, it is important to note that toxicity and biodegradability studies have been carried out separately. In the toxicity literature, LAS toxicity, reported by different studies, occurred at different concentrations, and are attributed to different environmental conditions [10–13]. Such environmental conditions include the microorganisms used in the studies, LAS concentrations, and time. In addition, these studies [14–17] focused on finding a single value that is identified as a lethal concentration (LC_{50}), while the other concentrations, bellow and above LC_{50}, have not gained attention in terms of deep understandings or investigation. In the biodegradability literature, LAS biodegradability studies focused on determining LAS biodegradability fraction [8,9,18–20] at certain predetermined concentrations without considering other possible concentrations.

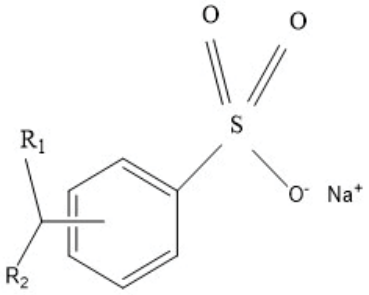
The linear economic model (LEM) views "used LAS" as waste that should be removed to void its negative impact. Circular economic model (CEM) views "used LAS" as resource that should be exploited. Thus, LEM has been looking for (1) a single value that represents the toxic concentration that should be avoided, and (2) the biodegradation potentials, in order to assess the impact of LAS on the environment. In contrast, this study argues that under the CEM, LAS toxic concentrations could be exploited to disinfect the pathogenic organism in wastewater to produce a better quality sludge and effluent. In addition, reclaimed wastewater containing toxic concentration of LAS can be used to disinfect the soil. Other potentials for LAS as a nonconventional resource under the CEM, is studying its hydrophobic/hydrophilic nature to find the potentials for improving sludge characteristics and WWTP performance. Furthermore, the biodegradability of LAS can be exploited as a source of carbon to soil microorganism to enhance soil conditions and structure.

Therefore, this study aims to investigate both the toxicity and the biodegradability of LAS by examining the interactions between LAS and the microorganism growth (fungi species; *Aspergillus niger* (*A. niger*) over a wide range of LAS concentrations during a period of time. The filamentous fungus *A. niger* is chosen because of being of the most abundant fungi in the environment compartments [21–23], in addition to being one of the constituents of WWTP microorganism consortium [24–26], has the potential to enhance the WWTP performance [27] and hence can advocate the potential of LAS as a nonconventional resource under the CEM.

2. Materials and methods

In order to test the toxicity and biodegradability of LAS in Fig. 1, [27]'s protocol was applied. The detailed protocol are given in the following subsections.

2.1. Solution preparation



$R_1+R_2=C_nH_{2n+1}$ where n=10-15

Fig. 1. Chemical structure of linear alkylbenzene sulfonate (LAS).

Potato dextrose broth (PDB); for *in vitro* diagnostics, manufactured by HiMedia Laboratories Pvt., Ltd., India and potato dextrose agar (PDA) for *in vitro* diagnostics, manufactured by Biolab Zrt., Hungary, were prepared by dissolving 24 g of the PDB and 39 g of the PDA, each in a 1 L of distilled water. Then each of the prepared solutions (24 g/L PDB and 39 g/L PDA) was autoclaved in a Tuttnauer-Autoclave-Steam sterilizer, model 3850, USA at 121°C and 4.1 bar for 15 min. The autoclaved agar, after cooling, was used for viable count. The pH of PDB and PDA solutions are 5.1 and 5.6, respectively.

LAS stock solution was prepared by dissolving 24 g of LAS; technical grade, manufactured by Sigma-Aldrich, Co., 3050 Spruce Street, St. Louis, MO 63103 USA 314-771-5765, in a 1 L of autoclaved water. The stock solution (24 g/L) was used to prepare a series of diluted LAS solutions, that is, 0.33, 0.65, 1.3, 2.6, 5.2, 6.25, 7.5, and 8.3 mL of LAS stock were diluted by autoclaved distilled water, so that the final volume of each diluted LAS solution is 50 mL. LAS dilutions were chosen according to a series of pre-experiments that relied on a trial and error, in order to (1) determine the concentrations of LAS with impacts on the growth of *A. niger* that could be recognized during the experiment period, that is, in terms of avoiding the potential of turbidity interference in addition to be able to control the growth rate of *A. niger* that was measured by an ELISA microplate reader (BioTek, model Elx808, USA). (2) Cover a wide range of LAS concentrations, 0.1–1,000 mg/L, while keeping the difference between the consecutive values as close as possible, specifically at low concentrations.

2.2. A. niger cultures

A. niger cultures were prepared by cultivating ATCC strain 6275 (Tech. Supplies Co./Lab Division, USA) in a PDB, which we call the old culture. Then fresh culture of *A. niger* was prepared by adding 3 mL of 30 d-old culture to 200 mL of

fresh PDB, which was incubated at 35°C for 5 d. In this study, the two *A. niger* cultures were used; the 30 d old culture that is also known as "starving culture" and the 5 d old culture, which is known as "fresh culture."

2.3. Experiment setup

The experiment consists of three batches that were conducted in parallel using, in each batch, two microtiter plates. Each microtiter plate consists of 96 *u*-shape wells "holes," out of these wells, 88 wells were used in the experiments. These wells hosted 88 concentrations of LAS that were examined in each plate; each LAS concentration was repeated one, two, three, or four times. Growth of *A. niger*, which was the investigated parameter, was monitored by measuring optical density (OD) via ELISA microplate reader at 650 nm wavelength. The three-batch experiment was carried out two times in sequence.

In each experiment, the three batches received the same concentrations of LAS. In contrast, they received different *A. niger* inoculums; starving culture was added to batch one and fresh culture was added to each of the batches; two and three. In addition, batch three received PDB as an external carbon source. In order for the three batches to have almost the same count number, that is, 18 ± 2 CFU of *A. niger*, both cultures; the old and the fresh were diluted to 10^{-1} and 10^{-2}, respectively.

Microtiter plates preparation (1) each well in the microtiter plate received a 100 μL of autoclaved distilled water if it represents batch one or batch two while received a 100 μL of PDB solution if it represents batch three. (2) Then each of the wells in column 1 received a 100 μL of LAS diluted solutions in the following order 4,000; 3,500; 3,000; 2,500; 1,250; 625, 313, and 156 mg/L from *A* to *H*, respectively. This applied to the three batches, (3) after mixing the content of each well-properly; a 100 μL was withdrawn from the well to be added to its neighboring well in column 2. (4) Step three was applied to each column from 2 to 10, where the solutions were withdrawn from each column's wells and added to their neighboring column's wells, and finally the 100 μL that was withdrawn from each well in last column; column 10 was disposed of. (5) Then each well of the microtiter plate received 100 μL of *A. niger* culture; (a) starving culture if the microtiter represents batch one and (b) fresh culture if it represents batch two or batch three. The 100 μL of an average of 18 CFU of *A. niger* culture was chosen based on pre-experiments, to avoid over- or under-growth of *A. niger*. (6) The wells in column 11 represents the control wells (blanks), which were prepared in a similar manner but without the addition of the LAS solutions. (7) The microtiter plates of the three batches were incubated at 35°C for 7 d, which was chosen to avoid the wells dryness. (8) The plates were monitored by measuring *A. niger* growth intensity via microplate ELISA reader on a daily basis over the working days. (9) Growth intensity per each LAS concentration was reported as an average value per that concentration.

3. Results and discussion

3.1. A. niger growth in the absence of LAS

A. niger grew in control wells, which contained autoclaved distilled water with 70 mg/L of COD. Growth intensity increased over the 7 d test period. The results are reported for the two tested culture of *A. niger*, fresh and starving. It was found that the intensity and the rate of growth of fresh culture are higher than that for starving culture. The growth in theses control wells is attributed to two factors (1) fungi utilization of its food storage in (glycogen) oil vacuoles [28], which is the main contributor to the growth of fresh culture and (2) fungi utilization of the nutrients traces that existed in the distilled water, which is the main contributor to the growth of starving culture. The higher growth intensity of fresh culture indicates that the stored food in the oil vacuoles of *A. niger* is more supportive to its growth than the traces of nutrients available in solution. To further test the effect of substrate on *A. niger* growth, PDB was added to the fresh culture of *A. niger* as an external carbon source. In these experiments, growth intensity of *A. niger* in control wells, which contained the autoclaved PDB also increased over the 7 d test-period. Furthermore, the growth intensity consistently exceeded the growth intensity when only autoclaved distilled water was used during all the time. This finding is explained PDB is considered as a substrate for fungi species [29]. This indicates that PDB is more supportive to the growth, including growth intensity and rate, of *A. niger* than its stored food. It can be concluded, from the above three batches that *A. niger* has the capacity to handle the stress conditions of food shortage, and *A. niger* growth is a function of the nature of the provided substrates. The latter is in line with the results reported by Taylor [15] on *Daphnia magna* that showed variations in growth in response to the different supplied substrates.

3.2. A. niger growth in the presence of LAS

The results of testing the effect of LAS on *A. niger*, both starving and fresh cultures, after 7 d of incubation, showed no-observed growth, that is, no growth was detected, at 125–1,000 mg/L. In contrast, the LAS no-observed effect concentrations (NOECs) on the growth of *A. niger* was found at 0.15, 0.3, 0.6, and 1.2 mg/L of LAS; in other words, the growth at these concentrations was similar to the growth that occurred in their equivalent control wells. These NOECs are comparable with the NOECs that were reported in the literature for LAS, for example, it was found that NOECs occurred at; 1.18 mg/L LAS $C_{11.8}$ and 0.57 mg/L of LAS C_{13} on *D. magna* [30], 0.4 mg/L LAS $C_{11.8}$ on *Mysidopsis bahia* [16], and 0.9 mg/L LAS $C_{11.8}$ and 0.15 mg/L LAS C_{13} on fathead minnow [11].

Interestingly, this study shows, and for the first time in the literature, that the NOECs of LAS are not limited to the above reported low concentrations range 0.15–1.2 mg/L, but NOECs are also observed at 10 mg/L for starve culture, 5 and 50–80 mg/L for fresh culture, and 2–80 mg/L for fresh culture in the presences of PDB (Fig. 2a). In addition, *A. niger* showed higher capacity, to handle 80 mg/L LAS with NOEC, than *D. magna* and *Ceriodophnia* sp. that showed NOEC, up to 10 mg/L LAS ($C_{11.8}$) [14] and 3 mg/L of $C_{11.7}$ LAS [16], respectively. In contrast to the literature, this study used a wider range of LAS concentrations of 0.15–1,000 mg/L LAS. Our results showed that even under the same experimental conditions, the NOEC of LAS on the growth of *A. niger* does not occur at one single concentration, but rather at several

concentrations of LAS, both low and high concentrations. Furthermore, these NOECs occur not only at discrete values but also at continuous intervals of LAS concentrations (Fig. 2a). Therefore, it could be argued that the variations in the NOECs results that were reported in the literature are not only due to the variations in the experimental conditions applied by the researchers [6]. In fact, our findings emphasize on the need for a detailed investigation of the effects of the different LAS concentrations on the growth of *A. niger* as will be discussed in the following sections.

3.2.1. Growth of starving vs. fresh cultures of A. niger

Starving culture is inhibited at concentrations of 2 up to 40 mg/L of LAS, except at 10 mg/L that is found to be an NOEC (Fig. 2a). Meanwhile, no growth is detected at concentrations of LAS > 40–1,000 mg/L. The inhibition is defined in this study as the growth intensity that is less than the growth intensity in the equivalent control well. The fresh culture is inhibited at concentrations of 2 and 110 mg/L of LAS, note that 2 mg/L inhibition is similar to the inhibition concentration of starving culture (Fig. 2a). Meanwhile, no growth of fresh culture is observed at concentrations of 125–1,000 mg/L of LAS, which shows a shift toward a higher lower bound value compared with the starving culture. Furthermore, contrary to starving culture results, where inhibition occurs between 2 and 40 mg/L of LAS excluding 10 mg/L, the 5–80 mg/L LAS concentrations have no negative impact on the growth of fresh culture; even more, LAS concentrations greater than 5 and less than 50 mg/L enhance *A. niger* growth to exceed the growth intensity that occurs in the equivalent control wells (Fig. 2a). This approves that *A. niger* utilizes LAS, that is, biodegrades LAS, as a substrate for growth, and the effect of LAS concentrations on the growth of *A. niger* is controlled by the test conditions that cause different types of interactions between LAS and *A. niger.* Further, the results of testing the effect of LAS, when accompanied by an external substrate "PDB," on the growth of fresh culture of *A. niger* show no growth at 220–1,000 mg/L concentrations of LAS after 7 d

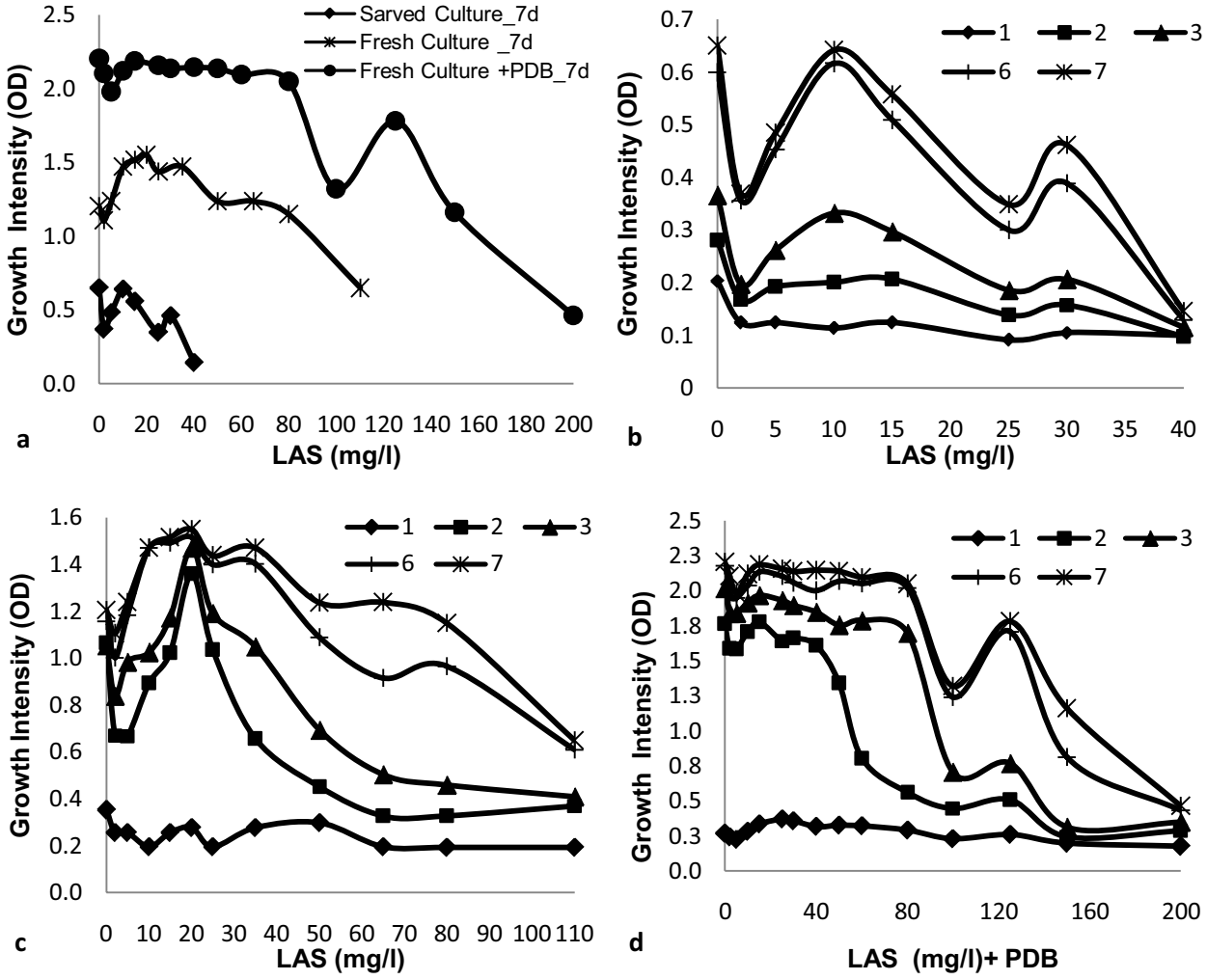


Fig. 2. *A. niger* growth measured, as an optical density, by ELISA microplate readers (BioTek, Elx808, USA) at 650 nm. (a) Growth on day 7 of *A. niger* cultures; starved, fresh, and fresh with PDB. Growth on days: 1, 2, 3, 6, and 7 of *A. niger* (b) starved cultures, (c) fresh culture, and (d) fresh culture with PDB.

incubation period. Meanwhile, growth is observed at LAS concentrations of 2–200 mg/L with higher intensity than the growth that occurs with LAS only (Fig. 2a). This indicates that PDB supports *A. niger* growth more than LAS and also PDB supports *A. niger* to grow at higher LAS concentrations >110–200 mg/L. The growth that occurs at 2–200 mg/L can be classified into two categories (1) NOECs are extended over 2–80 mg/L LAS; a range that is similar to their control wells (Fig. 2a). This provides robust evidence that LAS concentrations ≤80 are not toxic to *A. niger* but slow down the growth, based on the nature of LAS interactions with *A. niger*. (2) Inhibition is observed at LAS 100–200 mg/L compared with their equivalent control wells. This indicates that the inhibitory effect of LAS concentrations of >80 up to 200 mg/L is not permanent and can be attributed to the interactions between LAS and *A. niger*. Then, it is clear that the effects of LAS concentrations of 2–200 mg/L on the growth of *A. niger*, vary not only at different concentrations but also at the same concentration value. The variations in the effects could be attributed to the nature of the interactions between LAS and *A. niger*, which are determined by the experiment conditions that include LAS concentrations; available substrates; as well as the age/activity of *A. niger.*

Therefore, this study shows that there are variations in LAS effects on *A. niger*, which result from the variations in the interactions between LAS and *A. niger*. This is observed in the form of variations in the growth enhancement, NOEC, inhibition, and no growth values. All these effects of LAS are observed in the same experiment at different LAS concentrations and at the same LAS concentration but under different experimental conditions (Fig. 2a). Having no specific ranges for each of these effects and even more, the same concentration might be inhibitory and under other conditions, it enhances growth, NOEC, or shows no growth (Fig. 2a), emphasize that LAS effect on *A. niger* growth is not attributed to a specific LAS concentration, but rather to a specific interaction between LAS and *A. niger*. In contrast, the focus of [14–17], among others, was on identifying a single or a small range of LAS concentrations at which one of these effects like NOEC and/or lethal concentrations (LC_{50}) occur. Therefore, in order to understand the variations in the interactions between LAS and *A. niger* a further investigation on the effect of LAS on *A. niger* during the 7 d experiment was conducted.

3.2.2. Daily growth of starving culture of A. niger

The daily analysis of the effect of LAS on starving culture of *A. niger* shows the following:

- Day 1; shows no growth over LAS concentrations of 2, 5, 10, 15, 25, 30, 40, 55, 90, 125, 190, 250, 500, and 1,000 mg/L.
- Day 2 shows similar results of day 1 except a minor growth that is observed at LAS concentrations of 5, 10, and 15 mg/L.
- Growth increases on days 3, 6, and 7 and displays similar growth patterns, which are: inhibition at LAS concentrations of 2, 5, 15, 25, 30, and 40 mg/L, growth similar to control (NOEC) at 10 mg/L, and no growth at 55, 90, 125, 190, 250, 500, and 1,000 mg/L.
- Since day 3, *A. niger* has shown distinguishable daily growth trends, when depicted against different LAS concentrations (Fig. 2b), in two regions: the first is extended from 2 to 25 mg/L LAS and the second from >25 to 40 mg/L LAS. In the first region, the growth trend (Fig. 2b) peaks at 10 mg/L LAS; the critical point, which is preceded by an upward sloping growth curve (2, 5, and 10 mg/L) and followed by a downward sloping growth curve (10, 15, and 25 mg/L). The second region has a lower peak at 30 mg/L; the critical point, which is also preceded by an upward sloping growth curve (>25–30 mg/L) and followed by a downward sloping growth curve (>30–40 mg/L) growth (Fig. 2b).

The growth observed after day 2 approves that LAS is not toxic to *A. niger* but slows down its growth. Considering that the growth of starving *A. niger*, as mentioned earlier, relies mainly on the utilization of nutrients in the solution, then the slowdown of the diffusion of nutrients contributes to the slowdown of growth. The part of the first region, between 2 and 10 mg/L concentrations of LAS, which shows an upward sloping growth curve, provides evidence that LAS slows down the diffusion rate of nutrients, mainly due to its interaction with the nutrients in the solution rather than its interaction with the *A. niger* itself. The reason of the interaction could be the hydrophobicity of both nutrients and LAS. Furthermore, the upward sloping growth part of the curve indicates that *A. niger* utilizes LAS and hence explains the increase in the growth with the increase in the LAS from 2 to 10 mg/L. In sum, there are two effects for LAS at work with opposite impacts, have been identified in the upward sloping part of the growth curve in the first region; the first is the LAS interaction with the nutrients that slows down the diffusion rate of the nutrients and hence results in slowing *A. niger* growth, and the second is LAS utilization by the *A. niger*, which contributes and enhances *A. niger* growth. Based on that, Fig. 2b shows that the slowdown of the nutrients diffusion rate is the dominant interaction at 2–5 mg/L LAS, as it shows inhibition, while utilization of the LAS effect is the dominant interaction at >5–10 (NOEC) mg/L LAS.

In contrast, the downward sloping growth curve of the first region that occurs at LAS concentrations of >10–25 mg/L shows, in addition to the two effects at work in the upward sloping part, that there are two inhibitory effects associated with LAS that are at work: the first effect is the inhibition that is caused by intermediate (byproducts) products, which are produced by LAS degradation and the second effect is the inhibition by LAS composition. The toxicity of LAS biodegradation byproducts, that is, chains of sulfophenyl carboxylates (SPCs), is 10 times lower than the toxicity of the parent materials [31]. Based on the findings in [31], SPCs seem to affect *A. niger* growth in both the upward sloping part and the downward sloping part of the growth curve in the first region, the SPCs concentrations determine the level of toxicity, which is higher in the downward sloping part. Fig. 2b indicates that SPCs are the dominant factor, which influences *A. niger* growth at >10–15 mg/L, while LAS composition is the main factor that affects its growth at LAS concentrations >15–25 mg/L. The reason is that on day 3 at LAS concentration of 15 mg/L, growth is observed, while at LAS concentration of 25 mg/L no growth is detected.

In the second region, LAS composition is the main factor that contributes to the growing trend, both the upward

sloping and the downward sloping parts of the curve, that is, >25–40 mg/L LAS. This dominant effect of LAS composition is identified based on the manufacturers that were reported by the OECD SIDS [32] as, <2% <C_{10}, 1%–25% C_{10}, 7%–50% C_{11}, 20%–50% C_{12}, 5%–45% C_{13}, <1%–10% C_{14}, and <1 C_{15}. Increasing LAS concentration increases the concentrations of the longer chains, that is, C_{12}–C_{14}, which are known to have higher toxicity than the shorter chains [4,6]. However, longer alkyl chains have a greater ability of biodegradation [4,33]. The biodegradation of LAS long alkyl chain reduces their toxicity impact and contributes to growth, which is detected after 3 d of no-growth period at 30 and 40 mg/L LAS. These results prove that LAS is not only interacting with the nutrients but also with the *A. niger* itself. Therefore, the factors that contribute to LAS toxicity and biodegradability are interrelated and the interactions between these factors determine the dominant interaction that ultimately determines the LAS final effect. The factors that control the interactions in this experiment are LAS concentrations, LAS composition, starving *A. niger*, and time.

3.2.3. Daily growth of fresh A. niger

The results of daily analysis of LAS interaction with fresh culture of *A. niger* are presented in Fig. 2c which shows no growth on day 1 at 2–1,000 mg/L LAS that is similar to starving culture finding. In contrast, growth is observed on days 2, 3, 4, and 7. On day 2, *A. niger* growth occurs at 2–50 mg/L and continues on day 3 which also witnesses a new growth at LAS concentrations up to 80 mg/L. The growth continues on days 6 and 7 as well, and new growth occurs at 110 mg/L LAS. This emphasizes that concentration range of 2–110 mg/L LAS is not toxic to *A. niger* but it slows down its growth. Growth of fresh culture, even with inhibition, exceeds the growth of starving culture over the 7 d experiment period (Figs. 2b and c). In addition, through these 7 d, LAS concentrations of 10, 15, 20, and 25 mg/L constantly enhance the growth of fresh culture to exceed the growth in control wells (Fig. 2c). This provides further evidence that *A. niger* grows on utilization of LAS. The inhibitory effects of LAS at concentrations of 2, 5, 25, 35, 50, 65, and 80 mg/L that are found on days 2 and 3, disappeared on days 6 and 7 (Fig. 2c). These observations indicate that the initial period of incubation is critical in studying the ecotoxicology of LAS that was overlooked by many studies, which reported their results for 21 d [11,14–15], 7 d [10], in addition to other period including 6, 10, 30, 60, and 90 d [6].

The growth trend of fresh culture is similar to that of starving culture, that is, it follows the pattern upward–downward sloping with 20 mg/L concentration of LAS is the critical point "peak." This trend is observed over the 7 d experiment period (Fig. 2c). The difference between the growth patterns of fresh culture and starving culture relates to the daily trends of LAS concentrations range and the "smoothness" steepness of the curve, that is, growth intensity. Moreover, fresh culture shows only one region of upward–downward sloping compared with two regions for the starving culture, which are; 2–25 mg/L and >25–40 mg/L. The one region for the fresh culture is accompanied by a wider concentration LAS range; 2–110 mg/L, and a shifting in the critical point from 10 to 20 mg/L LAS. Therefore, fresh culture *of A. niger* shows higher potential than the starving culture in handling high LAS concentrations. The reason is attributed to the growth rate of the fresh culture *of A. niger* that is 2–4 times faster than that of the starving culture (Fig. 2a). Increasing the growth rate accelerates LAS utilization, especially the longer alkyl chains, as well as reduces the accumulation of the intermediate products (SPCs) that are the main contributor to LAS inhibition effect. Then the high growth rate of fresh culture, relative to starving culture, mitigates the inhibitory effects of LAS, therefore growth of *A. niger* occurs at higher LAS concentrations.

The upward–downward sloping growth trend of fresh culture (Fig. 2c) demonstrates, contrary to starving culture (Fig. 2b) as mentioned earlier, changes in the smoothness of the daily growth trends over different LAS concentrations. The changes in the smoothness are attributed to changes in the dominant factor(s) that affect the growth. These factors are very interrelated and include; LAS interactions with *A. niger* and the nutrients; such as PDB, that retards *A. niger* from accessing the nutrients, in addition to the inhibitory effects of both LAS's intermediate products (SPCs) and LAS's long alky chains. *A. niger* growth in the upward sloping part of growth curve at 2 mg/L LAS is less than that in control wells, because 2 mg/L of LAS is not enough to compensate the losses in growth due to LAS hindering the *A. niger* from accessing the nutrients. In contrast, the growth behavior that is observed at 5 mg/L LAS is similar to that in the control well; this is because 5 mg concentration of LAS is able to compensate for growth losses. Meanwhile, the dominant factor(s) that affect growth of *A. niger* between >5 and 25 mg/L concentrations of LAS are changing over time; on day 2, LAS enhances the growth of *A. niger*, while on day 3, SPCs, is the dominant factor and hence it slows down the growth, and on days 6 and 7 as the concentration of LAS decreases it limits the growth. In the downward-sloping part of the growth curve, the changes in the daily growth trend are attributed to the change in the dominant factor(s) that contribute to the inhibition of *A. niger* over the growth period. The change in the growth trend on both days; 2 and 3, occurs at 35 mg/L, where SPCs toxicity is the dominant factor (inhibitor)at 25–<35 mg/L LAS, while long alkyl chain is the dominant factor (inhibitor) at 35–110 mg/L LAS. Meanwhile, the downward-sloping part of the growth curve changes at 65 mg/L LAS, on both days; 6 and 7, where the toxicity of SPCs and toxicity of alkyl long chain are the main factors (inhibitors) affecting the growth at 35–65 mg/L LAS and >65–110 mg/L LAS, respectively. Therefore, the results of fresh and starving *A. niger* are in agreement on the factors that affect interactions between LAS and the microorganism growth. Moreover, fresh *A. niger* experiment emphasizes that the time is an important factor affecting these interactions and hence determines the dominant effect. This experiment further proves that the toxicity of a single LAS concentration varies, in the same experiment, over time due to the changes in the interactions.

3.2.4. Daily growth of fresh A. niger with external carbon source (PDB)

Supplementing LAS solution with PDB enhances the growth, both intensity and rate, of the fresh culture of *A. niger*, to exceed the growth in the presence of LAS only. Furthermore, PDB makes changes in the growth patterns

over LAS concentrations and time (Fig. 2d). The effect of substrate on the toxicity results of different chemical compounds has attracted the attentions of [15,34–38]. In our experiment, no growth of *A. niger* is observed on day 1, meanwhile growth is observed at 2–125 mg/L concentrations of LAS from day 2 up to day 7, and at >125–200 mg/L LAS on days 6 and 7 (Fig. 2d). Daily analysis of fresh culture growth patterns shows disappearance of the upward sloping- downward sloping – growth trend that is observed in the absence of PDB at 2–80 mg/L in Fig. 2c. However, an upward sloping-downward sloping growth trend is observed at >100–200 mg/L, from day 2 up to day 7, with a peak occurs at 125 mg/L with growth intensity increases with time. The upward sloping-downward sloping growth trend that is observed in Fig. 2c, while disappeared in Fig. 2d is replaced in Fig. 2d simultaneously by a downward sloping growth followed by a declining exponential growth over LAS concentrations of 2–80 mg/L; the range of LAS concentration that is covered by each of these patterns change with time (Fig. 2d). Day 2 shows declining pattern at 2–40 mg/L LAS and declining exponential pattern >40 to 125 mg/L, while no growth is observed at >125 up to 200 mg/L. Days 3, 5, and 7 show declining growth patterns extended over a wider range of LAS concentrations, that is, 2–80 mg/L, with the slope of the declining curve deceases with time. The extension of the declining growth pattern results in shifting the occurrence of the declining exponential growth patterns at LAS concentrations of >80 up to 125 mg/L LAS. These results indicate that PDB supports *A. niger* to cope with higher LAS concentrations by enhancing the organism, that is, *A. niger*, growth intensity and rate. This finding is consistent with Taylor [15] who reported a change in the toxicity effect of LAS $C_{11.3}$ on *D. magna*, in the presence of dietary substrates.

3.2.4.1. Declining growth and declining exponential growth parts

PDB effect is the dominant factor affecting *A. niger* growth at LAS 2, 5, 10, and 15 mg/L that enhances its growth to exceed growth occurs in the presence of LAS only and keeps growth intensity similar to the growth that occurs in PDB control wells. This proves that PDB contributes to *A. niger* growth, in presence of these LAS concentrations, and overcomes the negative impacts of LAS. At higher LAS concentrations; 25, 30, 40, and 50 mg/L, PDB effect is still the dominant factor enhancing *A. niger* growth, but to a lesser degree compared with the growth of *A. niger* that occurs in PDB control wells on days 2 and 3. However, the loss in growth is compensated for through days 5 and 7 (Fig. 2d). This provides further evidence that LAS concentrations of 25 up to 50 mg/L are not toxic but slow down *A. niger* growth; and the higher the LAS concentration, the slower the growth on days 2 and 3. The slowdown in the growth of *A. niger* is attributed to LAS interaction with *A. niger*, despite the fact that *A. niger* growth rate is many times higher in the presence of PDB than when only LAS is used (Figs. 2c and d). Furthermore, the higher the LAS concentration is the higher the interaction with *A. niger*, the greater hydrophobicity effect, and thus more fractions of *A. niger* are utilizing LAS as substrate, which decreases the total growth intensity in the solution. Increasing LAS concentration from >50 to <100 mg/L further slows down *A. niger* growth by acting as the main dominant substrate, which is accompanied by the inhibitory effects of both SPCs intermediate products and LAS compositions.

3.2.4.2. Upward sloping–downward sloping growth parts

Day 2 shows a minor growth of *A. niger* at 100 mg/L LAS, which indicates PDB contribution to growth is minimal and the inhibitory effect of LAS $C_{12–14}$ is the dominant factor, which is mitigated on days 3, 5, and 7. This is supported further by the growth results at LAS concentrations of 100, 125, 150, and 200 mg/L, as the growth follows the growth trend in the presence of LAS only but with shifting toward higher concentrations. The growth trend at these concentrations follows an upward sloping growth curve from 100 to 125 mg/L, where growth peaks at 125 mg/L, then followed by a downward sloping growth curve up to 200 mg/L LAS. Utilization of LAS as substrate for growth has the dominant effect in the upward sloping part of the growth curve, while SPCs inhibitory effect is the dominant factor in the downward sloping curve. So, the supplementary substrate "PDB" causes changes in the roles of which factors is the dominant factor affecting *A. niger* growth under specific growth conditions, that is, substrates concentrations including PDB and LAS, *A. niger* growth stage and time.

4. Conclusion

A. niger has the potential to survive under difficult conditions such as food shortage and *A. niger* growth is a function of the nature of the supplied substrate. Furthermore, *A. niger* has the capacity to handle 80 mg/L concentration of LAS as NOEC, which is higher than the NOECs reported in literature for LAS. The NOEC of LAS on the growth of *A. niger* is found at several concentrations of LAS; 0.15–1.2, 10, 5, 40–80, and 2–80 mg/L. These multiple values of NOEC of LAS are not only attributed to different experiment conditions, but are also found under the same experimental conditions. For example, for starve culture, NOECs occur at 0.15–1.2 and 10 mg/L, for fresh culture, NOECs occur at 0.15–1.2, 5, and 50–80 mg/L, and for fresh culture with PDB, NOECs occur at 0.15–1.2 and 2–80 mg/L.

This study is the first to apply a wide range of LAS concentrations. The use of a wide range of LAS concentrations is resulting in showing the LAS potential effects on *A. niger* which vary from growth enhancement, NOEC, inhibition, and no growth. Each of these effects, even under the same experiment conditions, does not occur at a single LAS concentration value but occur at – several concentrations. Moreover, different effects of LAS occur at the same LAS concentrations in different times and under the same experimental conditions.

The LAS effect is a consequence of LAS interactions with *A. niger*. On one hand, LAS behaves as a substrate; in terms of degradation and utilization, to *A. niger* on another hand, LAS behaves as a toxicant; in terms of composition and intermediate products in addition to reducing the availability of nutrients in the media to *A. niger*. The interaction between LAS and *A. niger* is a function of LAS concentrations, availability of the substrates, as well as the age/activity of *A. niger*, and time. Therefore, *A. niger* growth vs. LAS concentrations patterns show increasing–decreasing trends that reflect the different LAS effects on *A. niger* growth.

Acknowledgments

This work has been funded by The World Academy of Sciences (TWAS); grant number: 13–325 RG/ENG/AF/AC_C-UNESCO FR:3240279212. The University of Jordan/Deanship of Scientific Research: grant number: 146/2014-2015.

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