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ALGAL BACTERIAL PROCESSES FOR THE TREATMENT OF AGROINDUSTRIAL WASTEWATERS: A BIODEGRADABILITY SCREENING

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Que D. ESTHER POSADAS OLMOS ha realizado bajo su dirección el Trabajo Fin de Máster, del Máster en Investigación Ingeniería de Procesos y Sistemas, titulado ALGAL BACTERIAL PROCESSES FOR THE TREATMENT OF AGROINDUSTRIAL WASTEWATER: A BIODEGRADABILITY SCREENING.

Valladolid, 2 de julio de 2012

Fdo. TUTORES

Reunido el Tribunal designado por el Comité Académico del Máster en Investigación en Ingeniería de Procesos y Sistemas, para la evaluación de los Trabajos Fin de Máster, y después de estudiar la memoria y atender a la defensa del trabajo "ALGAL BACTERIAL PROCESSES FOR THE TREATMENT OF AGROINDUSTRIAL WASTEWATER: A BIODEGRADABILITY SCREENING", presentado por el alumno D. ESTHER POSADAS OLMOS, decidió otorgarle la calificación de _____.

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ALGAL-BACTERIAL PROCESSES FOR THE TREATMENT OF AGROINDUSTRIAL WASTEWATERS: A BIODEGRADABILITY SCREENING

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Abstract

The potential of photosynthetic oxygenation for the removal of carbon, nitrogen and phosphorus from 5 agroindustrial wastewaters (at several wastewater dilutions) was investigated in enclosed batch biodegradation tests using a mixed microalgae consortium and activated sludge as model microorganisms. The target wastewaters were obtained from potato processing (PW), fish processing (FW), animal feed production (MW), coffee manufacturing (CW) and yeast production (YW). The maximum final organic carbon (TOC) and nitrogen removal efficiencies were recorded for 2 fold diluted FW (64±2 % and 85±1%, respectively), while the maximum P-PO_4^{3-} removal was 89±0.01% for undiluted PW. Nitrogen removal via biomass assimilation was the main nitrogen removal mechanism since neither nitrification nor stripping were recorded regardless of the wastewater and dilution evaluated. The biodegradable TOC was the most common limiting component in the wastewaters evaluated. The C/N ratio of the biomass generated was not correlated with the initial C/N ratio of the wastewater.

Keywords

Algal-bacterial symbiosis, agroindustrial wastewaters, carbon and nutrients removal, photosynthetic oxygenation.

1. Introduction

Large volumes of wastewaters from industries processing agricultural and livestock raw materials are continuously disposed to aquatic ecosystems worldwide as a result of the increasing food demand (Dareioti et al., 2009; Bhatnagar and Sillanpää, 2010). In Spain, the total estimated volume of agroindustrial wastewaters produced in 2006 was 61.226.000 m^3 (INE, 2006). These agroindustrial effluents are mainly characterized by high concentrations of organic matter, nitrogen and phosphorus and a variable pH (Drogui et al., 2008). Both the wastewater flow rates and characteristics are industry specific and can vary significantly throughout the year because of the seasonal nature of the raw materials processing (Dareioti et al., 2009). The disposal of such effluents in natural water bodies results in surface and groundwater contamination and other environmental problems such as eutrophication and ecosystem imbalance (Drogui et al., 2008). Therefore, the development of cost-effective and environmentally friendly methods for the treatment of agroindustrial effluents is mandatory.

Although anaerobic digestion constitutes one of the most commonly used processes for agroindustrial wastewater treatment, its performance is often limited by a poor nutrients removal (Rovirosa et al., 1995; Wilkie and Mulbry, 2002). On the other hand, activated sludge processes require a high energy input for mechanical aeration while methods such as adsorption or ion exchange involve prohibitive operating costs (González et al., 2008; Bhatnagar and Sillanpää, 2010). In this context, algal-bacterial systems can overcome these limitations by supporting an *in-situ* oxygen production via photosynthesis and nutrients removal via assimilation into the algal-bacterial biomass in a simple operational process (De Godos et al., 2009). Thus, in the presence of sunlight microalgae consume the CO_2 released during the bacterial mineralization of the organic matter and produce in turn the O_2 required by bacteria for the above referred mineralization and NH_4^+ oxidation (Oswald, 1988; Muñoz et al., 2005). Another advantage of this technology nowadays derives from the large amounts of microalgae biomass produced, which constitutes a valuable feed stock for energy production (Rawat et al., 2010; Rusten and Sahu, 2011).

The first studies based on microalgal-bacterial symbiosis were carried out in California in the mid 1950s for the treatment of domestic wastewaters in high rate algal ponds (HRAP), and recent works have extended their application to livestock and industrial effluents (Muñoz and Guieysse, 2006; González et al., 2011). However, little attention has been given to the treatment of agroindustrial

wastewaters in microalgal-bacterial photobioreactors. In this context, González et al. (1997) revealed the potential of algae-based systems for the removal of ammonia and phosphorus in agroindustrial wastewaters using the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus.* Nevertheless, due to the large variability in the characteristics of agroindustrial wastewaters, a systematic evaluation of the performance of this technology on a significant number of these wastewaters is needed to confirm its cost-effectiveness. This need was stressed by a recent study by Bahr et al. (2011), who reported that pollutant biodegradation in algal-bacterial systems was intrinsically linked to the carbon oxidation-reduction state.

This study systematically evaluated the potential and limitations of microalgal-bacterial systems for the removal of carbon, nitrogen and phosphorus from five representative agroindustrial effluents in Castilla y León (Spain): potato processing (PW), fish processing (FW), animal feed production (MW), lyophilized coffee manufacturing (CW) and yeast production (YW). A mixed microalgae consortium from a HRAP and activated sludge were used as a model algal-bacterial consortium. Special attention was given to the elucidation of the factors limiting the biodegradation process for each agroindustrial wastewater and to the establishment of any potential correlation between the composition of the wastewater and the final biomass produced (the latter being relevant in the optimization of assimilatory nitrogen removal).

2. Materials and methods

2.1 Agroindustrial wastewater pretreatment and characterization

Five fresh wastewaters originated from different agroindustries in the region of Castilla y Leon (Spain) were used in this study: potato processing wastewater (PW), wastewater from fish processing (FW), wastewater obtained from an industry producing animal food (MW), lyophilized coffee manufacturing wastewater (CW), and wastewater from a yeast production factory previously subjected to anaerobic digestion (YW). Samples were collected in polypropylene bottles and kept at 4ºC prior to use. All agroindustrial wastewaters were pretreated by centrifugation at 10,000 rpm for 10 minutes at 23ºC (Sorvall/Legend RT, Thermo Scientific, USA) and filtered through 0.40 µm filters. Therefore, only the soluble fraction of carbon, nitrogen and phosphorus was considered in the present study (Table 1).

2.2 Microorganisms and culture conditions

A mixed bacterial consortium was obtained from the secondary settler of the biological nutrients removal wastewater treatment plant of Valladolid (Spain). A mixed microalgae consortium was collected from a HRAP treating diluted centrates at the Department of Chemical Engineering and Environmental Technology (University of Valladolid, Spain).

2.3 Photosynthetically oxygenated agroindustrial wastewater biodegradation

Unless otherwise specified, all tests below described were incubated at 30ºC (temperature controlled by a thermostatic water bath) under magnetic agitation (200 rpm) and diluted with tap water. Tests conducted with undiluted, two, four, ten, twenty and one hundred times diluted pretreated agroindustrial wastewater will be herein referred as 1×, 2×, 4×, 10×, 20× and 100×, respectively. Control tests deprived of biological activity will be refereed as B× and were performed in order to assess any potential abiotic wastewater degradation. All tests were cultivated under a 12:12 h light/dark illumination regime at 76±4 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and in duplicate.

Glass bottles of 1,250 ml (22 cm×10.5 cm height× diameter) were filled with 1,000 ml of undiluted, 2× and 4× diluted pretreated wastewater. The experiments with CW were carried out only at 2×, 10×, 20× and 100× due to the potential presence of toxic compounds for microalgae activity (Dinsdale et al., 1997). An additional test with 10× diluted wastewater was conducted with YW due to its high NH_4^+ concentration and therefore potential inhibition on microalgae (González et al., 2008). Control tests were carried out for each wastewater at 4× and at 20× in CW, by adding $CuCl_2$ as growth inhibitor at 200 mg Cu l^{-1}. The tests were inoculated by mixing 2.2 ml of activated sludge and 22 ml of microalgae culture, resulting in final concentrations of 8±1 mg volatile suspended solids l^{-1} (mg VSS l^{-1}) and 15±7 mg VSS l^{-1}, respectively. The bottles were flushed with helium, closed with butyl septa, and then sealed with plastic caps in order to ensure that the biodegradation of the agroindustrial wastewaters proceeded exclusively driven by photosynthetic oxygenation.

Parameters	Agroindustrial wastewaters				
	PW	FW	MW	CW	YW
TOC (mg l^{-1})	327	381	959	8,532	1,186
IC (mg l^{-1})	54	51	37	171	1,353
TN (mgN l^{-1})	69	82	197	766	703
N-NH_4^+ (mg l^{-1})	13	9	189	101	565
N-NO_2^- (mg l^{-1})	<D.L.	<D.L.	<D.L.	3	<D.L.
N-NO_3^- (mg l^{-1})	<D.L.	<D.L.	<D.L.	<D.L.	<D.L.
$P_{soluble}$ (mgP l^{-1})	6	6	27	59	7
pH	7.1	7.7	6.0	7.0	8.1
C/N/P (g/g/g)	100/18/2	100/19/1	100/20/3	100/9/1	100/28/0.3

Table 1.Composition of the soluble fraction of the 5 agroindustrial wastewaters evaluated. <D.L. = below the detection limit of the HPLC-IC method used: 1.1 mg N-NO_3^-·l^{-1}, 1.5 mg N-NO_2^-·l^{-1}.

Liquid samples were periodically withdrawn from the tests, centrifuged for 10 minutes at 5,000 rpm (Kubota 5000, Kubota, Japan) and filtered through 0.20 µm nylon filters to monitor the dissolved total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), N-NH_4^+, N-NO_2^-, N-NO_3^-, P-PO_4^{3-} and pH. Phosphorus concentration was measured at the beginning and end of the tests. Sampling during experimentation considered a maximum total volume withdrawal of 15% of the initial value. Gas samples of 100 µL were also taken using gas-tight syringes (Hamilton Co., Reno, Nevada) to record CO_2, O_2 and N_2 concentration in the flask's headspace. Test monitoring stopped when the TOC concentration remained constant for at least two consecutive samplings. The final biomass grown in each test was collected by centrifugation (10,000 rpm and 10 min) and dried at 105 ºC for 24 h in order to determine its carbon and nitrogen (C/N) ratio.

2.3.1 Systematic determination of the limiting component in the biodegradation tests

In order to elucidate the limiting component (C, N or P) in the biodegradation of each wastewater, phosphorus (KH_2PO_4) at 10 mg P l^{-1} was added from a stock solution in one of the duplicates tests at the end of the experiment when TOC was stable for two samplings. If the TOC concentration in this duplicate remained stable after KH_2PO_4 addition, ammonium (NH_4Cl) at 10 mg N l^{-1} was further added.

2.4 Analytical procedures

TOC, IC and TN (NH_4^+ + $N_{organic}$ + NO_3^- + NO_2^-) were determined using a Shimadzu TOC-5050A analyzer (Japan) equipped with a TN Chemiluminescence module. Based on the different wastewater characteristics (turbidity and matrix effect) 3 different N-NH_4^+ determination methods were used: an ammonia electrode Orion Dual Star (Thermo Scientific, The Netherlands) in PW; the Nessler analytical method using a Spectrophotometer U-2000 (Hitachi, Japan) at 425 nm in FW and MW, and by distillation using a Bütcher distiller (KlejFlex K-360, Spain) in CW and YW. N-NO_3^- , N-NO_2^- and P-PO_4^{3-} were analyzed via HPLC-IC (Hewlett Packard 5890 Series II). The soluble phosphorus concentrations were also determined spectrophotometrically (U-2000 spectrophotometer, Hitachi, Japan). All analyses were carried out according to *Standard Methods* (Eaton et al. 2005). A Crison micropH 2002 (Crison instruments, Barcelona, Spain) was used for pH determination. The gaseous concentrations of CO_2, O_2 and N_2 were analyzed using a gas chromatograph (Varian CP-3800, Palo Alto, CA, USA) coupled with a thermal conductivity detector and equipped with a CP-Molsieve 5A (15 m × 0.53 mm × 15 µm) and a CP-Pora-BOND Q (25 m × 0.53 mm × 15 µm) columns. Injector and detector temperatures were maintained at 150ºC and 175ºC, respectively. Helium was the carrier gas at 13.7 ml min^{-1}. The light intensity was measured with LI-250A light meter (LI-COR Biosciences, Germany).

The determination of the C/N biomass ratio was performed using a LECO CHNS-932 at the Instrumental Techniques Laboratory of Universidad Complutense de Madrid (Spain).

3. Results and discussion

The preliminary analyses of the agroindustrial wastewaters tested showed that they were mainly characterized by a wide range of concentrations for the different parameters evaluated (Table 1). Likewise, the biodegradability tests showed varied carbon and nutrients removal depending on the agroindustrial wastewater evaluated and the dilution tested. The optimal C/N/P ratio in the raw wastewater for microalgae growth was estimated in literature to 100/18/2 (Oswald, 1988), which corresponded to the C/N/P ratio of PW (Table 1) and also to the fastest carbon and nitrogen removal rates recorded among the five wastewaters. The C/N/P ratio in FW and MW was also similar to this optimal ratio and supported efficient carbon and nutrients removals. The CW presented a low C/N ratio, while YW exhibited both a low P content and C/N ratio, which could eventually limit the biodegradation process. The monitored parameters in the control tests remained constant regardless of the wastewater tested, which confirmed that both carbon and nutrients removal in the biotic tests took place exclusively supported by the symbiosis between microalgae and bacteria.

Dilutions	Agroindustrial wastewaters									
	PW		FW		MW		CW		YW	
	Est.	Emp.	Est.	Emp.	Est.	Emp.	Est.	Emp.	Est.	Emp.
1×	$C\ N_{0.15}\ P_{0.019}$	$C\ N_{0.15}$	$C\ N_{0.21}\ P_{0.017}$	$C\ N_{0.11}$	N.B.O.	N.B.O.	N.T.	N.T.	N.B.O.	N.B.O.
2×	$C\ N_{0.23}\ P_{0.097}$	$C\ N_{0.14}$	$C\ N_{0.22}\ P_{0.012}$	$C\ N_{0.13}$	$C\ N_{0.22}\ P_{0.017}$	$C\ N_{0.16}$	$C\ N_{0.05}\ P_{0.006}$	$C\ N_{0.16}$	N.B.O.	N.B.O.
4×	$C\ N_{0.22}P_{0.010}$	$C\ N_{0.13}$	$C\ N_{0.19}\ P_{0.018}$	$C\ N_{0.11}$	$C\ N_{0.38}\ P_{0.017}$	$C\ N_{0.16}$	N.T.	N.T.	$C\ N_{0.19}$	$C\ N_{0.16}$
10×	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	$C\ N_{0.06}$	$C\ N_{0.15}$	$C\ N_{0.20}$	$C\ N_{0.15}$
20×	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	$C\ N_{0.08}$	$C\ N_{0.15}$	N.T.	N.T.
100×	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	$C\ N_{0.11}$	$C\ N_{0.09}$	N.T.	N.T.

Table 2. Estimated (Est.) and empirical (Emp.) stoichiometric formula of the biomass produced from the biodegradation of each agroindustrial wastewater at the dilutions tested. N.B.O. = non biodegradation observed/ N.T.= non tested.
***** Due to the low amount of biomass formed in some biodegradation tests, the experimental determination of the P content in the biomass was not performed.

3.1 Potato processing wastewater biodegradation

Carbon and nutrients concentrations in potato processing wastewater were relatively low compared to the other agroindustrial wastewaters tested, despite of its neutral pH and optimal C/N/P ratio (Table 1). PW showed a fast carbon and nutrients removal regardless of the dilution applied. TOC removal took place in 1×, 2× and 4× within the first 40 h of experimentation, with total removal efficiencies (REs) of 31±8, 38±3 and 54±8 % (Fig. 1a), respectively. The TOC concentration remained nearly constant afterwards at 198 ±4, 80±6 and 35±2 mg l^{-1}, respectively. The rapid TOC removal and the increase in the cultivation pH, suggest an active microalgae population capable of satisfying the bacterial oxygen demand of the biodegradation process. Likewise, the TN concentration decreased within the first 86 h, with TN REs of 19±1, 52±0.1 and 60±0.4 % for 1×, 2× and 4×, respectively, and remained constant afterwards at, respectively, 54±1, 13±2 and 6±1 mg l^{-1}. The decrease in TN at 1× and 2× was not correlated with a $N\text{-}NH_4^+$ decrease likely due to the ammonification of the dissolved organic N (which constitutes the major fraction of TN) (Fig. 1b, d). Indeed, $N\text{-}NH_4^+$ concentration increased in 1× from 17 ±5 to 53±2 mg l^{-1} and in 2× from 6±1 to 18±2 mg l^{-1} within the first 40 h of experiment with a subsequent decrease to 34±9 and 2±1 mg l^{-1}, respectively. The low ammonification rate mediated by the low organic N concentration likely caused the steady $N\text{-}NH_4^+$ decrease in 4× from 3±0.1 to 1±0.1 mg l^{-1}. The fact that neither NO_2^- nor NO_3^- was detected in the biodegradation assays, together with the enclosed nature of the tests, suggests that most of the nitrogen was removed via assimilation into biomass. The lack of nitrification during the first stages of the biodegradation process was be likely due to both the low fraction of nitrifying bacteria added in the inoculum (15±7 mg VSS l^{-1} of total bacterial biomass), their low growth rate and the high initial oxygen demand of the TOC- degrading heterotrophic bacteria. Likewise, the high pHs prevailing in the last stages of the biodegradation process likely inhibited the nitrification process despite of the high O_2 concentrations. As a result of the high microalgae activity and low oxygen demand of the wastewater in 4×, the O_2 concentration in the flask's headspace of these tests increased to 206±3 g m^{-3} in the first 134 h, concomitantly with a complete CO_2 depletion and a steady pH increase to 11 likely mediated by the decrease in IC concentration (data not shown). In 2×, the O_2 concentration increased only at the end of the process to 49±3 g m^{-3}, while CO_2 was depleted by 86 h and the pH reached 9.6±0.2 at 183 h of experimentation. On the contrary, while negligible O_2 headspace concentrations were recorded in 1×, CO_2 increased from 40±2 to ≈ 100±11 g m^{-3} at the end of the experiment. The fact that O_2 concentration only increased significantly in 4×, and in a lower extent in 2×, despite the TOC removal process stopped after 50 h, suggest a partial inhibition of microalgae activity mediated by the wastewater. Phosphorus REs of 89, 80 and 87% were achieved in 1×, 2× and 4×, respectively. In order to elucidate the limiting component in the biodegradability test, phosphorus at 10 mg l^{-1} was added at the end of the process in one of the duplicates for each dilution, with an increase in the TOC and TN REs of 12 and 20%, respectively, in 1×, but no significant effects in 2× and 4× (data not shown). A further test supplementation with NH_4^+ at 10 mg N l^{-1} did not result in an enhanced TOC or TN removal in 2× and 4×. Therefore, it can be concluded that while the limiting component in 1× was phosphorus, the biodegradable TOC seemed to limit the biodegradation process in 2× and 4×. However, a potential inhibition mediated by the high pH values recorded in 2× and 4× was more likely to inhibit bacterial activity. Although similar estimated (based on C an N removal from the wastewater) and empirical (CHNS analyzer)

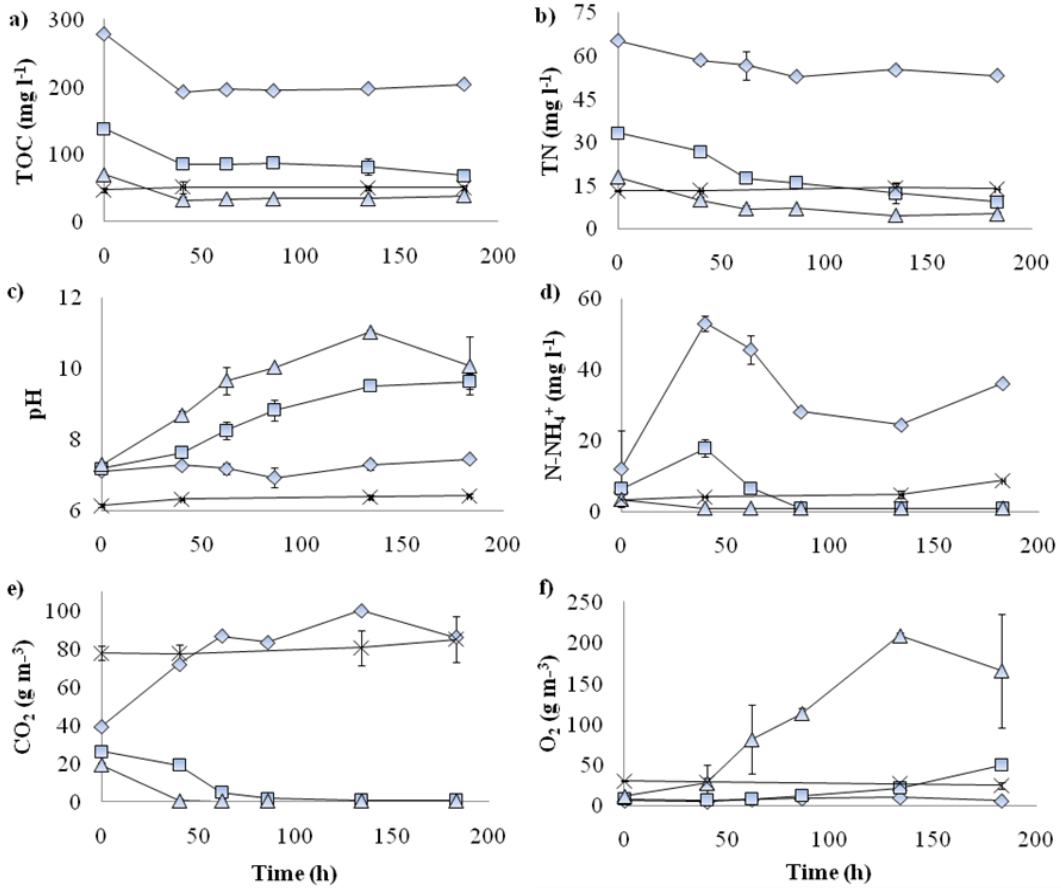

Figure 1.Time course of a) TOC, b) TN, c) pH, d) $N\text{-}NH_4^+$, e) CO_2 and f) O_2 headspace concentrations during PW biodegradation in algal-bacterial photobioreactors in undiluted ( ), 2 times diluted ( ), 4 times diluted ( ) and control tests ( ).

biomass formulas were obtained in 1×, the estimated C/N ratio was lower in the biomass formed in 2× and 4× (Table 2). Previous studies have reported a direct correlation between the nutrients concentration in the wastewater and the N and P content in the biomass formed, which itself determines nutrients removal by assimilation (Kebede-Westhead et al., 2004). These lower estimated C/N ratios might be due to the formation of N_2O since negligible NH_3 concentrations were present in the flask's headspace despite the high pH values attained. The excretion of N_2O from microalgal cultures was reported in the early 1980s (Weathers, 1984; Weathers and Niedzielski, 1986), and despite research on this topic is gradually increasing, the mechanisms underlying N_2O formation are still unclear (Florez-Leiva et al., 2010; Fagerstone et al., 2011).

3.2 Fish processing wastewater biodegradation

The initial characterization of the fish processing wastewater showed slightly similar characteristics than that of the potato processing wastewater, although its pH was slightly higher (Table 1). The TOC concentration steadily decreased from 368±2 to 162±20 mg l^{-1} in 327 h in 1×, from 195±2 to 70±3 mg l^{-1} in 279 h in 2× and from 75±1 to 45±2 mg l^{-1} in 156 h in 4× (Fig.2a), which corresponded to TOC-REs of 56±2, 64±2 and 40±1%, , respectively. Likewise, TN decreased concomitantly with TOC from 78±0.2 to 29±1 mg l^{-1} in 1×, from 43±1 to 6±0.1 mg l^{-1} in 2× and from 20±5 to 5±1 mg l^{-1} in 4×, resulting in TN-REs of 64±1, 85±1 and 74±1%, respectively (Fig.2b). $N\text{-}NH_4^+$ in 1× increased from 8±1 to 40±0.4 mg l^{-1} in 136 h as a result of organic nitrogen ammonification and decreased to 14±2 mg l^{-1} by the end of the test. A similar trend was recorded in 2× and 4×. The difference between TN and $N\text{-}NH_4^+$ concentrations and the absence of NO_2^- and NO_3^- clearly indicate that organic nitrogen was the main component of total nitrogen in FW. The reasons underlying the lack of nitrification in this test were likely similar to those discussed in PW and could be applied for each agroindustrial wastewater. Due to the low phosphorus concentration (≤6 mg l^{-1}) and the coloured nature of FW, the final $P\text{-}PO_4^{3-}$ removals were not experimentally determined. CO_2 concentration initially increased during the first hours of experimentation due to an intense bacterial

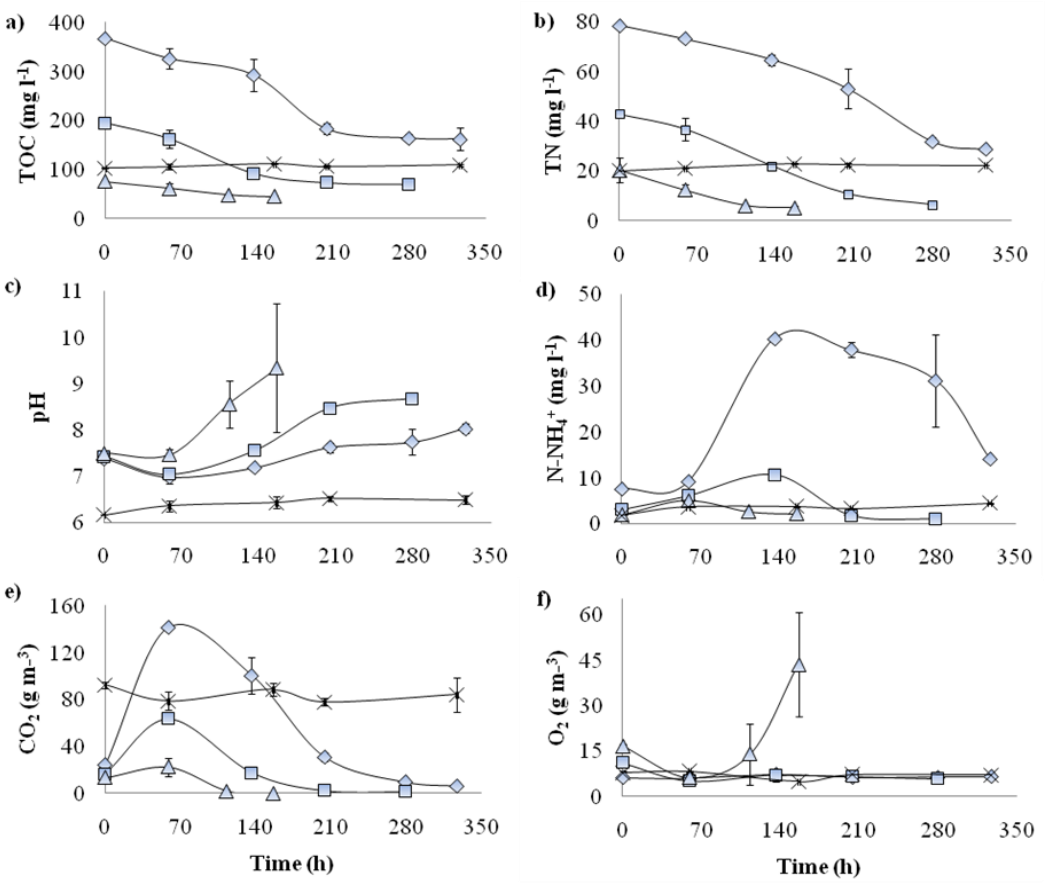

Figure 2 Time course of a) TOC, b) TN, c) pH, d) $N\text{-}NH_4^+$, e) CO_2 and f) O_2 headspace concentrations during FW biodegradation in algal-bacterial photobioreactors in undiluted ( ), 2 times diluted ( ), 4 times diluted ( ) and control tests ( ).

activity up to 142±2, 63±3 and 22±8 g m^{-3} for 1×, 2× and 4×, respectively, followed by a faster decrease until its complete depletion likely due to microalgae uptake. Oxygen was completely removed in 1× and 2× during the initial stage of the biodegradation process and its concentration remained close to zero during the entire test, while in 4× tests the oxygen concentration gradually increased from 13±1 to 44±1 g m^{-3} as a result of the high photosynthetic activity and low oxygen demand during the last stages of the biodegradation process at this dilution. The increase in the pH values was recorded concomitantly with the decrease in CO_2, reaching a maximum value of 9.3±1.4 in 4× tests. In order to determine the limiting component in the biodegradation, one of the duplicates in 1×, 2× and 4× tests was sequentially supplemented with $P\text{-}PO_4^{3-}$ and $N\text{-}NH_4^+$. The results showed no further evolution of both TOC and TN, which suggests that the biodegradable TOC was likely the limiting component of the process. The estimated C/N ratio based on carbon and nitrogen removal was again lower than the experimental values (Table 2).

3.3 Animal feed production wastewater biodegradation

Compared to the rest of agroindustrial wastewaters tested, the animal feed production wastewater was initially characterized by moderate carbon and nutrients concentrations, a favorable C/N/P ratio and a slightly acidic pH (Table 1).The biodegradation of MW diluted 2× and 4× in enclosed algal-bacterial systems was characterized by an initial lag phase with constant total organic carbon concentrations of 459±17 and 206±30 mg l^{-1}, respectively, followed by a rapid decrease after 134 h in 2× and after 69 h in 4×. This resulted in final TOC-REs of 49±1% and 42±2%, respectively (Fig.3a). TN and $N\text{-}NH_4^+$ concentrations were always similar and correlated through the entire biodegradation process, which indicate that $N\text{-}NH_4^+$ was the only contributor to TN. $N\text{-}NH_4^+$ steadily decreased in 4× from 51±2 to 5±1 mg l^{-1}, whereas it remained constant at 96±2 mg l^{-1} in 2× within the first 134 h and decreased to 33±7 mg l^{-1} by the end of the test. The final TN REs in 2× and 4× were 62±2% and 80±2%, respectively. The absence of O_2 in the bottle's headspace during most of the biodegradation assays likely inhibited the nitrifying activity of the activated sludge since neither NO_3^- nor NO_2^- were

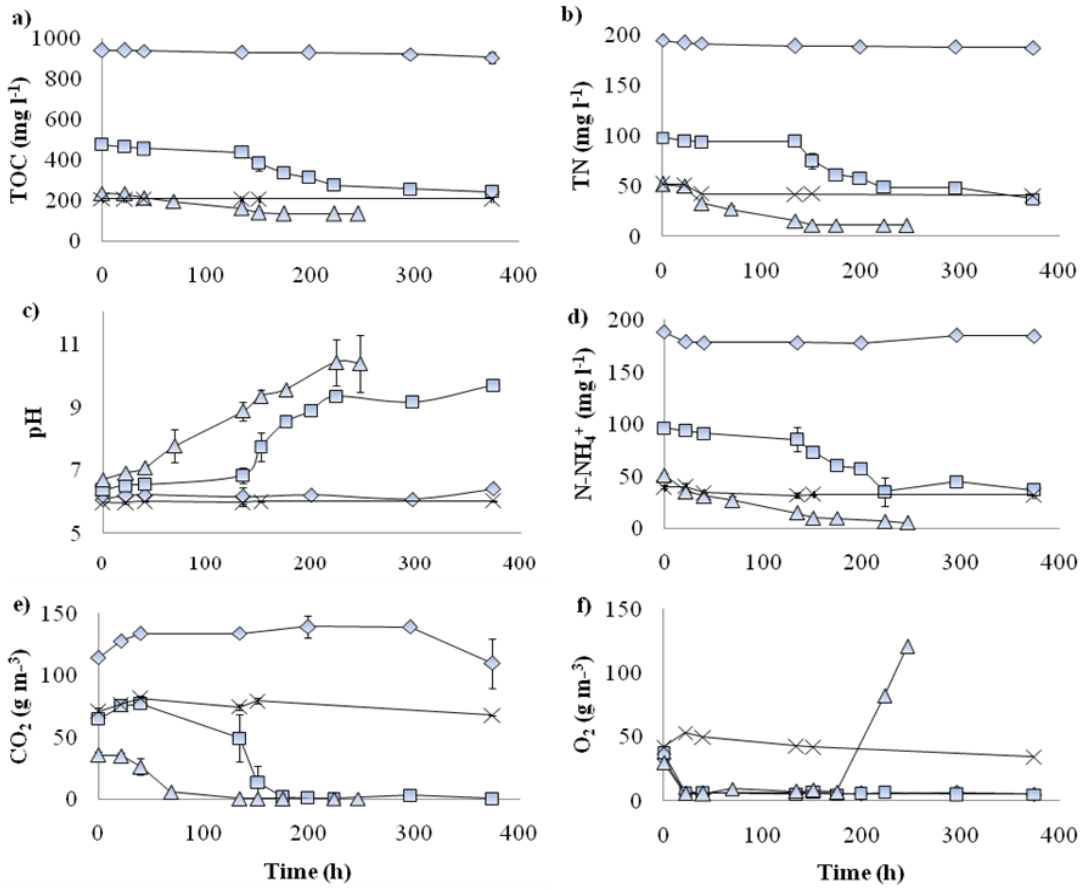

Figure 3.Time course of a) TOC, b) TN, c) pH, d) $N\text{-}NH_4^+$, e) CO_2 and f) O_2 headspace concentrations during MW biodegradation in algal-bacterial photobioreactors in undiluted ( ), 2 times diluted ( ), 4 times diluted ( ) and control tests ( ).

recorded in any of the tests. The initial pH values steadily increased from 6.7 to 10.4 and 9.6 in 4× and 2×, respectively (Fig.3c). This increase in the cultivation broth pH was mediated by the intense photosynthetic activity (CO_2 uptake) and a potential release of basic metabolites. The initial CO_2 present in the flask's headspace was completely removed after 134 and 175 h in 4× and 2 ×, respectively (Fig.3e). The O_2 concentration in the flask's headspace rapidly decreased during the first 22 h. This suggests that microalgae activity likely limited the biodegradation process during the initial stages of the process. A sudden increase in the O_2 concentration to 120±5 g m^{-3} was recorded in 4× after 175 h likely due to the depletion of all potential oxidizable substrates (TOC or NH_4^+) and to an active microalgal photosynthesis (Fig. 3f). Phosphorus removal in 2× and 4× achieved final REs of 83±5 and 57±9%, respectively. The faster TOC, N and P removal rates in 4× compared to 2×, together with the absence of an increase in the O_2 headspace concentration at the final stages of the biodegradation process, could be likely due a partial inhibition of microalgae in 2× by a combined effect of the high pH and $N\text{-}NH_4^+$ concentrations present in the cultivation broth (Muñoz and Guieysse, 2006). The high pH value recorded could have promoted $N\text{-}NH_4^+$ removal by abiotic mechanisms such as stripping or precipitation, respectively. However, the estimation of the flask headspace's NH_3 concentration revealed that $N\text{-}NH_4^+$ removal by stripping was negligible. No significant biological activity, estimated from the variation in TOC, TN, O_2 and CO_2 concentrations, was recorded in undiluted tests. The TOC, TN, phosphorous and CO_2 concentrations remained constant at 940±2 mg l^{-1}, 194±1 mg l^{-1}, 20±2 mg l^{-1} and 139 ±3 g m^{-3}, respectively. The high $N\text{-}NH_4^+$ concentrations (188±2 mg l^{-1}) or the presence of any inhibitory compounds might exert a detrimental effect on microalgae activity at 1× (and in a lower extent in 2× compared to 4 ×) and therefore on O_2 supply. The concentration of biodegradable TOC likely limited the biodegradation process in this particular wastewater. The estimated C/N ratio of the biomass formed was once again lower than the empirical ratio, which was similar to that measured for PW and FW (Table 2).

3.4 Lyophilized coffee manufacturing wastewater biodegradation

TOC and nutrients concentrations in the lyophilized coffee manufacturing wastewater ranked the highest among the five agroindustrial wastewaters tested. A neutral pH and a low C/N ratio were also recorded in the raw CW (Table 1). Organic matter biodegradation in CW tests was initially characterized by a rapid TOC decrease in the first 45 h from 4,758±5, 879±5, 433±6 and 96±2 mg l^{-1} to 4,293±160, 742±53, 423±30 and 75±8 mg l^{-1} in 2×, 10×, 20× and 100×, respectively (Fig. 4a). This biodegradation resulted in final TOC-REs of 18±2, 23±4, 21±8 and 56±2%, respectively. TN decreased concomitantly with TOC, with final REs of 8±2, 27±8, 32±13 and 80±4% for each dilution accordingly. The time course of N-NH_4^+ was characterized by an initial concentration decrease during the first stages of the biodegradation process likely due to assimilation into algal-bacterial biomass (based on the absence of nitrifying activity) followed by an slight increase due to the amonification of N-organic. Overall, N-NH_4^+ concentrations remained approximately constant at 55±4, 14±2, 9±3 and 6±2 mg l^{-1} for 2×, 10×, 20× and 100×, respectively, during the assays. Differences in TN and N-NH_4^+ concentration and the absence of NO_2^- and NO_3^- suggest that organic N was the main nitrogenous component in CW. The highest microalgae activity was recorded in 100×, where O_2 increased to 185±49 g m^{-3}, pH to 9.6±0.4, while CO_2 was depleted after 164 h. The highest bacterial activity was recorded in 2× where CO_2 increased to 1542±107 g m^{-3}, the pH decreased to 4.9±0.2 and O_2 was depleted after 95 h. A similar behavior was recorded in 10× and 20 ×. The high CO_2 concentrations recorded together with the absence of O_2 and the absence of a significant microalgal growth in 2×, 10 × and 20 × suggest the occurrence of anaerobic organic matter biodegradation, which could also explain the decrease in the pH of the cultivation broth.

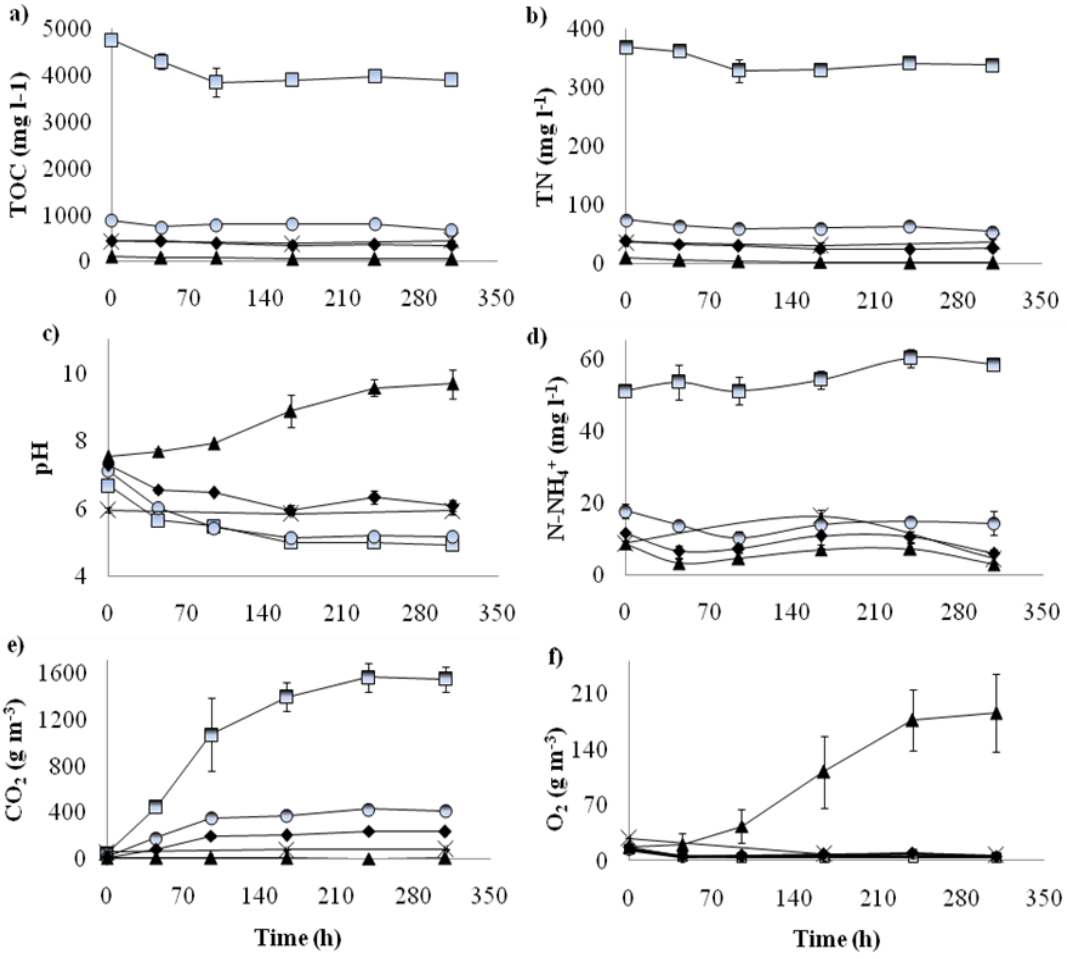

Figure 4. Time course of a) TOC, b) TN, c) pH, d) N-NH_4^+, e) CO_2 and f) O_2 headspace during CW biodegradation in algal-bacterial photobioreactors in 2 times diluted ( ), 10 times diluted ( ), 20 times diluted ( ), 100 times diluted ( ) and control tests ( ).

The spectrophotometric determination of phosphorus concentration was not possible due to the coloured nature of the sample and the high detection limit of our HPLC-UV method (1.6 mg P-PO_4^{3-} l^{-1}), which only allowed the determination of phosphorus concentration in 2×. However, the REs of phosphorus in 2× only accounted for 8±1%. At the end of the assay, the addition of P-PO_4^{3-} at 10 mg l^{-1} in one of the duplicates did not result in variation in TOC or TN. Based on its high concentrations, N-NH_4^+ was not supplemented. Therefore, it can be concluded that the biodegradable TOC was the limiting component of the biodegradation process regardless of the wastewater dilution. The low pH values recorded in 2×, 10× and 20× could have also inhibited microalgae activity. In this particular wastewater, the experimental nitrogen content in the biomass was always higher than the estimated value as a result of the low N assimilation rates (Table 2).

3.5 Yeast production wastewater biodegradation

The main characteristics of the yeast production wastewater compared to the rest of agroindustrial wastewaters were its low soluble phosphorus, moderate TOC and high N-NH_4+ and IC concentrations (Table 1). The biodegradation of YW was characterized by a constant TOC at 327±10 mg l^{-1} and 113±2 mg l^{-1} in 4× and 10×, respectively, during the first 323 h of experimentation. Low TOC REs of 27±10 and 33±2% were achieved in 4× and 10×, respectively. The N-NH_4^+ concentration decreased from 144±10 to 115 ±0.4 mg l^{-1} after 245 h. In 10× tests, TN and N-NH_4^+ concentrations were always correlated with final REs of ≈ 44±2%. Based on the absence of NO_3^- and NO_2^-, the results suggest that N-NH_4^+ and N-organic were the main contributors to TN (94.8 and 5.2%, respectively). The low O_2 concentrations in the headspace of 4× and 10× during the initial stages of the process suggest that photosynthesis likely limited the biodegradation activity.

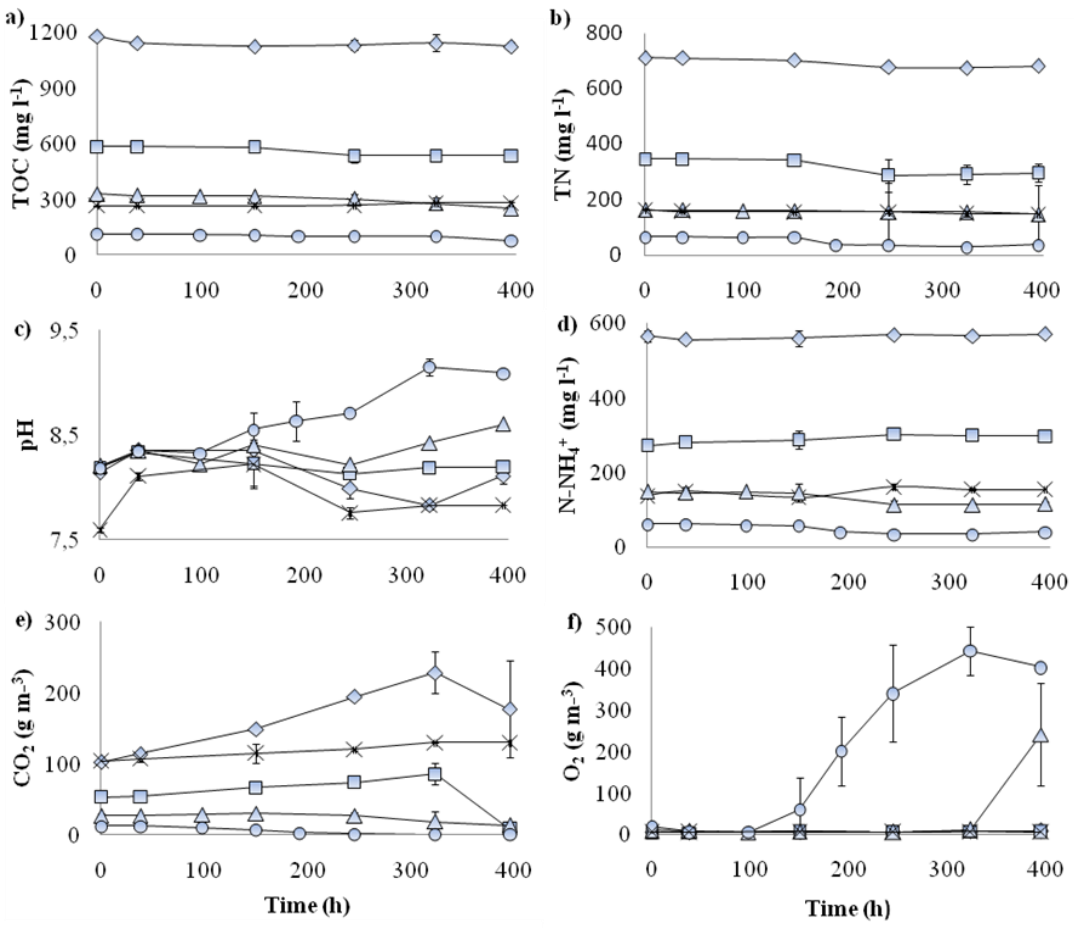

Figure 5. Time course of a) TOC, b) TN, c) pH, d) N-NH_4^+, e) CO_2 and f) O_2 headspace concentrations during YW biodegradation in algal-bacterial photobioreactors in undiluted ( ), 2 times diluted ( ), 4 times diluted ( ), 10 times diluted ( ) and control tests ( ).

The buffer capacity for 4× and 10× was the highest in comparison with the rest of the tests carried out with the other agroindustrial wastewaters at similar dilutions due to their high IC concentration (Table 1), which limited the pH increase from 8 to 8.6 in 4× and from 8.2 to 9.1 in 10×. This increase was correlated to the start-up of the photosynthetic activity, which was characterized by a CO_2 uptake from 27±0.2 to 15±5 g m^{-3} and from 13 ±0.1 to 1±0.1 g m^{-3}, respectively, concomitantly with an O_2 increase from 9±0.1 to 153±10 g m^{-3} and from 19 ±2 to 406±3 g m^{-3}, respectively. Similarly to CW, the spectrophotometric determination of phosphorus concentration was not possible due to the coloured nature of the sample. P-PO_4^{3-} at 10 mg l^{-1} was added in one of the duplicate in 4× and in 10× at the end of the biodegradability test, which resulted in an increase in the TOC-REs of 42 and 57%, respectively, and in TN of 22 and 60%, respectively. This confirmed that phosphorus was the limiting substrate in the biodegradation of this anaerobically treated wastewater. The more efficient TOC and TN biodegradation, faster pH and O_2 increase, and CO_2 decrease in 10× compared to 4× could be due to a partial NH_4^+-mediated inhibition of microalgae by the high N-NH_4^+ concentrations present in the tests. No significant variations in TOC, TN and O_2 concentrations were recorded in 1× and 2×. CO_2 increased slightly in 1× and 2× from 102±2 to 229±29 g m^{-3} and from 54±3 to 86±14 g m^{-3}, respectively, at 323 h with a sudden decrease at the end of the process. The high N-NH_4^+ concentrations present in both systems (565±14 and 271±1 mg l^{-1} in 1× and 2×, respectively) likely inhibited microalgae activity, and therefore the biodegradation process. However, the fact that the variations in CO_2 concentration were not correlated to a decrease in the TOC, TN or O_2 concentrations suggest that they were induced by the pH variations (Fig 5c). Despite the empirical C/N ratios were slightly higher than the estimated values, they were similar to those recorded for all final biomass harvested (Table 2).

4. Conclusions

In the absence of inhibitory compounds, the initial C/N/P ratio of the agroindustrial wastewater was correlated with its biodegradability, although no correlation was found with the C/N ratio of the algal-biomass formed. PW and FW were effectively treated in terms of carbon and nutrients removal. A low carbon and nutrients removal was recorded in CW and YW, the most recalcitrant wastewaters. Despite the low TOC removal efficiencies recorded, the biodegradable organic carbon was in most cases the limiting component in the biodegradation. Nitrogen removal via biomass assimilation was the main nitrogen removal mechanisms since neither nitrification nor stripping were significant.

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