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# Combination of nejayote and swine wastewater as a medium for *Arthrospira maxima* and *Chlorella vulgaris* production and wastewater treatment

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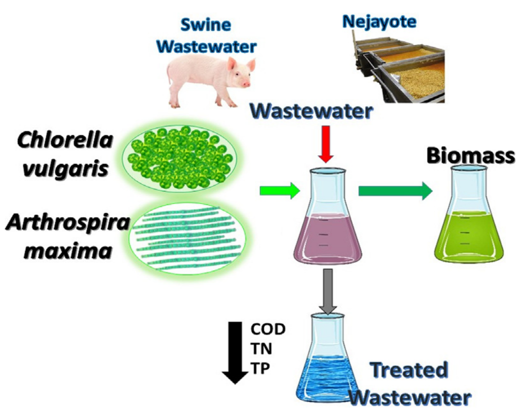
## HIGHLIGHTS

- The mix of nejayote and swine wastewater allows the microalgae cell growth.
- Kinetics of cell growth adjusted at logit mathematic model.
- Microalgae were able to grow in nejayote-based culture media.
- Microalgae removed TN, TP and COD in nejayote and swine wastewater.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Nejayote and swine wastewater are highly pollutant effluents and a source of organic matter load that sometimes released into water bodies (rivers or lakes), soils or public sewer system, with or without partial treatments. Nejayote is a wastewater product of alkaline cooking of maize, whereas, swine wastewater results from the primary production of pigs for the meat market. Owing to the presence of environmentally related pollutants, both sources are considered the major cause of pollution and thus require urgent action. Herein, we report a synergistic approach to effectively use and/or treat Nejayote and swine wastewater as a cost-effective culture medium for microalgae growth, which ultimately induces the removal of polluting agents. In this study, the strains *Arthrospira maxima* and *Chlorella vulgaris* were grown using different dilutions of Nejayote and swine wastewater. Both wastewaters were used as the only source of macronutrients and trace elements for growth. For *A. maxima*, the treatment of 10% nejayote and 90% of water (T3) resulted in a cell growth of $32 \times 10^4$ cell/mL

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Swine wastewater
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at 12 days ($\mu_{max} = 0.27$/d). While, a mixture of 25% swine wastewater, 25% nejayote and 50% water (T2) produced $32 \times 10^4$ cell/mL at 18 days ($\mu_{max} = 0.16$/d). A significant reduction was also noted as 92% from 138 mg/L of TN, 75% from 77 mg/L of TP, and 96% from 8903 mg/L of COD, among different treatments. For *C. vulgaris*, the treatment of 10% swine wastewater and 90% water (T1) gave a cell growth of $128 \times 10^6$ cell/mL ($\mu_{max} = 0.57$/d) followed by T3 yielded $62 \times 10^6$ cell/mL ($\mu_{max} = 0.70$/d) and T2 yielded $48 \times 10^6$ cell/mL ($\mu_{max} = 0.54$/d). Up to 91% reduction from 138 mg/L of TN, 85% from 19 mg/L of TP and 96% from 4870 mg/L of COD was also recorded. These results show that microalgae can be used to treat these types of wastewater while at the same time using them as a culture media for microalgae. The resultant biomass can additionally be used for getting other sub-products of commercial interest.



## 1. Introduction

Wastewater treatment systems produce essential amounts of greenhouse gases (GHG), which represent a huge environmental concern (Gupta and Singh, 2015). Consequently, the development of innovative and sustainable solutions to this problem is much needed. In this context, microalgae-based eco-technology represents a promising approach providing a dual action, by generating sustainable subproducts, such as polymers (polysaccharides and proteins), and simultaneously improving the quality of the water (Collotta et al., 2018; Wang et al., 2014). Examples of high-value products of microalgae are pigments, proteins, lipids, carbohydrates, vitamins and anti-oxidants, with applications in cosmetics, nutritional and pharmaceuticals industries. Microalgae has been used as bio-remediators or biosorbents with the capacity to remove carbon dioxide ($CO_2$), nutrients, including nitrogen (N) and phosphorus (P) and other hazardous contaminants (Abdel-Raouf et al., 2012; Bilal et al., 2018; Collotta et al., 2018; Tolboom et al., 2019). The last two can harm the environment, leading to eutrophication (Jämsä et al., 2017). Even though microalgae technology in wastewater treatment represents a sustainable and cost-effective approach, to implement this kind of processes efficiently, there are challenges to overcome. One major limitation is the lack of energy efficient harvesting method since this step alone represents up to 30% of total production cost (Lavrinovičs and Juhna, 2017).

The term wastewater defines any water that has a change in its chemical and physical composition due to previous use or exposure to pollutants (Mohamad et al., 2015). At least 2212 $km^3$ of wastewater are discharged every year into the environment (UNESCO, 2017). Wastewater can be chemically characterized in terms of oxygen demand (OD), chemical oxygen demand (COD), total organic carbon (TOC), total nitrogen (TN) and total phosphorus (TP) (Zhang et al., 2015). To allow these effluents to be discharged into water sources, a proper treatment is needed to reduce the contaminants as much as possible. The methods for wastewater treatment are classified as physical, chemical, biological and combinations (EPA, 1977; Tolboom et al., 2019). In addition to biological-based treatments, microalgae are another type of microorganisms that can be used for the treatment of wastewater (Samer, 2015). The wastewater treatment methods are of great importance due to the concentration and variety of emerging pollutants derived from anthropogenic processes (Bilal et al., 2017; Barrios-Estrada et al., 2018a, 2018b; Bilal and Iqbal, 2019). In this context, processes such as pig farming and nixtamalization of corn generate great amounts of wastewater with a high pollutant load, and for which treatment is necessary before discharging these effluents into the water bodies. The worldwide production of pork is near 114.6 million tons (USDA FAS PSD, 2018). For each kilogram of pork meat, around 4850 L l of water is required (Hoekstra and Chapagain, 2008). The wastewater originated from farms or breeding of pigs for the meat market contains organic waste, including stool and urine. The composition of swine wastewaters found in previous reports is depicted in Table S1 (Supporting Information).

One type of biological treatment for swine wastewater is the use of grass, like Napier, which can reduce up to 94% of biological oxygen demand (BOD) and 98% of TN (Klomjek, 2016). Despite the efficiency in the treatment, the amount of arable land that is used for this purpose makes this option highly expensive at the environmental level (Kirby, 2002). Also, activated sludge is used to remove the organic load to treat raw swine wastewater, there can be a reduction of P by 83%, copper (Cu) by 96% and zinc (Zn) by 95% (Suzuki et al., 2010).

Nejayote is the wastewater obtained in the process of nixtamalization of corn. Nixtamalization is the cooking of corn in a solution of lime for 2–8 h (Niño-Medina et al., 2009). Only in Mexico, 2,437,552 tons of corn per year is used to produce nixtamalized corn flour (Mexican Ministry of Economy, 2012). Some of the strategies that have been applied previously to reduce the pollutant load in nejayote include treatments in bioreactors, which resulted in 84% of contaminant removal. Other techniques include the use of chitosan to improve the flocculation and coagulation in order to reduce the TOC (Meraz et al., 2016; Pedroza-Islas and Durán de Bazúa, 1990). Examples of reported treatment methods of swine wastewater and nejayote are depicted in Table S2 (Supporting Information).

In earlier studies, some strains of microalgae have been employed to treat swine wastewater. For instance, *Scenedesmus* spp. removed completely TP within 3 days (Prandini et al., 2016) and *Chlorella vulgaris* reduce 80–96% of ammonium, 32–97% of TP, 72–89% of COD and 95–99% of TN (Amini et al., 2016; Cao et al., 2018; Nam et al., 2017). Moreover, *Coelastrella* sp. can reduce 90–100% of $NH_3$-N and 90–100% of TP (Luo et al., 2016), and the consortium of *Chlorella vulgaris, Scenedesmus obliquus,* and *Chlamydomonas reindhardtii* can totally deplete phosphates, 99% of ammonium and 43–64% of nitrogen (Molinuevo-Salces et al., 2016). To this date, *Arthrospira maxima* has not been used in this kind of studies, however, a study with *Arthrospira platensis*, a similar strain, has been disclosed showing a reduction by 41% of TP and 88% of COD (Mezzomo et al., 2010). Also, swine wastewater has been used as a culture media for microalgae. The strain *C. vulgaris* JSC-6 can reduce up to 70% of the COD and 90% of $NH_3$-N, as well as this treatment increases the microalgae carbohydrate content to 58% dry weight (Wang et al., 2015). In another study the microalga *Chlorella vulgaris* MBFJNU-1, after 12 days of treatment, undiluted swine wastewater slurry showed a reduction of 90% of TN and 91% of TP. This *Chlorella* strain, in particular, can survive in water with either COD of 30,000 mg/L or 2000 mg/L of TN (Wen et al., 2017).

In a study by Deng et al. swine wastewater after anaerobic digestion was used as culture media for *C. vulgaris*, which yielded 76 mg/L/day of carbohydrates, 257 mg/L/day of proteins, and 183 mg/L/day of lipids (Deng et al., 2018). There are other studies where strains of microalgae have been used for wastewater treatment (Table S3 Supporting Information). The present work aimed to determine nejayote and swine wastewater mixture as a culture media for two microalgae and the possible reduction of pollutants in both wastewaters. The specific use of these wastewaters is because of the large amount of wastewater that is produced from two productive processes in Mexico: 1) nixtamalization of corn for tortilla production and 2) pig farming for pork meat production. Mexico is the second importer of corn worldwide, tortilla being the second product in the basic food basket (Mexican Ministry of Economy, 2012), so the amount of nejayote produced within the country is considerable. Also, in Mexico, the pork meat exportation has increased an 18% from 2016 to 2017, and it is increased 4% more its national production in 2018 (FAO, 2018; SAGARPA, 2018). Thus, the mixture of both wastewaters was used, considering their high pollutant load, to demonstrate that they can be used as a culture media for microalgae and to analyze the effect that the mix of two types of wastewater can have on cell growth at the laboratory level.

## 2. Materials and methods

### 2.1. Sources and characterization of residual wastewater

Swine wastewater after primary treatment (separation of solids) was obtained from the swine farm "Ana Margarita", located in Nuevo Leon, Mexico. Nejayote was collected from the corn-processing company "Harimasa". Both wastewaters were stored at 4 °C and used for characterization purposes. Total nitrogen (TN) was analyzed using salicylic acid nitration method, total phosphorus (TP) by the ascorbic acid method, chemical oxygen demand by the reactor digestion method, dissolved oxygen (DO), settleable solids with Imhoff cones and the total solids (TS) with the method of total solids dried at 103–105 °C. All samples were analyzed in triplicate.

### 2.2. Measurements

The following instruments were used to measure: (1) pH with a Thermo Scientific™ Orion™ 3-Star Benchtop pH Meter, (2) dissolved oxygen with Thermo Scientific Orion Star A213 Advanced RDO/dissolved oxygen benchtop meter, settleables solids with Imhoff cones (USEPA, 1983) and total solids (TS) with the method of total solids dried at 103° - 105 °C (USEPA, 2001), (3) total nitrogen was analyzed with the method of salicylic acid nitration (Robarge et al., 1983), (4) Total Phosphorus with Ascorbic Acid Method (Total phosphorus, Total phosphorus TNTplus™, LR, HACH, CO, USA) (EPA, 1978) and (5) Chemical Oxygen Demand with Reactor Digestion Method (COD TNTplus™, LR, HACH, CO, USA) (USEPA, 1980). The spectrophotometric measurements were taken on a DR 5000™ UV–Vis Laboratory Spectrophotometer.

### 2.3. Conditions of microalgae growth

The microalgae species used were *Chlorella vulgaris* from UTEX (UTEX, Austin, TX, USA) and *A. maxima* provided by the Laboratory of "Fisiologia Vegetal de la Escuela Nacional de Ciencias Biológicas" (IPN, CDMX, MEX). Erlenmeyer flasks were placed with a brand OPTIMA 4.5-W pump with an air filter of 0.20 μm, 1.5 L/m$^2$/min of aeration, and a photoperiod of 12:12 (Hydro grow extreme LED lamps). The temperature was kept at 21 °C. The culture medium used for the growth of both strains was BG11, which contains $NaNO_3$ 1.5 g/L, $K_2HPO_4$ 40 mg/L, $CaCl_2 \cdot 2H_2O$ 36 mg/L, $MgSO_4 \cdot 7H_2O$ 75 mg/L, Citric Acid $\cdot$ $H_2O$ 6 mg/L, Ferric Ammonium Citrate 6 mg/L, $Na_2EDTA \cdot 2H_2O$ 1 mg/L, $Na_2CO_3$ 20 mg/L, $H_3BO_3$ 2.86 mg/L, $MnCl_2 \cdot 4H_2O$ 1.81 mg/L, $ZnSO_4 \cdot 7H_2O$ 0.22 mg/L, $Na_2MoO_4 \cdot 2H_2O$ 0.39 mg/L, $CuSO_4 \cdot 5H_2O$ 0.079 mg/L, and $Co(NO_3)_2 \cdot 6H_2O$ 0.04 mg/L.

### 2.4. Nejayote and swine wastewater as a co-culture media for A. maxima and C. vulgaris

A volume of 40 mL of microalgae culture at $33 \times 10^6$ cells/mL of *C. vulgaris* and $15 \times 10^5$ cells/mL of *A. maxima*, respectively, was used as seed culture and was inoculated into a 500 ml Erlenmeyer flask, which operates with 400 mL of total volume. The experimental design used was a mixture design of extreme vertices with five combinations of swine wastewater, nejayote, and water (Table 1). The experiment was maintained until the biomass growth curve reached the plateau. Samples of 5 mL were obtained every day to assess the biomass growth curve by cell count in a Neubauer chamber and to measure pH in all treatments without any adjustment. The conditions of growth in the experiments were the same as for the growth of the microalgae. A diagram of the methodologic strategy is shown in Fig. 1, where also the specifications of the treatments (T1-T5) are included.

**Table 1**
Experiments for the study of swine wastewater and nejayote as culture media for *A. maxima* and *C. vulgaris*.

| OrderEst | Run order | TipoPt | Bloques | Nejayote | Swine wastewater | Water |
|---|---|---|---|---|---|---|
| 12 | 1 | 1 | 1 | 0.00 | 0.10 | 0.90 |
| 10 | 2 | 0 | 1 | 0.25 | 0.25 | 0.50 |
| 7 | 3 | 1 | 1 | 0.00 | 0.10 | 0.90 |
| 14 | 4 | 1 | 1 | 0.10 | 0.00 | 0.90 |
| 13 | 5 | 1 | 1 | 0.90 | 0.00 | 0.10 |
| 8 | 6 | 1 | 1 | 0.90 | 0.00 | 0.10 |
| 11 | 7 | 1 | 1 | 0.00 | 0.90 | 0.10 |
| 4 | 8 | 1 | 1 | 0.10 | 0.00 | 0.90 |
| 2 | 9 | 1 | 1 | 0.00 | 0.10 | 0.90 |
| 15 | 10 | 0 | 1 | 0.25 | 0.25 | 0.50 |
| 5 | 11 | 0 | 1 | 0.25 | 0.25 | 0.50 |
| 1 | 12 | 1 | 1 | 0.00 | 0.90 | 0.10 |
| 3 | 13 | 1 | 1 | 0.90 | 0.00 | 0.10 |
| 6 | 14 | 1 | 1 | 0.00 | 0.90 | 0.10 |
| 9 | 15 | 1 | 1 | 0.10 | 0.00 | 0.90 |

### 2.5. Removal of total nitrogen, total phosphorus and chemical oxygen demand by A. maxima and C. vulgaris in nejayote and swine wastewater

The experiments were sampled every five days (10 mL) throughout all experiment to obtain the concentration of total nitrogen (TN), total phosphorus (TP) and chemical oxygen demand (COD) of each of the flasks. All the samples were centrifuged at 4000 rpm for 10 min, and then the supernatants were filtered with a 0.45 μm filter before analysis. After filtration, the samples were analyzed for total nitrogen, phosphorus total, and COD.

### 2.6. Determination of the optimal mixture of nejayote and swine wastewater for the growth of A. maxima and C. vulgaris for the reduction of the pollutants

The experimental design of mixtures with extreme vertices was performed with three objectives: 1) to determine the best mix of wastewaters for the reduction of pollutants, 2) to determine the best mix of wastewaters that can be used as a culture media for microalgae growth, and 3) to determine the best concentration of both wastewaters for the reduction of pollutants and cell growth. The results obtained provided the optimal concentrations of nejayote and swine wastewater for the optimization of the process.

### 2.7. Experimental design

The experimental results were analyzed with Minitab 18; a Dunnett Analysis was performed and the optimization of the responses of cell growth, total nitrogen, total phosphorus and COD with the mixture design of extreme vertices to know the concentration levels of nejayote and swine wastewater where the best cell growth was obtained and which had the better reduction of pollutants. In addition, a Graph of mixing surface was obtained for each variable. The logit regression model for each strain was done with STATISTICA software (stat soft).

## 3. Results and discussions

### 3.1. Characterization of residual wastewater

The results of the characterization of both wastewaters used in the present study are expressed in Table 2. There are other studies with

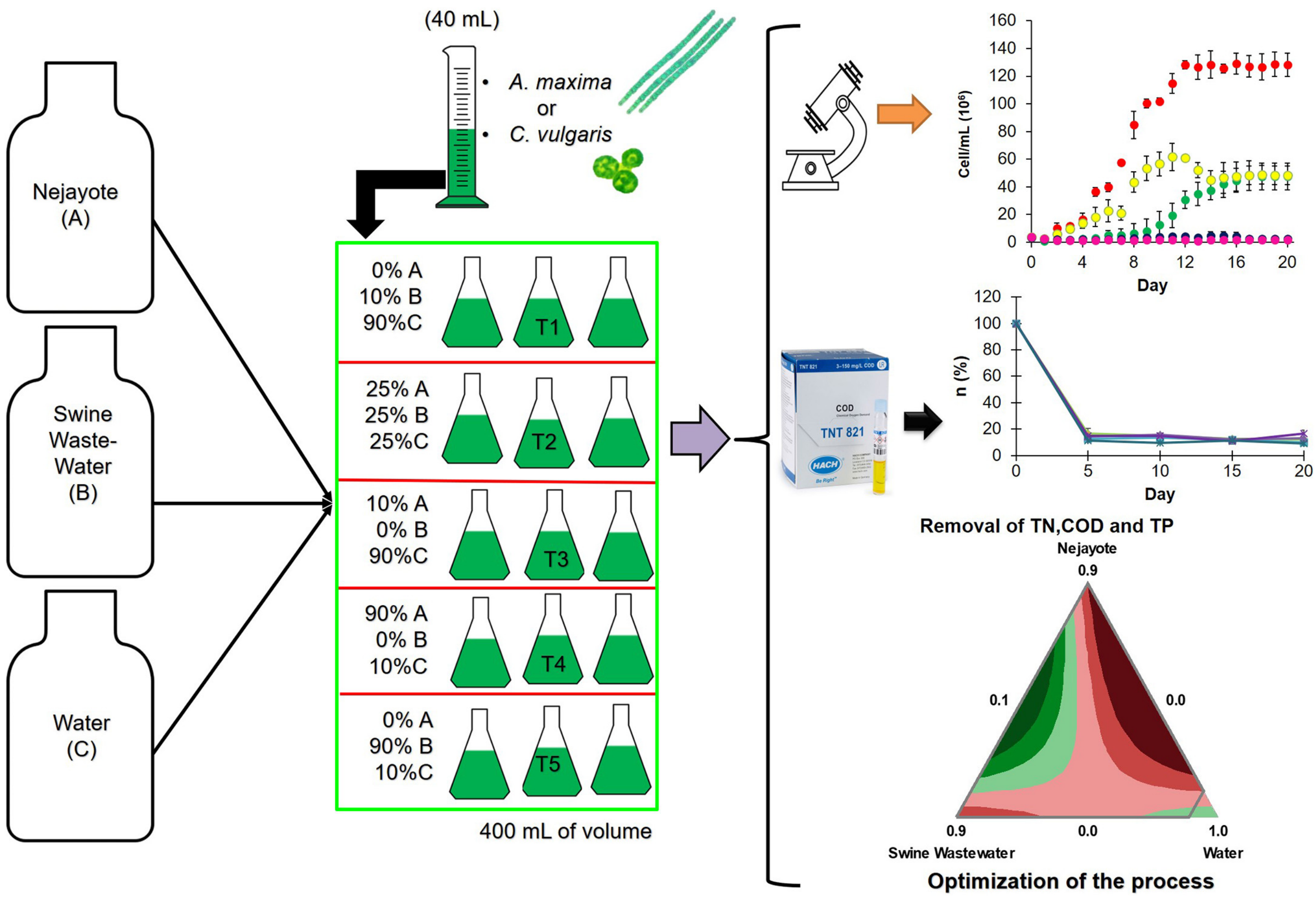


**Fig. 1.** Methodology strategy. Experiments were run with both strains individually (40 mL of microalgae). For every experiment, it was obtained the cell growth curve and the removal of COD and TP every five days. With the data obtained it was optimized the process with a statistical software (Minitab 18).

the characterization of nejayote, where the pH was reported at 9–14, total solids were 2.4–46.52 g/L, COD at 7500–28,450 mg/L, the TN was at 80–440 mg/L N, and the TP was at 6.4–1321 mg/L (Durán de Bazúa et al., 2007; España-Gamboa et al., 2018; García-Depraect et al., 2017; López-maldonado et al., 2017).

The characterization of the swine wastewater has been done, where the pH was reported at 6.89–9.50, total solids were 9.5–20 g/L, COD was at 2386–10,320 mg/ L, the TN was at 163–2582 mg/L N and TP was at 18.69–473 mg/L (Cao et al., 2018; Huang et al., 2016; Lim et al., 2016; Meng et al., 2019; Molinuevo-Salces et al., 2016; Rosa et al., 2017; Waki et al., 2018; Zhang et al., 2017).

### *3.2. Use of mixture of nejayote and swine wastewater as a culture media for A. maxima and C. vulgaris*

Before the cultivation of the microalgae in nejayote and swine wastewater mixture, both microalgae were grown in BG11 medium to know the behavior of these microorganisms under optimal conditions. The growth of both microalgae was monitored for 20 days. The growth trend curve (Fig. 2a) was similar to the one reported by Mishra and Prasad, however, in our study, it can be seen that it starts stabilizing after day 18, while in the report of Mishra and Prasad, stabilization occurred at day 14. Moreover, our growth kinetic showed similar as to the growth of *A. platensis* grown in modified Zarrouk's, Hiri's and Jourdan's media (Delrue et al., 2017; Mishra and Prasad, 2015). Additionally, the growth rate ($\mu_{max} = 0.30$) obtained was higher, than reported by Saeid and collaborators ($\mu_{max} = 0.19$) (Saeid, 2016). On the other hand, *C. vulgaris* reached a concentration greater than $100 \times 10^6$ cell/mL, around day 15 (Fig. 2b). The trend of the growth curve for *C. vulgaris* found in this study is similar to the reported in other studies (Ebrahiminezhad et al., 2016; Melo et al., 2018).

Varied mixtures of nejayote and swine wastewater (T1–5) were investigated in regard to their ability to function as a culture medium for *A. maxima* and promote the growth. The observed growth was different in each of the treatments (T1–5). Statistically, it was determined that T3 (10% nejayote +90% water +0% swine wastewater) showed the highest growth rate, providing up to $32 \times 10^4$ cell/mL, at day 12. The second-best biomass growth-promoting treatment was T2 (25% nejayote +25% water +50% swine wastewater) with $32 \times 10^4$ cells/mL, at day 18. T4 and T5 (90% wastewater) did not show good results as potential culture media for *A. maxima* (Fig. 2c).

In T3 the growth trend is similar to the growth curve with BG11 medium; however, in T3 the maximum growth point is reached around day 12 instead of day 18. Nevertheless, the amount of biomass obtained from all the treatments was lower than the amount that could be

**Table 2**
Characterization of nejayote and swine wastewater.

| Parameter | Nejayote | Swine wastewater | Unit |
|---|---|---|---|
| pH | 9.80 ± 0.005 | 6.83 ± 0.01 | – |
| DO | 4.80 ± 0.076 | 1.23 ± 0.01 | mg/L |
| TP | 41.16 ± 2.75 | 147.0 ± 12.0 | mg/L $PO_4$ |
| TN | 120.69 ± 10.0 | 163.40 + 12.0 | mg/L N |
| COD | 9153.30 ± 188 | 10,933 ± 252 | mg/L |
| Settleables solids | 3.86 ± 0.15 | 2.46 ± 0.05 | mL/L |
| TS | 9.06 ± 0.044 | 7.12 ± 0.04 | g/L |

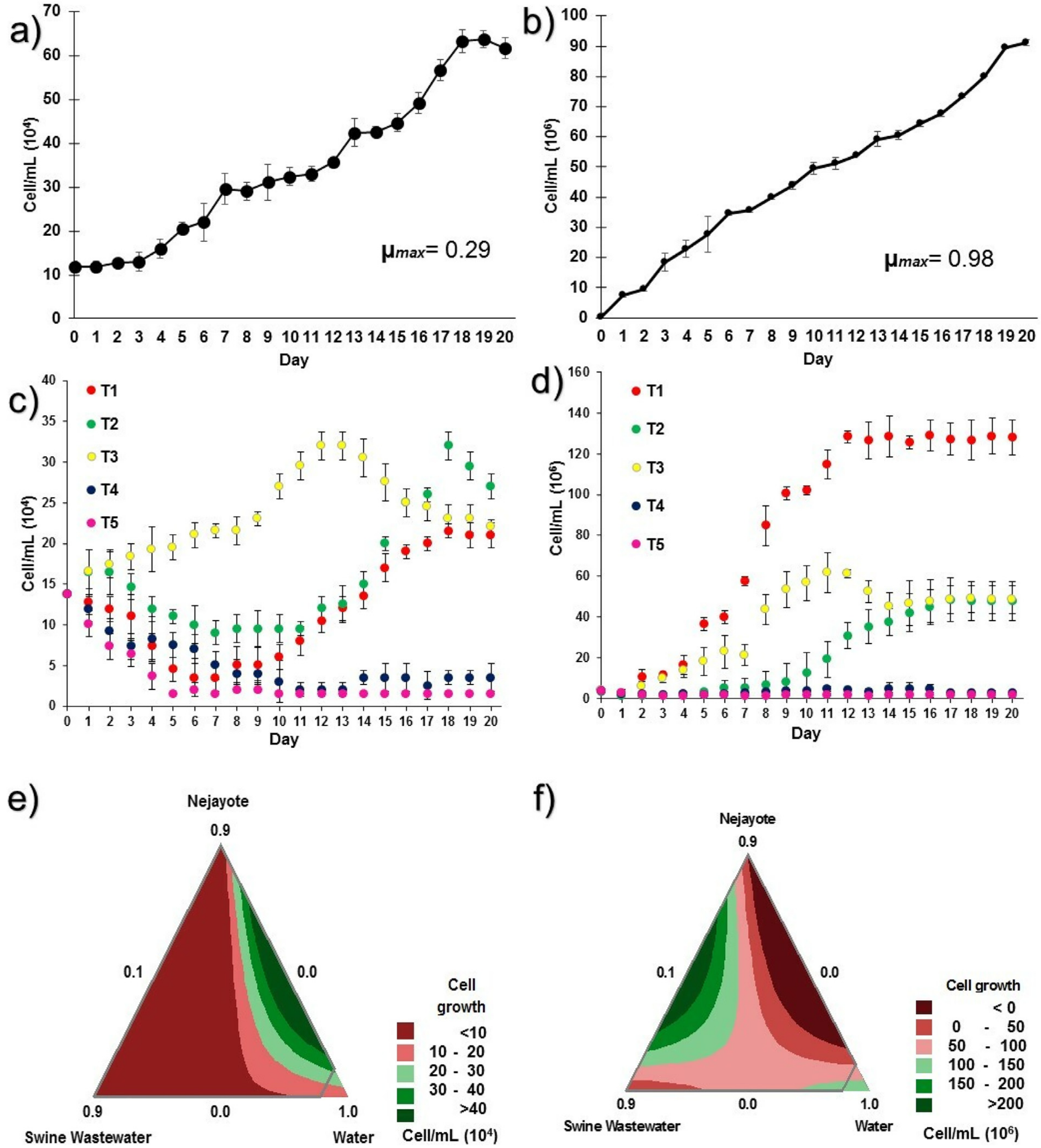


**Fig. 2.** a) The kinetic of the growth of *A. maxima* in BG11 medium. The growth curve followed a normal trend similar to other microalgae. It reached the stationary phase at day 18. All points were sampled by triplicate. b) The kinetic of the growth of *C. vulgaris* in BG11 medium. The growth curve followed a normal trend similar to other microalgae. It reached the stationary phase at day 19. All points were sampled by triplicate. c) Cell growth of *A. maxima* in treatments (T1–5) d) Cell growth of in *C. vulgaris* treatments (T1–5) e) Contour graphic of the behavior of cell growth variable using nejayote and swine wastewater as a culture medium for *A. maxima*. d) Cell growth of *C. vulgaris* in treatments (T1–5) e) Contour graphic of the behavior of cell growth variable using nejayote and swine wastewater as a culture medium for *A. maxima*. d) Contour graphic of the behavior of cell growth variable using nejayote and swine wastewater as a culture medium for *C. vulgaris*. The green area in contour graphics mean the best possible answer of the variable sought in the process. The points show the treatments in this experiment.

obtained with the BG11 medium. The results obtained are similar to the previously reported study employing *A. platensis* in removing the pollutants from swine wastewater (Cheunbarn and Peerapornpisal, 2010). However, the best result in that study was achieved with 10% of swine wastewater, providing $1.2 \times 10^5$ cells/mL, compared to our result of $3.2 \times 10^5$ cells/mL generated with T3. Similar to our study, the treatment with large amounts of wastewater (40–100%) did not provide good results, and the highest concentrations did not exceed $10 \times 10^4$ cells/mL (Cheunbarn and Peerapornpisal, 2010). The trend of the growth curves representing T1 ($\mu_{max} = 0.33$, Table 3) and T3 ($\mu_{max} = 0.27$, Table 3) are closest to the reported with *A.platensis* by Mezzomo, at $\mu_{max} = 0.46$ (Mezzomo et al., 2010).

The growth of *C. vulgaris*, was observed differently in each of the concentrations of nejayote and swine wastewater (T1 - T5). Statistically, the best result was obtained in T1, with up to $128 \times 10^6$ cell/mL in 12 days. The other concentration that had good results was T3, with $62 \times 10^6$ cell/mL at day 11, followed by T2 with $48 \times 10^6$ cell/mL at day 17. The treatments with 90% of both wastewaters did not show a statistically significant increase of cell growth when employed as a culture medium for *C. vulgaris* at any day, similar to the results obtained

**Table 3**
Logit regression model of cell growth of *A. maxima* and *C. vulgaris* per treatment of proportion of nejayote and swine wastewater a determinate time.

| Treatment | Period (d) | Model equation | $r^2$ | *K* (cell/mL) | x | $\mu_{max}$ (est) |
|---|---|---|---|---|---|---|
| | | *A.maxima* | | | | |
| T1 | 0–20 | $y = \frac{(1607744 \times 5.03) \times e^{(0.07 \times t)}}{(1607744 + 5.03 \times e^{(0.07 \times t)} - 1)}$ | 0.74 | 1,607,744 | 5.03 | 0.07 |
| T1 | 6–20 | $y = \frac{(24.17 \times 0.3334) \times e^{(0.3336 \times t)}}{(24.17 + 0.3340 \times e^{(0.3336 \times t)} - 1}$ | 0.99 | 24.17 | 0.33 | 0.33 |
| T2 | 0–20 | $y = \frac{(2518595 \times 7.786) \times e^{(0.06 \times t)}}{(2518595 + 7.786) \times e^{(0.6 \times t)} - 1}$ | 0.76 | 2,518,595 | 7.77 | 0.06 |
| T2 | 6–20 | $y = \frac{(58.83 \times 2.45) \times e^{(0.163 \times t)}}{(58.83 + 2.45) \times e^{(0.163 \times t)} - 1}$ | 0.94 | 58.83 | 2.45 | 0.16 |
| T3 | 0–20 | $y = \frac{(26.733 \times 13.23) \times e^{(0.279 \times t)}}{(26.733 + 13.23) \times e^{(0.279 \times t)} - 1}$ | 0.79 | 26.73 | 13.23 | 0.27 |
| T4 | 0–20 | $y = \frac{(0.1732 \times 14.14) \times e^{(0.003 \times t)}}{(0.1732 + 14.14) \times e^{(0.003 \times t)} - 1}$ | 0.95 | 0.17 | 14.14 | 0.003 |
| T5 | 0–20 | $y = \frac{(0.0001 \times 14.33) \times e^{(0.0001 \times t)}}{(0.0001 + 14.33) \times e^{(0.0001 \times t)} - 1}$ | 0.97 | 0.001 | 14.33 | 0.001 |
| | | *C.vulgaris* | | | | |
| T1 | 0–20 | $y = \frac{(129.03 \times 2.14) \times e^{(0.573 \times t)}}{(129.03 + 2.14) \times e^{(0.573 \times t)} - 1}$ | 0.99 | 129.03 | 2.14 | 0.57 |
| T2 | 0–20 | $y = \frac{(48.87 \times 0.089) \times e^{(0.546 \times t)}}{(48.87 + 0.089) \times e^{(0.546 \times t)} - 1}$ | 0.99 | 48.87 | 0.089 | 0.54 |
| T3 | 0–20 | $y = \frac{(51.77 \times 0.684) \times e^{(0.709 \times t)}}{(51.77 + 0.684) \times e^{(0.709 \times t)} - 1}$ | 0.95 | 51.77 | 0.684 | 0.70 |
| T4 | 0–20 | $y = \frac{(3.51 \times 2.025) \times e^{(0.187 \times t)}}{(3.51 + 2.025) \times e^{(0.187 \times t)} - 1}$ | 0.42 | 3.51 | 2.025 | 0.18 |
| T5 | 0–20 | $y = \frac{(1.481 \times 3.62) \times e^{(0.7188 \times t)}}{(1.481 + 3.62) \times e^{(0.7188 \times t)} - 1}$ | 0.89 | 1.481 | 3.62 | 0.71 |

Model equation: equation of logit regression model, where t = time in days. $\mu_{max}$ (est): $\mu_{max}$ estimated in the model.

with *A. maxima* (Fig. 2d). The growth trend in T1, T2, and T3 is similar to the growth curve with BG11 medium; however, in this case, the maximum growth point was reached around day 12, instead of day 20. The best result in that study was obtained with 20% of swine wastewater with 0.64 ± 0.01 g/L of biomass (Wen et al., 2017), and in this study, it was obtained better results due to the experiments carried out were obtained 1.70 g/L with the concentration of T1.

The growth curve for *C. vulgaris* is similar to the reported in another study, which used the swine wastewater at a pH of 4, 5 and 6 (Cao et al., 2018). Also, there is another study where the dilutions of 5 and 20 fold of swine wastewater help to the growth of the strain (Wang et al., 2015). To our knowledge, to this date, no studies have been found where microalgae is used to treat nejayote. For *C. vulgaris*, shown in Fig. 2d, T1 yielded a better result of $\mu_{max}$ compared against the cell growth in BG11 medium (Fig. 2b). In a study performed by Gao and collaborators, *Chlorella* sp. was grown to a $\mu_{max}$ of 0.15/d in wastewater from seafood processing, which was higher than the $\mu_{max}$ obtained for *Chlorella* in this study (Table 3) (Gao et al., 2018). Also, the treatments with 90% of either wastewater did not show any difference as a culture medium for *C. vulgaris*, so those are not the optimal concentrations of wastewater to promote microalgae growth.

The contour graph of mixture design for the cell growth (Fig. 2e) shows that the best option for the use of nejayote and swine wastewater as a culture media for *A. maxima* was achieved with a higher amount of nejayote than swine wastewater. Also, for *C. vulgaris*, the contour graph (Fig. 2f) shows that either wastewater can be used as a culture media of *C. vulgaris,* but the swine wastewater can be useful in a higher quantity to obtain more biomass. For all experiments, the use of pure water in the preparation of test media was essential for two purposes, firstly to allow the light to enter the culture flask, and secondly, to dilute the organic load to know the concentration in which both wastewaters yield the best result in terms of cell growth. Although the graph shows that a greater proportion of both wastewater than the used in this study can be used to obtain greater biomass growth, due to the turbidity and organic load not always the predicted data can be corroborated in the laboratory.

The kinetics of growth in both microalgae, presented in Fig. 2c–d, can be adjusted in a logit regression model (Table 3). For *A. maxima* it can be observed that the model adjusts in better from days 6 to 20 in T1 and T2, due to the decrease of the microalga in those culture flasks, this phenomenon possibly occurs due to competition and depredation of other microorganisms, as the protozoa and bacteria present in both wastewater (Wahi et al., 2018). Also, in the case of *C. vulgaris*, it was observed that in T1, T2, and T3 the model could explain the growth behavior of microalgae in both wastewaters. The pH value during the experiments remained within the range of 8 to 10 in all treatments, without any adjustment for cell growth for both microalgae.

### *3.3. Removal of TN during the culture of A. maxima and C. vulgaris in nejayote and swine wastewater*

The TN variable was measured to known the behavior of nitrogen in all the experiments, and how the microalgae can use this source to improve growth. Both microalgae treatments showed a decrease of TN against the initial value (Fig. 3), this reduction happened because the microalga uses this as a source of nitrogen for metabolism and growth (Kwangyong and Choul-gyun, 2002). In addition to the ability of microalgae to use nitrogen for their growth, there can be abiotic factors that remove TN such as stripping or possibly due to the presence of nitrifying bacteria, since none of the wastewaters were sterilized (Delgadillo-mirquez et al., 2016).

The major reduction of TN happens in the first five days with both microalgae, and these results are similar to the found in other studies with the strains: *C. vulgaris*, *Scenedesmus quadricauda*, and *Selenastrum gracile* (Lee et al., 2016). Also, this measure can be related to the decrease of concentration of (Delgadillo-mirquez et al., 2016). In the case of *A. maxima*, the reduction of this value is equated with the growth of the microalga in T3 day 10 (Fig. 3a). Statistically, there was no

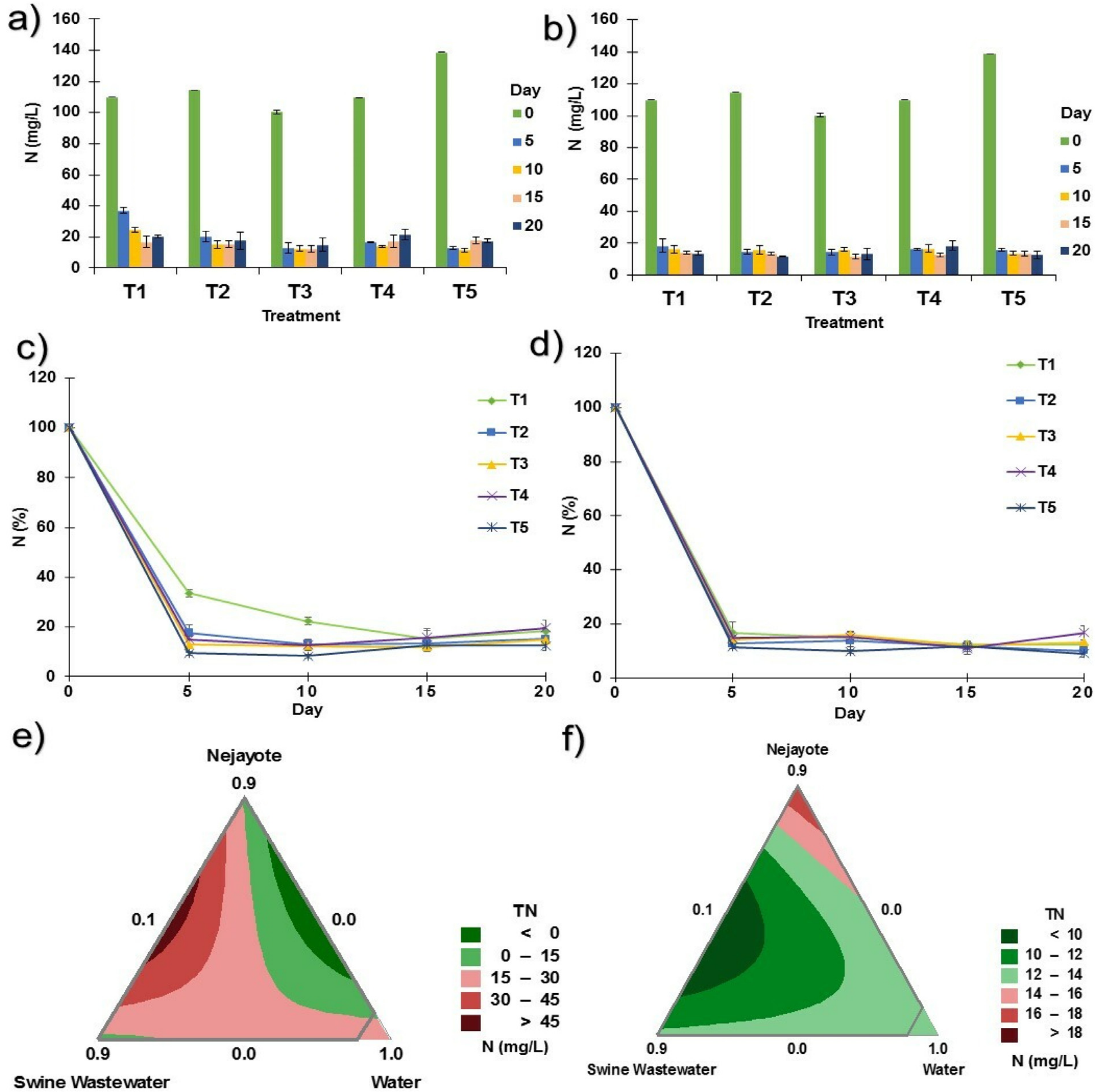

**Fig. 3.** Total nitrogen behavior during the culture of *A. maxima* and *C. vulgaris* in nejayote and swine wastewater. a) Quantification of TN in *A. maxima* cultured in nejayote and swine wastewater, in different proportions. The cultures were sampled by three replicate, every five days. b) Quantification of TN in *C. vulgaris* cultured in nejayote and swine wastewater, in different proportions. The cultures were sampled by three replicate, every five days. c) Interaction graph for TN in percentage (%) according to the initial value in *A. maxima* treatments. d) Interaction graph for TN in percentage (%) according to the initial value in *C. vulgaris* treatments. Initial values of both microalgae treatment: 1) T1 (109.88 mg/L), 2) T2 (114.50 mg/L), 3) T3 (100.23 mg/L), 4) T4 (109.67 mg/L) and 5) T5 (138.71 mg/L). e) Contour graphic of the behavior of TN variable using nejayote and swine wastewater as a culture medium for *A. maxima*. d) Contour graphic of the behavior of TN variable using nejayote and swine wastewater as a culture medium for *C. vulgaris*. The green area in contour graphics mean the best possible answer of the variable sought in the process.

difference between TN reductions of T2 and T5, which showed a greater reduction of 90% and 91%, respectively (Fig. 3c). ANOVA-Dunnett statistical analysis by treatment was carried out, taking day 0 as the control group, this analysis showed that there was a significant reduction of TN in T1, reducing up to 85% of 109.88 mg/L in 15 days, T2 reducing up to 87% of 114.50 mg/L in 10 days, in T3 reducing up to 88% of 100.23 mg/L in 15 days, in T4 reducing up to 87% of 109.67 mg/L in 10 days and in T5 reducing up to 92% of 138.71 mg/L in 10 days.

The contour graph in Fig. 3e indicates that the best option for the use of *A. maxima* in terms of TN reduction is to employ a higher proportion of nejayote than swine wastewater, providing values lower than 15 mg/L of TN at 20 days. Specifically, based on the shape of the surface, it can be observed that the process provides the lowest values in the area of nejayote and water.

In the case of *C. vulgaris*, TN removal was significant in most of the treatments (Fig. 3b), and there was a greater reduction with T5. Statistically, The ANOVA-Dunnett analysis, showed that there was a significant reduction of TN in T1 reducing up to 88% of 109.88 mg/L in 20 days, T2 reducing up to 90% of 114.50 mg/L in 20 days, T3 reducing up to 88% of 100.23 mg/L in 15 days, T4 reducing up to 89% of 109.67 mg/L in 15 days, and T5 reducing up to 91% of 138.71 mg/L in 20 days (Fig. 3d). In other studies, there are found the similar results did with wastewater, where *C. vulgaris* could reduce at least 83.3% of TN (Lee et al., 2016).

This contour graph (Fig. 3f) shows that the best option for reduction of TN of Nejayote and Swine wastewater with *Chlorella vulgaris* is with a media with a greater proportion of nejayote.

*3.4. Removal of TP during the culture of A. maxima and C. vulgaris in nejayote and swine wastewater*

Enduring the characterization, the TP decrease was monitored, where in the case of *A. maxima* the treatments T2, T4, and T5 showed a decrease of TP compared with the initial value (Fig. 4a). In T1 and T3 there was an increase of TP in day 5, indicating that the concentration of microalgae kept its concentration of the initial inoculum or reduced and subsequently when the microalgae started growing, the TP stared to reduce. The reduction of this value is equated with the growth of the microalga in the T1 and T2 that is around day 10. Also the reduction proportion of this value was different in each treatment (Fig. 4a). Thus, there was a greater reduction with the treatments T2 and T4. An increase of TP was observed in the days 1 to 5, when the microalgae was only present in the experiment with a concentration lower than the inoculum. It is well-known that not all the microalgae shows similar performance when exposed to a large load of pollutants, such as phosphorous(Patel et al., 2012).

Statistically, there are no differences between the TP reductions in the treatments T3 and T1, which were the treatments where higher cell growth was obtained. The ANOVA-Dunnett statistical analysis carried out taking as a control group day 0, showed that there was a significant reduction in TP in T2, T4, and T5, from the point when the microalga has stabilized. The treatments where there was a larger reduction was in the T2 and T4 reducing up to 75% from 77 mg/L and 52% from 19 mg/L, respectively. In other studies similar results were found regarding the growth of microalgae with swine wastewater, the strain *Arthrospira platensis* can reduce 42%(Mezzomo et al., 2010). The graph of contour in Fig. 4c indicates that the best option for the use of *A. maxima* in the reduction of TP is to employ more of nejayote than swine wastewater, providing values lower than 20 mg/L of TP. Specifically, based on the shape of the surface, it can be observed that the process provides the lowest values in the area of nejayote and water, and with a less proportion in the area of swine wastewater.

In the case of *C. vulgaris*, the total phosphorus removal was significant in most of the treatments; the reduction of this value is equated with the growth of the microalga in the treatment (Fig. 4b). Thus, where there was a greater reduction was in the treatment of T4. The ANOVA-Dunnett statistical analysis by treatment carried out taking as a control group day 0, showed that there was a significant reduction of TP in T1 reducing up to 81% of 13.69 mg/L in 15 days, in T2 reducing up to 84% of 77.7 mg/L in 5 days, in T3 reducing up to 51% of 2.2 mg/L in 15 days, in T4 reducing up to 85% of 19.09 mg/L in 15 days and in T5 reducing up to 59% of 27.9 mg/L in 5 days. In other studies, there are found the similar results did with swine wastewater, where *C. vulgaris* could reduce at least 95% of total phosphorus (Nam et al., 2017).

The contour graph (Fig. 4d) determines that the best option for to use of *Chlorella vulgaris* for the best reduction of total phosphorus of Nejayote and Swine wastewater is with greater use of Nejayote. These results in both microalgae are the accord with the observation that

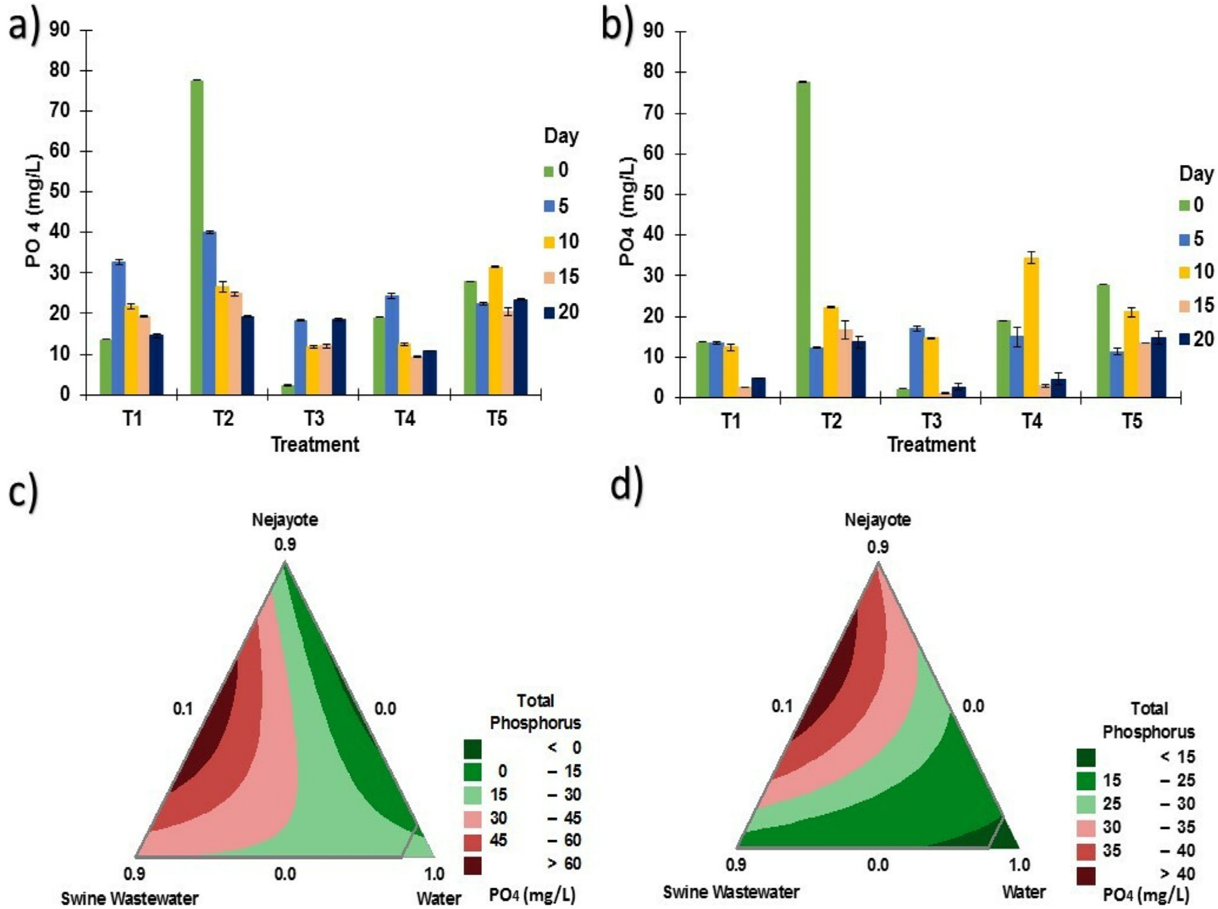


**Fig. 4.** Removal of TP during the culture of *A. maxima* and *C. vulgaris* in nejayote and swine wastewater. a) Quantification of TP in *A. maxima* cultured in nejayote and swine wastewater, in different proportions. The cultures were sampled by three replicate, every five days. b) Quantification of TP in *C. vulgaris* cultured in nejayote and swine wastewater, in different proportions. The cultures were sampled by three replicate, every five days. c) Contour graphic of the behavior of TP variable using nejayote and swine wastewater as a culture medium for *A. maxima*. d) Contour graphic of the behavior of TP variable using nejayote and swine wastewater as a culture medium for *C. vulgaris*. The green area in contour graphics mean the best possible answer of the variable sought in the process.

microalgae can increase the load of TP when the N/P ratio is inadequate in its culture media, due to the elemental composition of microalgae cells. Hence, the optimal ratio N/P in wastewater can be established, and it can also be determined by the Stumm empirical formula, that is $C_{106}H_{263}O_{110}N_{16}P$ (N/P ratio = 7.2:1), where the ratio depends on the strain and conditions of the culture (Kapdan, 2008; Xin et al., 2010).

### 3.5. Removal of chemical oxygen demand during the culture of *A. maxima* and *C. vulgaris* in nejayote and swine wastewater

The characterization of COD during the experiment in both microalgae showing a decrease in all the treatments (T1–5) (Fig. 5a–b). The higher reduction of COD was observed at the growth and stationary stages in one of the cultures of both strains, these results are similar to a previously reported study (Cheunbarn and Peerapornpisal, 2010). Also, in other reports it was shown that the time of aeration in the treatment of wastewater reduced the COD in a larger extent, this behavior can be seen in T4 and T5, where the cellular growth was not predominant in the media (Ibrahim, 2017).

The ANOVA-Dunnett statistical analysis by treatment carried out taking as a control group day 0, showed that there was a significant reduction of COD in T1 reducing up to 88% of 4340 mg/L in 10 days, in T2 reducing up to 84% of 4870 mg/L in 20 days, in T3 reducing up to 81% of 2278 mg/L in 5 days, in T4 reducing up to 75% of 6438 mg/L in 15 days

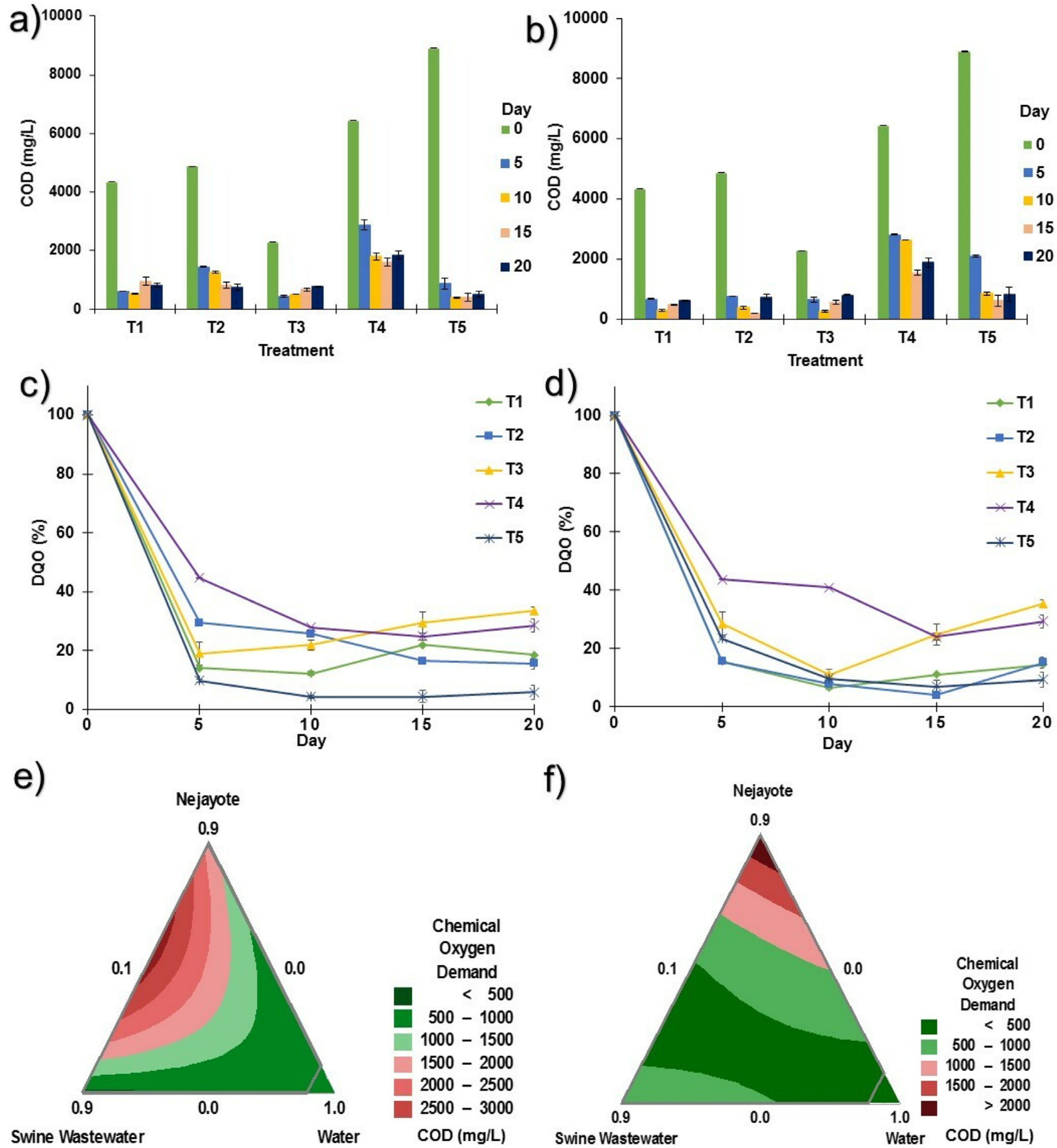


**Fig. 5.** Removal of COD during the culture of *A. maxima* and *C. vulgaris* in nejayote and swine wastewater. a) Quantification of COD in *A. maxima* cultured in nejayote and swine wastewater, in different proportions. The cultures were sampled by three replicate, every five days. b) Quantification of COD in *C. vulgaris* cultured in nejayote and swine wastewater, in different proportions. The cultures were sampled by three replicate, every five days. c) Contour graphic of the behavior of COD variable using nejayote and swine wastewater as a culture medium for *A. maxima*. d) Contour graphic of the behavior of COD variable using nejayote and swine wastewater as a culture medium for *C. vulgaris*. The green area in contour graphics mean the best possible answer of the variable sought in the process.

and in T5 reducing up to 96% of 8903 mg/L in 10 days (Fig. 5c). These results are comparable to the report by Mezzomo et al. (2010). The result obtained in this study was better compared to the one reported by employing *Arthrospira platensis*, where the COD had a reduction of 88.5% (Mezzomo et al., 2010). The graph of surface mixture design presented in Fig. 5e showed that lower values of COD where obtained in the area with an equal quantity of swine wastewater, water and nejayote. From this observation, the optimal selection of treatment composition for the use of *A. maxima* in the context of COD reduction would be the use of equivalent proportions of swine wastewater and nejayote providing a reduction of COD to ~1000 mg/L.

In the case of *C. vulgaris*, according to the statistical analysis carried out, there was a significant reduction in of COD in all the treatments. The treatments where there was a smaller reduction was in the T1, T2, and the T5. The treatment that obtained a greater reduction of the COD was in the T2 in day 15, with a reducing up to 96% of the 4870 mg/L (Fig. 5b). In other studies, there are similar results with swine wastewater, where *Neochloris aquatica* could reduce at least 82%, and *C. vulgaris* JSC-6 reduce nearly of 70–90% of COD (Wang et al., 2015, 2017). In the interaction graph (Fig. 5d) of the treatments regarding the removal of COD, the behavior of reduction of this value can be observed throughout the experiment, obtaining a better result of reducing of COD in the treatment of T2. The other treatment where it was a significant reduction of COD is the T5. The contour graph (Fig. 5e) determine that the best option for the use of *C. vulgaris* for the reduction of COD in Nejayote and Swine wastewater is with the use of an equal amount of Nejayote and swine wastewater. The proportion of both wastewaters with the area in green is optimal to reduce de COD value.

The ANOVA-Dunnett statistical analysis by treatment carried out taking as a control group day 0, showed that there was a significant reduction of COD in T1 reducing up to 93% of 4340 mg/L in 10 days, in T2 reducing up to 96% of 4870 mg/L in 15 days, in T3 reducing up to 89% of 2278 mg/L in 10 days, in T4 reducing up to 76% of 6438 mg/L in 15 days and in T5 reducing up to 93% of 8903 mg/L in 15 days. In both microalgae, the highest values of COD affected the cell growth in T4 (6438 mg/L) and T5 (8903 mg/L), where there were not cell growth, giving the idea that the COD concentration is a limiting factor to cell growth(Lee et al., 2016). Also, the major decrease of this variable is in the first five days, these results are similar to the found with the strain *C.vulgaris* in synthetic wastewater (Otondo et al., 2018).

### 3.6. Determination of the best mix of nejayote and swine wastewater for the growth of A. maxima and C. vulgaris and the reduction of pollutants

Based on all the above results three different information could be obtained for each strain 1) the optimal mixture of nejayote and swine wastewater for the reduction of pollutants, 2) the optimal mixture for the growth of *A. maxima* and 3) simultaneously the growth of the *A. maxima* and reduction of the pollutants (Table 4). This analysis determined that nejayote was the most suitable wastewater for the growth of *A. maxima* and the growth of *C. vulgaris* was swine wastewater since all responses (growth and reduction of contaminants and the combination of both) were optimal when each wastewater was used as a culture media for determinate microalgae. Also with these results, it can be shown that the results depend on the microalgae strain, so is better for *A. maxima* use one type of wastewater, and for *C.vulgaris* the mix of two types of wastewater can be used in cell growth at the laboratory level.

The treatment of both types of wastewater by *C. vulgaris* and *A. maxima* give a good result in terms of reducing COD and in the cellular growth and represents an attractive alternative for wastewater treatment. Despite the above limitations, the treatment of swine wastewater can be a good alternative for the employment of these residues since it generates some sub-products that can further be employed. One of these sub products that can be obtained from the biomass of this process is the production of biodiesel due to the possible concentration of lipids in the biomass(Sreekumar et al., 2018). Additional to the treatment with microalgae, another treatment stage could be needed, like a process of clarification. Usually, the possible treatments of this wastewater involve at least two types of treatments (Garzón-Zuñiga and Buelna, 2014).

## 4. Conclusions

The swine wastewater and nejayote have a considerable amount of organic and inorganic load that could have a negative environmental impact and must, therefore, be treated before discharging into the water. There are several limitations with current technologies, such as sub-products are extracted from those processes, not all of them are eco-technology, and they are also expensive. Therefore, alternative methods that address these limitations and offer efficient, low-cost approaches are highly desirable and must be implemented. One attractive option to overcome the limitations, thus providing a sustainable approach, is the employment of microalgae-based technology, which also can provide sub-products such as biofuels from the biomass, and it does not require a big proportion of land for implementing the technology and the handling is relatively straight forward.

In this present study, both swine wastewater and nejayote were demonstrated as a culture media for the strain *A. maxima* and *C. vulgaris*. Based on the results obtained, the best option for cellular growth is the employment of a larger amount of the nejayote than the swine wastewater in the case of *A. maxima*. The best result was the combination of 10% of Nejayote and 90% of water with at least $33 \times 10^4$ cell/mL and the combination of 25% of swine wastewater, 25% of nejayote and 50% of water provided similar results. Nevertheless, for the reduction of the COD, the optimal combination was 90% swine wastewater and 10% of water with 96% reduction from 8903 mg/L. For the reduction of TP, the combination 25% of nejayote, 25% of swine wastewater and 50% of water with a reduction of 75% from 77 mg/L and for the reduction of TN a mixture of 90% swine wastewater and 10% of water displayed the best efficiency with a reduction of 92% and 39%, respectively.

And in the case of *C. vulgaris* the better option for the cellular growth is to use swine wastewater rather than Nejayote, and the best result of

**Table 4**
The best mixtures for the optimization of the process by response optimizer of the mixture design.

| Process | Proportion of mixture (%) | | | Predicted responses | | | | |
|---|---|---|---|---|---|---|---|---|
| | Swine wastewater | Nejayote | Water | Cell growth (10^4) | COD (mg/L) | TP (mg/L) | TN (mg/L) | Error (%) |
| | | *A.maxima* | | | | | | |
| Cell growth | | 43 | 57 | 61.20 | – | – | – | 0.00 |
| Reduction of pollutants | | 15 | 85 | – | 492 | 7.80 | 5.80 | 4.88 |
| Growth of *A. maxima* and reduction of pollutants | | 15 | 85 | 41.15 | 492 | 7.80 | 5.80 | 2.88 |
| | | *C. vulgaris* | | | | | | |
| Cell growth | 45 | 45 | 10 | 268 | – | – | – | 0.00 |
| Reduction of pollutants | 25 | 37 | 38 | – | 574 | 19.25 | 10.70 | 3.65 |
| Growth of *C. vulgaris* and reduction of pollutants | 63 | 27 | 10 | 227 | 576 | 29.60 | 10.90 | 3.76 |

this is the combination of 10% of swine wastewater and 90% of water with at least $128 \times 10^6$ cell/mL in 12 days. Although Nejayote did not result in the best, the fact that in the concentrations with this wastewater if there was cellular growth, determines the potential of this waste be used as a culture medium for these microalgae. Nevertheless, for the reduction of TN is better the mix of 90% of swine wastewater and 10% of water where the reduction was of 91% of 138.7 mg/L and 54% of 12 mg/L, respectively, for the reduction of TP was the mix of 90% of nejayote and 10% of water with 85% of 19 mg/L and for the COD was the mix of 25% of nejayote, 25% of swine wastewater and 50% of water with reduction up to 96% of 4870 mg/L.

In conclusion, we have demonstrated the first-time valorization of two different wastewaters (swine wastewater and nejayote) as a culture media for two microalgae at level scale, obtained that nejayote is better for the *A. maxima* and the swine wastewater for *C. vulgaris*. A tunable technological platform has been disclosed where it can be directed towards the reduction of the pollutants, growth of the microalgae as a green source for high-value products or a combination of both intentions depending on the desired application.

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## Conflicts of interest

The authors declare no conflict of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.04.278.

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