Harvesting microalgae from wastewater treatment systems with natural flocculants: effect on biomass settleability and biogas production

Raquel Gutiérrez, Fabiana Passos, Ivet Ferrer, Enrica Uggetti, Joan García *

GEMMA - Group of Environmental Engineering and Microbiology, Department of Hydraulic, Maritime and Environmental Engineering, Universitat Politècnica de Catalunya·BarcelonaTech, c/ Jordi Girona 1-3, Building D1, E-08034, Barcelona, Spain.

E-mail addresses: raquel.gutierrez-martinez@upc.edu; fabiana.lopes.del.rei@upc.edu; ivet.ferrer@upc.edu,
enrica.uggetti@upc.edu; joan.garcia@upc.edu

* Corresponding author:

Tel: +34 934016464

Fax: +34 9340173574

E-mail: joan.garcia@upc.edu

19 **Abstract**

20 Research on new sources of bioenergy is nowadays driving attention to microalgae. Cost-effective biomass harvesting
21 and thickening pose a challenge for massive microalgae production for biofuels. In this study, coagulation-flocculation
22 and sedimentation with natural flocculant (*Ecotan* and *Tanfloc*) was evaluated on microalgae grown in an experimental
23 high rate algal pond treating urban wastewater. Jar tests showed how flocculants doses of 10 and 50 mg/L of *Ecotan* and
24 *Tanfloc* enabled over 90% biomass recovery. Furthermore, settling column tests showed that both flocculants increased
25 microalgae settling velocity, performing fast and efficient biomass recovery (> 90% recovery in 10-20 min). Thus, the
26 use of either flocculant would enhance microalgal biomass reducing the HRT and settler volume. Finally, the potential
27 toxicity of flocculant upon biomass production was assessed in biochemical methane potential tests. Results indicated
28 that doses of 10-50 mg/L of *Ecotan* and *Tanfloc* did not affect anaerobic digestion, leading to the same methane yield
29 (162-166 mL CH₄/g VS) with the same methane content (70%) as the control without flocculants. This study
30 demonstrates that *Ecotan* and *Tanfloc* flocculants would be appropriate for microalgae biomass harvesting and
31 subsequent biogas generation.

32

33 **Keywords**

34 Coagulation, Flocculation, Photobioreactor, Raceway pond, Algae, Wastewater

1. Introduction

Treatment of wastewater with microalgal cultures has the major advantage of producing biomass that can be valorized to produce bioenergy or molecules of interest. In fact, energy production and resources recovery have been identified as one of the main challenges for wastewater treatment systems of the future by relevant initiatives such as the recently created European Innovation Partnership on Water. However, microalgal wastewater treatment systems such high rate algal ponds (HRAP) have some bottlenecks like biomass separation [1,2]. Since the invention and development of HRAP in California in the 1950s, the problem of algal biomass separation has remained unsolved. The main constraint is related to the fact that wastewater is a product without market value, and therefore any added cost to the treatment system (such as the implementation of an intensive harvesting system) cannot be recovered. Nevertheless, this paradigm may change in the near future if biomass is valorized to obtain bioenergy or resources, since biomass will then have a market value.

Microalgal harvesting and thickening can be achieved by means of several techniques including coagulation-flocculation and sedimentation, flotation, centrifugation, magnetic separation and electrophoresis [3-7]. However, in the context of wastewater treatment, only low-cost techniques capable of managing large volumes of water and biomass can be applied, such as coagulation-flocculation followed by a solid/liquid separation. Indeed, coagulation-flocculation and sedimentation may lead to a solids concentration in microalgal biomass from 1 to 5% w/w [7], which is appropriate for downstream processes such as biogas production.

Coagulation consists of neutralising negative surface charges of colloidal particles (in this case microalgae), while flocculation is the aggregation of neutralized particles followed by flocs formation. Coagulants that have been traditionally used in water and wastewater treatment are salts of aluminum or iron. However, these substances have a limited application in microalgal systems because they can contaminate downstream products restricting biomass valorization [3,8]. This drawback may be overcome by using natural organic coagulants like tannin based polymers or modified starch which are being increasingly used since the 80s [9]. These types of coagulants (also referred to “flocculants”, as from now in the text) are becoming very popular in the field of water treatment as substitutes for polyacrylamide based flocculants due to health concerns [2]. Previous studies on microalgae coagulation-flocculation and sedimentation with different types of organic polymers have shown promising results in terms of separation efficiency (Table 1).

Table 1

Literature results on microalgal biomass harvesting by coagulation-flocculation and sedimentation with different types of organic polymers.

Microalgae	Flocculant	Dose	Biomass recovery	Reference
<i>Tetraselmis suecica</i>	Zetag 7650 + $\text{Al}_2(\text{SO}_4)_3$	5-50 mg/L (Zetag 7650) + 50 mg/L ($\text{Al}_2(\text{SO}_4)_3$)	~100 %	[3]
<i>Parachlorella</i>	Cationic starch (Cargill C*Bond HR 35.849)	120 mg/L	>95%	[2]
<i>Scenedesmus</i>	Cationic starch (Greenfloc 120)	20 mg/L	>90%	[2]
<i>Scenedesmus dimorphus</i>	Cationic starches	10-100 mg/L	70 to 95%	[10]
Microalgal-bacteria consortia	Drewfloc 447, Flocudex CS/5000, Flocusol CM/78, Chemifloc CV/300 and Chitosan	25-50 mg/L	58 to 99 %	[5]
<i>Microcystis aeruginosa</i>	Chitosan + Fe_3O_4	1.6 mg/L (Chitosan) + 4-6 mg/L (Fe_3O_4)	99%	[11]
<i>Spirulina</i> , <i>Oscillatoria</i> and <i>Chlorella</i>	Chitosan	15 mg/L	90%	[12]
Microalgal-bacterial consortia	Chitosan	214 mg/L	92 %	[13]
<i>Chlorella Sorokiniana</i>	Chitosan	10 mg/L	90%	[14]
<i>Phaeodactylum tricornutum</i>	Chitosan	20 mg/L	80-90%	[15]

68 In the field of wastewater treatment, biogas production is perhaps the most straightforward option for microalgal
69 biomass valorization [16, 17]. Indeed, anaerobic digestion has a long tradition in the context of wastewater treatment
70 and this expertise fully justifies the use of microalgae for this purpose. Nevertheless, if microalgae are separated and
71 thickened with coagulation-flocculation and sedimentation it is evident that flocculants should not be toxic or inhibit the
72 anaerobic digestion process. Natural organic flocculants could meet this requirement; to our knowledge though it has
73 yet to be confirmed.

74 The objective of the present study is to evaluate two tannin-based cationic flocculants for coagulation-
75 flocculation and sedimentation of microalgae grown in experimental HRAP for wastewater treatment. In particular the
76 study aimed at: 1) determining the optimal flocculants doses with jar tests, 2) studying the settleability of formed flocs
77 using settling column tests, and 3) assessing the effect of flocculants on biomass anaerobic digestion by means of
78 biochemical methane potential tests. To the best of our knowledge, this is the first time that natural flocculants are
79 evaluated not only on their efficiency, but also on their effect on downstream processing.

80

81 **2. Material and Methods**

82 *2.1. Microalgal biomass*

83 Experiments were carried out at the laboratory of the GEMMA research group (Universitat Politècnica de
84 Catalunya-BarcelonaTech, Barcelona, Spain). Microalgal biomass was grown in an experimental plant that had been in
85 continuous operation for more than 1 year. Urban wastewater was pumped from a nearby municipal sewer and
86 conveyed to a primary settler. Following, primary treated wastewater was continuously fed (60 L/d) to an experimental
87 HRAP; a raceway pond with a volume of 0.47 m³ and a nominal hydraulic retention time of 8 days. Average loading
88 rates of the HRAP were 24 g COD/m²·day and 4 g NH₄-N/m²·day. Microalgal biomass grown in the HRAP was
89 separated in a clarifier connected in series with the HRAP (without coagulation-flocculation). A detailed description of
90 the wastewater treatment system and its operation and performance may be found elsewhere [18].

91 In the present study, microalgal biomass term is referred to the microalgal-bacterial biomass grown in the
92 HRAP. The biomass concentration of the HRAP mixed liquor ranged from 0.06 to 0.6 g TSS/L over the year and
93 consists of consortia of microalgae as well as bacteria, microalgae accounting for much of the biomass (over 90% of the
94 biomass according to [19]). Average microalgal biomass production was 9.4 g TSS/m²·d. However, without flocculants,
95 harvested biomass corresponds to approximately 5 g TSS/m²·d, since 45 % of the produced biomass escaped from the
96 settler. The biomass was characterized by an average VS/TS ratio of 60 % VS/TS, being most of the organic matter in
97 particulate form as indicated by the low VSS/VS (0.89 %) and CODs/COD (0.72 %) ratios. During the experimental
98 period, microalgal population was mainly composed by green algae belonging to genus *Monoraphidium* sp.,
99 *Scenedesmus* sp. and *Stigeoclorium* sp. and the diatoms *Nitzschia* sp., *Navicula* sp. and *Amphora* sp. (Figure 1).

Fig. 1. Microalgae identified in the mixed liquor of the experimental HRAP **a** *Monoraphidium* sp. (elongated fusiform twisted cells) and *Scenedesmus* sp. (coenobia with elliptical cells). **b** *Monoraphidium* sp. and *Amphora* sp. (two adhered cells can be observed) **c** General view of *Stigeoclonium* sp. **d** Detail of *Stigeoclonium* sp.

Samples were collected from the HRAP on a weekly basis and analyzed in triplicate. Total solids (TS), total suspended solids (TSS), volatile solids (VS), volatile suspended solids (VSS), chemical oxygen demand (COD) and soluble chemical oxygen demand (CODs) were determined according to Standard Methods (APHA AWWA-WPCF, 1999). Moreover, microalgae images were taken with an optic microscope (Axioplan Zeiss, Germany), equipped with a camera MRc5, using the software Axioplan LE. Microalgae genus were identified using conventional taxonomic books [21, 22].

2.2. Natural polymeric flocculants

Harvesting properties of two cationic tannin-based flocculants were investigated on the samples of the HRAP mixed liquor. Ecotan AR® (Servyeco, Spain) and Tanfloc SG® (Tanac SA, Brazil) are natural cationic flocculants extracted

from the bark of *Acacia mearnsii* having strong coagulating properties. None of the flocculants modifies the pH of the medium significantly and both of them are effective over a pH range of 4.5-8 (9 for *Ecotan*). *Ecotan* was provided in liquid form with a concentration of 0.3 g/L, while *Tanfloc* was supplied as a dry product that was dissolved in water until complete solution. Both flocculants are suitable for wastewater treatment applications, and were conceived to replace metal-based products with aluminum and iron chlorides.

Stock solutions of 1000 mg/L were prepared for each flocculant prior to jar tests, column settling tests and biochemical methane potential (BMP) tests.

2.3. Jar tests

Jar tests were used to determine the optimal dose of each flocculant following standard protocols employed in the water and wastewater treatment fields using common jar test equipment [23]. During one week, HRAP liquor samples were taken and two jar tests were carried out for each flocculant in order to determine the optimal concentration for coagulation-flocculation and sedimentation tests. Flocculants concentrations were: 10, 20, 30, 40, 50 and 60 mg/L. Altogether, five jar test replicates were performed for each flocculant. In each experiment aliquots of 500 mL were placed in six beakers. Increasing flocculant concentrations were simultaneously added to each beaker, intensively stirred (200 rpm) for 1 minute, stimulating the coagulation process. Following, beakers were gently stirred (35 rpm) for 15 minutes, enhancing the flocculation process. Finally, formed flocs were allowed to settle (without stirring) for 15 minutes (sedimentation process). Images of the three jar test steps are shown in Figure 2. At the end of the process, supernatant liquid samples were taken from each beaker; turbidity and pH were measured with a HI93703 Hanna Instruments Turbidimeter and a Crison 506 pH-meter, respectively. Turbidity and pH were also measured from the mixed liquor without flocculants addition. Biomass recovery (RE) was calculated based on the initial (T_i) and final (T_f) turbidity measurements (Eq. 1).

$$RE (\%) = (T_i - T_f) / T_i * 100 \quad (1)$$

Fig. 2. Different steps of the jar test after flocculant addition at doses ranging from 10 to 60 mg/L. **a** Coagulation step (200 rpm, 1 min); **b** Flocculation step (35 rpm, 15 min); **c** Sedimentation, initial stage (1 min); **d** Sedimentation, final stage (15 min).

2.4. Sedimentation tests

In order to evaluate the settleability of flocculated biomass, static column settling tests were conducted using standard procedures employed in the water treatment field [23]. The column had a total height of 45.5 cm, an internal diameter of 8.5 cm and four sampling ports at intermediate depths (at 12.5, 20, 30 and 40 cm). Optimal doses found in jar tests were used to perform four test replicates per flocculant.

The test was carried out as follows: 1) HRAP mixed liquor aliquots were coagulated and flocculated in the jar test device, adding optimal dose of each flocculant; 2) the resulting sample (2.65 L) was gently poured into the sedimentation column to prevent breakage of formed flocs; 3) samples of 10 mL were withdrawn from the four sampling ports along the column at different time intervals over 24 hours (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 120, 150, 180, 210, 240, 270 min and 24 hours). The turbidity of each sample was immediately measured. Thus, biomass recovery (RE) was calculated for each column's depth and sedimentation time according to Eq. 1. [23].

2.5. Biochemical methane potential tests

Biochemical methane potential (BMP) tests were used to compare microalgae anaerobic biodegradability with and without flocculant addition. For this reason, BMP tests were performed in triplicate with samples of flocculated microalgal biomass with *Ecotan* and *Tanfloc* (with the optimal dose for each flocculant), together with a sample without flocculant (control). Blank trials containing only inoculum were also performed. Digested sludge from a full-scale anaerobic reactor located in a municipal wastewater treatment plant near Barcelona (Spain) was used as inoculum at a substrate/inoculum ratio of 0.5 g COD/g VS, defined as the optimal ratio by [24].

Serum bottles had a total volume of 160 mL and a useful volume of 100 mL. The concentration of microalgal biomass after jar test was 23.8 g COD/L and 20.1 g COD/L corresponding to 21.0 and 25.0 g microalgal biomass/bottle for *Ecotan* and *Tanfloc*, respectively. Following, inoculum was added to each trial (42 g VS/ bottle) and bottles were filled with distilled water to reach the useful volume (100 mL). Afterwards, bottles were flushed with Helium gas, sealed with butyl rubber stoppers and incubated at 35 °C until biogas production ceased. Biogas production was periodically determined by measuring the pressure increase in the headspace volume with an electronic manometer (Greisinger GMH 3151). After each measurement gas was released until atmospheric pressure. Samples from the headspace volume were taken every 2-3 days to determine biogas composition (CH₄/CO₂) by gas chromatography (GC Trace, Thermo, Finnigan) following the procedure described by [18].

Accumulated volumetric methane production (mL) was calculated from the pressure increase and methane content in biogas, expressed under standard conditions. The net values of methane production and yield were obtained by subtracting the endogenous production of blank trials, containing only inoculum.

3. Results and Discussion

3.1. Optimal doses of flocculants

In order to improve microalgal biomass recovery, coagulation-flocculation was tested with two natural cationic flocculants, *Ecotan* and *Tanfloc*. During the experimental period, microalgal biomass turbidity varied from 277 to 573 NTU. However, for each jar test, the same biomass (with the same turbidity) was used with both flocculants. Biomass recovery ranged from 91.8 to 99.4% and from 51.6 to 93.3% with *Ecotan* and *Tanfloc*, respectively (Table 2). The optimal dose (boldfaced in Table 2) was based on the lowest concentration of flocculant ensuring over 90% biomass recovery. Regarding *Ecotan*, 91.8% of microalgal biomass was removed with a concentration of 10 mg/L. In contrast, a higher *Tanfloc* concentration (50 mg/L) obtained a similar efficiency 90.2%. Note that flocculation efficiency depends on biomass concentration [25], for this reason tests with both flocculants were carried out using the same microalgal biomass concentration.

202 **Table 2**

203 Jar test results with *Ecotan* and *Tanfloc* flocculants (n=5). Microalgal biomass recovery was calculated from residual
 204 turbidity values. The optimal dose is the minimum leading to more than 90% biomass recovery (highlighted in bold).

Flocculant	Dose (mg/L)	Turbidity (NTU)	Biomass recovery (%)	pH
<i>Ecotan</i>	0 (without flocculant)	385.0 (142.0)	-	8.4 (0.2)
OPTIMAL DOSE				
	10	35.0 (25.5)	91.8 (3.0)	7.7 (0.3)
	20	8.3 (7.7)	98.2 (1.1)	7.2 (0.3)
	30	1.9 (1.7)	99.6 (0.3)	7.1 (0.3)
	40	0.9 (0.7)	99.8 (0.2)	6.9 (0.4)
	50	0.9 (0.6)	99.7 (0.2)	6.8 (0.4)
	60	2.2 (1.3)	99.4 (0.5)	6.7 (0.5)
<i>Tanfloc</i>	0 (without flocculant)	385.0 (142.0)	-	8.4 (0.2)
	10	181.2 (151.2)	51.6 (10.1)	8.4 (0.2)
	20	119.8 (69.9)	70.5 (91.3)	8.2 (0.4)
	30	68.7 (43.5)	83.3 (6.3)	8.1 (0.4)
	40	48.1 (29.0)	88.2 (4.2)	8.0 (0.4)
OPTIMAL DOSE				
	50	40.7 (27.6)	90.2 (4.4)	7.9 (0.5)
	60	27.6 (18.2)	93.3 (2.7)	7.7 (0.5)

205

206

207

208

Golueke and Oswald [26] were the first studying coagulation-flocculation of microalgae grown in wastewater. The authors reported that polyvalent organic polymers were successful at flocculating *Scenedesmus* sp. and *Chlorella* sp. Authors tested *Puriflocs 601/02* and *Sondellite*, using optimal doses of 10 mg/L in all cases. Both flocculants led to 100% microalgal biomass recovery. De Godos et al. [5] assessed the coagulation-flocculation efficiency of five different organic polymers on a *Chlorella* sp. consortium with bacteria. Three of the tested coagulants (CHEMIFLOC CV-300, Drewfloc 447, Flocusol CM-78) achieved microalgal biomass recoveries similar to our study (94, 99 and 93% respectively). Flocculant dosage was 50 mg/L, except for one of the flocculants (Flocudez CS-5000) which enabled 95% biomass recovery with 25 mg/L. In the same study, de Godos et al. [5] compared microalgae recovery with ferric metal salts and found that 5 to 6-times lower doses were needed for organic polymers in comparison with tested conventional chemical flocculants. Notwithstanding, optimal concentrations for all flocculants (25-50 mg/L) were similar to *Tanfloc* but higher than *Ecotan* (10 mg/L) doses found in the present study. However, comparisons are not straight forward since recovery efficiencies strictly depend on biomass concentration.

Beyond enhancing biomass recovery efficiency, flocculants should not hinder downstream biological conversion techniques for bioenergy generation. Indeed, conventional chemical flocculants, such as ferric and aluminum salts, consume alkalinity and decrease pH. This may affect the final effluent quality and biomass reuse. However, the flocculants used in this study have a cationic character, which may prevent alkalinity consumption; reducing pH values by less than 1 point (Table 2). Indeed for the optimal dose of *Ecotan* and *Tanfloc* (10 mg/L and 50 mg/L, respectively) the pH values decreased by 0.7 and 0.5 with *Ecotan* and *Tanfloc*, respectively. Propitiously, pH values stabilized to 7.7 and 7.9, within the appropriate range for anaerobic digestion (6.0-8.3) [27].

3.2. Settleability of formed flocs

Microalgae settleability was evaluated in settling column tests with and without flocculants addition. Firstly, a column test was performed without flocculants (control). Afterwards, two tests per week (one for each flocculant) were performed over 1 month, obtaining 4 replicates for each flocculant. Mixed liquor turbidity varied over the experimental period, average values of 294 and 378 NTU were obtained for *Tanfloc* and *Ecotan* samples, respectively. As described in the methods section, recovery percentages at different time intervals were calculated from turbidity measurements before and after settling at different times and column depths.

Fig. 3. Biomass recovery along for the four column depths (without flocculant addition).

Figure 3 represents the biomass recovery at different sampling time; each curve corresponds to a sampling depth (12.5, 20, 30 and 40 cm). Subsequently, for each curve corresponding to one sampling depth, the time required to attain a certain biomass recoveries (80, 85 and 90%) was obtained (Table 3). Thus, curves of isorecovery were plotted in relation with sampling depth and settling time for the control (Figure 4), *Ecotan* (Figure 5) and *Tanfloc* (Figure 6). Each curve traces the settling velocities of the biomass and shows the time needed to reach a certain biomass recovery. For instance, 80% biomass recovery was achieved within 80 min and 90% biomass recovery within 50 min at the port corresponding to 20 cm depth. From these curves, the settling velocities for each biomass recovery (80, 85 and 90%) were determined by dividing the column depth (d_x) by the time elapsed (t_x) (Table 4). The settling velocity is one of the key parameters for settler design (i.e. surface loading rate).

Table 3

Time interval to attain each biomass recovery at different column depths (without flocculant addition). Time intervals are obtained from isorecovery curves for the 4 sampling depth (Figure 4).

Column depth (cm)	Time (min)		
	80%	85%	90%
$d_1=12,5$ cm	18.3	21.33	40
$d_2=20$ cm	21.15	30	48.67
$d_3=30$ cm	26.42	29.63	65.53
$d_4=40$ cm	32.63	65.53	79

Fig. 4. Microalgal biomass isorecovery curves (80, 85, and 90%) without flocculant addition. Curves are calculated from data summarized in Table 3.

Fig. 5. Microalgal biomass isorecovery curves (80, 85, 90, and 95 %) after coagulation-flocculation with an *Ecotan* dose of 10 mg/L. Each figure corresponds to one of the replicates carried out over one month.

Fig. 6. Microalgal biomass isorecovery curves (80, 85, 90, and 95 %) after coagulation-flocculation with a *Tanfloc* dose of 50 mg/L. Each figure corresponds to one of the replicates carried out over one month.

Table 4

Velocities to attain 90% biomass recovery at different column depths (with and without flocculants addition).

Column depth (cm)	Velocity (mm/s)		
	Control	<i>Ecotan</i>	<i>Tanfloc</i>
	(without flocculant)		
d ₁ =12,5 cm	0.05	0.21	0.16
d ₂ = 20 cm	0.07	0.29	0.22
d ₃ = 30 cm	0.08	0.42	0.29
d ₄ = 40 cm	0.08	0.56	0.35

293 It is relevant that without flocculant addition the velocities obtained along the column were fairly constant
294 (0.05-0.08 mm/s) and much lower than those obtained with flocculated samples (0.21-0.56 mm/s with *Ecotan* and 0.16-
295 0.35 mm/s with *Tanfloc*). Comparing flocculants, higher velocities along the column (with a maximum of 0.56 mm/s at
296 40 cm), were obtained with *Ecotan* (10 mg/L). In contrast, higher doses of *Tanfloc* (50 mg/L) yielded slower settling
297 velocities of about 0.35 mm/s. A conventional gravity settler should be designed in order to retain 90% of the biomass
298 with a surface loading rate of 0.3 mm/s [23]. Considering the settling velocities obtained in this study (Table 4), a
299 velocity of 0.3 mm/s was obtained at column depths of 20 and 30 cm for *Ecotan* and *Tanfloc*, respectively. Therefore,
300 settler surface area with *Ecotan* would be 2-times smaller than *Tanfloc* and 8-times without flocculant.

301 As can be seen by comparing Figures 4, 5 and 6, samples with an optimal dose of *Ecotan* (Figure 5) and
302 *Tanfloc* (Figure 6) had much more higher settling velocities than samples without flocculant (Figure 4). For instance,
303 without flocculant, 90% of the biomass was settled at port d₄=40 cm after 80 min by adding (Figure 4), while after
304 *Ecotan* and *Tanfloc* addition, 90-95% biomass recovery took place after less than 20 and 10 min, respectively (Figures 5
305 and 6).

306

307 3.3. Anaerobic digestion of flocculated microalgal biomass

308 BMP tests were performed with flocculated algal biomass under optimal doses of each coagulant. The same mixed
309 liquor sample was used for both flocculants (160 NTU). As shown in Figure 7, none of the studied natural flocculants
310 showed a clear effect on the anaerobic biodegradability of flocculated microalgal biomass in BMP tests. Accumulated
311 methane yield was 163 mL CH₄/g VS for the control (gravity settled microalgae) and 162 and 166 mL CH₄/g VS after
312 coagulation-flocculation with *Ecotan* and *Tanfloc*, respectively. The methane content in biogas was the same in all
313 cases (70 %). The methane yield was comparable with that achieved in our previous studies with microalgal biomass
314 grown in domestic wastewater [24, 28].

Fig. 7. Methane yield of microalgal biomass without flocculants (control) and after coagulation-flocculation with the optimal dose of *Ecotan* (10 mg/L) and *Tanfloc* (50 mg/L) (n= 3).

To date, there are no literature results on the role of organic flocculants on the anaerobic digestion of harvested microalgae, and the results are here compared with flocculated waste activated sludge. According to Chu et al. [29], synthetic polyelectrolyte flocculants may have a different effect depending on the digestion stages. The authors observed how flocculated biomass presented higher solubilisation and methane production (about 55 gCH₄/kgTS) in comparison with the control sample (about 45 gCH₄/kgTS) during the first 6 days; while after 10 days of digestion the methane production seemed to be inhibited. This was attributed to the greater size of flocs, which may impede efficient bacteria movement within the sludge flocs, hindering the subsequent acidogenic and the methanogenic stages to produce methane.

As previously discussed, the pH remained within the optimum range for anaerobic digestion after adding optimal doses of both flocculants. Furthermore, the flocculants used in this study were natural polymers, which are neither corrosive nor toxic. This was attested by the BMP test, which indicates that *Ecotan* and *Tanfloc* do not show any effect on the anaerobic digestion of harvested biomass.

4. Conclusions

In this study microalgal biomass produced in an experimental HRAP treating urban wastewater was used to test the harvesting efficiency of two natural flocculants. The natural flocculants *Ecotan* and *Tanfloc* attained microalgal biomass recoveries over 90% with low concentrations (10 and 50 mg/L for *Ecotan* and *Tanfloc*, respectively). With this dose sedimentation of flocculated biomass was achieved within 10 minutes for *Ecotan* and 20 minutes for *Tanfloc* in a 40 cm column, compared to 80 minutes for microalgal biomass sedimentation without flocculants. This means that larger volumes of microalgae suspension could be recovered in less time and/or smaller settlers. None of the studied flocculants showed negative impacts in terms of pH and anaerobic biodegradability as shown in BMP tests with flocculated biomass (162-166 mLCH₄/gVS).

Acknowledgements

The authors would like to thank the Spanish Ministry of Economy and Competitiveness for financial support to this project (DIPROBIO, CTM2012-37860). Raquel Gutiérrez kindly acknowledges her PhD scholarship (2013FI_B 01096). Fabiana Passos appreciates her PhD scholarship funded by the Coordination for the Improvement of Higher Level Personal (CAPES) from the Brazilian Ministry of Education. The authors acknowledge Albert Francia from UPC for his contribution to the experiments, Mariona Hernández-Mariné from the University of Barcelona for her valuable help on microscopic images, and José Cabeza from Servyeco for providing *Ecotan* flocculant.

349 **References**

- 350 [1] L. Christenson, R. Sims. Production and harvesting of microalgae for wastewater treatment, biofuels, and
351 bioproducts. *Biotechnol. Adv.* 29 (2011) 686-702.
- 352 [2] D. Vandamme, I. Foubert, B. Meesschaert, K. Muylaert. Flocculation of microalgae using cationic starch. *J Appl*
353 *Phycol.* 22 (2010) 525-530.
- 354 [3] M.K. Danquah, L. Ang, N. Uduman, N. Moheimani, G.M. Forde. Dewatering of microalgal culture for biodiesel
355 production: exploring polymer flocculation and tangential flow filtration. *J Chem Technol Biotechnol.* 84 (2009) 1078-
356 1083.
- 357 [4] M.R. Granados, F.G. Acién, C. Gomez, J.M. Fernández-Sevilla, E. Molina-Grima. Evaluation of flocculants for the
358 recovery of freshwater microalgae. *Bioresour Technol.* 118 (2012) 102-110.
- 359 [5] I. de Godos, H.O. Guzman, R. Soto, P.A. García-Encina, E. Bécares, R. Muñoz, V.A. Vargas. Coagulation
360 flocculation-based removal of algal-bacterial biomass from piggery wastewater treatment. *Bioresour Technol.* 102
361 (2011) 923-927.
- 362 [6] S. Salim, R. Bosma, M.H. Vermuë, R.H. Wijffels. Harvesting of microalgae by bio-flocculation. *J Appl Phycol* 23
363 (2011) 849-855.
- 364 [7] B.T. Smith, R.H. Davis. Sedimentation of algae flocculated using naturally-available magnetic-based flocculants.
365 *Algal Research* 1 (2012) 32-39.
- 366 [8] H. Zheng, Z. Gao, J. Yin, X. Tang, X. Ji, H. Huang. Harvesting of microalgae by flocculation with poly (γ -glutamic
367 acid). *Bioresour Technol.* 112 (2012) 212-220.
- 368 [9] D. Vandamme, I. Foubert, I. Fraeye, B. Meesschaert, K. Muylaert. Flocculation of *Chlorella vulgaris* induced by
369 high pH: Role of magnesium and calcium and practical implications. *Bioresour Technol.* 105 (2012) 114-119.
- 370 [10] P.A. Hansel, R.G. Riefler, B.J. Stuart. Efficient flocculation of microalgae for biomass production using cationic
371 starch. *Algal Research* 5 (2014) 33-139.
- 372 [11] D. Liu, L. Fengtin, Z. Bingru. Removal of algal blooms in freshwater using magnetic polymer. *Wat. Sci. Tech.* 59
373 (2009) 1085-1091.
- 374 [12] R. Divakaran, V.N.S. Pillai (2002) Flocculation of algae using chitosan. *J Appl Phycol* 14:419-422.
- 375 [13] B. Riaño, B. Molinuevo, M.C. García-González. Optimization of chitosan flocculation for microalgal-bacterial
376 biomass harvesting via response surface methodology. *Ecol Eng* 38 (2012) 110-113.

377 [14] Y. Xu, S. Purton, F. Baganz. Chitosan flocculation to aid the harvesting of the microalga *Chlorella sorokiniana*
378 *Bioresour Technol.* 129 (2013) 296-301.

379 [15] S. Sirin, R. Trobajo, C. Ibanez, J. Salvadó. Harvesting the microalgae *Phaeodactylum tricornutum* with
380 polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. *J. Appl. Phycol.* 24 (2011)
381 1067-1080.

382 [16] M. Salermo, Y. Nurdogan, T.J. Lundquist. Biogas Production from Algae Biomass Harvested at Wastewater
383 Treatment Ponds (2009). ASABE Conference Presentation. Seattle, Washington (2009)

384 [17] A.J. Ward, D.M. Lewis, F.B. Green. Anaerobic digestion of algae biomass: A review. *Algal Research.* 5 (2014)
385 204-214

386 [18] F. Passos, J. García, I. Ferrer. Biogas production from microalgae grown in wastewater: Effect of microwave
387 pretreatment. *Appl Energy* 108 (2013) 168-175.

388 [19] J. García, B.F. Green, T. Lundquist, R. Mujeriego, M. Hernández-Mariné, J. Oswald. Long term diurnal variations
389 in contaminant removal in high rate ponds treating urban wastewater. *Bioresour Technol.* 97 (2006) 1709-1715.

390 [20] APHA-AWWA-WPCF (1999) Standard Methods for the Examination of Water and Wastewater (20th ed.)
391 Washington.

392 [21] M. Palmer. Algas en los abastecimientos de agua (1962). Ed. Interamericana SA (1^a ed.).

393 [22] Bourelly P. Les algues d'eau douce (1966). Editions N Boubée cie (2th ed.)

394 [23] Metcalf and Eddy. Wastewater Engineering. Treatment and Reuse (2003) .4 edn. McGraw-Hill, New York.

395 [24] F. Passos, M. Solé, J. García, I. Ferrer. Impact of low temperature pretreatment on the anaerobic digestion of
396 microalgal biomass. *Bioresour Technol.* 138 (2013) 79-86.

397 [25] J. A. Gerde, L. Yao, Y.I. Lio, Z. Wen, T. Wang. Microalgae flocculation: Impact of flocculant type, algae species
398 and cell concentration. *Algal Research* 3 (2014) 30-35.

399 [26] C.G. Golueke, W.J. Oswald. Harvesting and processing sewage grown planktonic algae. *J Water Pollut Control*
400 *Fed* 37 (1965) 471-498.

401 [27] I. Angelidaki, W. Sanders. Assessment of the anaerobic biodegradability of macropollutants. *Rev. Environ. Sci.*
402 *Biotechnol.* 3(2) (2004), 117.

403 [28] F. Passos, S. Astals, I. Ferrer. Anaerobic digestion of microalgal biomass after ultrasound pretreatment. *Waste*
404 *Management* 34 (2014) 2098-2103

405 [29] C.P. Chu, D.J. Lee, B-V Chang, C.H. You, C.S. Liao, J.H. Tay. Anaerobic digestion of polyelectrolyte flocculated
406 waste activated sludge. *Chemosphere* 53 (2003) 757-764.

Acknowledgements

The authors would like to thank the Spanish Ministry of Economy and Competitiveness for financial support to this project (DIPROBIO, CTM2012-37860). Raquel Gutiérrez kindly acknowledges her PhD scholarship (2013FI_B01096). Fabiana Passos appreciates her PhD scholarship funded by the Coordination for the Improvement of Higher Level Personal (CAPES) from the Brazilian Ministry of Education. The authors acknowledge Albert Francia from UPC for his contribution to the experiments, Mariona Hernández-Mariné from the University of Barcelona for her valuable help on microscopic images, and José Cabeza from Servyeco for providing *Ecotan* flocculant.

OPTIMAL DOSE

Biomass harvesting poses a challenge for massive microalgae production for biofuels

Coagulation-flocculation and sedimentation with natural flocculants was evaluated

Jar tests showed how low natural flocculants doses enabled over 90% biomass recovery

Settling column tests showed that flocculants increased microalgae settling velocity

Biochemical methane potential indicated no flocculants' effect anaerobic digestion