

## BIODEGRADATION OF LINEAR ALKYL BENZENE SULFONATE (LAS) BY *CHLORELLA SOROKINIANA*

Tainá C. I. Silva<sup>1</sup>, Marcos G. Machado<sup>1</sup>, Ana M. Oliveira<sup>2</sup> & Enio N. O. Junior<sup>2\*</sup>

<sup>1</sup> BSc. in Bioprocess Engineering, Universidade Federal de São João Del Rei, Ouro Branco, MG, Brazil.

<sup>2</sup> Department of Chemistry, Biotechnology, and Bioprocess Engineering, Federal University of São João del-Rei, Ouro Branco, Brazil.

\* Corresponding author's email address: [eniobrasil@gmail.com](mailto:eniobrasil@gmail.com), [tainacnacio@gmail.com](mailto:tainacnacio@gmail.com)

### ABSTRACT

Linear alkylbenzene sulfonates (LAS) are the surfactants most widely used as active agents in cleaning products and may be present as hazardous contaminants in the environment. The biodegradation of sodium dodecylbenzene sulfonate was performed according to a factorial experimental design, considering the effects of the concentrations of LAS and mineral medium (Inthorn). The biodegradation process was tested in an aerobic culture experiment employing a synthetic medium, with *Chlorella sorokiniana* as the microbial agent. The results showed a high percentage of surfactant removal from 53.50 to 88.08% depending on the conditions used for biodegradation, suggesting that the technique could be used in activated sludge wastewater treatments.

**Keywords:** Surfactant. Microalgae. Cleaning products.

## 1 INTRODUCTION

Due to their exceptional detergent properties and relatively low cost, LAS are the most commonly used anionic surfactants.<sup>1</sup> The use of these compounds as key components in cleaning products accounts for over 80% of their consumption.<sup>2</sup> Compared to nonionic and cationic surfactants, LAS has a higher concentration in sludge.<sup>3</sup> Under aerobic conditions, LAS are regarded as biodegradable, and aerobic processes in wastewater treatment plants have been reported to achieve 97-99% biodegradation.<sup>4</sup> However, the transportation of substantial amounts of these chemicals can lead to inefficient breakdown processes in anaerobic zones.<sup>5</sup> Domestic sewage has been reported to have LAS concentrations that range from 0.001 g/L to 0.0022 g/L.<sup>6</sup> The reason for these higher values is that biodegradation pathways are only effective when oxygen is present. Microalgae have been widely used as raw material in many processes due to a range of products resultants from its metabolism and the need to find substitutes to the usual biomass sources. The microalgae growth has advantages which make it a potential solution to energy and environmental problems.<sup>7</sup> The application of these microorganisms in effluent treatment process has been used once is possible to associate the production of microalgae biomass to effluent contaminants removal. Many species of microalgae are able to grow in wastewater because they can be cultivated under mixotrophic conditions.<sup>7</sup> Mixotrophy is a trophic culture method in which microalgae can grow in both photoautotrophic and heterotrophic conditions utilizing both inorganic and organic carbon sources that provide higher biomass and lipid productivities than cultivation under photoautotrophic conditions.<sup>8</sup> The objective of this study was to assess the efficiency of LAS biodegradation in a synthetic medium using microalgae in a batch biological treatment system, given concerns about its persistence. The optimal conditions for the biodegradation process were identified through the application of an experimental design methodology, which involved a limited number of experiments.

## 2 MATERIAL & METHODS

An experimental apparatus was made for realization of the designed experiments. It was composed of 07 transparent and cylindrical glass bottles. The flow through the bottles was maintained in a rate of 1 L/min with the aid of compression pump, silicone aquarium hoses, aquarium valves and 07 air diffusers. Luminosity on the PETs walls was maintained at  $300 \pm 5$  klux in all the experiments with the aid of 3 fluorescent bulbs. The experiments were conducted at room temperature. The cultivation of microalgae in the photobioreactors was carried out with variable surfactant (0.006 – 0.018 g/L) and Inthorn v/v (10 – 80%) according to the experimental design shown in Table 1. The results of analysis of variance (ANOVA) and figures were obtained by using the software Statistica.<sup>9</sup>

**Table 1** Configuration of independent variables.

Run	Surfactant (g/L)	Inthorn v/v (%)
1	0.006 (-1)	10 (-1)
2	0.006 (-1)	80 (+1)
3	0.018 (+1)	10 (-1)
4	0.018 (+1)	80 (+1)
5	0.012 (0)	45 (0)
6	0.012 (0)	45 (0)
7	0.012 (0)	45 (0)

Linear alkylbenzene sulfonate (LAS) was quantified in the medium using the methylene blue method (MBAS) that was modified from the standard procedure.<sup>10</sup>

The surfactant removals (%) were determined according to equation (1):

$$\text{Surfactant removal (\%)} = \frac{\text{Concentration (Final-Inicial)}}{\text{Inicial}} \times 100 \quad (1)$$

### 3 RESULTS & DISCUSSION

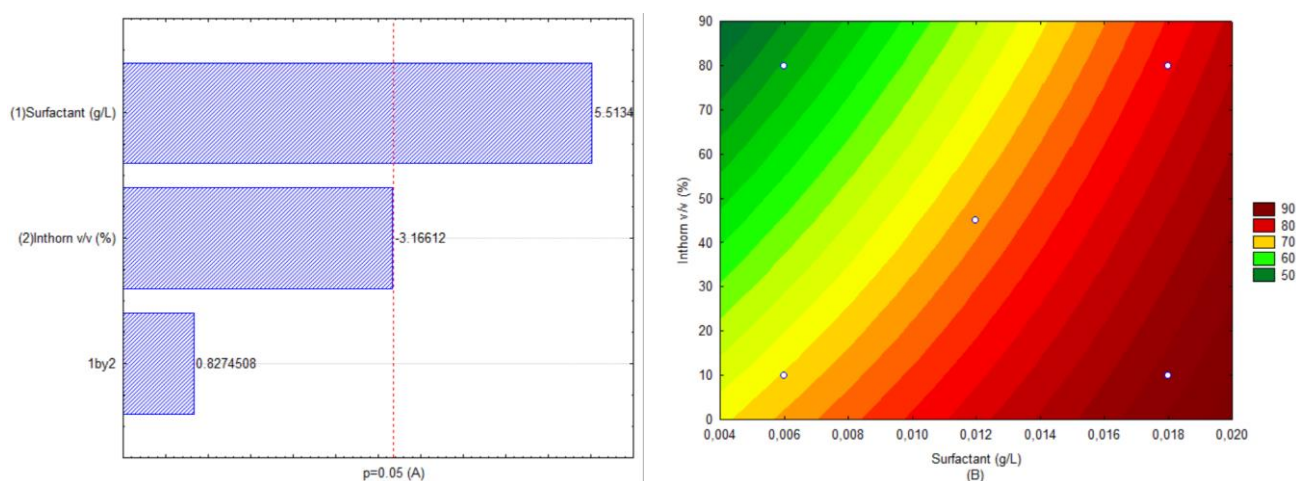
Aerobic biodegradation of sodium dodecylbenzene sulfonate surfactant in a synthetic medium was done using *Chlorella sorokiniana* in this study. Table 2 shows that trial 3 achieved the highest surfactant removal. These results indicate that the microalgae in question can degrade LAS in large quantities without the need for an ideal medium to survive. The efficiency of surfactant removal is significantly impacted by the high volume of Inthorn. The yield values observed in the central point of the 07 experiments were generally consistent, with an average removal rate of approximately 69.6%.

**Table 2** The matrix of the central composite rotatable design with the real and coded values (in parenthesis) for the response surfactant removal.

Run	Surfactant (g/L)	Inthorn v/v (%)	Surfactant removal (%)
1	0.006 (-1)	10 (-1)	69.41
2	0.006 (-1)	80 (+1)	53.50
3	0.018 (+1)	10 (-1)	88.08
4	0.018 (+1)	80 (+1)	78.76
5	0.012 (0)	45 (0)	69.40
6	0.012 (0)	45 (0)	73.78
7	0.012 (0)	45 (0)	65.58

Figure 1A indicates that the surfactant is the only variable showing statistical significance, surpassing the established significance level of  $p = 0.05$ . Although Inthorn experimentally causes a reduction in surfactant removal, it did not show statistically significant impacts on the results. This indicates that Inthorn variation did not cause substantial effects to distinguish them from experimental noise. The Inthorn factor did not exert a notable influence on the observed alterations in the response variables, remaining below the statistical significance threshold.

According to the data presented, the most significant influence in this study was the limited presence of Inthorn, which was equivalent to 10% of the total volume. In Experiment 2, which is depicted in the upper left corner of Figure 1B, the Inthorn concentration was elevated while the surfactant removal percentage was low, registering only 53.50%. Conversely, under similar surfactant concentration conditions and less Inthorn, we achieved higher efficiency, with a surfactant removal rate of 69.41%.



**Figure 1** Pareto chart of surfactant and Inthorn effects on the surfactant removal (A) and contour diagrams of surfactant removal (%) as a function of Inthorn v/v (%) and surfactant (g/L) (B).

Furthermore, the production of sludge remains a significant challenge for the activated sludge process, particularly in the post-treatment phase.<sup>11</sup> Low biomass production and a high ability to remove recalcitrant compounds are some of the advantages of the method developed in this study. The topic of biomass production was not addressed in this study and will be the subject of a future publication.

In addition, the microorganism utilized in this study demonstrated the capacity to tolerate LAS at concentrations exceeding those commonly observed in wastewater, thereby substantiating its potential for utilization as a biological agent for the treatment of effluent contaminated with sodium dodecylbenzene sulfonate.<sup>6</sup>

## 4 CONCLUSION

The capacity of *Chlorella sorokiniana* to degrade linear alkylbenzene sulfonate in a synthetic medium was considerable. The remarkable potential of this microalgae for biological wastewater treatment was evident by the high biodegradation rate, which exceeded 88%. Also, it was determined that surfactant removal was significantly greater at high LAS concentration and low Inthorn concentration (88.07%), whereas the opposite led to lower removal (53.50%). The results vary depending on the amount of Inthorn used. Surfactant removal was not significantly affected by changes in Inthorn concentration levels in the tested central conditions, with an average of 69.6%. In conclusion, the study demonstrates that the microalga *Chlorella sorokiniana* is capable of efficiently biodegrading surfactants in industrial wastewater with high LAS loads. Moreover, the biotechnological process described in the present work should therefore be of considerable environmental interest.

## 5 REFERENCES

- <sup>1</sup> VYMAZAL, J., 2014. Eng. 73, 724–751.
- <sup>2</sup> CORADA-FERNÁNDEZ, C., GONZÁLEZ-MASO, E., LARA-MARTÍN, P.A., 2018. J. Hazard. Mater. 360, 24–31.
- <sup>3</sup> STASINAKIS, A.S., 2012. Biores. Tech. 121, 432–440.
- <sup>4</sup> HENAU, H., MATTHIJS, E., NAMKUNG, E., 1989. In: QUAGHEBEUR, D., TEMMERMAN, I., ANGELETTI, G. (Eds.), Organic Contaminants in Waste Water, Sludge and Sediment. Elsevier Applied Science, London, pp. 10–21.
- <sup>5</sup> MERRETTIG-BRUNS, U., JELEN, E., 2009. Materials 2, 181–206.
- <sup>6</sup> HOLT, M.S., WATERS, J., COMBER, M.H.I., ARMITAGE, R., MORRIS, G., NEWBERY, C., 1995. Water Res. 29 (9), 2063–2070.
- <sup>7</sup> SILVA, N. F. P., 2014. Crescimento de Microalgas em águas residuais: Produção de Biomassa e Remoção de Nutrientes. Master's thesis. Porto: Mestrado integrado em Engenharia do Ambiente.
- <sup>8</sup> SALATI, S.; D'IMPORZANO, G.; MENIN, B.; VERONESI, D.; SCAGLIA, B.; ABBRUSCATO, P.; MARIANI, P.; ADANI, F., 2017. Biores.Tech, n. 230, p.82-89.
- <sup>9</sup> MONTGOMERY, D. C.; RUNGER, G. C.; CALADO, V. 2000. Estatística aplicada e probabilidade para engenheiros. Grupo Gen-LTC.
- <sup>10</sup> APHA - AMERICAN PUBLIC HEALTH ASSOCIATION, 1998. Standard Methods for the Examination of Water and Wastewater, twentieth ed. American Public Health Association, American Water Works Association, Water Environmental Federation, Washington.
- <sup>11</sup> Semblante, G.U., Hai, F.I., Ngo, H.H., Guo, W., You, S., Price, W.E., Nghiem, L.D., 2014. Biores. Tech. 155, 395–409.

## ACKNOWLEDGEMENTS

This research was supported by the Brazilian agencies Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).