Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal

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HIGHLIGHTS

▶ Microalgal production is economically viable when coupled to wastewater treatment.
▶ Testing wastewater effluents at 5 distinct stages of depuration for growing microalgae.
▶ Combining industrial approaches enhances microalgae production with economic gain.

ARTICLE INFO

ABSTRACT

The streams from municipal wastewater treatment plants (WWTP) have been considered a valuable medium for mass cultivation of algal biomass. The aim of this work is to test and compare the performance of *Chlorella vulgaris* on several streams from five stages, from two different WWTP. The results showed biomass yields ranging from 39 to 195 mg dry-weight l^{-1} days^{-1}. The best performance as biomass production was obtained with the centrate (effluent from drying the anaerobic sludge). After testing a wide range of N/P ratios with centrate, the highest productivity and growth rates were obtained with the original N/P ratio (2.0) of this stream. The highest removal rates were of 9.8 (N) and 3.0 (P) mg l^{-1} - days^{-1}, in the centrate. Finally, this research also suggests that microalgal production seems to be a promising process when coupled to wastewater treatment.

1. Introduction

The demand for clean water is currently a worldwide priority. Currently, the main challenge of a wastewater treatment plant (WWTP) is not only to produce reusable clean water, but it is also to find resources for supporting those new developments. The current global investment in water management is about €150 bilion per year, of which 95% is public capital (Krozer et al., 2010). WWTPs commonly offer a treatment composed of several stages based on physical, chemical and biological methods (Carey and Migliaccio, 2009). Nevertheless, they can often remove only a fraction of the total nitrogen and phosphorous present in the effluents (Rawat et al., 2011).

New treatment systems have been developed to improve depuration of water, but there are still not cost competitive (Rawat et al., 2011). For instance, improved technology for removing nutrients would require an increase in energy consumption of about 60–80% (Lam and Lee, in press). The total global amount of investments is still considered insufficient to meet present needs, especially in low-income municipalities or in under developed countries (Krozer et al., 2010). A novel and revolutionary approach, however, would be to combine wastewater treatment with the production of renewable energy, from algal biomass. Biomass can be applied for energy production in two types of utilization: direct (heat and electricity) and indirect (transportation fuel conversion) (Mizsey and Racz, 2010).

The costs of biomass production can be significantly reduced if another source of nutrients is used instead of the expensive artificial amendments (Sheehan et al., 1998). Although, identified as ideal sources of such nutrients (Rawat et al., 2011), WWTP streams must be considered by their complex and variable nature and potential toxicity effects at distinct stages of the treatment. Thus, it is

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very important not only to investigate algal toxic response and biomass yields when using such streams, but it is also paramount to test distinct streams, which are commonly obtained at different stages of the wastewater treatment process.

This innovative approach may represent a more sustainable alternative for this sector (Rawat et al., 2011; Park et al., 2011; Chan et al., 2011; Ras et al., 2011). In this model, the cost of treatment is counterbalanced with the production of biomass that can generate energy and valuable products, that in turn can also cause a significant economic impact in the society (Rawat et al., 2011; Mizsey and Racz, 2010). However, such studies are still limited (Chan et al., 2011) and there is very limited knowledge in regard to the biological potential and process operation of such living systems. The goal of this research is to contribute to the advancement of this area.

The aim of this work is to evaluate the effect of different WWTP streams as a source of nutrients for producing biomass of the green microalgae *Chlorella vulgaris* SAG211-12. This is an organism known for being capable of producing byproducts, which are valuable for the biofuel industry (Nascimento et al., 2012); and it has also already been used to improve effluent treatment in WWTP (Kumar et al., 2011; Ruiz et al., 2011). Thus, this research is targeting an ideal organism that is capable of bridging the gap between improving nutrient removal of WWTP systems and the potential production of renewable energy.

2. Methods

2.1. Microorganism and culture conditions

For this experiment *C. vulgaris* strain SAG211-12 was used, acquired from Sammlung von Algenkulturen, Pflanzenphysiologisches Institut (Universität Göttingen, Germany). The inoculum was prepared batchwise using sterilized Combo medium (Kilham et al., 1998). The inoculum was maintained in 2 l borosilicate bioreactors. The operational conditions were: constant aeration and mixing (1 v/v/m) with filtered air (0.2 μm), photoperiod of 14:10 light:dark cycles, 150 μmol/m^2/s of luminance and incubated at a controlled temperature of 20 ± 1 °C.

2.2. Experimental set-up

The trials were carried out in 2 l borosilicate bioreactors, operated at constant aeration and mixing (1 v/v/m) with filtered air (0.2 μm), with a photoperiod of 14:10 light:dark cycles, 150 μmol/m^2/s of luminance and incubated at a controlled temperature of 20 ± 1 °C. All samples of WWTP streams were used the way they were supplied from the WWTPs, immediately after chemical characterization.

The experimental planning can be overviewed in Fig. 1, which shows that each trial was carried out with distinct streams from different treatment processes; such as: (i) the pretreated effluent from WWTP 1 and 2, (ii) anaerobic treatment effluent from WWTP 2 (the WWTP1 does not have this step of treatment), (iii) the final disposing effluent (WWTP 1 and 2), (iv) the effluent from primary settler (EPS) from WWTP1, and (v) the effluents from the centrate, WWTP2; (i.e. from the drying sludge).

The first trial was designed to evaluate the effect of different wastewater streams at distinct treatment stages from two wastewater treatment plants (WWTP) operating in the Provinces of Cadiz (1) and Sevilla (2), Spain, respectively (Fig. 1).

The second trial was carried to compare the growth kinetics of microalgae within a consortium including inoculated bacteria from activated sludge in the effluent from the primary settler. The third trial evaluated the feasibility of using the centrate, a stream with very high concentration of nutrient, as medium and also to

evaluate the effect of distinct N/P (from 0.8 to 15) ratios over the cultivation.

2.3. Analytical methods

Algal biomass was assessed daily by means of optical density (680 nm) and samples were diluted with the appropriate ratios in order to ensure that the measured optical density values were assessed within a range of 0.1–1. Algal dry weight was determined (daily) gravimetrically according to standard method 2540-D (APHA, AWA, WPCF 1992). The reactors were monitored daily for qualitative microscopic evaluation (*C. vulgaris*) along with pH and temperature. Total suspended solids were also analyzed for the composition ratios of C, H, N, S in duplicate using an elementary analyser (LECO CHNS-932, Leco Corporation). The phosphorus content was analysed by means of acid digestion of the dry biomass (in duplicate) in a microwave digester (ETHOS 1600, Milestone); and total phosphorus was determined by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES, Iris intrepid, Thermo Elemental).

Wastewater was sampled at the start and at the end of the experiments (Table 1) for the analyses of ammonium (N-NH$_4$), nitrate (N-NO$_3$), nitrite (N-NO$_2$), total nitrogen (TN), phosphate (P-PO$_4$) and total phosphorus (TP). Samples were filtered through a fiber filter of 0.7 μm pore diameter (WHATTMAN, GF-F) to separate the biomass. Soluble COD was determined according to Standard Methods 5220-D (APHA, AWWA, WEF, 1992). Total nitrogen and total phosphorus were measured colorimetrically as nitrate (N-NO$_3$) and phosphate (P-PO$_4$) after the sample had been oxidized. The determination carried out by dissolving 1.5 microspoons of Oxisolv® (Merck KGaA, Darmstadt, Germany) in 10 ml of sample, then incubating at 100 °C for 1 h to completely oxidize the phosphorus and nitrogen (APHA, AWA, WPCF 1992). After being allowed to cool, nitrate determinations were performed using a Spectroquant® test kit (Cod. 1.14773.0001 (Merck)), and phosphates were determined according to the method 4500-P E (APHA, AWWA, WEF, 1992).

2.4. Data analysis

Growth kinetics were resolved in SigmaPlot® v.12 using a sigmoidal curve model (Chinalia et al., 2008). The software is also equipped with a statistical package for testing the fitness of the used model, and the results are expressed as probability ($p < 0.05$). The kinetic parameters were also crosschecked using linear regression of the exponential phases of each curve individually. This approach was applied on the experimental data and their natural logarithmic (Ln) transformed values, for crosschecking the calculations of productivity and growth kinetics, respectively.

For statistical analysis it was also applied linear regression (with at least five points) for assessing correlations between variables (R^2 available in the text). The estimated market values were calculated with references presented in Table 4 and were used to estimate the investments and gains at a full-scale scenario. The productivities (y^{-1}) were calculated based on the results carried out in the present work scaled-up with full-scale scenario estimates. Ruiz et al. (2013), stated that batch cultures can be used for estimation under continuous conditions with satisfactory prediction.

3. Results and discussion

3.1. Biomass production

In order to compare the performance of *C. vulgaris* SAG211-12 under all experimental conditions above described (Fig. 1), growth
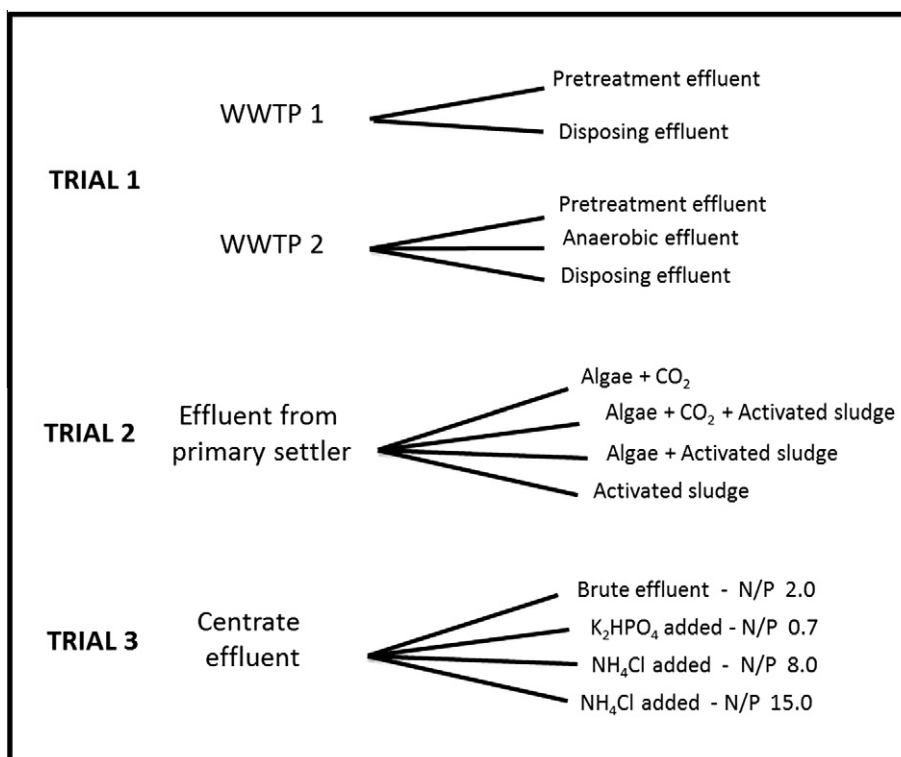
Fig. 1. Experimental design of the trials carried out in the present work. The acronyms refer to wastewater treatment plants (WWTP), nitrogen (N) and phosphorus (P).

Table 1
Results of the physical and chemical parameters analysed in different wastewater streams.

	Pretreated urban wastewater WWTP1	Pretreated urban wastewater WWTP2	Anaerobically treated wastewater WWTP2	Disposing effluent WWTP1	Disposing effluent WWTP2	Effluent from primary settler (EPS-WWTP1)	Centrate I (WWTP2)	Centrate II (WWTP2)	Centrate III (WWTP2)	Centrate IV (WWTP2)
pH	8.0	8.1	7.8	7.5	8.1	7.2	6.9	7.1	6.5	6.5
Conductivity ($\mu S\ cm^{-1}$)	1112	1073	1141	995	700	961	1939	1939	1939	1939
SS (mg l^{-1})	95.00	359.0	65.0	21.0	19.0	400.0	140.0	140.0	140.0	140.0
Turbidity (NTU)	113.0	183.0	95.0	8.00	7.00	55.0	73.0	73.0	73.0	73.0
COD (filtered) (mg l^{-1})	150.0	180.0	90.0	90.0	100.0	160.0	675.0	675.0	675.0	675.0
TP (raw) (mg l^{-1})	8.91 ± 0.38	8.81 ± 0.15	9.07 ± 0.24	2.72 ± 0.07	0.75 ± 0.04	5.08 ± 0.2	180.81	60.49 ± 1.7	60.49 ± 1.7	60.49 ± 1.7
TP (filtered) (mg l^{-1})	6.07 ± 0.26	5.93 ± 0.18	7.51 ± 0.16	2.38 ± 0.1	0.76 ± 0.02	3.20 ± 0.1	175.33	55.01 ± 1.0	55.01 ± 1.0	55.01 ± 1.0
P – PO$_4^{3-}$ (filtered) (mg l^{-1})	4.93 ± 0.06	4.89 ± 0.12	7.69 ± 0.39	2.12 ± 0.08	0.68 ± 0.01	1.7 ± 0.1	154.41	35.3 ± 1.5	35.3 ± 1.5	35.3 ± 1.5
TN (raw) (mg l^{-1})	88.47 ± 3.18	52.08 ± 9.48	64.14 ± 7.83	34.61 ± 1.25	9.79 ± 0.42	35.6 ± 1.0	130.1 ± 1.4	130.1 ± 1.4	471.00	909.9
TN (filtered) (mg l^{-1})	84.42 ± 2.65	41.96 ± 5.47	65.65 ± 1.43	36.44 ± 1.93	10.03 ± 0.33	33.9 ± 0.83	123.9 ± 1.5	123.9 ± 1.5	464.80	903.7
N-NH$_4^+$ (filtered) (mg l^{-1})	80.5 ± 6.62	39.55 ± 4.21	48.79 ± 5.46	23.34 ± 2.04	4.06 ± 1.23	30.6 ± 0.1	125.1 ± 2.1	125.1 ± 2.1	466.04	904.9
N-NO$_3^-$ (filtered) (mg l^{-1})	2.94 ± 0.60	<0.5	<0.5	7.23 ± 0.9	7.03 ± 0.23	<0.5	<0.5	<0.5	<0.5	<0.5
N-NO$_2^-$ (filtered) (mg l^{-1})	0.18 ± 0.23	0.02 ± 0.01	0.02 ± 0.01	1.26 ± 0.21	0.27 ± 0.01	<0.02	<0.02	<0.02	<0.02	<0.02
N/P	10.0	6.0	7.0	13.0	13.0	7.0	0.7	2.0	8.0	15.0

curves were plotted, with the graphs showing the values of biomass (as dry weight) versus time (in days) (Fig. 2). In all treatments, the microalgae showed a typical batch growth with an exponential phase of about 5–8 days during the 12 days of

incubation (Fig. 2). It was assumed that the results do not show a significant lag phase for any of the trials because the inocula were carefully prepared before each experiment (Fig. 2). The slopes of the curves suggested that EPS effluents were the less suitable for biomass production generating lower yields and growth rates (39.28–87.4 mg l^{-1} days^{-1} and 0.11–0.19 days^{-1}, respectively, Table 2). The microalgae responded rapidly to the centrate effluents with maximum growth rates between 0.23 and 0.38 days^{-1} within the period of 5–6 days during incubation (Table 2). It was also observed that microalgae had high productivity rates with pretreated effluents (WWTP 1 and 2) and the effluent of the anaerobic digester (115.7–128.2 mg l^{-1} days^{-1}, Table 2). In general, C. vulgaris showed in the best conditions yields varying from 115 to 195 mg l^{-1} days^{-1} with streams from pretreatment, from anaerobic digester and the centrate. Feng et al. (2011) reported values from 44 to 147 mg l^{-1} days^{-1} for C. vulgaris grown in artificial wastewater. Thus, this study indicates that C. vulgaris SAG211-12 may excel the prediction made using artificial media. This is a positive result for the feasibility of large-scale production of microalgae, once the utilization of a residue as substrate is proposed. Regarding the heat production from algal biomass, it may show calorific values of about 29 kJ g^{-1} (Iiiman et al., 2000). If intended, the generation of energy from methane, anaerobic digesters operated with such biomass at 1 g COD l^{-1} may produce it at a rate of 240 mL g$^{-1}_{vss}$ (Ras et al., 2011). Scaling-up predictions such those in as Chisti (2008) stated that, for well design microalgae cultivation systems, it is possible to reach biomass production close to 1535 kg m^{-3} days^{-1} in tropical zones. Assuming an average of 30% oil content in algal biomass (123 m^3/ha/year), the author estimated a biodiesel production of 98.4 m^3/ha/year.

The best productivities were observed with centrate effluent II and III. Centrate II effluent was used in nature (N/P ratio of 2, initial ammonium at 130 mg l^{-1}) and centrate III was amended with nitrogen in order to test a N/P ratio of 8 (ammonium at final concentration of 471 mg l^{-1}). The results suggested that nutrient amendments are not necessary. Centrate effluents are produced during the drying period of the anaerobic sludge and they are often reintroduced into the aerobic phase. This effluent is therefore considered as inappropriate for direct disposal and it may also affect the aerobic treatment as an additional organic and nutrients loading. Therefore, an alternative use for this effluent may represent both a financial and an environmental advantage for the management of a WWTP in order to improve the results of the treatment. Wang et al. (2010) carried out a similar experiment with Chlorella sp. and distinct WWTP streams, including centrate, and reported growth rates of 0.95 days^{-1}, for the former effluent. This highlights the significant variation in the quality of the centrate from one WWTP to another, but it also suggests that centrate effluents are to be considered as significant media for supporting algae growth, despite its elevated nutrient loads.

The trials with the EPS effluents are valuable for comparing the effect of CO$_2$ injection within the photoreactors. The highest growth rates were observed when 4% CO$_2$ was supplemented with atmospheric air (EPS I, Table 2). This effect was also observed by other authors (Ruiz et al., 2011; Park et al., 2011). CO$_2$ supplementation may represent an advantageous strategy for bioenergy production, once it can be used to mitigate previous emissions and offset fossil fuel emissions when biomass is used to replace fossil fuels (Rawat et al., 2011; Chisti, 2007). The carbon dioxide represents a way of "feeding" the system, since microalgae use it for their photosynthetic metabolism. It is possible that in some cases the activity of capturing CO$_2$ for this purpose may represent an economic disadvantage. On the other hand, this economic burden would not occur if algal biomass production is carried in parallel to a process such as anaerobic digestion in WWTP.
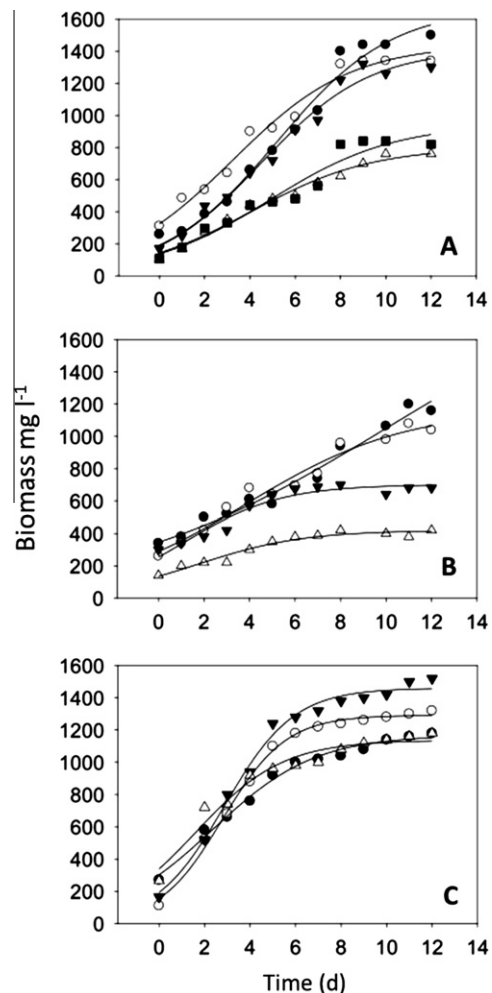

Fig. 2. Growth curves of Chlorella vulgaris at: pretreatment effluent WWTP1● pretreatment effluent WWTP1○ anaerobic treated effluent WWTP2▼ disposing effluent WWTP1n disposing effluent WWTP1△ (A), effect of different cultivation conditions with effluent from primary settler (EPS): EPS I (microalgae + CO$_2$) ●, EPS II (microalgae + CO$_2$ + sludge) ○, EPS III (microalgae + sludge) ▼, EPS IV (sludge) △ (B), and growth in the centrate at different N/P ratios: I (N/P 0.7) ●, II (N/P 2.0)○, III (N/P 8.0) ▼, IV (N/P 15.0)△ (C).

3.2. Nutrient removal

To evaluate the potential of microalgae system as a complement/replacement for wastewater treatment, the nutrient uptake in each reactor was assessed. A substantial reduction on nutrient concentrations (N and P) was recorded at the end of each trial (Table 2). High removal rates of N were observed with pretreatment WWTP 1 and 2, AD and disposing WWTP 2 streams (from 6.6 to 1.6 mg l^{-1} days^{-1}, respectively). Nutrient removal rates from centrate effluents were higher than the former, but the final concentration of nutrients were still considerable, while the initial ones were comparatively higher (from 4.0 to 9.8 mg l^{-1} days^{-1} of N and from 1.1 to 3.0 mg l^{-1} days^{-1} of P). Therefore, the lowest 6% nitrogen removal ratio observed in centrate IV is similar to the other experiments (i.e. about 4.0 mg l^{-1} days^{-1}, for centrate IV). The optimum conditions for nitrogen removal were observed in the trials with centrate I and II (9.7 and 9.8 mg l^{-1} days^{-1}, respectively). These correspond to trials with distinct N/P ratios (0.7 and 2, respectively). Thus, in the present conditions, C. vulgaris SAG 211-12 was efficient in removing nitrogen from the tested effluent. The European Directive 98/15/EC establishes a limit of 10 mg l^{-1} of nitrogen in the effluent for disposal and such a value was achieved

Table 2
Verlhust kinetic parameters of *Chlorella vulgaris* grown in different wastewater treatment streams. The model was fitted using SigmaPLOT® software v.8 with a $R^2 > 0.80$ and a *p*-value < 0.05.

	Pretreated urban wastewater WWTP1	Pretreated urban wastewater WWTP2	Anaerobically treated wastewater WWTP2	Disposing effluent WWTP1	Disposing effluent WWTP2	EPS I (microalgae + CO2)	EPS II (microalgae + activated sludge + CO2)	EPS III (microalgae + activated sludge)	EPS IV (activated sludge)	Centrate I (N/P 0.7)	Centrate II (N/P 2.0)	Centrate III (N/P 8.0)	Centrate IV (N/P 15.0)
X_o (mg SS l^{-1})	108.0	110.0	107.0	100.0	108.0	140.0	206.0	200.0	140.0	107.0	110.0	105.0	105.0
X_M (mg SS l^{-1})	1500	1340	1300	760	820	1160	1040	680.0	420.0	1180	1320	1520	1180
R^2	0.96	0.95	0.98	0.98	0.97	0.93	0.93	0.93	0.95	0.98	0.99	0.92	0.92
μ (day^{-1})	0.22	0.18	0.21	0.26	0.28	0.11	0.19	0.15	0.15	0.23	0.38	0.30	0.25
Productivity (mg SS l^{-1} d^{-1})	115.7	117.0	128.2	79.82	73.88	55.65	87.42	69.42	39.28	124.8	195.1	193.7	138.2
TN removal rate (mg l^{-1} d^{-1})	6.63	3.97	5.20	1.60	0.73	2.77	2.70	2.80	1.60	9.7	9.8	6.9	4.0
TP removal rate (mg l^{-1} d^{-1})	0.68	0.68	0.73	0.20	0.05	0.39	0.39	0.39	0.25	3.00	1.14	105.0	1.18
COD removal rate (mg l^{-1} d^{-1})	5.00	6.25	3.75	2.50	2.08	5.83	10.0	10.8	11.2	42.5	31.6	1520	23.7
Exponential phase (days)	7	6	8	5	5	6	5	5	6	5	5	6	5

Abbreviations: X_o = initial biomass; X_m = maximum biomass; μ = microalgae growth rate; EPS = effluent from the primary settler; centrate = effluent from drying the activated sludge.

in most of the trials with exception of WWTP 2, centrate I, II, III and IV. Nonetheless, centrate I and II reached values very close to 10 mg l^{-1} from a nitrogen starting point of 130 mg l^{-1}. This was considered a significant achievement, particularly considering the cost the centrate represents to a WWTP for its proper treatment (increase in the load of secondary lagoon).

In all experiments the removal of P was higher than 92%. The rate of P removal varied from 0.05 to 3 mg l^{-1} days^{-1} for disposing WWTP 2 and centrate I, respectively (Table 2). With the exception of the trials with centrate, the initial concentrations of phosphorus varied from 0.75 to 9 mg l^{-1}, corresponding to removal rates from 0.73 to 6.6 mg l^{-1} days^{-1}. Phosphorus removal rates positively correlated with nitrogen removal rates (R^2 0.85). However, such correlation was not observed when including the results of the experiments with centrate effluents, probably because of the initial nitrogen and phosphorus concentrations, which were comparatively higher (from 55 to 175 mg l^{-1} of P and from 123 to 903 mg l^{-1} of N). Therefore, the observed range
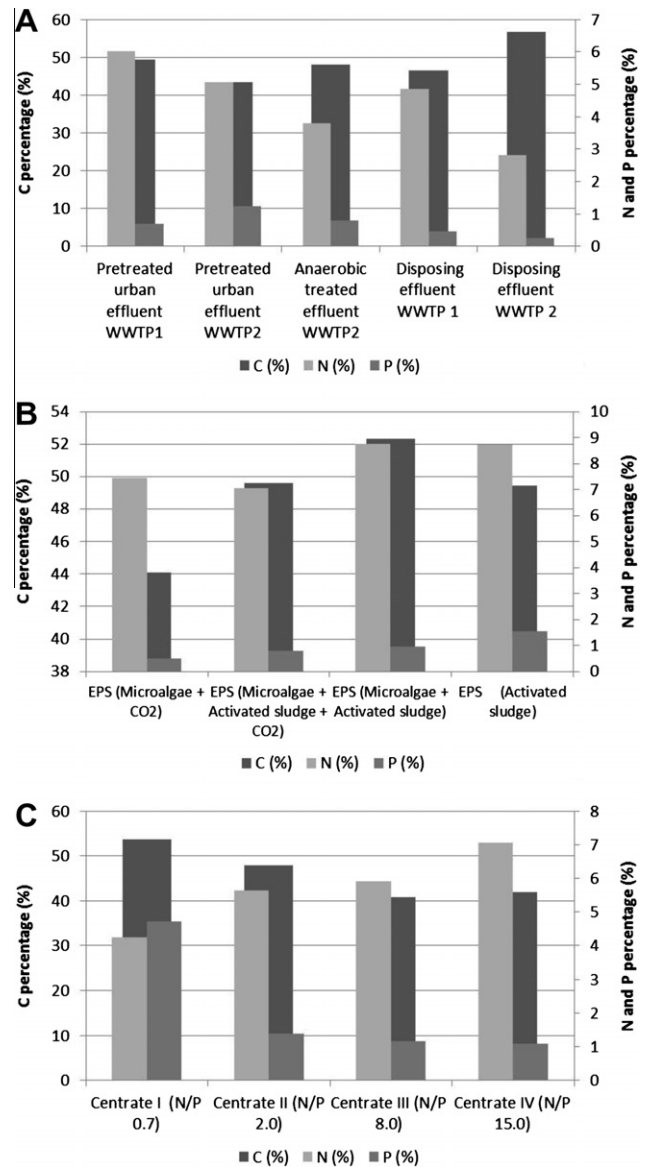

Fig. 3. Chemical composition as C (primary Y axis), N, and P (secondary Y axis) percentages in biomass: in different effluents from two WWTP (A), effect of different cultivation conditions with effluent from primary settler (EPS) (B), and growth in the centrate at different N/P ratios (C).

of linear correlation suggests that, at high concentrations of N and P (130 and 55 mg l^{-1}, respectively), distinct ratios (N/P) may not improve removal rates of these nutrients. This represents a positive result for growth in the centrate, as our results suggest that, despite the very high working concentrations of nutrients, no amendments would be necessary to guarantee the production of biomass with the centrate medium. The European Directive 98/15/EC establishes a limit of 1 mg l^{-1} of phosphorus in the effluent for disposal and this value was achieved in most of the trials with the exception of centrate I, II, III and IV. It is worth noting that, despite the still elevated values of nutrients in the centrate effluent, the presented result shows an alternative treatment process, which can be counterbalanced by the generation of valuable products.

COD removal was high in experiments with *C. vulgaris* cultivated in consortium with bacteria (75%), but such event is not linked with significant improvements of N and/or P removal rates (Table 2). It has been previously reported that microalgae grown in consortium with bacteria often show a COD reduction of about 75% (González et al., 2008; Kumar et al., 2011). COD removal was less pronounced in trials without CO_2 supplementation, suggesting that such event favored carbon phototrophic fixation by the algae and reduced available oxygen for bacterial metabolism. Conversely, in such conditions, algal biomass yields were considerably lower. Wang et al. (2010) carried out an experiment with *Chlorella* sp. grown in centrate effluents with a N/P ratio of 0.36 and the authors reported a removal ratio of 78%, 85% and 83% for N, P and COD, respectively (initial N:P:COD of 131:201:2250 mg l^{-1}). Kong et al. (2010) observed a similar response when working with *Chlamydomonas reinhardtii*. In the present experiment COD removal correlated negatively with increasing N/P ratios (R^2 equals 0.71 with four points). It should be stressed that the initial COD concentration was higher (675 mg l^{-1}) in the experiments with high nitrogen supplementation, when compared to the other reported trials (between 90 and 180 mg l^{-1}). Algal growth supported by centrate I effluent generated results that attend to the European Directive 98/15/EC (nutrient and COD removal of 10 and 1 mg l^{-1}, for N and P, and 75% COD, respectively). It has been reported that,

although some microalgae-based systems are negatively influenced by high COD values, this was not the case in these specific trials. In this experiment, however, high production rates were observed in the trials with centrate where COD values were above 600 mg l^{-1}. Thus, this observation suggests that operators may not need to dilute the centrate effluents for the preparation of microalgae cultivation as suggested elsewhere (Kumar et al., 2011; González et al., 2008). Nonetheless, the centrate effluents may also vary considerably from a WWTP to another and also in time.

3.3. Biomass content and market value estimates

The effect of the nutrient availability was accessed as elementary analyses of biomass (C, N and P, as percentage of dry weight), data shown in Fig. 3. The carbon content in the final biomass ranged from 43% to 56% in the current experiment. Such results shows that even when cultivated in different wastewater samples the carbon content of the final biomass is within the expected values of 40–60% (Grobelaar, 2004). The second most important nutrient for algal growth is nitrogen, usually found between values of 1% and 10% within the algal biomass (Grobelaar, 2004). Nitrogen content was observed in the expected range for all reactors (Fig. 3). The same was observed for phosphorus. However, the reactors with centrate wastewater have shown values above the common expected 1% for phosphorus (Grobelaar, 2004). Such findings, 4% of phosphorus as dry weight at centrate I (Fig. 3), indicate the possibility of applying microalgae-systems for recycling phosphorus. It is believed that the cultivation of microalgae may improve the recycling of phosphorus (Park et al., 2011). The phosphorus is an environmental problem due to eutrophication, but also may represent a severe economic problem in the future because of its decreasing supply for industrial processes, especially as agricultural fertilizers for food production. The current consumption of phosphate is about 15 million tons per year (Cordell, 2011). This is expected to become an issue in 60–100 years, when the supplies are expected to be exhausted (Cordell, 2011). Considering the 3.5 million tons of phosphorus accounted as food (effectively
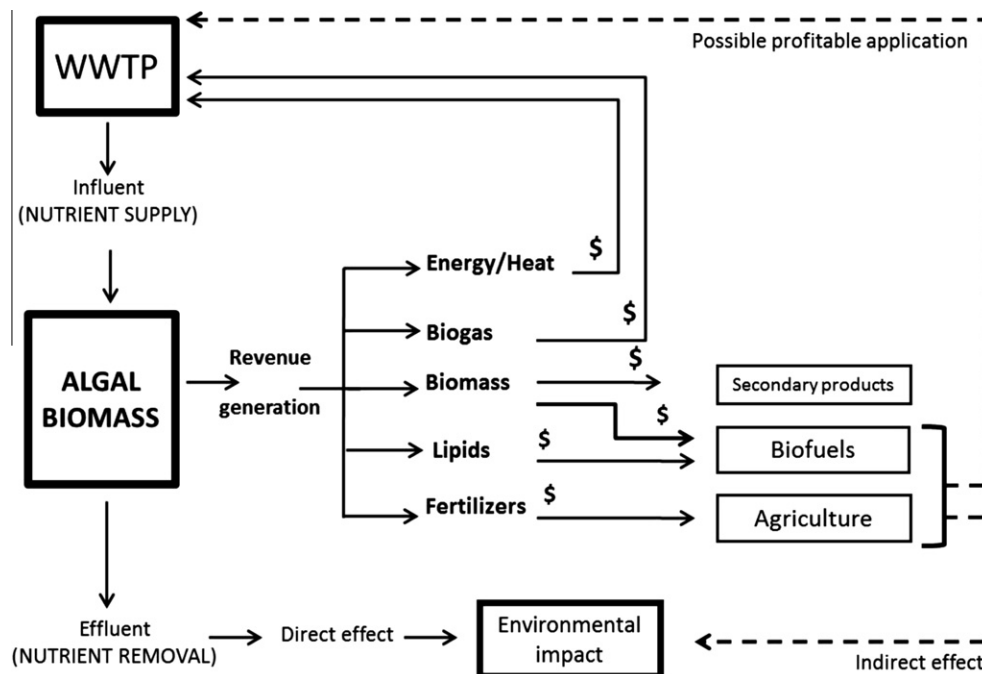

Fig. 4. Cleaner production system based on microalgae cultivation biotechnology for improving nutrient removal of WWTP effluents and upgrading such refuses with the generation of valuable products.

absorbed by crops), the approximate amount would be expected to reach the wastewater systems worldwide. For this matter, to develop processes to recycle this quantity of phosphorus from wastewater could lead to a 20% economy in P consumption (Cordell, 2011). In regard to hydrogen and sulfur, they were also found in values between the ones predicted in the specialized literature (2.9–10.0% and 0.15–1.6%, respectively) (Grobelaar, 2004).

The current applied concept defining a modern industrial activity is the minimization of second-hand goods. Therefore, each material or refuse present in the process should be considered as potential resources for other complementing and profitable activities. WWTP can also operate within such an ideology. Fig. 4 describes some alternatives for improvements in this sector, particularly in regard to nutrient removal and the upgrading of refuses. The main suggestion is to link nutrient removal to distinct profitable industrial activities using algal biomass production. In order to support such an idea, Table 3 shows estimated productivity and market values observed with the operation of microalgae systems. As previously discussed, in addition to providing a service of nutrient removal from streams, algal biomass can also be used for the production of several valuable materials such as proteins, biofuels and fertilizers. Biofuels can be obtained not only from the algal lipids, which are useful for the generation of biodiesel, but it is also an important organic resource for methane generation through anaerobic digesters (Ras et al., 2011). Therefore, the diagram shown in Fig. 4 is designed to identify opportunities, which may emerge from combining such apparent distinct processes. The goal is to demonstrate that there is a cleaner production approach to be implemented for combining enhancements in nutrients removal from WWTP streams and other industrial activities. The costs and operation of such a system can be supported by the generation of revenue obtained with such a link (Fig. 4). This combination is not only important to fulfill the prerequisite of a clean production model, but it is also a means to understand the potential of the best economic routes for achieving such goals with new biotechnologies.

The agricultural sector, for instance, is avid for nutrients such as N and P and related reports show that the USA consumption rates alone is of about 20 million tons per year (USDA, 2012). On the other hand, only the WWTP, which is the object of this research, must work to remove 52 and 24 tons of N and P respectively from domestic effluent on a yearly basis (taking the amount of nutrient available from Table 3 as example). Soil amendments with WWTP sludge are being practiced, but with significant drawbacks in regard to potential for soil organic and microbiological contamination (Chan et al., 2011). The production of microalgae biomass is a viable and cleaner alternative for supporting such practice of nutrient recycling (Fig. 4). This work shows that a modest algal system for this specific WWTP could yield about 7.8 tons of biomass per year (Table 3). Such biomass production can contribute to 780 and 7.8 kg of N and P, respectively. If this value is extrapolated for encompassing 90% of all WWTP present in the region, it would be enough to significantly impact the local market, allowing an economy of about US$36,000 per year in the investments for enhanced N and P removal (Table 3). Such an economy would have also a reflection on the wastewater treatment process as it is also a potential source of revenue; but it would also indirectly contribute to diminishing the impact caused by mining of nutrients. Therefore, WWTP nutrient recycling is expected to cause an indirect environmental impact for reducing the exploitation of mineral sources of fertilizers (Fig. 4).

Furthermore, the production of algal biomass is also considered as potential source of renewables in the form of energy and biofuels. Algal biomass can be directly associated to significant amounts of lipids that are applicable for biodiesel production. In addition, rejects of such process can be used as organic feed for anaerobic digestion, supporting the generation of methane. The combination of such activities can be very advantageous and profitable (Table 3). It has been estimated that each kg of biomass can generate 0.9 kg of biodiesel and the residual biomass can generate 168 l of methane (Chisti, 2007). The whole process can make an energy economic impact of US$12.203 per m^3 per year (Table 3). For instance, income for sustaining improvements of nutrient removal from WWTP streams can be generated from biodiesel and methane production (Fig. 4). It has been estimated that current new improvements for nutrient removal from WWTP streams may cost around US$17–23.000 per year (Table 3). Therefore, Fig. 4 shows cost-effective alternatives that, once developed, will offset invest-

Table 3
Comparison of microalgae systems efficiencies measured as production (p) or removal (r) rates of substances or energy, and their respective market values (data shown in first two columns). The last two columns show projections of a potential full-scale system operated in the specific condition encountered at the WWTP object of this research (a centrate production of 200 m^3 days^{-1}). This study shows that, in such a condition, C. vulgaris biomass production may reach 7.8 T per year, approximately (considering a total of 200 working days).

Product of microalgae systems	Market values and investments (US$)	Full-scale scenario estimatives	Estimative of annual production (p) (kg m^{-3} y^{-1}) or removal (r) rates
Protein (from total biomass)	50–250 kg^{-1b}	Average 40% DW[a]	15.6 (p)
Energy and heat (from total biomass)	9.52 MWh^{-1d}	29×10^3 kJ kg^{-1c}	$51{,}909 \times 10^3$ kJ (p)
Biogas (methane)	2.6 MMBtu^{-1f}	240 l kg^{-1e}	9360 l (p)
Lipids	1–300 g^{-1h}	Average 30% DW[g]	11.7 (p)
Fertilizer substitution	(P) 0.67 kg^{-1j}	10% as N[i]	3.9 (N) (p)
	(N) 0.53 kg^{-1j}	1% as P[i]	0.3 (P) (p)
Nutrient removal (N and P)	(P) 23.278 y^{-1l}	9.8 mg N l^{-1} days^{-1k}	1.96 g N l^{-1} days^{-1} (r)
	(N) 17.578 y^{-1m}	1.1 mg P l^{-1} days^{-1k}	0.22 g P l^{-1} days^{-1} (r)

[a] FAO (2010).
[b] FAO (2010) and market survey by authors.
[c] Iiiman et al. (2000).
[d] Bloomberg (2012).
[e] Ras et al. (2011).
[f] Bloomberg (2012).
[g] Chisti (2007).
[h] FAO (2010), Pulz and Gross (2004) and market survey by authors.
[i] Grobelaar (2004).
[j] USDA (2012).
[k] Current paper.
[l] Estimation of total costs (200 m^3 days^{-1}) for improvement of P removal to 1.0 mg l^{-1}, Cleland and Hartman (2007).
[m] Estimation of total costs (200 m^3 days^{-1}) for improvement of N removal mg l^{-1}, Cleland and Hartman (2007).

ments by upgrading low cost refuses (nutrients) into a higher hierarchal value level by means of clean recycling. For instance, a full-scale microalgae system at this particular WWTP can be used to generate 15 and 11 kg m^3 y^{-1} of proteins and lipids, respectively; which can be, in turn, used for food-like derivatives and biodiesel production. Alternatively, each kg of biomass may generate 29 MJ of energy; which may significantly balance the WWTP operating costs (Table 3). Therefore, algal biomass can connect distinct industries by supporting naturally complementing activities (Fig. 4) and shows potential for economic gains (Table 3).

4. Conclusion

The productivity observed with C. vulgaris showed that the system is a successful mean for treating WWTP streams, being at the same time suitable for biomass production. Another advantage of applying microalgae-based systems for treating streams from WWTP is that it can be achieved attached directly at the effluent discharge, without any major alteration in the design of the WWTPs. Thus, this research shows that microalgae based systems are viable and profitable biotechnologies if coupled to wastewater treatment processes.

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