BIOREMEDIATION OF SYNTHETIC PRODUCED WATER AND BIOMASS PRODUCTION WITH MICROALGAE *CHLORELLA vulgaris*

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ABSTRACT

The produced water (PW) generated during crude oil extraction contains substances that interfere with the equilibrium of the surrounding ecosystem if discharged inappropriately. In this sense, this study aims at employing the microalga *Chlorella vulgaris* in the treatment of synthetically produced water containing salt and cyclohexanecarboxylic (CHC) acid, a naphthenic component commonly found in this wastewater. Firstly, the microalga was preadapted to a culture medium with different fractions of PW. Secondly, a factorial design was applied to evaluate the effect of pH medium and PW concentration on the production of the bioproduct chlorophyll-a. The microalga presented an increase in biomass production until the PW fraction of 20% and, from the experimental design, chlorophyll-a production was maximized at pH 7.7 and PW fraction of 18% showing that *Chlorella vulgaris* could assimilate CHC matrix as a carbon source.

Keywords: Microalgae. Cyclohexanecarboxylic acid. Chlorophyll-a. Biomass growth. Wastewater treatment.

1 INTRODUCTION

According to the Brazilian Agency of Oil, Natural Gas, and Biofuels, the world's consumption of crude oil was 94.1 million barrels/day in 2021. In Brazil, the number attained 2.3 million barrels/day, which corresponds to 2.4% of the total world consumption[1]. During extraction from the oceanic bed, water is injected into the wells to expel the crude oil and bring it to the surface. This water, also known as produced water, presents hydrocarbon contaminants and must be treated before discharging into the ocean[2]. The volume of produced water (PW) is also not constant since it depends on geographic location, extraction technology employed, and aging of the well. It is estimated that the ratio between produced water and crude oil extracted is 3 to 1, which may increase to 50 to 1 in petroleum wells near exhaustion[3]. The produced water composition is complex and varies according to geographic location and geological formation. It carries within organic and inorganic compounds from the petroleum well, such as dissolved and dispersed oil, heavy metals, radioactive minerals, dissolved gases, and salts[4]. Due to low biodegradability, the concentration of these substances needs to be reduced to mitigate deleterious effects on nearby marine ecosystems.

Biological processes are normally considered for treating contaminants present in water. Microalgae cultivation consists in a promising alternative due to their aptitude for assimilating multiple substances and the versatile applications of their biomass. Once the essential nutrients for microalgae growth, such as nitrogen and phosphorous, and the organic compounds could be used as a source of carbon, a microalgae approach is an alternative for produced water treatment[4]. In the present work, the effects of different concentrations of cyclohexanecarboxylic (CHC) acid, a naphthenic acid, on *Chlorella vulgaris* growth were evaluated. Furthermore, the production of chlorophyll-a according to pH medium and synthetically produced water concentration was accessed by a full 2^2 factorial design.

2 MATERIAL & METHODS

The synthetically produced water was formulated with cyclohexanecarboxylic acid (500 $mg.L^{-1}$) and sodium chloride (100 $g.L^{-1}$), and its effect on biomass growth and adaptability of *C. vulgaris* was tested. The experiments were performed in an incubator with the Erlenmeyer containing 300 mL of BBM medium, where a fraction of this volume (0 – 40%) was correspondent to the synthetically PW[5]. Initially, the microalga at growing phase was inoculated at pH 6.8 with no PW, the experiment was carried out through 14 days, under 12:12 photoperiod, agitation 150 rpm, temperature 30 °C, and the $OD_{680\ nm}$ of the medium was monitored. At the end of the kinetic, the medium was used as inoculum in the subsequent assays with higher PW fractions.

After the adaptation study, a full 2^2 factorial design was performed aiming at defining the best conditions for chlorophyll-a production. The variables covered were PW fraction (15.8 – 44.2%) and initial pH of the medium (4.87 – 9.13), and the chlorophyll-a content was quantified by methanol extraction methodology[6]. The experiments were carried out under the same conditions of light exposition, temperature, and agitation, and the $OD_{680\ nm}$ was measured after 10 days. Figure 1 shows a schematic representation of the methodology.

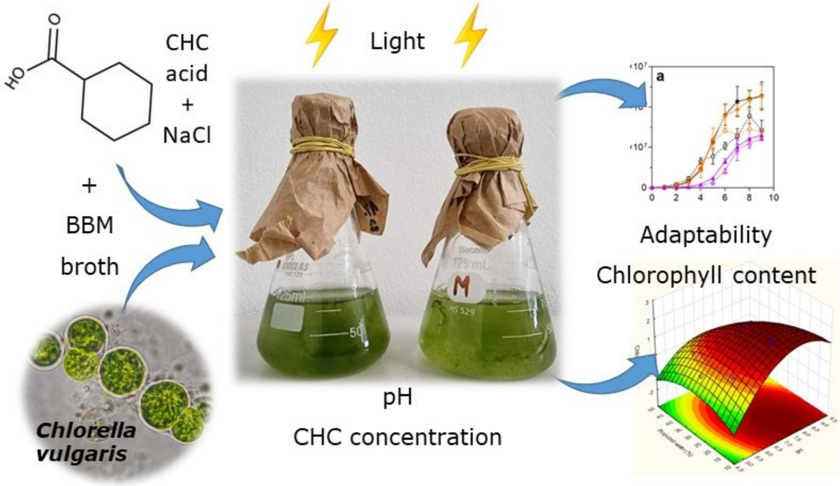

Figure 1: Schematic representation of experimental methodology.

3 RESULTS & DISCUSSION

The results for adaptation are presented in Figure 2, where the data is normalized according to the maximum growth attained with no contaminant. The microalga in study presented good adaptability for kinetic conducted with 10 and 20% of produced water in the medium, resulting in a relative biomass growth of 145 and 177%, respectively, at 14 days. It indicates that the naphthenic acid was assimilated and used as a carbon source during microalgae development, and the resulting salt concentration did not promote osmotic stress in the cells. Furthermore, these results demonstrate a gradual growth until the sixth day of the assay, when the exponential phase began and extended until the end of the 14 days. On the other hand, when the produced water in the medium reached a concentration of 40% the biomass growth only attained 38% at 14 days when compared to pure BBM medium conditions. At this point of the study, it is possible to infer that the simultaneous increase of CHC acid and salinity in the medium resulted in deleterious effects on microalgae cells.

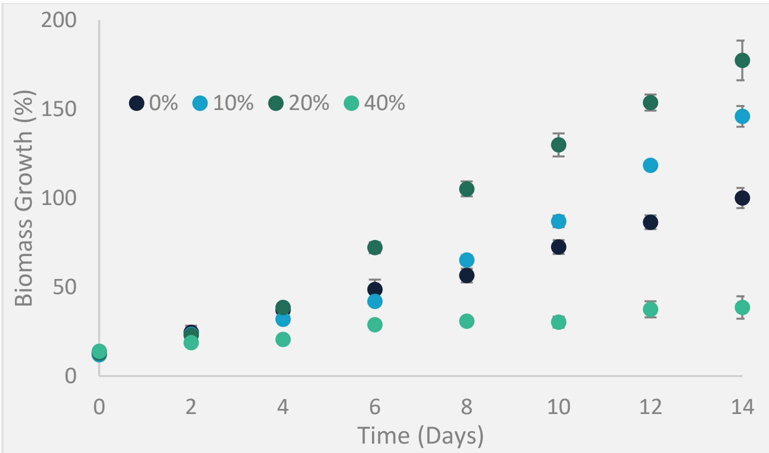

Figure 2: Microalgae kinetic growth in the presence of synthetically produced water.

The levels for each variable and the outcomes from the full 2^2 factorial design are presented in Table 1. The negative influence of higher fractions of produced water in the growing medium is clear. Experiments conducted with 40% of produced water (Runs 1 and 3) resulted in reductions around 60 and 20% in the production of chlorophyll-a in comparison with the fraction of 20% of PW (Runs 2 and 4), respectively. Similarly, an abrupt reduction of 70% is observed between axial points (Runs 7 and 8), which indicates the negative effect of salinity and the naphthenic acid in *C. vulgaris* growth. The initial pH of the medium also presented a significant effect. At the lower pH axial point (Run 6) the minimum chlorophyll- content was obtained. Conversely, experiments performed at pH 5.50 (Runs 3 and 4) led to a reduction of around 53 and 77% when compared to pH 8.50 (Runs 1 and 2), respectively. It signposts the neutral/slightly alkaline pH is the best condition for this microalga.

Table 1: Matrix of experimental design (real values) and outcomes for chlorophyll a content.

Run	pH	Produced water (%)	Chlorophyll a (mg.L^{-1})
1	8.50	40.0	0.631
2	8.50	20.0	1.591
3	5.50	40.0	0.291
4	5.50	20.0	0.365
5	9.13	30.0	0.864
6	4.87	30.0	0.145
7	7.00	44.2	0.622
8	7.00	15.8	2.036
9	7.00	30.0	1.647

The data in Table 1 were statistically analyzed to evaluate the effects of each variable on the response. A quadratic model (Eq. 1) was proposed to describe experimental data and predict chlorophyll-a content for non-performed conditions. The proposed model was validated by analysis of variance (ANOVA) as presented in Table 2 ($F_{0.90; 9; 7} = 5.30 < F_{calculated} = 8.80$).

$$Chlorophyll\ a\ (mg.L^{-1}) = -17.0851 + 4.48708 * pH - 0.273519 * pH^2 + 0.189377 * [PW] - 0.0020645 * [PW]^2 - 0.0147687 * pH * [PW] \quad (1)$$

Table 2: ANOVA Table for chlorophyll a results.

Source of variation	Sum of squares	Degree of freedom	Mean square	$F_{calculated}$
Regression	3.467597	5	0.693519	8.80
Residual	0.236261	3	0.078754	
Total	3.703858	8		

Regression Coefficient: R = 0.90; $F_{0.90; 9; 7} = 5.30$

The counterplot in Figure 3 shows the predicted results from modeling, which highlights the higher chlorophyll a production at slightly alkaline pH and lower produced water fractions. The model also predicts the critical values of pH 7.71 and produced water fraction of 18% that maximizes the response.

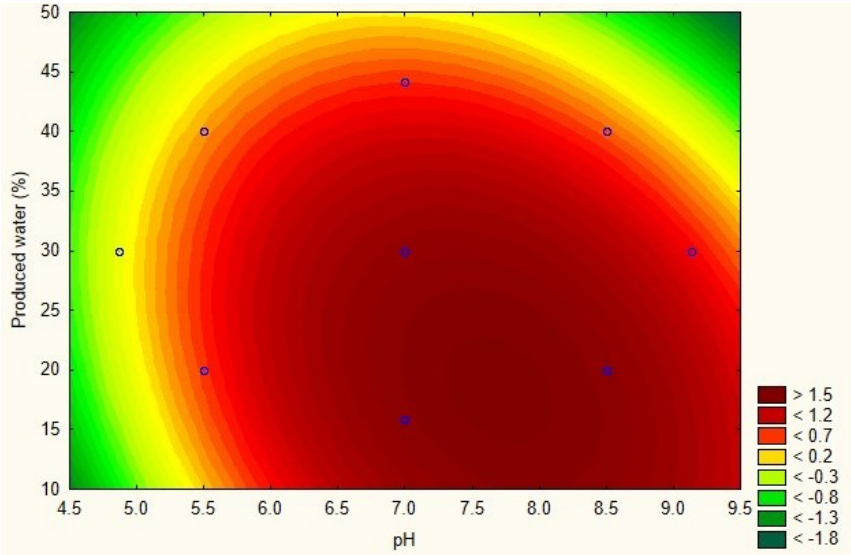

Figure 3: Counterplot for chlorophyll-a content.

4 CONCLUSION

Chlorella vulgaris showed great adaptability, with an increase in biomass production, to synthetically produced until 20% (100 $mg.L^{-1}$ of CHC acid and 20 $g.L^{-1}$ of NaCl), which indicates the ability of this microalga to assimilate the CHC acid matrix and convert it to potentially recoverable bioproducts. The experimental design that covered the pH of the medium and produced water content resulted in a validated model that indicates the slightly alkaline pH and lower fractions of produced water as the best conditions for chlorophyll a production. This microalga presents the potential of assimilating the naphthenic CHC acid and converting it into viable components.

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ACKNOWLEDGEMENTS

This study was financially supported by Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina – FAPESC (Project number 2023TR000275) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES (Finance Code 001). The authors also thank LABSIN/LABMASSA – Mass Transfer Laboratory, LAFIC – Phycology Laboratory and the Federal University of Santa Catarina – UFSC for the infrastructure provided.