Experimental mixture design for the recovery of residues from the food industry in the cultivation of *Scenedesmus acutus*

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***Abstract* — Nejayote and food leachate are highly polluting effluents and a source of important organic matter load that is sometimes released into bodies of water such as rivers or lakes, soils or public sewers. Both residues are considered to cause pollution, which is why urgent measures are required. The objective of this research was to determine the optimal combination of nejayote and food leachate to be used as a culture medium for the microalgae Scenedesmus acutus. The initial concentration of this microalgae was 0.5 g/L, the experiments lasted 20 days and the treatments had concentrations of 0, 10, 25, 50 and 90% of nejayote, food leachate and Bold medium. In the experiments, a biomass concentration higher than 6 g/L was achieved, an uptake of ammonium and orthophosphates up to 83 and 89 respectively. These results show that microalgae can be used to treat this type of liquid waste and, at the same time, used as a culture medium for microalgae. The resulting biomass can be used additionally to obtain other by-products of commercial interest.**

***Keywords* — nejayote, leachate, microalgae, recovery, *Scenedesmus acutus*.**

I. Introduction

Microalgae are a source of biomolecules for pigments, animal feed, biofuels, cosmetics, even therapeutic molecules. However, due to its complex biological structure and the high costs of the process, it causes the production and distribution to be restricted at an industrial level. Seaweed grown under controlled conditions can produce twenty times more oil per hectare than terrestrial soybean and canola crops [1]. Although microalgae are photoautotrophic microorganisms, the most popular cultivation strategy is one that relies on solar energy and carbon dioxide (CO_2). Sunlight is the key in the growth of microalgae, and the rate of light supply is what determines the productivity of the photobioreactor or pond [2].

The use of microalgae for wastewater treatment has gained worldwide attention. This interest is due to the fact that in addition to purifying nutrients from wastewater, it also generates microalgae biomass [3]. These microorganisms possess the ability to survive in different environmental conditions, as well as grow in various sources of wastewater, eg mixed municipal, industrial, domestic, agricultural wastewater and saline systems. Microalgae absorb toxic nutrients and heavy metals present in wastewater that aid in their growth, resulting in wastewater treatment [4].

Recently, possible solutions have been sought for the use of leachate by extracting its organic content and nutrients. Some of the approaches of interest that leachate has had is the production of microalgae biomass as a source of renewable energy. In addition to the reduction in nutrients from the leachate due to the growth of microalgae, the biomass generated is a by-product that can be marketed to produce biofuels, cosmetics, feed, fertilizers, among others. Unfortunately, this is limited by its pathogenic properties, so disinfection of the leachate is necessary [5]. In various studies carried out, the production of Chlorella sp. in a culture medium with different concentrations of leachates and a reduction in the growth of microalgae was found in concentrations greater than 10%. Other studies stressed the importance of the initial concentration of biomass in a culture medium with leachate from a sanitary landfill to obtain greater efficiency in biomass productivity, nutrient removal, Chemical Oxygen Demand (COD) and phenol [6]. Therefore, the objective of this study was to determine the optimum combination of nejayote and food leachate as a culture medium for the microalgae *Scenedesmus acutus.*

II. Materials and methods

A. Microalga culture

The green microalga Scenedesmus acutus was used. The culture used Bold medium as nutrient medium (Nichols and Bold, 1965), which consisted of (mg/L): $NaNO_3$ (750), $CaCl_2$ $2H_2O$ (12.5), $MgSO_4$ $7H_2O$ (150), $FeSO_4$ (6.27) , K_2HPO_4 (62.4), KH_2PO_4 (225), NaCl (0.341), H_3BO_3 (5), $MnSO_4$ (0.72), $ZnSO_4$ $7H_2O$ (17.64), KOH (15.5), NaCl (12.5), $CuSO_4$ $7H_2O$ (1.06), $NaMoO_3$ (0.6), $CoCl_2$ (0.2). Microalgae growth was assessed with optical density measurements at 680 nm using a UV/vis spectrophotometer. All experiments were performed with the same batch of *Scenedesmus acutus* to maintain uniformity.

B. Experimental system

The experiments were carried out in glass flasks with a capacity of 1 liter and with a working volume of 500 mL, at

room temperature and with an initial concentration of 0.5 g/L. The microalgae recovery efficiency was calculated based on the decrease in the optical density of the culture (measured at 680 nm with a UV-vis spectrophotometer, Thermo GENESYS). Samples were collected at regular time points (t) during the experimentation. The experimental runs are shown in table 1, where the residues and medium bold proportions are expressed as a percentage of the working volume. The experimental results were analyzed with Minitab 19, a Dunnett analysis and the optimization of cell growth, ammonium and orthophosphate responses were performed with the design of mixtures of extreme vertices.

TABLE I
EXPERIMENTS TO STUDY THE USE OF NEJAYOTE RESIDUES AND FOOD LEACHATE AS CULTURE MEDIA FOR SCENEDESMUS ACUTUS.

Runs	**Experimental values**		
	Leached (%)	***Nejayote (%)***	***Bold (%)***
1	0	90	10
2	90	0	10
3	0	10	90
4	0	10	90
5	0	90	10
6	10	0	90
7	25	25	25
8	25	25	25
9	25	25	25
10	0	90	10
11	90	0	10
12	10	0	90
13	10	0	90
14	90	0	10
15	0	10	90

C. Analytical methods

The biomass was estimated as dry weight from the standard curve that was built based on Abs_{680} versus dry biomass. The determination of Nitrogen in the form of Ammonium ($N\text{-}NH_4^+$) was carried out with the phenol method according to Standard Methods (4500-NH_3). For the determination of Phosphorus in the form of Orthophosphates ($P\text{-}PO_4^{-3}$) the ascorbic acid method was used according to Standard Methods (4500-P). To determine the carbohydrate content, the anthrone colorimetric method was used, where H_2SO_4 hydrolyzes the glycosidic bonds and the anthrone reacts with the resulting monosaccharide to produce a colored product. This method measures only the total carbohydrates and destroys the biopolymer in the hydrolysis process. For the extraction of lipids from microalgae, the Bligh and Dyer method was used. The extraction of liposoluble pigments was analyzed spectrophotometrically at 510, 630, 664 and 480 nm. Total protein quantification was carried out using the Bradford method.

III. RESULTS AND DISCUSSION

A. Waste characterization

The characterization of the two residual residues used as cell growth medium in the present study are expressed in table 2.

Parameter	**Waste**	
	nejayote (%)	***leachate (%)***
COD (mg/L)	4598.33 ± 43	8260.50 ± 19.68
Nitrates (mg/L)	146.25 ± 18	64.20 ± 0.08
Nitrites (µg/L)	1927.73 ± 59	1401.36 ± 1.85
Total solids (g)	10.04 ± 0.08	2.17 ± 0.02
pH	8.6	4.9
EC	3.028 ± 0.0	5.71 ± 0.01
Hardness (mg/L)	2050.00 ± 173.20	6400.00 ± 24.94
Turbidity (UNT)	944 ± 47.09	717 ± 2.86
Alkalinity (mg/L)	2466.66 ± 400.04	20000 ± 81.65
Ammonium (mg/L)	133.91 ± 8.66	206.53 ±1.45
Nitrates (mg/L)	146.25 ± 17.78	64.20 ± 0.08
Nitrites (µg/L)	1927.73 ± 59.44	1401.36 ± 1.85
Total solids (g)	10.04 ± 0.088	2.17 ± 0.02
pH	8.6	4.9

B. Nejayote and food leachate as a culture medium for *Scenedesmus acutus*

Before cultivating the microalgae in the waste mixture, the Scenedesmus acutus microalgae were cultivated in Bold medium under optimal growth conditions to determine the behavior without organic matter stress. This growth of the microalgae was monitored for 20 days, it was observed that began to stabilize around day 16, for its part, the growth rate was 0.16.

Varied mixtures of nejayote and feed leachate (T1–5) were investigated for their ability to function as a culture medium for the microalga *Scenedesmus acutus* and promote growth. The observed growth was different in each of the treatments (T1-5). Statistically it was determined that T4 (0% nejayote + 90% bold + 10% leachate) presented the highest growth rate, contributing up to 6.34 ±1.07 g L-1, on day 20. The second best biomass growth promoting treatment was T1, T2 and T3 (90% nejayote + 10% Bold + 0% leachate, 10% nejayote + 90% Bold + 0% leachate and 0% nejayote + 10% Bold + 90% leachate) with 4.09 ± 1.21, 4.45, ± 1.75, 4.14 ± 0.81 g L-1 at day 20, respectively. T5 (25% nejayote +50% Bold + 25% leachate) with 1.79 ± 0.69 g L^{-1} did not show good results as a potential culture medium for *Scenedesmus acutus* (Figure 1) showing a growth below the control that was 2.54 ± 0.09 g L^{-1}, so these mixtures are not the optimal residue concentrations to promote the growth of microalgae in this study.

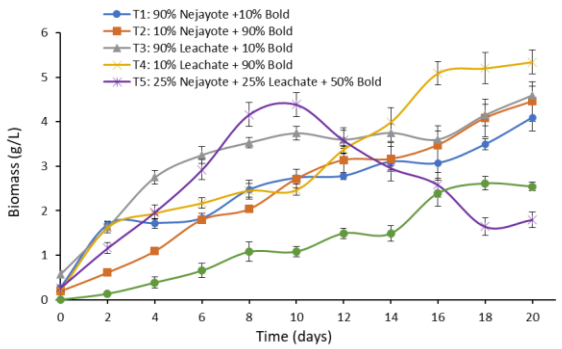

Fig. 1. Cell growth of *Scenedesmus acutus* through the treatments (T1–T5).

In T4 the growth trend is similar to the growth curve with Bold medium; but in T4 the maximum point of growth is reached on day 20 with a quantity 2.5 times greater. However, the amount of biomass obtained in the treatments T1-T4 was greater than the amount that could be obtained with the Bold medium.

The contour plot of the mixture design for cell growth (Figure 2) shows that the best option for the use of nejayote and food leachate as culture medium for *Scenedesmus acutus* was achieved with a higher amount of food leachate than the nejayote.

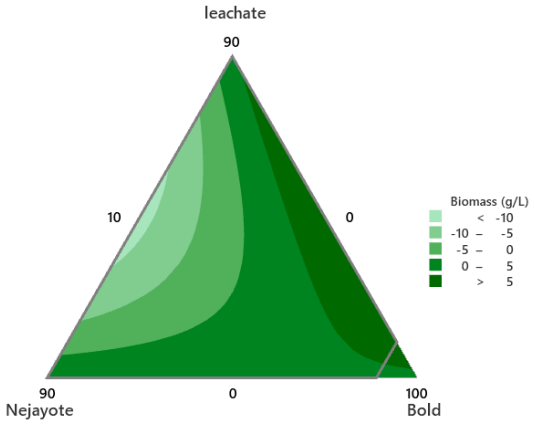

Fig. 2. Contour graph of the behavior of the variable cell growth using nejayote and food leachate as culture medium for *Scenedesmus acutus.*

The use of bold medium in the preparation of the test media was essential for two purposes, firstly, to allow light to enter the culture flask and secondly to dilute the organic load and thus know the concentration in which both residues produce the best result in terms of cell growth. The pH value during the experiments was maintained within the range of 7 to 9 in all treatments, without any adjustment for cell growth by microalgae.

C. Nutrient removal

The ammonium variable was measured to know the behavior of nitrogen in all the experiments, and how microalgae can use this source to improve growth. Treatments T3, T4 and T5 with microalgae showed a decrease in ammonium compared to the initial value, this reduction is due to the fact that the microalga uses it as a source of nitrogen for its metabolism and growth. The greatest reduction of ammonia occurred in the first eight days in the T3, T4 and T5 treatments, reaching an elimination of 71.3, 71.9 and 82.5%, respectively, on the other hand, the T1 and T2 treatments did not show a significant elimination, probably due to to the low concentration present in the initial mixture.

During the experimentation the decrease of orthophosphates was monitored, all the treatments showed a decrease with respect to the initial concentration, in the treatments T4 and T5 a greater decrease of orthophosphates of 89 and 83% with respect to the initial value, respectively, was shown. In T1, T3 and T5 there was a reduction that was 28, 61 and 41% respectively. This demonstrated what is well known that not all microalgae show similar behavior when exposed to a large load of pollutants, such as phosphorus.

D. Biochemical composition

A biochemical characterization of the biomass resulting from each experiment was carried out, the variables of carbohydrates, lipids, proteins and chlorophyll were evaluated at the end of each run. The amount of carbohydrates was found to be 66% in the control experiment, the amount was decreasing to a low value of 26% in treatment 3, this shows that a high concentration of leachate influences decreasing the amount of carbohydrate in the sample. For its part, the amount of lipids resulted in an increase when using treatment 3 with 27%, contrary to what happened with the amount of carbohydrates, the minimum value found in lipids was 7%. In the case of proteins, the highest value was again the control experiment with 47% in the rest of the experiments the value was in the range of 11-28%. The chlorophyll pigment is important in the industry of natural dyes, in our experiments the control treatment resulted in 11.4 mg/l, while the rest of the treatments showed a significant decrease when adding liquid residue, obtaining values of a maximum of 5mg/l.

IV. Conclusion

In the present study, both liquid residues and food leachate and nejayote were demonstrated as culture media for the *Scenedesmus acutus* strain. Based on the results obtained, the best option for cell growth is the use of a greater amount of food leachate than nejayote. The best result was the combination of 10% leachate and 90% medium bold with at least 6 g/L and the combination of other percentages gave results of around 4 g/L. For the reduction of ammonium, the combination that contained the highest initial concentration

showed the highest percentages of capture and for the reduction of orthophosphates a mixture of 10% food leachate and 90% bold and 25% food leachate, 25% nejayote and 50% bold showed the best efficiency with a reduction of more than 80%.

In this work, the valorization of two liquid residues as a culture medium for microalgae was demonstrated. A tunable technology platform has been unveiled where one can target pollutant reduction, microalgae growth as a green source for high-value products, or a combination of both depending on the desired application.

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