

Phycoremediation and microalgae production using poultry effluent from anaerobic digestion as a culture medium

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ABSTRACT

Biological treatment of effluents with microalgae can be a sustainable and effective technology. It is mainly useful for the meat industry which produces large volumes of effluents from the animal slaughter, meat processing and the cleaning of their facilities. The objective of this paper was to determine the optimal operating conditions of the *Scenedesmus obliquus* culture for the removal of contaminants from poultry effluent and recovering valuable nutrients for biomass production. Experiments were performed at laboratory scale using poultry effluent from anaerobic digestion without dilution and the microalga *Scenedesmus obliquus*. The evaluation was based on four dilution rates from 0.20 to 0.35 1/day on semi-continuous growth bioreactor. Results demonstrated that the highest biomass productivity of 0.57 g_{biomass}/L day was achieved at 0.30 1/day. However, the highest nutrients removal efficiency was obtained at 0.20 1/day. In these conditions, the removal efficiency exceeded 80% for nitrogen and phosphorus and 99% for *E. coli*, at all dilution rates tested and was above 90% for COD. Also, using a dilution rate of 0.20 1/day, a quantum yield of up to 0.24 g/E and nutrient coefficient yields of up to 41 mg_N/g_{biomass} and 32.32 mg_P/g_{biomass} were achieved. In conclusion, cultivation of *Scenedesmus obliquus* in poultry effluents can be adjusted based on the objective. That is to say, a dilution rate of 0.30 1/day is recommended for biomass production and 0.20 1/day is suggested for tertiary treatment. The production of microalgae coupled with biological treatment allows recovery of nutrients, generating biomass and treating the industry effluents before their final disposal.

KEYWORDS

dilution rate; phycoremediation; poultry effluent; productivity; quantum yield; *Scenedesmus obliquus*

1. INTRODUCTION

Microalgae have been employed for a wide range of applications, such as feed, biofertilizers, biofuels and pharmaceutical and nutraceutical products. The reasons for elevated usage of microalgae in different applications include their high growth rate, valuable biochemical composition, ability to grow in widely different environments and non-requirement of

fertile land or usable water (Chisti, 2013). However, microalgae are only produced on a small scale; overall worldwide biomass productivity of microalgae cultures does not exceed 150 tn/ha year (dry matter) and production costs exceed 10 €/kg (Benemann, 2013; Acien et al., 2012). In addition, to obtain high productivity values large amounts of nutrients are required. Nitrogen, phosphorus and CO₂, are the major nutrients required for microalgae biomass production. For example, to produce 100 tn of microalgae biomass

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up to 5 tn of nitrogen, 1 tn of phosphorus and 200 tn of CO₂, are needed. Generally, nitrogen and phosphorus are supplied as nitrate and phosphate salts, respectively. On the other hand, CO₂ can be supplied as pure food grade CO₂ or as CO₂ from industrial flue gases without pre-treatment (Li et al., 2013; Acién et al., 2012). Chemical fertilizers are usually utilized to supply nitrogen and phosphorus. However, utilizing these nutrients reduces the sustainability of microalgae production. This is because fertilizers are based on fossil energy and their production is associated with high energy consumption and CO₂ emissions (Lardon et al., 2009). To solve this problem the production of microalgae coupled to the treatment of effluents and the utilization of flue gases is necessary (Acién et al., 2012; Olgúin, 2012). Thus, nutrient recovery from effluents used in microalgae cultivation results in two economic benefits, which makes it a profitable process: microalgae biomass production and pollutant elimination (Park and Craggs, 2011). Discharge of untreated domestic and industrial effluents into natural aquatic resources poses a serious eutrophication threat, leading to a slow degradation of the water resources (Renuka et al., 2015). Effluents from food-industry are rich in nitrogen, phosphorus and organic matter, and even contain several compounds, such as heavy metals and toxins, which are potentially toxic to microalgae. The nitrogen and phosphorus are the main nutrients required for microalgae production. In effluents, nitrogen is present as ammonia, urea or organic nitrogen whereas phosphorus is normally found as phosphate or in organic compounds. Therefore, effluents contain the majority of compounds required to produce microalgae. However, it is important to study each case due to perturbations such as effluent variability and seasonality (Renuka et al., 2015; Rawat et al., 2011). The use of microalgae for the removal of contaminants from effluents offer additional advantages because microalgae are effective in removing *E. coli* and coliform bacteria, heavy metals as well as emerging contaminants (López-Serna et al., 2019; Hernández et al., 2013; Muñoz and Guieysse, 2006). This guarantees several advantages, such as production cleaner effluents with high dissolved oxygen concentrations, the reduction of energy consumption as well as reduction of greenhouse gases emissions.

In the poultry effluent treatment plants, one of the richest streams of nitrogen and phosphorus content comes from the digestate. These streams are produced during the anaerobic digestion of sludge in the conventional biological wastewater treatments (secondary treatment). However, anaerobic digestion

does not provide an efficient removal of contaminants and nutrients, so it is necessary to recirculate the effluent inside the effluent treatment plant, to avoid the phenomenon of eutrophication when the depurated water is released (Rosso et al., 2008). This increases the total operation cost by 45-75%, mainly due to the energy consumption. The utilization of poultry effluent as the sole nutrient source in microalgae production was previously reported (Uggetti et al., 2014; Rosso et al., 2008). Usually concentrations of ammonia nitrogen and phosphates in the effluent are in the ranges of 65–200 mg/L and 10–100 mg/L, respectively. These concentrations are adequate to obtain high biomass productivities from most freshwater microalgae strains (Li et al., 2012; McGinn et al., 2011). However, is important to consider the variability of inorganic N/P ratio in the different fractions of effluent within a poultry effluent treatment plant (Wang et al., 2010a). The optimal N/P ratio for microalgal growth should be between 7-10 (Cheng et al., 2015; Wang et al., 2010a). Effluent may also contain several compounds, such as organic acid, phenols, urea and pesticides, that are potentially toxic and inhibit to microalgae growth. Therefore, high concentrations of these compounds in the effluents might limit their use for the microalgae production (Djelal et al., 2014). So in each case, it is necessary to study, the optimal effluent concentration that can be used as the nutrient source in microalgae production. Strains as such *Scenedesmus*, *Oscillatoria*, *Nitzschia* and *Chlorella* are the most pollution-tolerant microalgae and hence they are commonly used in effluent treatment systems (Palmer, 1969). Thus, any species with high growth rate with tolerance to organic pollutants and external conditions can be tested. Nevertheless, the use of poultry effluent as the culture medium for microalgae production must be studied. It is also desirable to determine the optimal dilution rate and effluent percentage that can be mixed with freshwater for microalgae production. It should also be noted that in previous studies, in our laboratories we have optimized the isolation of *Scenedesmus obliquus* from freshwaters of the Embalse Salto Grande (Jiménez-Veuthey et al., 2018).

In this context, the aim of this work was to determine the optimal operating conditions and the feasibility of producing freshwater microalgae using poultry effluent from anaerobic digestion as a culture medium. The experiments were performed indoor, at laboratory scale, to analyse the efficiency of selected microalgae in removing nutrients as a prior step to the development of process on a large scale.

2. MATERIALS AND METHODS

2.1. Microorganisms

The microalga strain used in this study was *Scenedesmus obliquus* (IOACo81F), isolated from Embalse Salto Grande, a region of North-East Argentina that has been polluted with agro-industrial run-off from companies in Argentina and Uruguay for several decades (Jiménez-Veuthey et al., 2018). *Scenedesmus* genus is characterized by rapid growth and success in large-scale systems. The inoculum was cultured in batch mode, using Allen & Arnon culture medium enriched with 0.850 g/L of NaNO₃ (Arnon et al., 1974). The exponential growth of culture was maintained at temperature of 25 ± 1 °C, aerated at 0.20 v/v min, with CO₂ injected on demand (pH = 8.0 ± 0.1), a light intensity of 150 µmol/m² s and a 12:12 h light/dark cycle.

2.2. Culture media

Effluent from real poultry effluent treatment plant located in San José in Entre Ríos (32°11'45.2" S, 58°11'02.5" W - Argentina) was used as culture medium. The effluent was collected after active sludge treatment (secondary treatment), not being subjected to any further treatment or sterilization process.

2.3. Ability of effluent as culture medium

The ability of poultry effluent to be used as a culture medium for microalgal growth was studied using 3 bubble-column bioreactors, filled with 200 mL of secondary undiluted poultry effluent and 50 mL of microalgae inoculum. The culture is operated in batch until reaching the stationary phase. The three bioreactors were installed in an incubator chamber 400H (MGC, China) with control of temperature, photoperiod and relative humidity (RH). The culture temperature and RH were kept at 25 ± 1 °C and 65%, respectively. Each bioreactor was aerated at 0.20 v/v min. The pH of the cultures was maintained at 8.0 ± 0.1 using CO₂ injection on-demand. The cultures were artificially illuminated using eight 36 W fluorescent tubes, simulating 12 h light and 12 h darkness. The average irradiance during the light period was 870 µE/m² s; this was measured using a spherical SQS- LI 192SA sensor (LICOR, USA).

2.4. Bioreactors and culture conditions

Experiments were performed at laboratory scale in 12 bubble-column-type bioreactors of spherical-bases, which were filled with 250 mL of secondary undiluted poultry effluents and inoculated with 20% of total volume with microalgae inoculum. The twelve bioreactors were installed in an incubator chamber 400H (MGC, China) with control of temperature, photoperiod and RH. The culture temperature and RH were kept at 25 ± 1 °C and 65%, respectively. Each bioreactor was aerated at 0.20 v/v min. The pH of the cultures was maintained at 8.0 ± 0.1 using CO₂ injection on-demand. The cultures were artificially illuminated using eight 36 W fluorescent tubes (Osram Lumilux T8). Illumination was performed using a circadian cycle (12:12 light/dark), with an average irradiance during the light period of 870 µE/m² s. The irradiance value was measured using a spherical SQS- LI 192SA sensor (LICOR, USA).

Each assay was performed triplicate, thus in each trial four different dilution rates (0.20; 0.25; 0.30 and 0.35 1/day) were tested. Experiments were performed simultaneously on all bioreactors, which were operated in batch mode for four days and then in a semi-continuous mode by harvesting 20 to 35% of the volume daily and replacing it with fresh secondary effluent. The cultures were operated in semi-continuous mode until the culture volume was renewed at least three times and the biomass concentration remained constant for at least 3 days. Harvested biomass and supernatant were used for analytical determinations.

2.5. Cell density and biomass concentration

Cell density was measured every 48 h with Neubauer chamber using a Leica DMLS microscope (Leica Corp., Germany). The specific growth rate (μ) was calculated using Eq. (1):

$$\mu = (\ln N - \ln N_0) / (t - t_0) \quad (1)$$

where N and N₀ are the cell number concentration (cell number/L), at the beginning (t₀, d) and at the end of the exponential growth phase (t, d), respectively.

The biomass concentration (C_b) was measured by filtering 50 mL of culture through 0.45 µm pore diameter filters (MSI, Type A/E) and drying the filtered biomass in an oven at 80 °C ± 1 °C for 24 h. Thus, biomass productivity was calculated as the product of biomass concentration by the imposed dilution rate (0.20; 0.25; 0.30 and 0.35 1/day).

2.6. Light availability and quantum yield

Absorbance in the visible range (400 – 700 nm) was measured every 48 h using a UV-Vis Hach DR600 spectrophotometer (Hach Corp., Germany) and the biomass extinction coefficient (K_a) was calculated by dividing the average absorbance value by the biomass concentration (C_b) and light path of the cuvette (p) as below:

$$K_a = \text{Abs} / (C_b p) \quad (2)$$

The average irradiance, which cells are exposed inside a culture (I_{av}) in the range of photosynthetically active radiation (PAR), was calculated as a function of the irradiance at the surface (I_o), the biomass extinction coefficient (K_a), the biomass concentration (C_b), and the light path inside the bioreactor (p). It can be calculated by using Eq. (3) (Molina-Grima et al., 1997):

$$I_{av} = I_o / ((K_a C_b p)) [1 - \exp(-K_a C_b p)] \quad (3)$$

The amount of biomass generated by a unit of radiation absorbed by the culture is defined as quantum yield (Ψ_E). It is possible to calculate using Eq. (4) (Molina-Grima et al., 1997) as a function of the volumetric biomass productivity (P_b) and the photon flux absorbed in the volume unit (F_{vol}).

$$\Psi_E = P_b / (F_{vol}) \quad (4)$$

Volumetric biomass productivity was calculated multiplying biomass concentration (C_b) by the set dilution rate (D) using Eq. (5).

$$P_b = C_b D \quad (5)$$

The photon flux absorbed by the microalgal culture can be obtained from the average irradiance (I_{av}) on a culture volume basis using Eq. (6) (Molina-Grima et al., 1997).

$$F_{vol} = I_{av} K_a C_b \quad (6)$$

2.7. Nutrient removal efficiency and nutrient removal capacity

The nutrient removal was analysed using two variables: removal efficiency (R_e) and removal capacity (R_c). The removal efficiency was calculated as the ratio between the concentration outlet (C) and inlet (C_o) in the

bioreactors as below,

$$R_e = ((C_o - C) / C_o) \times 100 \quad (7)$$

The net system capacity can be calculated as the total amount of nutrient removed per time and culture volume as below,

$$R_c = (C_o - C) \times D \quad (8)$$

2.8. Nitrogen and phosphorus coefficient yields

The nitrogen ($Y_{b/N}$) and phosphorus ($Y_{b/P}$) coefficient yields were determined as the amount of biomass produced per unit of nitrogen and phosphorus adsorbed by the microalgae culture. The nitrogen removed was calculated as the difference between the total nitrogen concentration (TN) in the bioreactor inlet and outlet streams, at the imposed dilution rate (D) as below,

$$Y_{b/N} = (([TN]_{inlet} - [TN]_{outlet}) / P_b) \times D \quad (9)$$

The phosphorus removed was calculated as the difference between the total phosphorus concentration (TP) at the inlet and outlet of the culture at the imposed dilution rate (D) as below,

$$Y_{b/P} = (([TP]_{inlet} - [TP]_{outlet}) / P_b) \times D \quad (10)$$

2.9. Analytical methods

The chemical compositions of inlet and outlet water samples from the bioreactors were analysed using the Standard methods for the examination of water and wastewater (APHA (American Public Health Association), 2012). Water samples from the bioreactor outlet streams were filtered through 0.45 μ m pore diameter filters (MSI, Type A/E) to separate biomass. The pH and electrical conductivity (EC) were measured with a pH meter AD1030 (Adwa, China) and a conductivity meter GLP31 (Crison, Spain), respectively. Total phosphorus (TP) and dissolved reactive phosphorus (DRP) were measured by visible spectrophotometry through the phospho-vanado-molybdate complex. Chemical oxygen demand (COD), total nitrogen (TN), total organic carbon (TOC) and total inorganic carbon (TIC) were determined by spectrophotometric measurement using Hach kits. Total kjeldahl nitrogen (TKN) was determined by wet oxidation of nitrogen using sulfuric acid and digestion

catalyst. Ammonia was measured titrimetrically according to standard method (4500-NH₃ C). Nitrate was quantified colorimetrically according to standard method (4500-NO₃ B). A UV-Vis DR600 (Hach Corp., Germany) spectrophotometer was used. Nitrite and the organic form of nitrogen were calculated as the difference between total nitrogen and the sum of the rest of species analysed. Sodium (Na), calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn) and manganese (Mn) were quantified by atomic absorption spectroscopy using a Shimadzu ASC-6100 atomic absorption spectrometer. AccuStandard standard solutions were used for calibration. In addition, in the bioreactor inlet and outlet streams, *E. coli* and coliform bacteria were counted using the Compact Dry EC method.

3. RESULTS

To determine the optimal conditions for microalgae

production and nutrient removal using poultry effluent as the nutrient source, experiments were performed at laboratory scale in semi-continuous mode at a dilution rate between 0.20 to 0.35 1/day under controlled conditions simulating the outdoors. In addition, secondary effluent was used without any dilution due to the proper N/P ratio, previously determined. The differences in composition between Allen & Arnon culture medium with supplemented nitrate and poultry effluent are shown in Table 1. Operating in a semi-continuous mode, steady states were obtained. Under this condition the strain yields were evaluated in terms of biomass production, cell density, light utilization efficiency, nutrient removal efficiency and capacity, all of which are essential to adequately evaluate the performance of the strain in the culture mediums used. Both mediums contained relevant concentrations of minor elements such as Fe, Mg, Mn; where all of them are necessary for microalgae production. Regarding major compounds, the Allen & Arnon medium contained high concentrations of nitrogen and phosphorus,

Table 1. Chemical composition of Allen & Arnon medium with supplemented nitrate and poultry effluent.

Parameters	Units	Allen & Arnon	Poultry effluent
pH		7.23 ± 0.01	6.95 ± 0.01
EC	µS/cm	1821 ± 10	1376 ± 12
COD	mg/L	35.00 ± 1.00	559.00 ± 3.00
TN	mg/L	120.00 ± 0.01	122.00 ± 1.00
TKN	mg/L	31.74 ± 0.00	58.92 ± 0.45
Nitrate	mg/L	75.41 ± 0.35	0.29 ± 0.00
Nitrite	mg/L	12.85 ± 0.00	62.79 ± 0.45
Ammonium	mg/L	1.29 ± 0.00	54.15 ± 0.00
Organic Nitrogen	mg/L	30.45 ± 0.00	4.78 ± 0.45
TP	mg/L	75 ± 0.00	82.00 ± 1.00
DRP	mg/L	46.36 ± 0.01	78.00 ± 0.00
Na	mg/L	174.29 ± 4.51	34.21 ± 0.65
K	mg/L	224.51 ± 4.33	35.38 ± 1.41
Ca	mg/L	61.10 ± 2.23	4.03 ± 0.23
Mg	mg/L	7.26 ± 0.10	5.48 ± 0.00
Fe	mg/L	4.51 ± 0.00	1.10 ± 0.03
Zn	mg/L	0.13 ± 0.00	0.01 ± 0.00
Mn	mg/L	0.43 ± 0.01	0.09 ± 0.00
TOC	mg/L	0.01 ± 0.00	20.00 ± 0.07
TIC	mg/L	50.00 ± 2.00	55.00 ± 2.10
Coliform bacteria	CFU/mL	-	2.20E+04 ± 8.48E+02
<i>E. coli</i>	CFU/mL	-	3.40E+04 ± 1.06E+03

at 120 and 75 mg/L, respectively; nitrate being the predominant nitrogen form. Analogous in the poultry effluent, the phosphorus content was 122 mg/L and the nitrogen, 82 mg/L; with nitrogen mainly in forma of nitrite. In the case of COD, poultry effluent contained

high concentrations, at 559 mg/L. Conversely, in the Allen & Arnon medium the COD concentration was much lower, at 35 mg/L. This correlates with low concentration of total organic carbon in the Allen & Arnon medium, at 0.01 mg/L, and high concentration

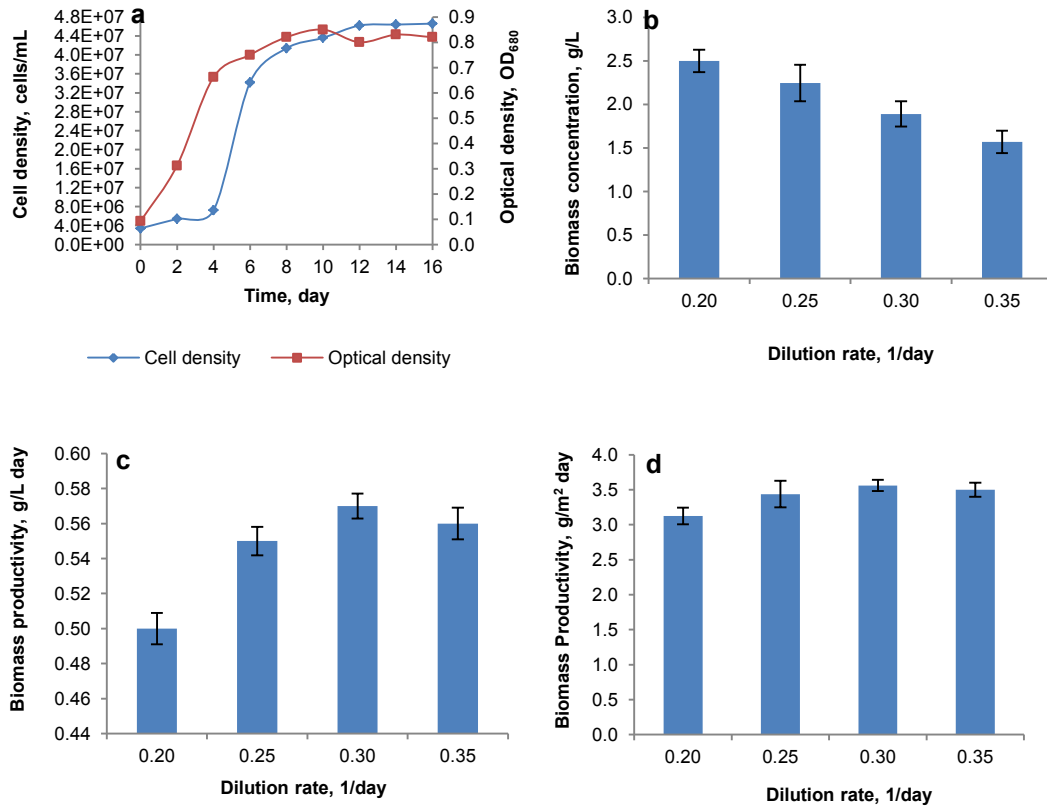


Figure 1. Influence of the poultry effluent on the microalga growth (a), variation in biomass concentration (b) and biomass productivity (c, d) in the selected strain as a function of the culture medium used and the imposed dilution rate (0.20 – 0.35 1/day).

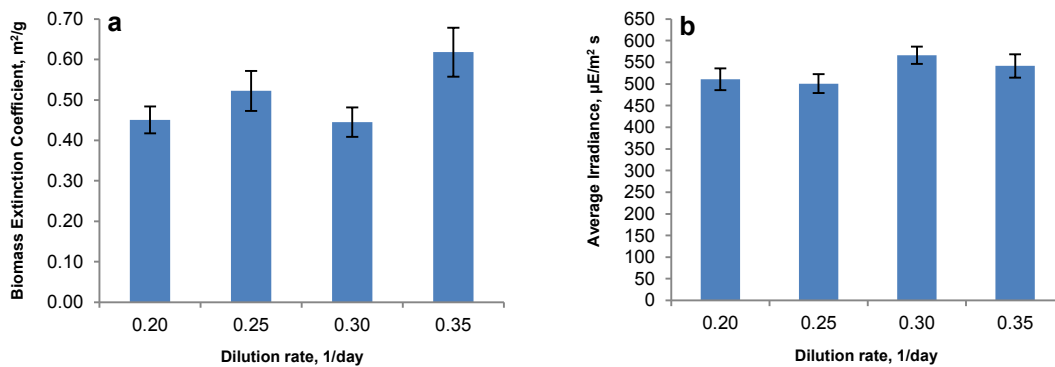


Figure 2. Influence of the imposed dilution rate on the extinction coefficient of the biomass and the average irradiance inside the culture in indoors bubble-column-type bioreactors. Extinction coefficient of the biomass (a), average irradiance (b) to which the cells are exposed inside the cultures.

in the poultry effluent, at 20 mg/L. Regarding total inorganic carbon, both Allen & Arnon medium and poultry effluent contained similar concentrations of 50 and 55 mg/L, respectively. According to these values, the C/N/P ratio of Allen & Arnon medium was 100/240/150, whereas for poultry effluent, this ratio was equal to 100/221/149. By comparing these values with the proximate composition of the biomass (100/4/1), it was concluded that both culture mediums contained excess of nitrogen and phosphorus as well as low carbon concentrations. Thus, the cultures might be carbon limited if additional carbon is not supplied by CO₂ injection on-demand. Results show that *S. obliquus* could be produced in a semi-continuous mode using only poultry effluent without dilution as the nutrient source (Figure. 1).

Analysing the microalga growth through the logarithmic curve presented in Figure. 1a, it is possible to confirm that poultry effluent does not contain anything toxic to microalgae growth and that the minor component concentrations are sufficient to support the growth of *S. obliquus*. The culture grown in poultry effluent presented typical growth curve, the batch culture had an initial cell density over 4×10^6 cells/mL and it was increased until it reached an optical density over 0.8 (the cell density was around 4.8×10^7 cells/mL), which occurred after 10 – 12 days of incubation (stationary phase). Also, a lag phase was observed the first 2 days. Maximum specific growth rate (μ_{max}) was calculated from the logarithmic growth phase between 4 and 8 days batch culture. Under the conditions tested, maximum specific growth rate of *S. obliquus* was 0.44 1/day.

Both the biomass concentration and productivity varied as a function of the dilution rate set in the culture medium (Figure 1 b, c and d). The biomass concentration achieved in a steady state was higher (2.50 ± 0.13 g_{biomass}/L) when the dilution rate was 0.20 1/day, which reduces as the set dilution rate increased. At dilution rate of 0.35 1/day, the biomass concentration was reduced by approximately 35% (1.6 ± 0.13 g_{biomass}/L) (Figure 1b). These results indicate that a culture in semi-continuous mode at dilution rate above 0.30 1/day can affect the performance of the cultures. This is important because biomass concentration influences biomass productivity; but also, high biomass concentrations improve culture stability, which is necessary for large-scale production. In terms of biomass productivity, a maximal biomass productivity of 0.60 ± 0.007 g_{biomass}/L day (3.56 ± 0.02 g_{biomass}/m² day) was achieved using a dilution rate of 0.30 1/day, but this was reduced to 0.50 ± 0.009

g_{biomass}/L day (3.13 ± 0.01 g_{biomass}/m² day) when using a dilution rate of 0.20 1/day. However, at dilution rate of 0.25 and 0.35 1/day, similar biomass productivity was observed, ranged from 0.55 ± 0.008 g_{biomass}/L day to 0.60 ± 0.007 g_{biomass}/L day (3.44 ± 0.02 g_{biomass}/m² day to 3.56 ± 0.02 g_{biomass}/m² day), respectively (Fig 1 c,d).

To determine light-use efficiency, light attenuation and light availability by the cells were studied (Figure 2a and b). This was determined by measuring the biomass extinction coefficient and the average irradiance inside the cultures. The extinction coefficient quantifies the attenuation of light caused by the cells, whereas the average irradiance is the light availability required by the cells to maintain the imposed dilution rate. Data confirm that when the set dilution rate is manipulated; the optical properties of the biomass can be modified (Figure 2a).

Thus, the biomass extinction coefficient increased from 0.44 to 0.62 m²/g when the dilution rate increases from 0.20 to 0.35 1/day. The extinction coefficient might increase due to reduction in biomass concentration. Also, it was observed that at dilution rates below 0.35 1/day, the biomass extinction coefficients are similar. An increased extinction coefficient implies greater light attenuation, which is potentially a negative behavior. Theory suggests that biomass with a lower extinction coefficient is more appropriate for large-scale production because greater light availability inside the cultures can be achieved with the same external irradiance.

Regarding light availability, the average irradiance (I_{av}) with no-culture was 870 $\mu\text{E}/\text{m}^2 \text{ s}$ but this value decreased until 500-566 $\mu\text{E}/\text{m}^2 \text{ s}$ due to the presence of cells (Figure 2b). Data shows that the average irradiance remained constant when the dilution rate was lower than 0.30 1/day; however, above this value, the biomass concentration in the cultures were reduced and the average irradiance greatly increased indicating the low light utilization efficiency of cultures. A decrease in average irradiance usually correlates with an increase in culture efficiency, but due to changes in the biomass extinction coefficient and biomass productivity, a direct correlation cannot be established.

Finally, the chemical composition and colony-forming units (CFU) of *E. coli* and coliform bacteria in the outlet stream of bioreactor at the imposed dilution rate were determined (Table 2). Results demonstrated that when using poultry effluent as culture medium, the nitrogen was in the form of ammonium. No relevant nitrate amounts were measured at the outlet in experiments using poultry effluent, thus nitrification

Table 2. Chemical and microbiological composition of the bioreactor outlet stream, at the imposed dilution rate

Parameters	Units	Outlet			
		Dilution rate, 1/day			
		0.20	0.25	0.30	0.35
pH		8.04 ± 0.01	8.16 ± 0.01	8.01 ± 0.01	7.88 ± 0.01
EC	µS/cm	751 ± 3	805 ± 6	805 ± 8	806 ± 6
TN	mg/L	20.00 ± 1.00	32.00 ± 1.00	35.00 ± 1.00	43.00 ± 1.00
TKN	mg/L	13.64 ± 0.10	23.22 ± 0.98	22.18 ± 0.49	23.56 ± 0.49
Ammonium	mg/L	11.06 ± 0.01	16.29 ± 0.49	20.10 ± 0.00	20.44 ± 0.49
Organic nitrogen	mg/L	2.58 ± 0.01	6.93 ± 1.47	2.08 ± 0.49	3.12 ± 0.98
Nitrate	mg/L	0.23 ± 0.00	0.18 ± 0.00	0.12 ± 0.00	0.25 ± 0.00
Nitrite	mg/L	6.13 ± 0.71	8.6 ± 0.98	12.71 ± 0.49	18.68 ± 1.20
COD	mg/L	57.00 ± 1.00	77.00 ± 0.00	85.00 ± 1.00	103.00 ± 1.00
TP	mg/L	1.20 ± 0.06	15.15 ± 0.21	38.20 ± 0.57	40.90 ± 0.14
DRP	mg/L	1.00 ± 0.71	15.00 ± 0.11	38.15 ± 0.07	39.75 ± 0.35
TOC	mg/L	8.00 ± 0.00	12.00 ± 0.00	13.00 ± 0.00	15.00 ± 0.00
TIC	mg/L	19.20 ± 0.12	33.00 ± 0.10	36.00 ± 0.10	41.00 ± 0.15
Na	mg/L	82.42 ± 4.12	70.76 ± 3.11	64.60 ± 1.11	65.50 ± 0.97
K	mg/L	52.48 ± 4.61	43.60 ± 0.21	41.95 ± 0.14	45.03 ± 1.40
Ca	mg/L	6.04 ± 0.61	5.46 ± 0.12	5.76 ± 0.11	6.95 ± 0.34
Mn	mg/L	4.61 ± 0.24	4.25 ± 0.05	4.72 ± 0.01	4.99 ± 0.02
Zn	mg/L	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Fe	mg/L	0.44 ± 0.01	0.40 ± 0.01	0.09 ± 0.00	0.11 ± 0.00
Mn	mg/L	0.06 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Coliform bacteria	CFU/mL	200 ± 17	181 ± 3	134 ± 1	76 ± 1
<i>E. coli</i>	CFU/mL	8 ± 1	7 ± 0	6 ± 0	1 ± 0

did not take place. Total nitrogen concentration is used to evaluate the system performance. Results showed that by increasing the dilution rate in the bioreactor, the nitrogen outlet concentration increased from 20.00 ± 1.00 to 43.00 ± 1.00 mg/L. Argentina Ministry of Works and Public Services (Ministerio de Obras y Servicios Públicos, 2009) established the water release limit of 35 mg/L for nitrogen. Thus the nitrogen concentration outlet achieved high values, up to 40 mg/L, only when the dilution rate was about 0.35 1/day, thus confirming the inability of the system to remove the nitrogen supplied to the system when dilution rates are above at 0.30 1/day.

Regarding phosphorus and COD, a similar trend was observed. Argentina Ministry of Works and Public Services (Ministerio de Obras y Servicios Públicos, 2009) establishes the requirements for urban

wastewater discharges in sensitive areas, setting total maximal of 1 mg/L for phosphorus and 250 mg/L for COD. The phosphorus outlet concentration increased from 1.20 ± 0.06 to 40.90 ± 0.14 mg/L upon increasing the dilution rate in the bioreactor. Consequently, only by using the dilution rate at 0.20 1/day this limit can be accomplished; using a dilution rates above 0.20 1/day, the phosphorus concentration exceeds this limit. Results of the COD values at the outlet of the cultures showed a variation from 57.00 ± 1.00 to 103.00 ± 1.00 mg/L for 0.25 1/day and 0.35 1/day, respectively. Nonetheless, in all cases, the COD values measured at the outlet were below the permitted limit regulating the release of water into the environment.

In the case of *Escherichia coli*, the results showed that the number of this bacterium decreases when the dilution rate increases, being 8 ± 1 CFU/mL

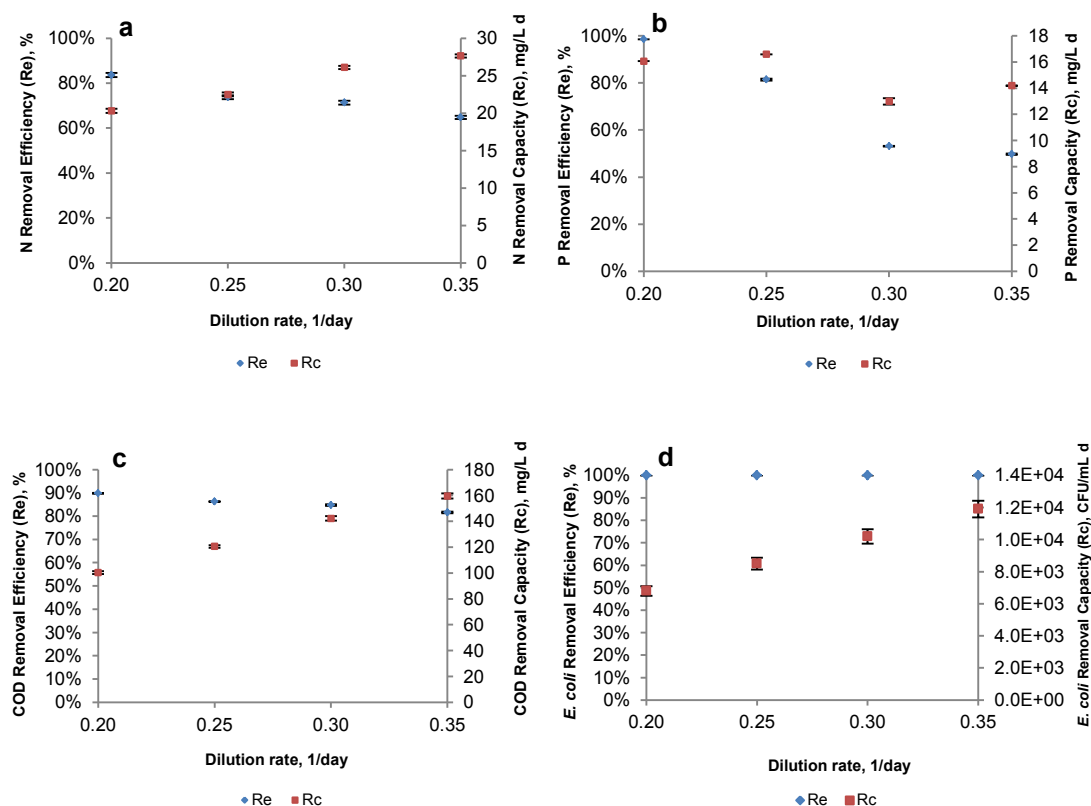


Figure 3. Influence of the imposed dilution rate on nutrient and *Escherichia coli* removal efficiency and capacity in bubble-column-type bioreactors at laboratory scale. Total nitrogen (a), total phosphorus (b), chemical oxygen demand (c) and *Escherichia coli* (d).

for a dilution rate of 0.20 1/day and 1 ± 0 CFU/mL for a dilution rate of 0.35 1/day. However, in all cases, *E. coli* cells were reduced by 4 logarithmic cycles.

In terms of treatment capacity, the results show that nitrogen removal efficiency was higher than 80% when using a dilution rate of 0.20 1/day, reduces to 65 – 74% when using a dilution rates between 0.35 – 0.25 1/day (Figure 3a). Moreover, the nitrogen removal capacity increased from 20.30 ± 0.28 to 27.65 ± 0.21 mg/L day by increasing the dilution rate in the bioreactor, despite the adverse effect of an excess dilution in the culture medium. Regarding phosphorus, a similar trend was observed as in total nitrogen. Thus, the removal efficiency was higher than 80% when using < 0.25 1/day dilution rate in the culture medium but was reduced to 50% when using a dilution rate above 0.30 1/day (Figure 3b). In terms of removal capacity, values were higher at 0.20 and 0.25 1/day than at 0.30 and 0.35 1/day, increasing to 16.59 ± 0.00 mg/L day. The COD removal efficiency was 90% when using a dilution rate of 0.20 1/day; however, reduced to 80 – 85% when using a dilution rate above 0.25 1/day (Figure 3c). Moreover, the COD removal capacity increased from 100 ± 0.57 to 160 ± 1.98 mg/L day by

increasing the dilution rate in the bioreactor. The *E. coli* removal efficiency, it was generally above 99.98%, and exhibited a similar trend as that of total nitrogen and COD (Figure 3d). The results showed that the removal capacity of this bacterium increases with the dilution rate, being 6800 ± 300 CFU mg/L day for a dilution rate of 0.20 1/day and 12000 ± 525 CFU mg/L day for a dilution rate of 0.35 1/day.

4. DISCUSSION

The sustainability and profitability of microalgae production can be increased with the production of large amounts of biomass at low cost; however, this requires large volumes of water and nutrients. Hence, recycling of nutrient from effluents is strongly recommended, permitting the nitrogen and phosphorus contained within them to be reused. To produce such a huge amount of biomass, it would be necessary to supply $153 \text{ g}_N/\text{kg}_{\text{proteins}}$ and $16.5 \text{ g}_P/\text{kg}_{\text{proteins}}$. Additionally, considering that approximately 7% of biomass is nitrogen and that to produce 1 kg of NH_3 , more than 10

kW h of energy is required, it is important to not that consumption of fertilizers implies approximately 20% of total production costs (Ación et al., 2012; Ministerio de Obras y Servicios Públicos, 2009). At the same time, effluent treatment plants manage large volumes of water containing nitrogen and phosphorus. These plants have the obligation to develop more efficient processes which comply with discharged water quality requirements imposed by environment regulations as well as being obliged to use less energy and enhance nutrient recovery from the effluent. Consequently, the possible way to increase process sustainability and profitability for microalgae biomass production is to use waste from other industries such as poultry effluent treatment. Thus, it makes sense to consider develop hybrid microalgae production and effluent treatment technology, and more specifically the utilization of poultry effluent as the nutrient-rich solution for culture medium preparation; along with the added benefit that the effluent can be remediated during the process (De Godos et al., 2010). Microalgae can bioremediate the effluents by removing the excess nitrogen and phosphorus, thus avoiding eutrophication problems as well as oxidizing any remaining compounds as a result of the oxygen oversaturation that takes place in the microalgae cultures. The results obtained in the present study indicate that *S. obliquus* can be produced with secondary undiluted poultry effluent as the only source of nutrients. Biomass productivities achieved using a dilution rates in the range of 0.25 to 0.35 1/day are similar, with values of up to $0.60 \pm 0.01 \text{ g}_{\text{biomass}}/\text{L day}$ were measured (Figure 1c).

Secondary poultry effluent contains large amount of nitrogen, phosphorus and carbon, as well as other micronutrients required for microalgae production (Table 2). However, in order to optimize process performance, it is important to determine the optimal operating conditions, such as chemical composition, optimal dilution rate and effluent dilutions for microalgal production (De Godos et al., 2010; Singh and Gu, 2010). The high biomass productivity achieved demonstrated that no fertilizers are needed.

The main problem of using poultry effluent as a culture medium for the microalgae production is its high ammonium content instead of nitrate. Ammonium assimilation varies with the pH of culture and can become toxic at a high pH, so pH is a very important factor in the cultivation of microalgae (Ledda et al., 2015; Sepúlveda et al., 2015). At pH values above 9, most of the ammonium is in the form of ammonia, this leads to the generation of oxidation

reactions and as a consequence O_2 is produced. The use of NH_3 -tolerant microalgae strain can improve the process (Collos et al., 2005). It was also reported that ammonium concentrations of 364 mg/L inhibit growth in *Scenedesmus sp.* (Posadas et al., 2015), whereas *Chlorella sorokiniana* was reported as tolerating up to 400 mg/L, while *Spirulina platensis* was almost completely inhibited at 198 mg/L (Ogbonna et al., 2000). In this work, the ammonium concentration at the bioreactor inlet was $54.15 \pm 0.00 \text{ mg/L}$ using poultry effluent without dilution and no inhibition was observed for *S. obliquus*.

High temperatures and high pH values influence ammonia stripping (Ruiz-Marin et al., 2010). However, as the pH is maintained at 8, the amount of nitrogen that can be removed by stripping is low. Although P content in biomass is less than 1%, it is essential for microalgal growth as it is involved in many cellular processes. Previous study indicated that up to 90% of the N and 80% P were removed from sewage wastewater using *Chlorella vulgaris* (Lau et al., 2014). Using anaerobic digestate dairy manure, the ammonium was completely removed with *C. vulgaris* (Wang et al., 2010a), whereas using *Neochloris oleoabundans*, ammonium removal was 90 – 95% (Yang et al., 2011; Wang et al., 2010b). A phosphorus removal rate of between 63% and 75% was previously reported with *Chlorella sp.* with anaerobic digestate from dairy manure (Wang et al., 2010a), while phosphorus removal from urban wastewater was 80% for *C. vulgaris* and 83% for *S. obliquus* (Ruiz-Marin et al., 2010). With agro-industrial wastewater from the poultry industry, *S. obliquus* achieved results higher than 97% removal efficiency for both ammonium and phosphate (Oliveira et al., 2018). Using secondary treated effluent from municipal wastewater, a dilution rate of 0.40 1/day and the microalga *Chlorella sp.*, the highest removal of total nitrogen, total phosphorus and COD from the liquid phase were 94%, 96.6% and 90%, respectively; also, total coliform and *E. coli* removal exceeded 99% regardless of the dilution rate (Pereira et al., 2019). Tolerance to ammonium is a strain-specific response, thus from the data reported here, it is clearly observable that *S. obliquus* has a high tolerance to ammonium concentration. For the experiments performed using *S. obliquus* and a dilution rate of the culture medium of 0.20 1/day, the cultures performing well with high biomass concentration and nutrients (nitrogen and phosphorus) and COD removal efficiency (Figure 3a, b and c). However, using *S. obliquus* and a dilution rate above of 0.25 1/day, the biomass concentration, the N and P removal efficiencies and COD were found to be

decreasing. For all dilution rates, reduction in *E. coli* was above 99% (Figure 3d).

In microalgae production, the composition of the culture medium has a great effect on its performance. One example is the extinction coefficient; this parameter is specific to the microalgae strain and changes with the medium composition, thereby affecting cell weight and biomass concentration (Figure 2a). Experimental data showed an increase in the average irradiance inside the bioreactors when the value of dilution rate was above 0.30 1/day (Figure 2b).

To quantify performance of a strain when using secondary poultry effluent as a culture medium it is necessary to include the quantum yield and the nutrient coefficient yield. These parameters allow determining the behavior and the most efficient microalgae strain to be used on a large scale in external environmental conditions. Results show that under nutrient-sufficient conditions, *S. obliquus* was very efficient; quantum yield was higher when operating at the dilution rate of 0.30 1/day, with values up to $0.33 \pm 0.03 \text{ g}_{\text{biomass}}/\text{E}$ (Figure 4). Moreover, quantum yield increases with increasing the dilution rate in the culture. The increase of cells photosynthetic performance indicated the ability of the culture medium to absorb the photons of radiation.

It was identified that quantum yield reached maximum values of $0.85 \text{ g}_{\text{biomass}}/\text{E}$ are reached when culturing *Scenedesmus obtusiusculus* under N-replete conditions (Cabello et al., 2015), reducing to $0.39 \text{ g}_{\text{biomass}}/\text{E}$ under N-deplete condition. This indicates that the strain is less energy efficient under these conditions. Therefore, this parameter is relevant for scaling-up purposes and to select the most robust strain for outdoor production. Consequently, the efficiency and robustness of *S. obliquus* is confirmed.

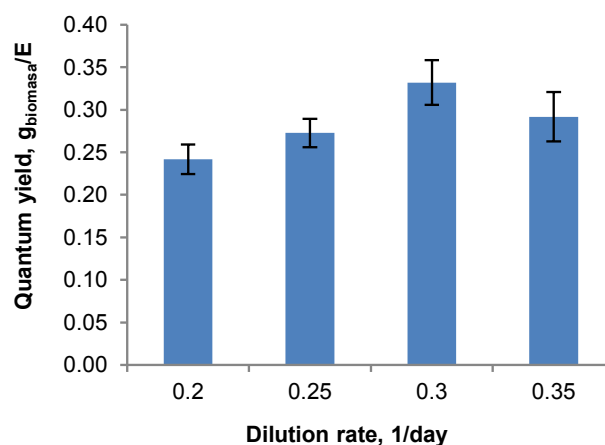


Figure 4. Influence of the imposed dilution rate on the quantum yield in indoors bubble-column-type bioreactors.

Regarding the nutrient coefficient yield for nitrogen and phosphorus under the experimental conditions tested, this was calculated as the amount of biomass produced per mass unit of nitrogen and phosphorus taken up (Figure 5). Results showed that the nitrogen coefficient yield for *S. obliquus* continuously increased when the dilution rate was increased in the culture medium (Figure 5a). Thus, the nitrogen coefficient yield showed the highest value of $49.38 \pm 2.00 \text{ mg}_\text{N}/\text{g}_{\text{biomass}}$ for a dilution rate of 0.35 1/day. In contrast, phosphorus coefficient yield exhibited an inverse trend, the highest value of $32.32 \pm 0.75 \text{ mg}_\text{P}/\text{g}_{\text{biomass}}$ was observed at dilution rate of 0.20 1/day (Figure 5b). When considering nitrogen and phosphorus content in the biomass of 5 and 1% d. wt., respectively, the nitrogen and phosphorus coefficient yield has to be $50 \text{ mg}_\text{N}/\text{g}_{\text{biomass}}$ and $10 \text{ mg}_\text{P}/\text{g}_{\text{biomass}}$, respectively. Thus, experimental values are close to these under nutrient-

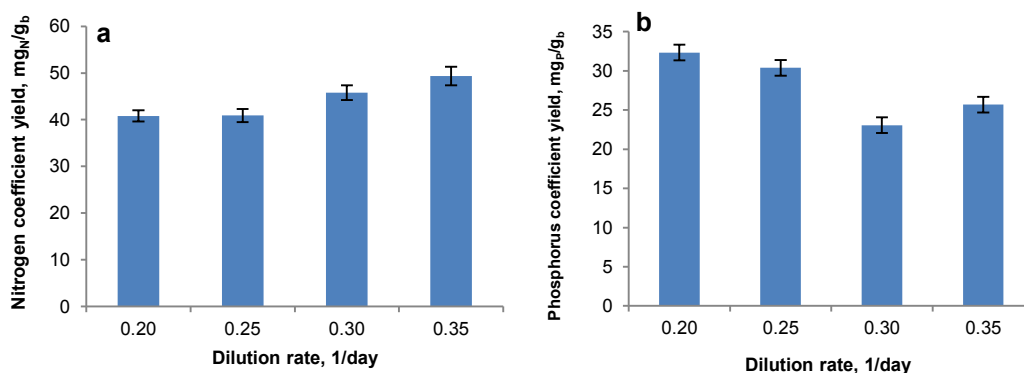


Figure 5. Influence of the imposed dilution rate in the bioreactor on the nitrogen and phosphorus removal in semi-continuous mode. Nitrogen coefficient yield (a) and phosphorus coefficient yield (b).

sufficient conditions independently the dilution rate tested. The data indicates that *S. obliquus* is capable of producing larger amounts of biomass, making it very efficient in terms of nutrient utilization.

With respect to nutrient removal, COD and *E. coli*, the data obtained from the present study demonstrated that *S. obliquus* is capable of removing nitrogen, phosphorus, COD and *E. coli* from the culture medium. Thus, maximal values of 27.65 ± 0.21 mg/L day of nitrogen, 16.59 ± 0.00 mg/L day of phosphorus, 160 ± 1.98 mg/L day of COD and 12000 ± 525 CFU/mL day of *E. coli* were determined for *S. obliquus*. No large data are available regarding the net amount of nutrients, COD and *E. coli* removed from waste, although maximum removal of 8.5 mg/L day were reported for *Chlorella* cultures using diluted effluent 10 times; this value can be increased to 27.65 mg/L day of nitrogen under optimal conditions (Uggetti et al., 2014). A similar trend has been reported for pig manure, with nitrogen removal ranging from 0.5 to 12 mg/L day of nitrogen (Sevrin-Reyssac, 1998). In other case, using *Chlorella* sp. and effluents from municipal wastewater treatment at dilution rate of 0.40 1/day, the highest removal rates of total nitrogen and phosphorus from the liquid phase were 13.0 and 1.4 mg/L day and the maximal decay rate for *E. coli* was 2.9 1/day (Pereira et al., 2019). In order to produce high amount of microalgae biomass at low production costs, it is necessary to use cheap but productive bioreactors and recycle the nutrients from a widely-available source. Therefore, this research demonstrated that for the production of large amounts of *S. obliquus*, poultry effluent from anaerobic digestion can be used as the only nutrient source, achieving high nutrient removal rates and productivities if the system is operated adequately.

5. CONCLUSIONS

It is feasible to produce *S. obliquus* in semi-continuous mode using poultry effluent from anaerobic digestion without dilution as the only source of nutrients. The highest biomass productivity (0.57 g/L day) was achieved at a dilution rate of 0.30 1/day. However, the highest nitrogen, phosphorus, COD and *E. coli* removal rates was achieved at dilution rate of rate 0.20 1/day, operating in semi-continuous mode. In this sense, the selection of dilution rates for microalgae cultivation should be carried out according to the objectives and cost-benefit of the process when both effluents treatment and biomass production are desired.

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