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Influence of volumetric loading rate on aerobic sewage treatment for indigenous algal growth

L. Mendoza, M. M. Aray-Andrade, R. Bermudez, J. Amaya, L. Zhang and C. Moreira

ABSTRACT

Many rural areas of Latin America and the Caribbean (LAC) region are economically depressed. Rural sewage treatment in most areas of LAC is deficient or non-existent. Consequently, the possibility of generating economic revenue from treated sewage is an attractive option for deprived areas of developing countries. Given its peculiar characteristics, rural sewage may be coupled with biological systems such as algae for nutrient cycling. Acceptable algae growth and nutrient elimination were obtained from rural sewage whose treatment may have fallen short of current disposal standards. In this study, aerobic systems working on an 8-month cycle at three different volumetric loading rates (Bv) were assessed in relation to the lifetime growth of three algae strains native to Ecuador. Results indicate *Chlorella* sp. M2 as the optimal algal strain, with the highest growth rate at Bv of 1 g COD L^{-1} d^{-1} and a removal of organic-N (30%), PO_4^{3-}-P (87%) and NH_4^+-N (95%). Concomitantly, the kinetic constants of the sewage resulted in a low biomass yield coefficient, making the proposed system highly suitable for developing countries. Finally, the proposed partial recovery stream method, combining nutrient recovery with economic resource generation, appears to contain great potential.

Key words | aerobic pure sewage treatment, circular economy, nutrient cycling, nutrient removal

L. Mendoza (corresponding author)
M. M. Aray-Andrade
R. Bermudez
J. Amaya
C. Moreira
ESPOL Polytechnic University, Escuela Superior Politécnica del Litoral, ESPOL, Environmental and Chemical Sciences Department (DCQA),
Faculty of Natural Science and Mathematics (FCNM), Center of Renewable and Alternative Energy (CERA), Faculty of Mechanical Engineering and Production Science (FIMCP), Faculty of Maritime Engineering and Sea Sciences (FIMCM),
Campus Gustavo Galindo Km. 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil,
Ecuador
E-mail: *mlmendoz@espol.edu.ec*

L. Mendoza
ESPOL Polytechnic University, Escuela Superior Politécnica del Litoral, ESPOL, Environmental and Chemical Sciences Department (DCQA),
Faculty of Natural Science and Mathematics (FCNM),
Campus Gustavo Galindo Km. 30.5 Vía Perimetral, P.O. Box 09-01-5863, Guayaquil,
Ecuador

L. Zhang
State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resources and Environmental Engineering,
East China University of Science and Technology,
Shanghai 200237,
China

INTRODUCTION

Rural areas of the Latin America and the Caribbean (LAC) region have limited capacities in sewage treatment systems and suffer from a lack of opportunities to expand their economy (Sparkman & Sturzenegger 2016). In developing countries, up to 90% of sewage is discharged without proper treatment, affecting mainly the poor. Water is a very important concern as it is interwoven with sustainable development issues. Therefore, any possibility of obtaining economic revenue from treated sewage of emergent nations is worth investigating.

Sewage treatment has been widely studied, both from the perspective of the chemical and biological processes involved and with reference to the source; that is, urban, synthetic, separated and mixed pipelines, contaminated with agricultural runoff or industrial water, among others (Aiyuk & Verstraete 2004; Aiyuk *et al.* 2004). Nevertheless, only a few studies touch upon the behaviour of the aerobic microbial population growing in rural domestic sewage of developing countries. The organic fraction of the solid waste stream in these countries is considerably higher (from 40% to 80% of the total) compared to those from developed nations (Hoornweg & Bhada-Tata 2012; Ozcan *et al.* 2016). This strongly suggests an organic sewage composition derived mainly from kitchen food residues and

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preparation. The widespread use of non-processed organics, mainly fruits and vegetables, in developing countries results in sewage with less oxidized organic matter, higher biochemical and chemical oxygen demand (BOD/COD) ratio, peculiar micronutrients and vitamin concentrations (folic acid being of special interest) (Selimoğlu *et al.* 2015). This may eventually affect kinetics, and particularly, the biomass yield (Velho *et al.* 2016).

Algae have become a potential raw material in the production of locally valuable resources (animal feed, starch, pigments). There is, consequently, some economic potential in coupling traditional biological wastewater treatments with native algae cultivation. From Abdel-Raouf *et al.* (2012), micro-algae culture offers a promising tertiary bio-treatment step, involving the production of potentially valuable biomass. Still, optimising volumetric organic loading rates (Bv) in sewage treatment to favour algae growth is currently an underresearched area (Zamalloa *et al.* 2012). The present study aims to identify a particular Bv that promotes optimal algae growth via coupling of partially biologically treated rural sewage. Importantly, the algae used should be resistant to the local solar energy radiation, as well as to the presence of competitors.

MATERIALS AND METHODS

Wastewater characteristics and collection

The sewage used in the present study comes from a countryside community (27 °C mean temperature and 140 m.a.s.l) located 100 km east of Guayaquil, Ecuador. Due to cyclic variations in its organic load, samples were taken from the community collector during a 1.5-hour daily interval representative of the highest COD load (352 ± 32 mg COD L^{-1}). Thus, in case of extrapolation of this study to a pilot or industrial scale bio-refinery facility, bulk water with lower organic load (80 ± 50 mg L^{-1}) could be treated with any other less energy-demanding technique.

The physical-chemical characteristics of rural sewage (Table 1) were determined according to *Standard Methods for the Examination of Water and Wastewater* APHA/AWWA/WEF (2012): 5220 B for COD; 5210 B for BOD; 2320 B for alkalinity; 2540 B for total solids (TS); 2540 D for total suspended solids (TSS) and total dissolved solids (TDS); 2540 E for total fixed solids (TFS), fixed suspended solids (FSS), fixed dissolved solids (FDS), total volatile solids (TVS), volatile suspended solids (VSS) and volatile dissolved solids (VDS); 4500-P D for phosphorus; 4500-NO_3^- E and 4500-NO_2^- B for nitrate and nitrite, respectively; 4500-NH_3 C for ammonia; 4500-N_{org} B and direct colorimetric nesslerization for organic nitrogen. pH values were measured using a 510 series Oakton benchtop meter.

Table 1 | Physical-chemical characteristics of the rural sewage samples under study ($n = 32$)

Parameter	Unit	Value
pH	–	7.3 ± 0.3
COD	mg L^{-1}	352 ± 32
BOD	mg L^{-1}	330 ± 21
sCOD[a]	mg L^{-1}	196 ± 34
Alkalinity	mg L^{-1}	310 ± 19
TAN[b]	mg L^{-1}	32 ± 3.7
Nitrate	mg L^{-1}	0.4 ± 0.2
Nitrite	mg L^{-1}	<0.01[c]
TKN[d]	mg L^{-1}	51 ± 4
TS	mg L^{-1}	438 ± 23
TDS	mg L^{-1}	355 ± 35
VSS	mg L^{-1}	39.3 ± 8
PO_4^{-3}-P	mg L^{-1}	8.21 ± 2

[a] Soluble COD.
[b] Total ammonia nitrogen (NH_4^+-N + NH_3-N).
[c] The lowest detection limit.
[d] Total Kjeldahl nitrogen (organic, NH_3 and NH_4^+ nitrogen).

COD, TS, TDS, and TSS were compared with those values of urban sewage.

Treatment of rural sewage with the highest COD

The experimental design for the treatment of rural sewage is shown in Figure 1.

Aerobic systems setup

Three aerobic systems were constructed (S1, S2 and S3). For each system (Figure 1, from c to f), the homogenisation compartment was equipped with overflow and the secondary clarifier with a siphon for the discharge of treated water. The 10 L aerobic compartment (34.5 cm (L) × 20.5 cm (W) × 14 cm (H)), was equipped with three air diffusers located at the bottom and arranged in parallel to keep the oxygen level above 2 mg O_2 L^{-1}. Additionally, the diffusers were covered with a perforated plate to ensure a uniform distribution of bubbles.

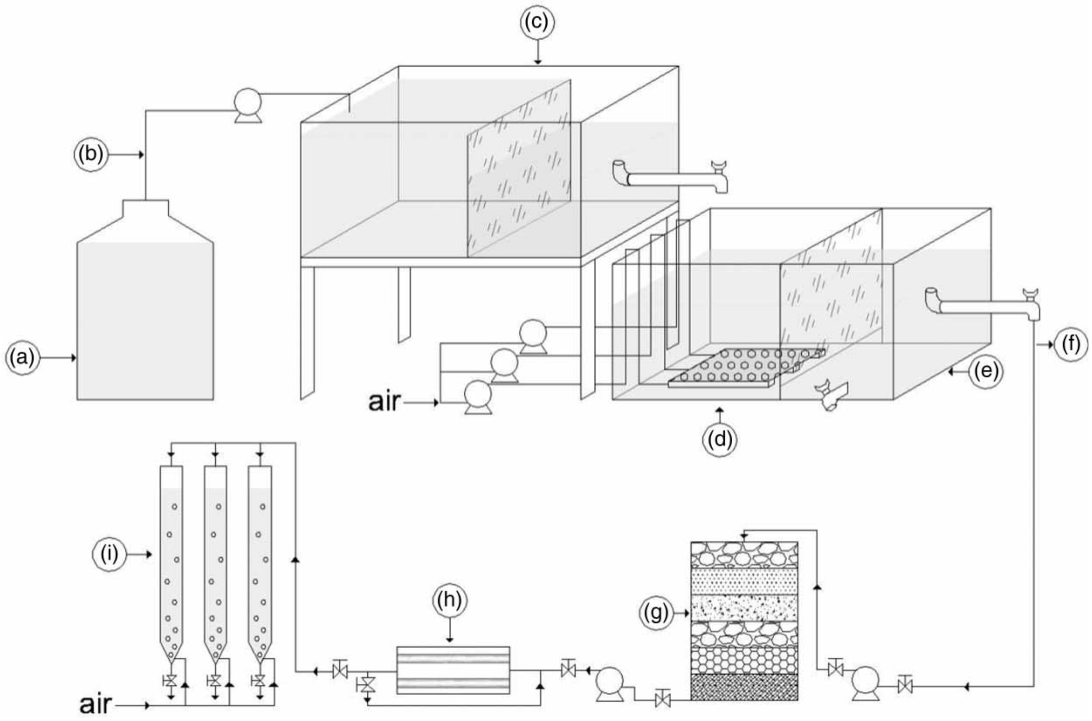

Figure 1 | Schematic setup of the integrated treatments used in the present study. (a) Storage tank, (b) influent, (c) homogenization compartment, (d) aerobic compartment, (e) secondary clarifier, (f) effluent, (g) multilayer filter, (h) UV-light chamber, (i) photobioreactors.

Aerobic systems operation

S1, S2 and S3 operated simultaneously at 25 ± 2 °C for about 8 months in continuous-flow mode involving extended aeration. In order to select the range at which COD removal rates took place, a wide range of Bv were tested in advance. Finally, the chosen values were 0.7, 1.0 and 1.4 g COD $L^{-1}d^{-1}$; the mixed liquor volatile suspended solids (MLVSS) were 1,390, 1,360 and 1,290 mg VSS L^{-1}, for S1, S2 and S3, respectively. The pH in the aeration tanks was adjusted to 7.6 ± 0.1.

The kinetics of sewage were computed based on the usual imposed and measured parameters.

Sludge characteristics and excess biomass (ΔX)

In the initial stages, S2 and S3 showed negligible biomass growth, while S1 showed an appreciable growth (from 39 to 7,600 mg VSS L^{-1}). After 3 weeks, the activated sludge appeared fluffy, sticky and highly settleable in all systems. In order to have similar MLVSS concentrations, the sludge from S1 was equitably distributed in S2 and S3.

Due to non-homogeneity and physical irregularities of the sludge flocs, the excess biomass (ΔX) was determined by measuring the entire biomass present in each system (own method). For that, the total biomass was poured into Imhoff cones, decanted over 20 minutes, and the surplus sludge taken away, thus achieving the required MLVSS. The sludge density (1.12, 1.1 and 1.0 g cm^{-3}, for S1, S2 and S3, respectively) was previously determined and correlated with the VSS levels.

Use of partially treated wastewater as a medium for growth of native algae

A multilayer filter (Figure 1) composed of quartz, activated carbon, white, red and medical sandstone was used to remove debris from the aerobic effluents. Afterwards, UV-light was used to eliminate ciliates. Effluents S1, S2 and S3, free from debris and ciliates, were used as a medium to grow three algal strains native from Ecuador, viz. *Chlorella* sp. M2, *Chlorella* sp. M6 and *Scenedesmus* sp. R3 (NCBI accession number: MF677855, MF677856 and MF677857, respectively). Previously, they were isolated and propagated as described in Aray-Andrade *et al.* (2019).

The algae were cultivated in triplicate for 7 days, outdoors, with an initial concentration of 630 algal cells ml^{-1}, using 3-liter glass-made cylindrical (115 cm in height

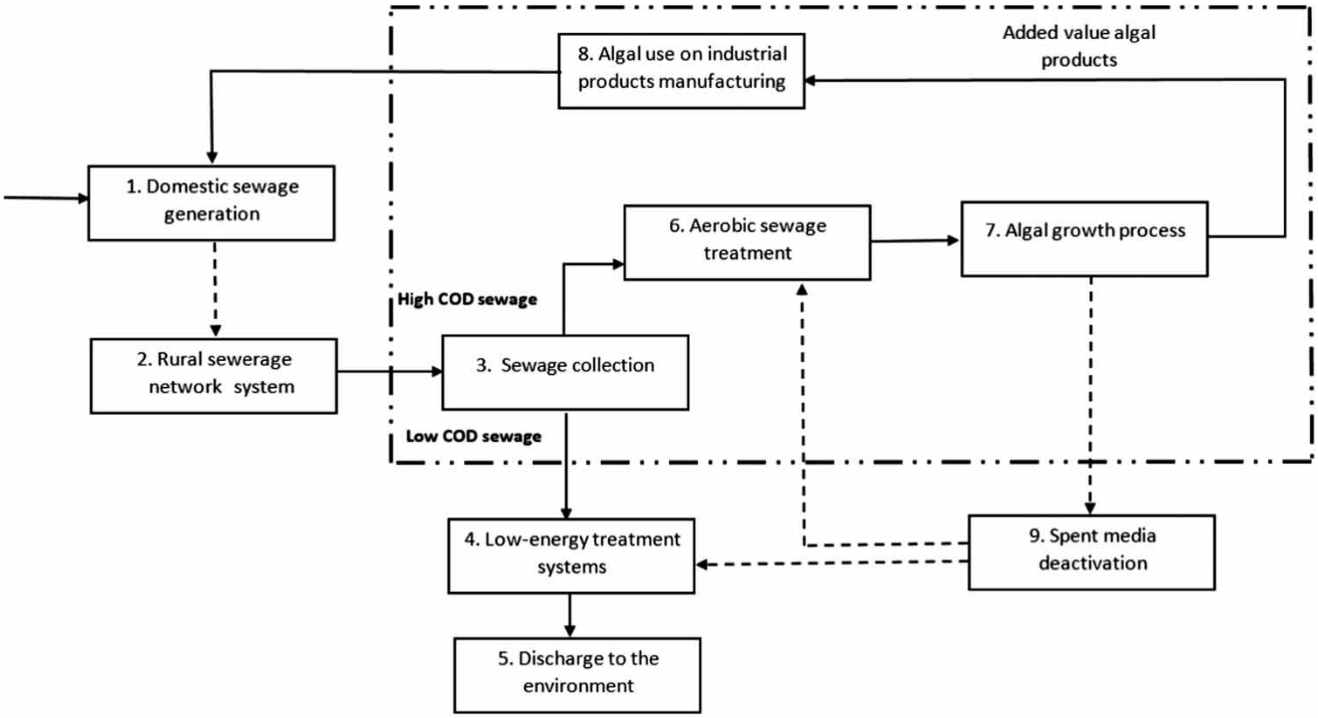

Figure 2 | Proposed stages in the partial stream recovery concept for rural sewage in developing countries.

and 7 cm in diameter) photo-bioreactors. Growth rate was determined by microscopic counting and expressed as cell $\mathrm{ml^{-1}}$. Agitation was provided by air bubbling, using a 1 L $\mathrm{min^{-1}}$ flow. Algae biomass was harvested by filtration using glass microfiber filters PALL 61631 Type A/E and a Whatman GF/D (pore size 1 and 27 μm, respectively), according to cell size.

The mean temperature registered for the site was 37 °C; however, the greenhouse effect raised it inside the photo-bioreactor to about 46 °C. Conversely, in summer, the temperature decreased to about 18 °C.

Evaluating the potential of coupling biological systems for conversion of rural sewage to resources

This cleaner production approach differs from the typical linear sequence of sewage treatment (i.e. collection, low energy demand biological treatment, clarification and disposal) in that it includes the cycling of sewage nutrients for algae production, as shown in Figure 2. In view of this, stage 6 must work at the selected Bv optimal for algal growth and consequently, to a high organic load. The spent media in process 9 must be adequate to fulfil the

Table 2 | Influent and effluent COD and nutrients levels of the sewage entering the systems of study

	System 1		System 2		System 3	
Parameter	**Level mg $\mathrm{L^{-1}}$**	**Removal (%)**	**Level mg $\mathrm{L^{-1}}$**	**Removal (%)**	**Level mg $\mathrm{L^{-1}}$**	**Removal (%)**
$\mathrm{COD_i}$	352 ± 38.2	89	352 ± 38.2	72	352 ± 38.2	43
$\mathrm{COD_e}$	39 ± 2.2		98.5 ± 6.2		151 ± 8.3	
$\mathrm{NH_4^+\text{-}N_i}$	32.2 ± 3.7	76	32.2 ± 3.7	62	32.2 ± 3.7	46
$\mathrm{NH_4^+\text{-}N_e}$	7.7 ± 0.7		12.2 ± 1.3		17 ± 3.4	
$\mathrm{Org\text{-}N_i}$	12.2 ± 2.1	98	12.2 ± 2.1	96	12.2 ± 2.4	93.7
$\mathrm{Org\text{-}N_e}$	0.28 ± 0.02		0.45 ± 0.1		0,76 ± 0.1	
$\mathrm{PO_4^{3-}\text{-}P_i}$	8.21 ± 1.2	99	8.21 ± 1.2	98.2	8.21 ± 1.2	97.3
$\mathrm{PO_4^{3-}\text{-}P_e}$	0.1 ± 0.03		0.15 ± 0.02		0.22 ± 0.01	

i: influent. e: effluent.

above-mentioned parameters in order to be recycled to stage 6 or sent to either stage 4 or stage 5 directly. Finally, stages 8 and 9 would depend on the location, on further processes applied to the cultivated algae, and on the characteristics of the spent media, which were not part of the present study.

RESULTS AND DISCUSSION

Solid analysis of rural sewage

From the TS mean value (438.3 ± 43 mg L^{-1}, Table 1), 123.3 ± 9 mg L^{-1} (28.2%) corresponded to TFS and 315 mg L^{-1} (71.8%) to TVS. TSS was 83.3 mg L^{-1} (19%) and TDS, 355 mg L^{-1} (81%). Roughly, 53% of TSS was VSS (44 mg L^{-1}). TDS resulted in 56.85 and 43.1% for VDS and FDS, respectively; indicating that most of the solids were soluble and organic. In addition, the distribution of organic and inorganic matter in TSS was roughly 50%. Rural TS values scored lower than urban sewage values (652 ± 34 mg TS L^{-1}), which approximated to those reported by Van Haandel & Lettinga (1994), studying sewage in Latin American cities. In the present study, urban sewage TS were 48.5% TVS, which means a lower content in organics, compared to rural sewage. Moreover, the percentage of urban TDS was higher than TSS, but much less than rural sewage. These differences are attributable to the presence of more stabilized organic matter as sewage stays longer in the main collectors.

COD, Bv and nutrients in the systems

From Table 1, COD values of rural sewage (high COD load in the present study) were much lower than those found for urban sewage (620 ± 53 mg L^{-1}); urban sewerage systems could be exposed to other kinds of organic matter.

An inverse correlation was observed between both the influent Bv (in a range from 0.7 to 1.4 g COD L^{-1} d^{-1}) and organic loading rate (Bx, from 0.5 to 1.1 g COD g^{-1} DW d^{-1}), with the effluent COD values, attributable to the effect of higher biomass-substrate contact.

From Table 2, the mean NH_4^+-N removal levels of 76%, 62% and 46% obtained for S1, S2 and S3 respectively, are a balance between aerobic transformation of organic-N to ammonium, and the remaining ammonium levels initially present. Ammonium levels were not greatly reduced, contrary to what might be expected in extended aeration, perhaps owing to the high ambient temperature typical of tropical countries. On the other hand, S1, S2 and S3 showed significant organic-N removal rates.

Table 3 | Influents and effluents nutrient values for microalgae cultures using cylinder type photo-bioreactors at 32 ± 2 °C

	NH_4^+-N (mg L^{-1})				Organic-N (mg L^{-1})				PO_4^{3-}-P (mg L^{-1})			
		E[(2)]				E				E		
	I[(1)]	*Chlorella* sp. M2	*Chlorella* sp. M6	*Scenedesmus* sp. R3	I	*Chlorella* sp. M2	*Chlorella* sp. M6	*Scenedesmus* sp. R3	I	*Chlorella* sp. M2	*Chlorella* sp. M6	*Scenedesmus* sp. R3
S_1	7.7 ± 0.3	0.52 (93.2)[(3)]	0.54 (93.0)	0.60 (92.2)	0.30	<0.05[(4)]	<0.05	<0.05	0.1	0.03 (70)	0.03 (70)	0.05 (50)
S_2	12.2 ± 3	0.67 (94.5)	0.67 (94.5)	0.28 (97.7)	0.4	0.28 (30)	0.3 (25)	1.5	0.15	0.02 (86.6)	0.03 (80)	0.16 (8.8)
S_3	17.2 ± 3	4.65 (72.9)	5.60 (67.4)	0.8 (95.3)	0.76	0.65 (14.5)	0.224 (70.5)	1.2	0.22	0.08 (63.7)	0.22	0.1 (54.5)

(1) Influent (2) Effluent (3) Percentage of removal (4) The lowest detection limit.

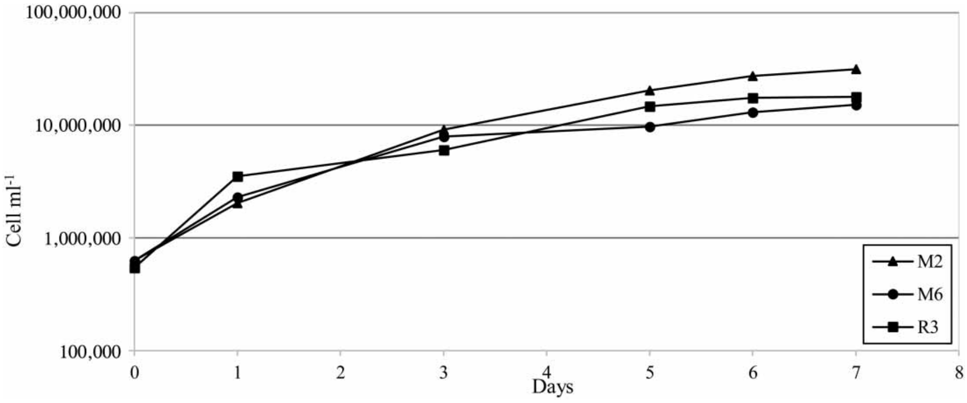

Figure 3 | Growth curve of *Chlorella* sp. M2, *Chlorella* sp. M6 and *Scenedesmus* sp. R3.

The lowest COD and NH_4^+-N removal rates were observed for S3 at the highest Bv of the study. Thus, this system reflected the low solids retention time applied and consequently a poor bacteria-substrate contact. As with COD and NH_4^+-N, a high removal of P was also observed for all the systems, with S3 the least effective in this respect (from 8.21 ± 1.2 mg to 0.2 mg PO_4^{3-}-P L^{-1}).

Kinetic constants

The organic loading rate (ϴx) applied to the systems ranged from 0.51 to 1.1 g COD g $DW^{-1}d^{-1}$, resembling an extended aeration mode. However, atypically for this mode, good sludge settleability was observed in the secondary clarifier. The maximum substrate utilization rate (q_{max}), half-saturation constant (K_s) and decay coefficient (k_d) of rural sewage were 0.69 d^{-1}, 0.021 g L^{-1} and 0.136 d^{-1}, respectively. The growth yield coefficient, 0.32, was lower than the usual values from urban sewage (0.4–0.6) commonly reported (El-Seddik 2017). Velho *et al.* (2016) obtained low Y values for sewage rich in vitamins and minerals, especially after folic acid addition. Senorer & Barlas (2004) found higher efficiency in treatments after progressive folic acid dosage to sewage. Moreover, from Fujii *et al.* (2010), algae harvesting effluents contain amino acids, vitamins and folates, among others, that would magnify bacterial metabolic activity with consequent improved COD removal.

Algal growth

From Table 3, NH_4^+-N removal was the highest (97.7%) when *Scenedesmus* sp. R3 grew on the S2 effluent. On the other hand, organic-N showed the highest removal rate, 70.45%, when *Chlorella* sp. M6 was fed on the S3 effluent; also, PO_4^{3-}-P removal rate was the highest when Chlorella sp. M2 was fed on the S2 effluent. *Scenedesmus* sp., growing on wastewater, reported 96% P removal and 97% NH_4^+-N removal (Acevedo *et al.* 2017). Praveen & Loh (2016) reported a NH_4^+-N removal efficiency of 93% by *Chlorella vulgaris* using wastewater as growth medium at 6.16 mg NH_4^+-N L^{-1}. This initial value appears close to that found in S1. Similarly, Chan *et al.* (2014) using *Chlorella* sp. reported higher ammonia removal in secondary sewage at initial concentrations of 12 mg L^{-1}, comparable to S2 and a PO_4^{3-}-P removal rate of 50% at initial levels nearly to 0.2 mg L^{-1}, similar to S1. P levels in effluents are very low. Interestingly, Bowman *et al.* (2007) showed that low *P*-values in the range of 0.1–5.6 μg L^{-1} did not interfere in the attainment of good algae growth.

Table 3 also shows the lowest removal rates for NH_4^+-N when effluent S3 (highest Bv) was used as growth media. Probably, this was due to bacterial mediated algal cellular lysis (Bolch *et al.* 2017) or excretion of small organic molecules, which may affect nutrient removal rates (Wang *et al.* 2009). Contrarily, the removal rate of organic-N does not follow a pattern and varies depending on the algal specie and the effluent used. The growth rate of the three algal species used was consistent among species (Figure 3). The growth rate of *Chlorella* sp. M2 ($\mu = 0.56 \pm 0.02$ day^{-1}) was comparable to *Chlorella* sp. (0.53 day^{-1}, 26 ± 1 °C) (Min *et al.* 2011). *Chlorella* sp. M6 ($\mu = 0.45 \pm 0.04$ day^{-1}) growth rate was comparable to C. Zofingiensis ($\mu = 0.49$ day^{-1}) when using piggery wastewater with an initial COD of 400 mg L^{-1} as media (Zhu *et al.* 2013). Finally, the growth rate of *Scenedesmus* sp. R3 ($\mu = 0.50 \pm 0.01$ day^{-1}) was similar to the findings of Latiffi *et al.* (2017) in *Scenedesmus* sp. ($\mu = 0.44$ day^{-1}). Thus, the suitability of the collected, isolated, propagated and cultivated native species is remarkable; growth rates of native algae on the selected medium with a range of temperatures between 18 and 46 °C were very similar to those of closely related species, growing at a controlled temperature.

CONCLUSIONS

Of the three algal strains tested, *Chlorella* sp. M2, cultured in cylindrical-type photo-bioreactors, showed the highest growth rate. For this, the optimal Bv was 1 g COD L^{-1} d^{-1} attaining a removal of 72% COD, 30% organic-N (30%), 87% PO_4^{3-}-P, and 95% NH_4^+-N. Interestingly, the proposed system resulted in a low biomass yield coefficient of 0.3, possibly due to the peculiar characteristics of the substrate.

The partial stream recovery concept proposed in this study presents itself as a promising strategy for nutrient capture and reduction of organic load peaks. The method allows for extraction of the highest organic portion of daily sewage (advantageously present in low volume) for algae growth while leaving the lower bulk contamination for less energy demanding treatments. To maintain suitable parameters for algal growth, the spent media could be either re-circulated or sent straight to a low energy final treatment.

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