Please cite this article as:

Gutiérrez, R., Ferrer, I., Uggetti, E., Arnabat, C., Salvadó, H., García, J. (2016) Settling velocities distribution of microalgal biomass from urban wastewater high rate algal ponds. Algal Research, 16, 409-417.

http://dx.doi.org/10.1016/j.algal.2016.03.037

Settling velocities distribution of microalgal biomass from urban wastewater high rate algal ponds

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Abstract

The aim of this study was to evaluate the settling velocities distribution of microalgal biomass with and without flocculant (*Tanfloc* SG). Microalgal biomass was obtained from two experimental wastewater treatment high rate algal ponds (HRAPs) operated with 4 and 8 days of hydraulic retention time. Two sets of dynamic sedimentation tests were carried out using a water elutriation apparatus. In the first set, most of the biomass of the 8 days-HRAP (63%) had settling velocities between 16.5 and 4 m/h, while most of the biomass of the 4 days-HRAP (65%) had settling velocities between 16.5 and 1 m/h. In the second set, most of the biomass from both HRAPs (60% from the 8 days-HRAP and 80% from the 4 days-HRAP) had settling velocities between 6.5 and 0.4 m/h. In this second set, settling velocities <0.4 m/h were reached by 20% and 40% of the biomass from 4 days-HRAP and 8 days-HRAP, respectively. The addition of flocculant at optimal doses ranging from 20 to 40 mg/L had impressive effects on the settling velocities distribution in this second set. 70% and 84% of biomass reached velocities >6.5 m/h, compared to 10% and 14% of microalgal biomass without flocculant for the 8 days- and 4 days-HRAPs, respectively. With flocculant, a very small amount of biomass (3% for the 4 days-HRAP and 8% for the 8 days-HRAP) had settling velocities <0.4 m/h. Microscopic examination of samples from sedimentation tests showed how an important amount of microalgae settled in the system. Indeed, less than 1,500 microalgae individuals/mL were found in all outlet samples from the elutriation apparatus (inlet samples > 10^5 microalgae individuals/mL). According to our results, a settler designed with a critical settling velocity of 1 m/h would reach biomass recoveries as high as 90-94% with flocculant compared to 77-88% without flocculant.

1. Introduction

Microalgae-based wastewater treatment systems constitute an alternative technology to conventional wastewater treatment plants (WWTP) which has aroused a growing scientific interest in the last years [1]. These systems, while removing contaminants from wastewater, allow the production of microalgal biomass, which can be valorised for example as substrate for anaerobic digestion to produce biogas and biofertiliser. However, a necessary condition to achieve self-sufficient systems from an energy perspective is to ensure efficient and cost-effective microalgal biomass harvesting techniques.

Indeed, biomass harvesting is probably the main bottleneck hampering the application of full-scale microalgae-based wastewater treatment systems [2,3]. Usual solids separation techniques applied in WWTP such as conventional sedimentation (without coagulation-flocculation) have low harvesting efficiency (60-70%) [4]. In the case of microalgae production for high value-added compounds, where very high harvesting efficiencies are required (>99%), sophisticated techniques are used and they represent 20-30% of the total production costs [5–8].

Small size (few micrometres), relatively intrinsic low concentration (0.2-2 g/L) and their colloidal stability are the main reasons that make microalgae difficult to recover. Nowadays great research efforts are being conducted to develop efficient and cost-effective harvesting technologies [9–11]. The most suitable technology for each particular application depends mostly on the required moisture content of harvested biomass, and on its cost [12,13]. While centrifugation and rapid filtration may be feasible for producing high value-added compounds that require a very concentrated biomass, the combination of coagulation-flocculation followed by sedimentation may be the most suitable technique for low-cost products such as biogas. In fact, coagulation-flocculation and sedimentation is regarded by different authors as the unique cost-effective and easily scalable technique for microalgae-based wastewater treatment systems [11,12,14,15].

In coagulation-flocculation and sedimentation processes, the surface charge of microalgae cells is neutralised and therefore dispersed single cells can aggregate to form flocs which settle by

74 gravity. Both metal-based coagulants (i.e. aluminium sulphate or iron chloride) and organic
75 polymeric compounds (i.e. chitosan or starch) have been studied in the context of microalgae
76 harvesting [2,9,16–20]. However, the use of metal-based coagulants can make the biomass useless
77 for downstream processes [21–23]. In contrast, organic compounds are usually biodegradable and
78 do not hamper processes such as anaerobic digestion [19,20]. For instance, some polyelectrolytes
79 such as tannin-based and starch-based polymeric flocculants widely employed in the water
80 treatment industry, have shown promising results in terms of anaerobic digestion performance and
81 biogas production [19,20,24,25].

82 Nevertheless, information on settling properties of flocculated microalgae with polymeric
83 flocculants is completely lacking [26]. Only a few studies have investigated specific microalgae
84 physical characteristics (i.e. settling velocity, floc size and concentration factor) [26,27]. However, a
85 deep characterization of the settling velocities distribution and the microalgae species composition
86 of the microalgae population cultivated in wastewater is missing in literature. The settling velocities
87 distribution of flocculated microalgal biomass is a crucial factor for designing cost-effective gravity
88 settlers for biomass recovery. Therefore, the objectives of the present study are on the one hand to
89 evaluate microalgal biomass settling velocities distribution and, on the other hand, to improve this
90 velocity by adding a polymeric flocculant. Dynamic sedimentation tests were used to achieve this
91 goal. The main advantage of these tests over classical settling column tests is that settling velocity is
92 evaluated under real dynamic conditions. Also, microscopic examination of samples from
93 sedimentation tests was conducted to help interpret the results.

94

95 **2. Material and Methods**

96 *2.1 Microalgae-based wastewater treatment system*

97 Microalgal biomass was obtained from the mixed liquor of two experimental high rate algal
98 ponds (HRAPs) located outdoors at the laboratory of the GEMMA research group (Universitat
99 Politècnica de Catalunya·BarcelonaTech, Barcelona, Spain). Note that in HRAPs mixed populations

100 of microalgae, bacteria, protozoa and small metazoans coexist spontaneously, forming flocs with
101 different size and settling velocities [4,28]. Microalgae represent most of the biomass (80-90%)
102 [29,30]. The experimental HRAPs were operated uninterruptedly for 3 years prior to the
103 experiments here presented. The HRAPs were open raceway ponds (0.47 m^3 of volume each, and
104 0.3 m of depth), equipped with paddle-wheels for mixing and fed with primary treated wastewater.
105 Daily, urban wastewater was pumped from a near municipal sewer to a 1 m^3 homogenisation tank.
106 After that, wastewater was treated in primary settlers (7 L of volume and 0.9 h of hydraulic
107 retention time (HRT)) and then drawn in each HRAP by means of two peristaltic pumps. Each
108 HRAP was fed with a different continuous flow of wastewater: 60 L/day and 120 L/day, giving as a
109 result different hydraulic retention times (theoretical HRT of 8 and 4 days, respectively) and
110 consequently microalgal biomass with different properties. The effluent of each HRAP was
111 conveyed to secondary settlers for biomass recovery. Further details of this pilot wastewater
112 treatment system, operation and performance may be found in Passos et al. [31].
113
114 *2.2. Dynamic sedimentation test*
115 Dynamic sedimentation tests were carried out using a water-current separation technique in
116 which biomass flocs are washed out according to their relative density, volume and form, under
117 dynamic conditions [19,32,33]. The water elutriation apparatus consisted of two identical plastic
118 tanks (inlet and outlet, 30L each) and three glass settling columns (50, 100 and 200 mm of nominal
119 diameter) interconnected in series from the smaller to the larger diameter (Figure 1). The cross
120 sectional area and volume of the 3 critical settling columns were 1,923, 7,854 and 51,416 mm^2; and
121 2.3, 4.26 and 8.8 L, respectively. In each test, the elutriation apparatus was initially filled with
122 water. Then, 25 L of mixed liquor samples were poured to the 30 L inlet tank, which was kept under
123 continuous stirring to avoid microalgal biomass sedimentation. Samples of 25 L of HRAP mixed
124 liquor were then pumped from the inlet tank by means of a peristaltic pump located at the
125 downstream side of the elutriation apparatus, which forced samples to pass through the columns by

suction. HRAPs mixed liquor entered each column near the bottom and exited near the top (as seen in the detail of Fig.1). Note that the critical settling velocity decreased progressively in successive columns due to the gradual increment in column diameter, and therefore biomass flocs were retained in different columns depending on their settling velocities. In this manner, flocs with a settling velocity equal to or higher than the critical settling velocity of a given column were retained, while flocs with a settling velocity lower than the critical settling velocity escaped to the following column. Flocs with a settling velocity lower than the critical velocity of the third column were not retained in any column, and were thus collected in the outlet tank.

In this apparatus, the critical settling velocity of each column was obtained by dividing the flow rate through the apparatus by the area of the column (Eq. 1).

$$v_i = \frac{Q}{S_i} \quad \text{(Eq. 1)}$$

where v_i is the critical settling velocity in column "i" (m/h), Q is the flow rate (m^3/h) and S_i is the area of column "i" (m^2).

2.3. Experimental procedures

Experiments were carried out in two periods; during two weeks in summer (July) and during two weeks in autumn (October). Primary effluent and HRAPs mixed liquor samples were taken daily for evaluating temperature, pH, DO (dissolved oxygen) and turbidity, and weekly for measuring VSS (volatile suspended solids), COD (chemical oxygen demand) and ammonium nitrogen (NH_4-N). The main properties of the primary effluent and of the mixed liquor of both HRAPs are summarised in Table 1.

In summer, dynamic sedimentation tests were carried out in order to determine the HRAPs mixed liquor settling velocities distribution without flocculant. Along 6 days of experiment, three samples of mixed liquor (25 L each) were collected from each HRAP at 12 pm. During this period, the flow rate through the apparatus was set at 0.54 L/min based on a previous study [33]. This

generated critical settling velocities within the range of 1 - 16.5 m/h. The first column retained flocs with a settling velocity ≥16.5 m/h, the second one between 16.5 and 4 m/h, and the third one between 4 and 1 m/h, while flocs with a settling velocity of <1 m/h were collected in the outlet tank.

In autumn tests were conducted to determine the settling velocities distribution when a flocculant was added to improve the microalgal biomass settling properties. Since the sedimentation test carried out in summer showed low variability among replicates, in autumn the experiments were conducted without replicates, in order to minimize the time-lapse between samples. Therefore, in the first week of October, two samples of mixed liquor (25 L each) were collected from the 4 days-HRAP and tested one with flocculant and the other one without flocculant. The following week, the same process was repeated with the 8-days HRAP mixed liquor. The optimal dose of flocculant was determined with jar tests described below. In this case microalgae species populations were also assessed. The flocculant was a cationic tannin-based substance extracted from the bark of the tree *Acacia mearnsii*, which is nowadays widely used in the water and wastewater treatment sectors (*Tanfloc* SG). This flocculant is effective over a pH range from 4.5 to 8 and does not significantly modify the pH of the medium. *Tanfloc* SG was supplied by Tanac SA (Brazil) and had a cost of 1.7 $/kg. The flocculant, provided as dry product, was dissolved in water until complete solution. Stock solutions of 1000 mg/L of flocculant were prepared prior to jar tests. Jar tests were carried out using common jar test equipment, following standard protocols employed in the water and wastewater treatment sectors [34]. Prior to dynamic sedimentation test, 6 L of the same HRAP mixed liquor were used to perform the jar tests. Duplicate experiments were carried out to determine the optimal dose of flocculant for each HRAP mixed liquor, subsequently used in the dynamic sedimentation test. The steps followed in jar tests along with calculations may be found elsewhere [20].

For sedimentation tests with flocculant, samples from the mixed liquor were firstly mixed with *Tanfloc (*at the optimal doses obtained in jar tests) inside the 30 L inlet tank simulating a coagulation-flocculation process. After 15 min of flocculation, the mixed liquor was pumped

177 through the elutriation apparatus. The flow rate in these tests was set to 0.21 L/min in order to have
178 a range of critical settling velocities (0.4 - 6.5 m/h) more similar to those used in secondary settlers
179 (0.7 – 1.3 m/h according to Metcalf and Eddy [34]). Consequently, the first column retained flocs
180 with a settling velocity ≥6.5 m/h, the second one between 6.5 and 1.6 m/h, and the third one
181 between 1.6 and 0.4 m/h, while flocs with a settling velocity <0.4 m/h were in the outlet tank had.
182 At the end of each test, flocs retained in each column were collected by emptying the
183 volume retained in each column in 10 L plastic tanks. Afterwards, samples collected were
184 homogenously mixed and analysed for volatile suspended solids. The mass of microalgal biomass
185 settled in each column and outlet tank (expressed as grams and percentage of VSS) was then
186 obtained from the equations described in Table 2.
187 Due to the dynamic conditions of the experiment, a correction factor was taken into account
188 not to overestimate the results. This correction factor corresponds to the term in brackets in
189 equations to calculate W_i in Table 2. The term is used to consider the fraction of microalgal biomass
190 that did not reach the column corresponding to its settling velocity and remained in the previous
191 column.
192 The experimental error was calculated as an indicator of the reliability of the test
193 considering the amount of solids retained in each column and in the outlet tank divided by the
194 amount of solids pumped to the water elutriation apparatus (Eq. 2).
195 $$\text{Experimental error (\%)} = \frac{W_{C1}+W_{C2}+W_{C3}+W_{outlet}}{W_{inlet}} * 100 \quad \text{(Eq.2)}$$
196 where W_i is the mass of VSS retained in each column "i" and outlet tank (g VSS) and W_{inlet} is the
197 mass of VSS in the inlet tank (g VSS).
198
199 *2.3. Analytical methods*
200 Volatile suspended solids, total and soluble chemical oxygen demand, and ammonium
201 nitrogen were analysed according to Standard Methods [35]. Water temperature and dissolved

202 oxygen were measured *in situ* in the HRAP at 12 PM with an YSI 58 oxymeter. Turbidity was
203 determined with a Hanna Microprocessor Turbidity Meter HI93703 and pH with a Crison Portable
204 506 pH-meter.
205 Microalgae species populations were determined as follows. Two replicates of 25µL of each
206 sample were examined by bright and contrast phase microscopy using a Zeiss microscope Axioskop
207 40. Microalgae species were identified *in vivo* using conventional taxonomic books [36,37].
208 Microalgae were counted 100 and 400 magnification using coverslides of 20 mm side [38].
209 Microalgal biomass images were taken to complement quantification.
210
211 **3. Results and Discussion**
212 *3.1 Settling velocities distribution of microalgal biomass*
213 Wastewater organic loading rate, seasonal environmental conditions and potential
214 microorganisms interactions are known to influence the microalgal biomass properties (solids
215 concentration, chemical composition and microalgae population) [4,39]. The impact of these
216 parameters on floc characteristics is an important issue related to flocculation efficiency that should
217 be considered in a pre-concentration harvesting step. From this point of view, the settling velocity of
218 microalgal biomass is a key parameter in the design of full-scale sedimentation units [27]. Thus, the
219 settling velocities distribution of the mixed liquor from two experimental HRAP was initially
220 evaluated without flocculant. Note that the mixed liquor microalgal biomass concentration (mg
221 VSS/L) was higher in the 8 days-HRAP than in the 4 days-HRAP (Table 1). Thus, different
222 microalgal biomass concentrations were used in sedimentation tests. The results of these tests are
223 shown in Table 3, where the amount of microalgal biomass collected in each settling column and
224 outlet tank is summarized along with the amount of biomass pumped through the system (inlet
225 tank). In the last two columns biomass recovery is calculated as absolute mass (sum of columns and
226 outlet tank) (g VSS) and the experimental error as an indicator of the reliability of tests (%). The
227 average biomass pumped through the system was 5.97 g (±1.30) for the 4 days-HRAP and 11.22 g

228 (±0.90) for the 8 days-HRAP. Dynamic sedimentation tests results for the three samples of each
229 HRAP were very similar with experimental errors between 93-99%. The deviation of biomass
230 recovery from 100% is equivalent to the experimental error of the test. In general, the higher the
231 amount of biomass pumped, the higher the biomass recovery and subsequently, the lower the
232 experimental error. Thus, summer tests with higher concentration of biomass lead to lower
233 experimental error (1 to 7%) than autumn tests (2 to 30%).

234 Data in Table 3 were used to plot the settling velocities distribution of microalgal biomass
235 from both HRAPs (Figure 2). Each pair of bars refers to the amount of microalgal biomass with a
236 certain settling velocity. As it can be seen, only a small percentage of biomass (<13%) had settling
237 velocities ≥16.5 m/h in both HRAPs. Most of the biomass from the 8 days-HRAP (63%) had
238 settling velocities between 16.5 and 4 m/h, while most from the biomass of the 4 days-HRAP (65%)
239 had settling velocities between 16.5 and 1 m/h. 23% of the microalgal biomass from the 4 days-
240 HRAP had a settling velocity <1 m/h, and only 12.5% from the 8 days-HRAP. From these results it
241 can be estimated that dimensioning a settler with a critical settling velocity of 1 m/h (which is the
242 usual value in secondary settlers [34]) would attain a biomass recovery of 77% and 87.5% for the 4
243 and 8 days-HRAPs, respectively. Therefore, consistent different settling velocities distribution
244 between both HRAPs put into evidence the different microscopic properties of the flocs of the
245 mixed liquor from each HRAP in relation with their different HRT. On the whole, this experiment
246 highlights the importance of HRT on the settling properties of biomass.

247

248 *3.2 Settling velocities distribution of microalgal biomass with flocculant*

249 Microalgae harvesting by flocculation has been mostly investigated in terms of biomass
250 recovery [2,17,40]. However, the settling velocity is an important parameter which is affected by
251 the size, structure and density of microalgal biomass flocs, and very few studies have focused on its
252 relevance [26,27]. Indeed, only a few results of microalgae settling velocities using organic
253 flocculants are reported in literature [19,27].

In order to determine the optimal flocculant dose, a jar test was carried out and results are shown in Table 4. The optimal dose was established as the lowest dose of flocculant ensuring over 90% biomass recovery. In the 4 days-HRAP the optimal dose of flocculant was 20 mg/L, while in the 8 days-HRAP was 40 mg/L. These results are in accordance with other studies reporting a positive relation between microalgae concentration and dose of flocculant, where the higher the biomass concentration, the higher the flocculant dose needed to obtain the same biomass recovery [18,41,42].

Table 5 shows the results obtained in the four dynamic sedimentation tests, two without flocculant (control) and two with the optimal dose of *Tanfloc* SG. Experimental errors were slightly variable, probably due to the low biomass concentration in both HRAPs mixed liquor in comparison with the experiments in summer. As expected, differences in microalgal biomass characteristics were observed between summer and autumn samples [29]. Higher solids concentration was obtained in summer than in autumn due to more favourable environmental conditions (e.g. high solar radiation and temperature). Indeed, the influence of environmental conditions on microalgal biomass evolution has been widely discussed [30,39].

Figure 3 shows the percentage of microalgal biomass with a certain settling velocity (calculated from the results in Table 5). In the control sample (without flocculant) from the 4 days-HRAP the majority of the biomass (80%) had settling velocities ranging from 6.5 and 0.4 m/h, while 20% of the biomass had settling velocities <0.4 m/h. The addition of flocculant had an impressive effect since most of the biomass (84%) had a settling velocity ≥6.5 m/h. Only a 3% of the biomass had a settling velocity <0.4 m/h. In this case, a settler designed with a critical settling velocity of 1 m/h would allow a biomass recovery greater than 94% (estimated from the percentages corresponding to the ≥6.5 m/h and 6.5-1.6 m/h bars in Figure 3 (a)).

In the control from the 8 days-HRAP, around half of the biomass (60%) had settling velocities between 6.5 and 0.4 m/h. Only 10% of the microalgal biomass had settling velocities ≥6.5 m/h, and 40% of the biomass had velocities <0.4 m/h. Again, when the flocculant was added,

280 results were impressively affected, with 70% of biomass with a settling velocity ≥6.5 m/h (the same
281 trend as in the 4 days-HRAP). Only an 8% of the biomass had settling velocities lower than 0.4
282 m/h. In this case, a settler designed with a critical settling velocity of 1 m/h would allow a biomass
283 recovery greater than 90%. Note that microalgal biomass with low settling velocities would result in
284 higher settler's surface and/or higher HRT in settlers. With flocculant, higher biomass recovery may
285 be accomplished, leading to design more compact settlers.
286
287 *3.2.1 Microscopic examination*
288 The biomass settling ability is highly dependent on the microalgae species populations
289 present in the HRAPs mixed liquor [5,11]. In autumn, microalgae identification and quantification
290 were carried out from the inlet tank samples (HRAPs mixed liquor) and outlet tank samples of the
291 elutriation apparatus. In general, the dominant microalgae identified in both HRAPs were the green
292 algae *Chlorella* sp. and the diatoms *Navicula* sp. and *Nitzschia* sp. Indeed, *Chlorella* sp. and
293 *Nitzschia* sp. species are often classified in the top 10 most tolerant microalgae [1,7]. Although less
294 abundant, *Micractinium* sp., *Scenedesmus* sp., *Chlamydomonas* sp. and *Desmococcus* were also
295 present in all samples. The main difference between microalgae populations present in the two
296 HRAPs was driven by differences in the HRT. Even if the same microalgae species were observed
297 in the two HRAPs, *Chlorella* sp. and diatoms were more abundant in the 8 days-HRAP than in the 4
298 days-HRAP (56% more *Chlorella* sp. and 16% more diatoms). Indeed, the influence of HRAPs
299 operational parameters (such as HRT) and environmental conditions (e.g. solar radiation and
300 temperature) on shifts in microalgae dominance and abundance was previously reported [4,43,44].
301 Figure 4 shows the distribution of the main microalgae species in the inlet tank and the
302 outlet tank for the control and flocculated samples. Microalgal biomass images are shown in
303 Figures 5 and 6. Diatoms had a similar abundance in the inlet tank samples of both HRAPs. In
304 samples without flocculant, diatoms were lowered by more than 90% between the inlet and the
305 outlet tanks. The diatoms observed in this study are benthic organisms normally linked to floc

306 aggregates, so these microalgae were not expected to be found in outlet tank when flocculant was
307 added. Accordingly, once flocculant was added, almost 100% of diatoms were retained in the
308 apparatus. *Chlorella* sp. was 35% more abundant in the 8 days-HRAP than in the 4 days-HRAP.
309 Without flocculant, the percentage of recovery was higher in the 4 days-HRAP (94%) than in the 8
310 days-HRAP (83%). The lower amount of *Chlorella* sp. in the outlet tank of the 4 days-HRAP
311 sample may be attributed to an enhanced floc formation in this HRAP with higher flow rate (120 vs.
312 60 L/d), where more bacteria were likely to grow as a result of the higher organic loading rate (23 g
313 COD/m^2d in the 4 days-HRAP vs. 12 g COD/m^2d in the 8 days-HRAP). In fact, the presence of
314 bacteria enhances spontaneous flocs formation [45]. This behavior did not correspond to the one
315 observed in summer, when microalgal biomass flocs of the 8 days-HRAP had higher settling
316 velocities than those of the 4 days-HRAP. This demonstrates the complexity of the bioflocculation
317 process due to the large number of biological interactions between microorganisms and wastewater.
318 As expected, after flocculant addition, *Chlorella* sp. cells were mostly aggregated in flocs (see
319 Figures 5c and 6c). Indeed, the high recovery of individuals after flocculant addition (around 99%
320 of *Chlorella* sp. and almost 100% of diatoms) resulted in less than 1,500 individuals/mL in all
321 outlet tank samples. Microscopic images supported this finding (see Figures 5d and 6d).

322 Images of the inlet tank mixed liquor samples (Figures 5a and 6a) indicated that the initial
323 biomass was composed by flocs aggregates of different sizes and dispersed single cells in both
324 HRAPs. Once passing through the elutriation apparatus, mostly single cells and some smaller flocs
325 (<100 μm) were identified (Figures 5b and 6b). After coagulation-flocculation, most single cells
326 were aggregated, leading to larger flocs (Figures 5c and 6c). Comparing outlet tank images with and
327 without flocculant, the reduction of single cells and flocs aggregates can be clearly observed
328 (Figures 5d and 6d).

329 Therefore, low concentrations of *Tanfloc* (20-40 mg/L) were not only effective in terms of
330 biomass recovery, but also in terms of settling velocity. These parameters are important for the
331 design of secondary settlers by achieving fast settling and high concentrated microalgal biomass in

a pre-concentration step.

3.3. Economic assessment

Chemical flocculation followed by gravity sedimentation is considered a cost-effective harvesting method as low energy and no extra materials (e.g. membrane or electrode used for membrane filtration and electro-flocculation, respectively) are required [7]. Regarding energy requirements, microalgal biomass harvesting by gravity sedimentation needs less energy (0.9 kWh/ton TSS) than conventional harvesting methods such as centrifugation, tangential flow filtration and/or dissolved air flotation (>50 kWh/ton TSS) [46,47]. The viability of microalgal biomass flocculation with chemicals will ultimately depend on the flocculant cost, since the low energy requirement for mixing (around 1.5 kWh/ton TSS) does not hamper the viability of the process. The feasibility of flocculation with *Tanfloc* is compared to other commercial inorganic and organic coagulants/flocculants based on the cost of flocculating a ton of TSS microalgal biomass (Table 6). Notice that this calculation is only based on the flocculant cost, considering the optimal flocculant dose and the initial microalgal biomass concentration. In general, optimal doses of *Tanfloc* (0.02 – 0.04 g/L) fit within the range of other organic flocculants (e.g. starch-based flocculants, tannin-based flocculants, chitosan or polyacrylamides) and are low in comparison with metal-based coagulants (normally >0.10 g/L) [42,48]. However, considering the biomass concentration, *Tanfloc* would demand higher doses (0.1-0.2 ton of flocculant/ton TSS) than the rest of organic flocculants (0.02-0.1 ton of flocculant/ton TSS). As shown in Table 6, the cost of flocculating a ton of microalgal biomass with *Tanfloc* would be around 170-340 $/ton TSS, which is similar to the cost of cationic starch (120-370 $/ton TSS) and lower than conventional metal-based coagulants (e.g. 160-1000 $/ton TSS for aluminium salts). Furthermore, metal-based coagulants cause contamination of microalgal biomass, which may interfere in downstream processes like biogas production; while most organic flocculants do not modify the properties of the microalgal biomass [19,20] and the low flocculant doses needed would decrease the operational

358 costs of harvesting in comparison with metal-based coagulants.

359 Indeed, the economic viability of microalgal biomass production for low added-value
360 applications (e.g. biofuels) involves reducing biomass production costs to 400-750 $/ton TSS [49].
361 Taking into account that biomass harvesting accounts for 20-30% of the total costs of biomass
362 production, the cost of harvesting one ton biomass should range from 100 to 200 $/ton TSS [7].
363 Even if *Tanfloc* cost is slightly high (170 – 340 $/ton TSS), the low energy required for flocculation
364 (1.5 kWh/ton TSS) along with the low contamination risk of microalgal biomass and high biomass
365 recovery (>90%) at low doses (20-40 mg/L), make *Tanfloc* an efficient and cost-effective flocculant
366 for microalgal biomass harvesting, which represents a promising alternative to metal-based
367 coagulants.

368

369 **4. Conclusions**

370 Two sets of dynamic sedimentation tests were carried out in this study (summer and
371 autumn). In the first set, most of the biomass of the 8 days-HRAP (63%) had settling velocities
372 between 16.5 and 4 m/h, while most of the biomass of the 4 days-HRAP (65%) had settling
373 velocities between 16.5 and 1 m/h. In the second set, most of the biomass of the 8 days-HRAP
374 (80%) and 4 days-HRAP (60%) had settling velocities between 6.5 and 0.4 m/h. In this second set,
375 20% of the biomass of the 4 days-HRAP and 40% of the 8 days-HRAP had velocities <0.4 m/h. The
376 addition of flocculant (*Tanfloc* SG) at optimal doses ranging from 20 to 40 mg/L had impressive
377 effects on the settling velocities distribution in this second set. 70% and 84% of biomass reached
378 velocities >6.5 m/h, compared to 10% and 14% of microalgal biomass without flocculant for the 8
379 and 4 days-HRAPs, respectively. With flocculant, a very small amount of biomass (3% for the 4
380 days-HRAP and 8% for the 8 days-HRAP) had a settling velocity <0.4 m/h. Microscopic
381 examination of samples from sedimentation tests revealed that after passing through the elutriation
382 apparatus less than 1,500 of microalgae individuals/mL were detected in all outlet tank samples
383 (inlet samples > 10^5 individuals/mL). According to our results, a settler designed with a critical

384 settling velocity of 1 m/h (typical from secondary settlers) would reach a biomass recovery of 90-
385 94% with *Tanfloc,* while only 77-88% of the biomass would be recovered without flocculant.
386
387 **Acknowledgements**
388 This research was funded by the Spanish Ministry of Economy and Competitiveness
389 (Project DIPROBIO, CTM2012-37860). Raquel Gutiérrez is grateful to the Generalitat de
390 Catalunya for her PhD scholarship (2013FI_B 01096). The authors kindly acknowledge Aitor
391 Padilla from UPC and Rocío López from UJAT for their contribution to the experiments.
392

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532

533

Table 1

Main properties of the primary effluent and the mixed liquor of both high rate algal ponds (HRAPs) in summer and autumn. Mean values (standard deviation) for daily (n=10) and weekly (n=3) samples taken at 12 PM.

Parameter	Primary effluent	4 days-HRAP	8 days-HRAP	
Summer				
Temperature (ºC)	29.1 (2.6)	23.1 (3.1)	23.0 (3.0)	Daily
pH	8.02 (0.17)	8.85 (0.21)	9.12 (0.16)	
DO (mg/L)	1.3 (0.4)	8.3 (0.7)	8.7 (0.9)	
Turbidity (NTU)	94 (44)	106 (9.0)	204 (18)	
VSS (mg/L)	-	240 (9)	361 (68)	Weekly
COD (mg/L)	159 (55) *	55 (5) **	54 (11) **	
NH_4^+-N (mg/L)	34.7 (1.40)	0.60 (0.33)	0.47 (0.52)	
Autumn				
Temperature (ºC)	25.9 (4.01)	23.12 (3.14)	23.03 (3.02)	Daily
pH	7.81 (0.09)	8.51 (0.44)	8.9 (0.4)	
DO (mg/L)	2.2 (1.8)	9.2 (1.8)	11 (2.23)	
Turbidity (NTU)	104 (81)	96 (35)	187 (28)	
VSS (mg/L)	-	152 (12)	249 (34)	Weekly
COD (mg/L)	296 (165) *	62 (13) **	57 (18) **	
NH_4^+-N (mg/L)	22.7 (10.1)	1.68 (0.88)	0.45 (0.18)	

* Total COD

** Soluble COD

Note: DO: dissolved oxygen. VSS: volatile suspended solids. COD: chemical oxygen demand and NH_4^+-N: ammonium nitrogen.

534

Table 2

Equations required to calculate the mass of biomass (expressed in g VSS and %VSS) in the inlet tank, retained in each column (C1, C2 and C3) and collected in the outlet tank.

Tank/column	Microalgal biomass (as g VSS) (W_i)	Microalgal biomass (as VSS %)
Inlet tank	$VSS_{inlet} * V_{inlet}$	
50 mm-column (C1)	$VSS_{C1} * V_{C1}$	$\frac{W_{C1}}{W_{C1} + W_{C2} + W_{C3} + W_{outlet}} * 100$
100 mm-column (C2)	$VSS_{C2} * V_{C2} * (1 - \frac{V_{C1}}{V_{inlet}})$	$\frac{W_{C2}}{W_{C1} + W_{C2} + W_{C3} + W_{outlet}} * 100$
200 mm-column (C3)	$VSS_{C3} * V_{C3} * (1 - \frac{V_{C1} + V_{C2}}{V_{inlet}})$	$\frac{W_{C3}}{W_{C1} + W_{C2} + W_{C3} + W_{outlet}} * 100$
Outlet tank	$VSS_{outlet} * V_{outlet} * (1 - \frac{V_{C1} + V_{C2} + V_{C3}}{V_{inlet}})$	$\frac{W_{outlet}}{W_{C1} + W_{C2} + W_{C3} + W_{outlet}} * 100$

where V_{inlet} is the volume of mixed liquor pumped (L); V_{C1}, V_{C2}, V_{C3} are the volumes of each column (C1, C2 and C3) (L); V_{outlet} is the sum of the volumes of each column (C1, C2 and C3) and V_{inlet} (L); VSS_{inlet}, VSS_{C1}, VSS_{C2}, VSS_{C3}, and VSS_{outlet} are the volatile suspended solids concentrations (g/L) measured in the samples collected from inlet tank, columns C1, C2 and C3 and outlet tank, respectively; W_{inlet}, W_{C1}, W_{C2}, W_{C3} and W_{outlet} are the mass of microalgal biomass (g VSS) in inlet tank, columns C1, C2 and C3 and outlet tank, respectively.

546 **Table 3**
547 Dynamic sedimentation test results in summer (without flocculant) from both HRAPs (4 and 8 days
548 of hydraulic retention time).

Sample		Microalgal biomass (as VSS)					Biomass recovery (g)	Experimental error (%)
		Inlet tank (g)	50 mm-column (g)	100 mm-column (g)	200 mm-column (g)	Outlet tank (g)		
1	4 days-HRAP	6.38	0.81	2.48	1.52	1.33	6.13	96.1
	8 days-HRAP	10.45	1.00	5.18	2.03	1.90	10.10	96.7
2	4 days-HRAP	4.51	0.47	1.42	1.38	0.96	4.23	93.7
	8 days-HRAP	11.00	1.11	6.87	1.49	0.98	10.44	94.9
3	4 days-HRAP	7.02	0.84	2.10	2.20	1.65	6.80	96.8
	8 days-HRAP	12.20	1.30	8.60	1.08	1.14	12.11	99.3

549
550

Table 4

Results of jar tests with *Tanfloc* SG (n=2). Microalgal biomass recovery was calculated from turbidity values. Mean values (standard deviation) from the HRAP with (a) 4 days and (b) 8 days of hydraulic retention time. The optimal dose (here grayed) is the lowest dose leading to a biomass recovery above 90%.

(a)

Concentration (mg/L)	Turbidity (NTU)	Biomass recovery (%)	pH
0	133.0 (17.4)		8.4 (0.2)
10	14.5 (6.1)	88.7 (6.1)	8.3 (0.3)
20	8.5 (1.9)	93.5 (2.3)	8.3 (0.3)
30	5.2 (0.2)	96.1 (0.7)	8.1 (0.3)
40	4.0 (0.8)	97.0 (1.0)	8.1 (0.3)
50	3.0 (0.1)	97.7 (0.2)	8.0 (0.3)
60	1.4 (0.3)	98.9 (0.4)	7.9 (0.3)

(b)

Concentration (mg/L)	Turbidity (NTU)	Biomass recovery (%)	pH
0	219.3 (27.8)		8.4 (0.2)
10	50.5 (20.5)	77.4 (6.5)	8.4 (0.2)
20	40.2 (16.7)	82.0 (5.3)	8.4 (0.1)
30	27.3 (8.7)	87.7 (2.4)	8.3 (0.1)
40	17.8 (3.3)	91.9 (0.5)	8.2 (0.1)
50	15.1 (0.3)	93.1 (0.8)	8.1 (0.2)
60	7.3 (1.9)	96.7 (0.4)	8.0 (0.3)

Table 5

Dynamic sedimentation test results in autumn without flocculant (control) and with flocculant (*Tanfloc* SG).

Sample		Microalgal biomass (as VSS)					Biomass recovery (g)	Experimental error (%)
		Inlet tank (g)	50 mm-column (g)	100 mm-column (g)	200mm-column (g)	Outlet tank (g)		
Control	4 days-HRAP	4.25	0.41	0.91	1.07	0.60	2.99	70.2
	8 days-HRAP	4.40	0.44	0.87	1.30	1.70	4.32	98.2
Tanfloc SG	4 days-HRAP	4.17	3.71	0.46	0.12	0.11	4.41	105.8
	8 days-HRAP	5.86	4.62	1.18	0.26	0.52	6.58	112.3

573 **Table 6**
574 Operational cost of different coagulants/flocculants used for microalgae harvesting and wastewater
575 treatment.
576

	Ecotan[1]	**Starch**[2]	***Tanfloc***[3]	**Poly γ-glutamic acid**[4]	**Chitosan**[5]	**PAC**[6]	**Aluminium sulphate** [6]
Optimal dose of flocculant (mg/L)	10	25-40	20-40	20	10-15	60	60-250
Biomass concentration (mg/L)	400	200-500	100-400	400	400-600	150	150-900
Dose (ton/ton TSS)	0.02	0.07-0.1	0.1-0.2	0.02	0.02-0.03	0.4	0.2-0.8
Flocculant cost ($/ton TSS)	1.05	1-3	1.7	5	25-70	0.4-1.4	0.9-2.1
Contamination risk	Low	Low	Low	Medium	Medium	High	High
Operational cost ($/ton TSS)	<50	120-370	170-340	250	500-1400	160-560	300-1000

577 [1][20],[2][18,19], [3]This study and [20], [4][22], [5][9,48], [6][48]

578

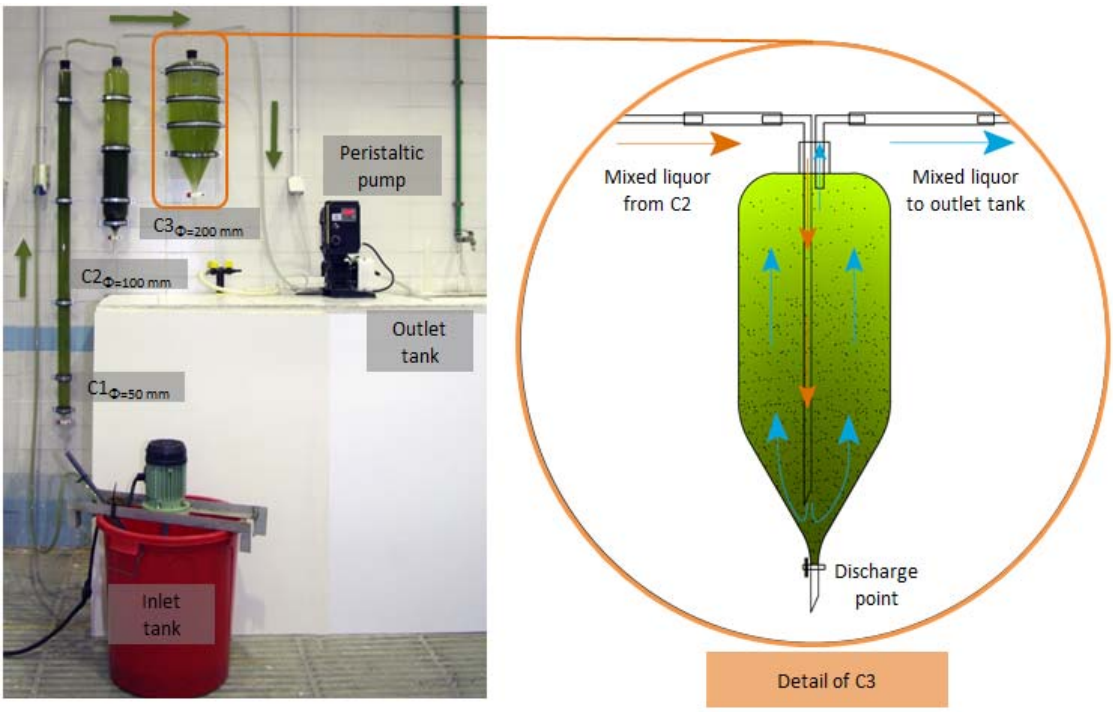
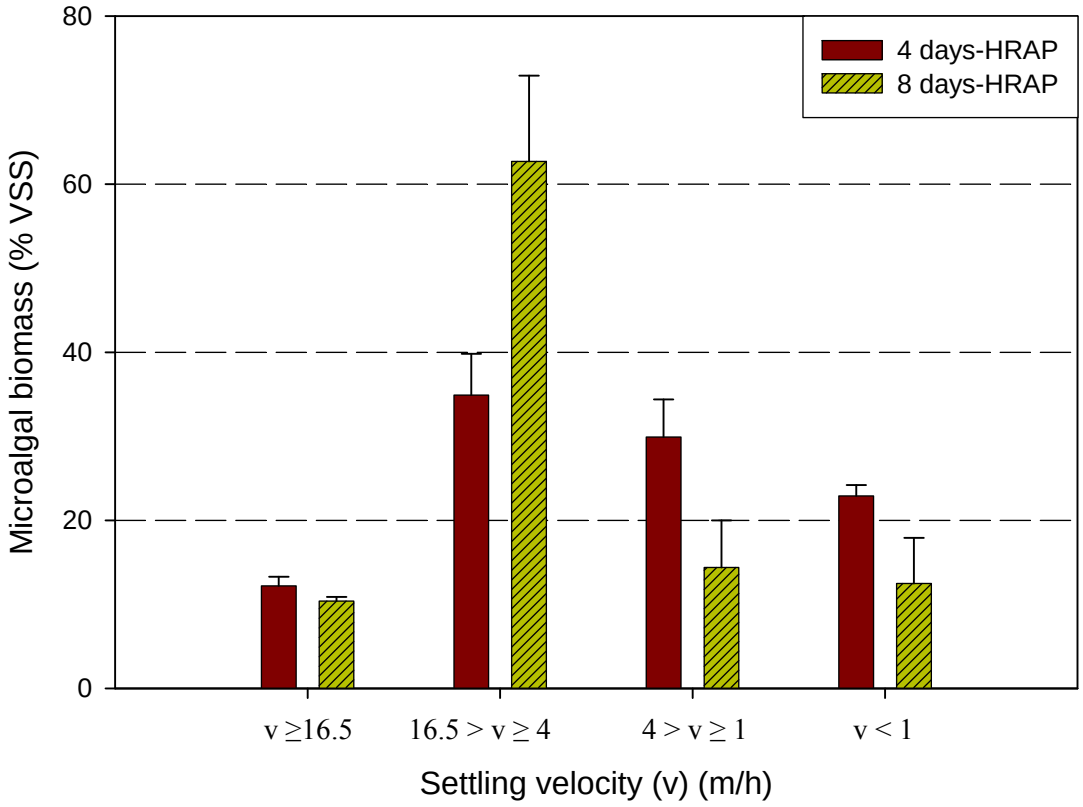
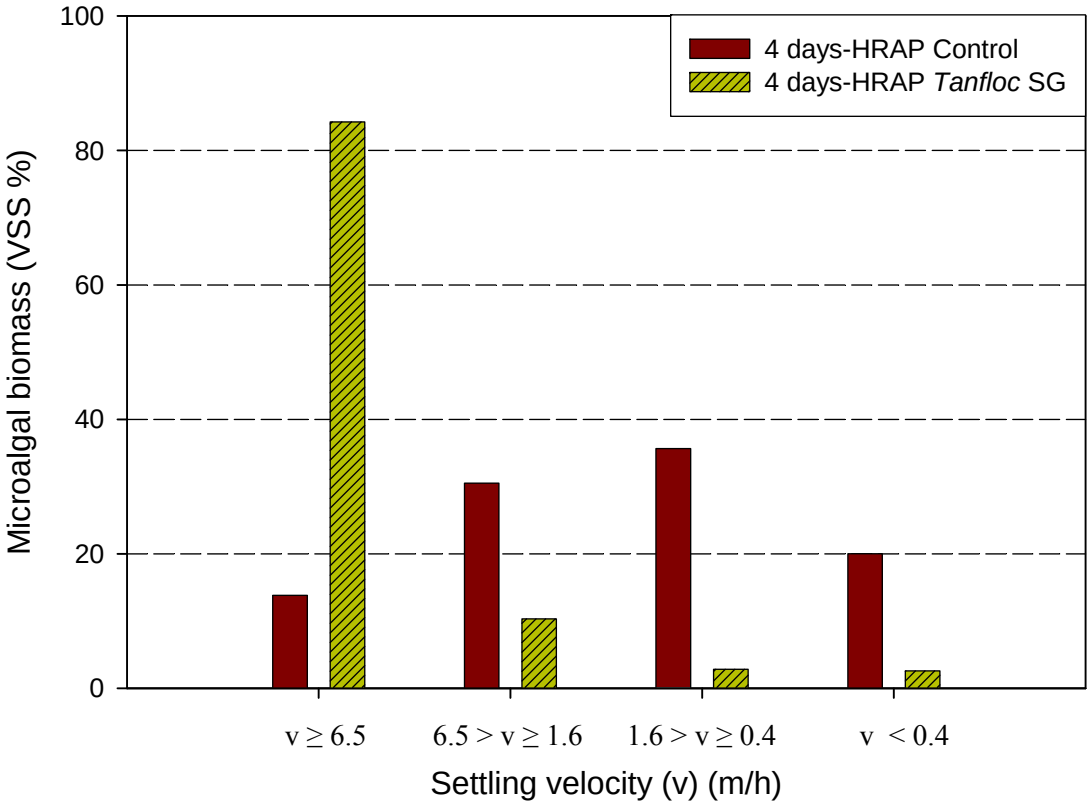


Fig. 1. Water elutriation apparatus used for the dynamic sedimentation test. C1, C2 and C3 are the interconnected settling columns. The discharge point located at the bottom of each column was used to collect microalgal biomass at the end of the experiment.

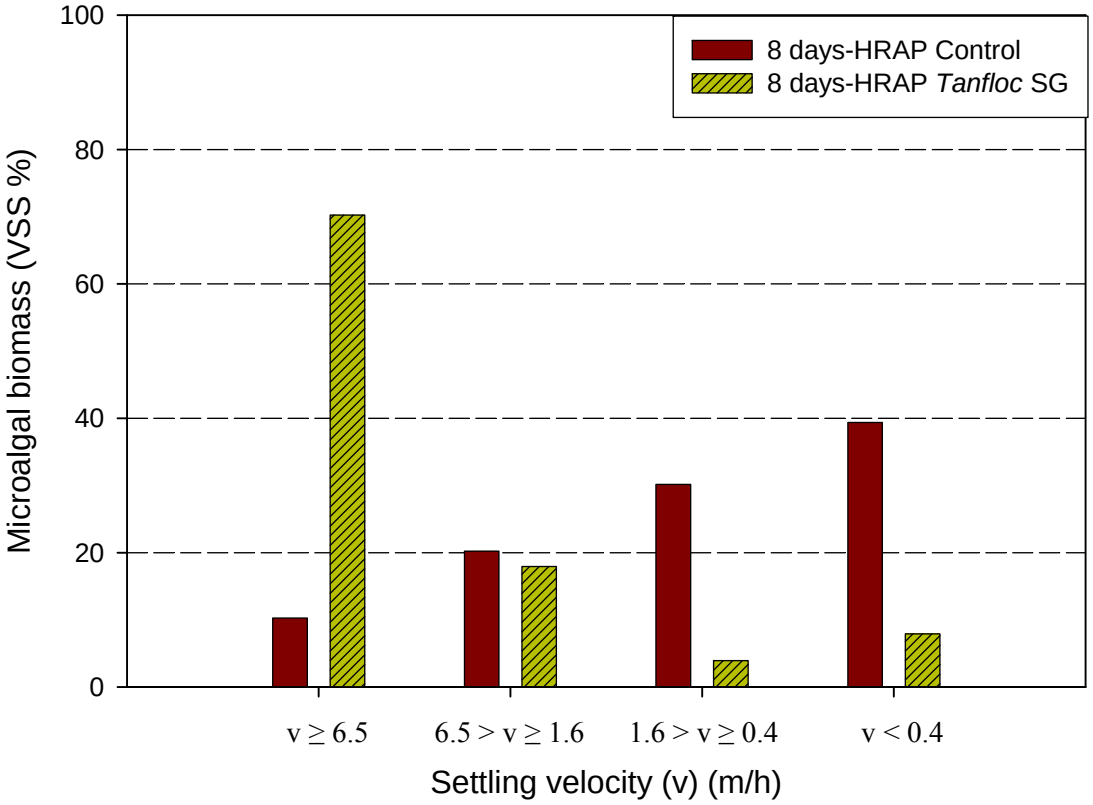


585
586
587 **Fig. 2.** Average percentage of microalgal biomass with a given settling velocity distribution
588 (without flocculant) in both HRAPs (4 and 8 days of hydraulic retention time) (n=3). Error bars
589 represent standard deviations.
590

591 (a)

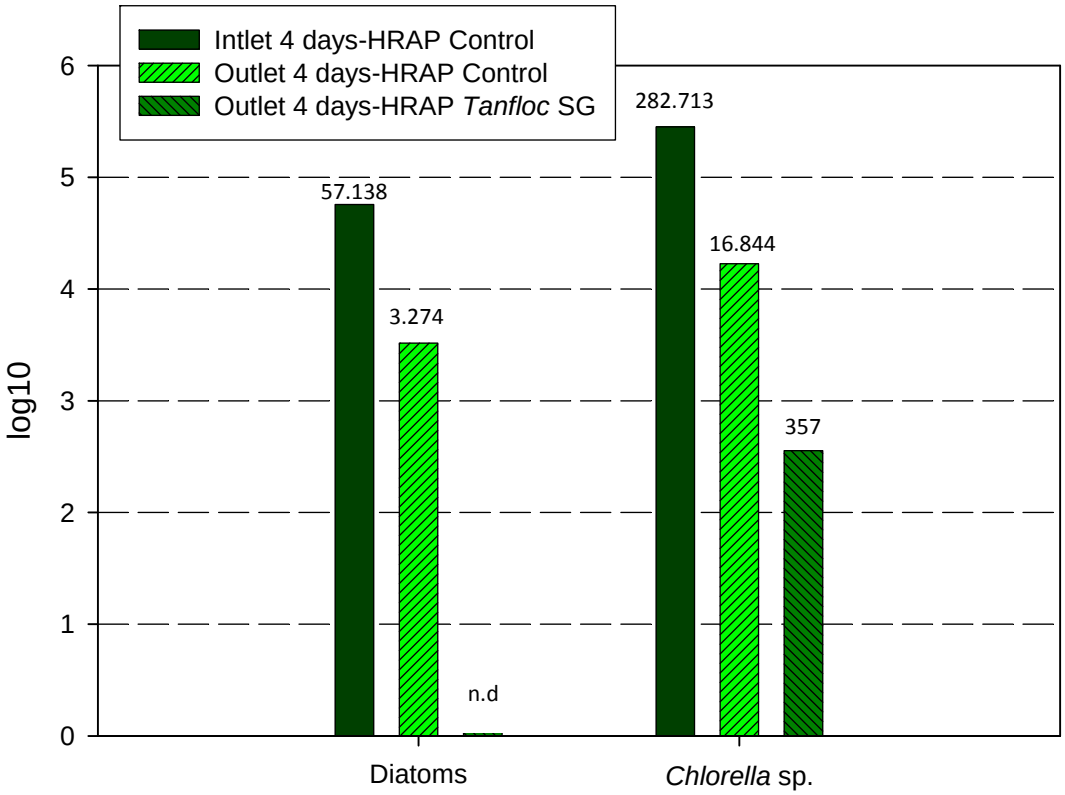


592
593 (b)
594



595
596
597 **Fig. 3**. Average percentage microalgal biomass with a given settling velocities distribution in
598 autumn (with and without flocculant) in the 4 days-HRAP (a) and in the 8 days-HRAP (b).

(a)



(b)

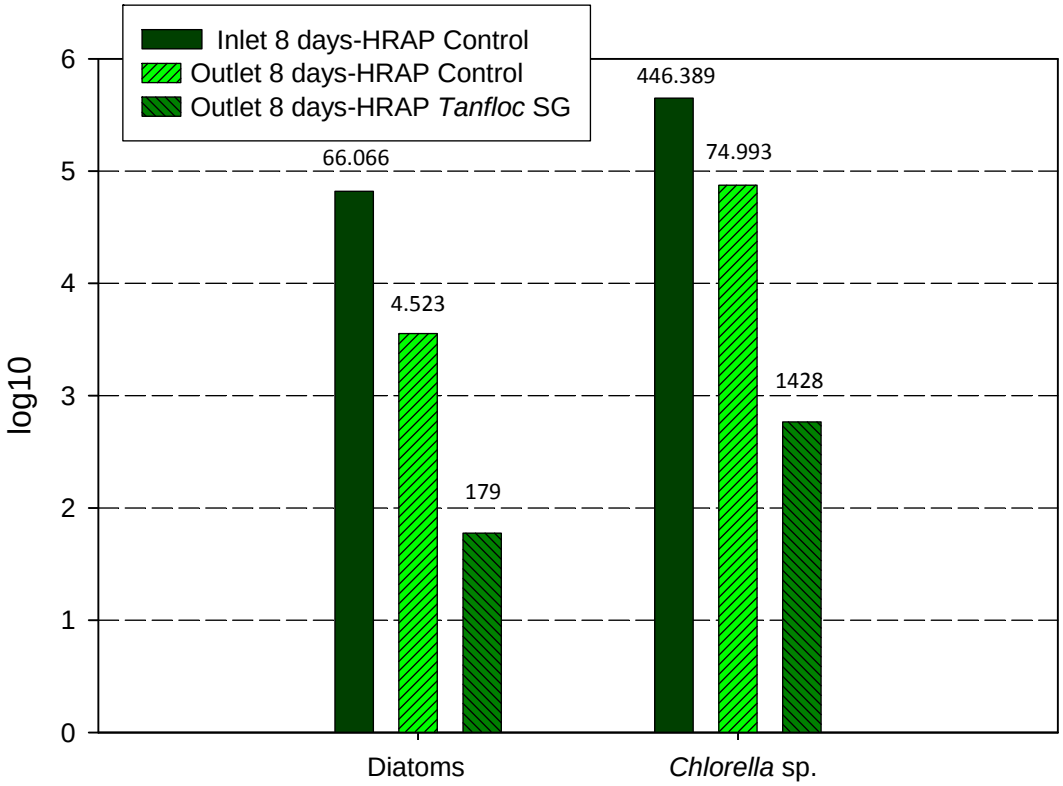
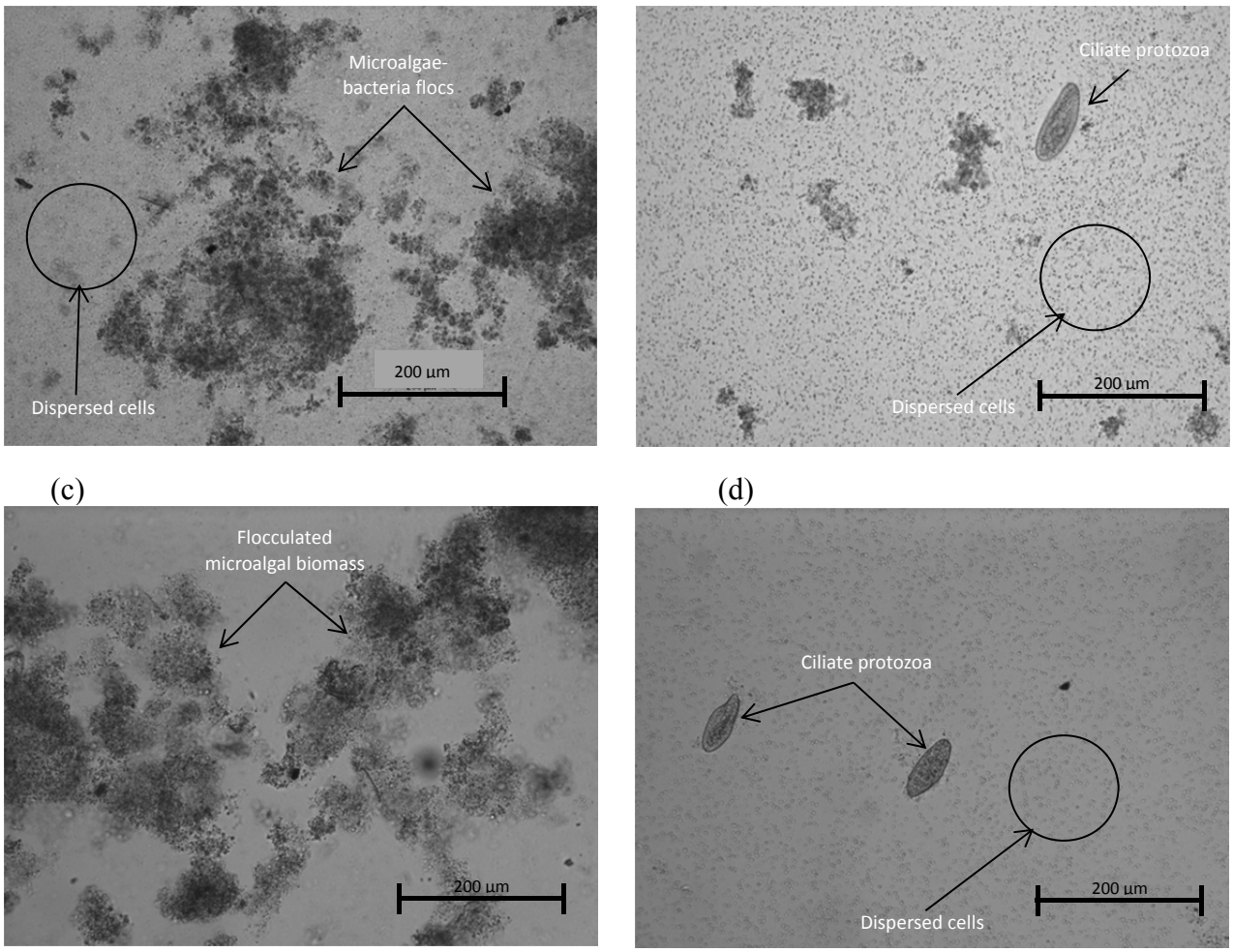


Fig. 4. Distribution of the main microalgae populations in the inlet tank and the outlet tank (with and without flocculant) from the 4 days-HRAP (a) and 8 days-HRAP (b). n.d: non-detected

606 (a) (b)



607 (c) (d)

608
609
610
611 **Fig. 5.** Images of 4 days-HRAP mixed liquor samples before and after dynamic sedimentation tests.
612 (a) Inlet tank sample without flocculant. (b) Outlet tank sample without flocculant. (c) Inlet tank
613 sample with flocculant. (d) Outlet sample with flocculant.
614
615

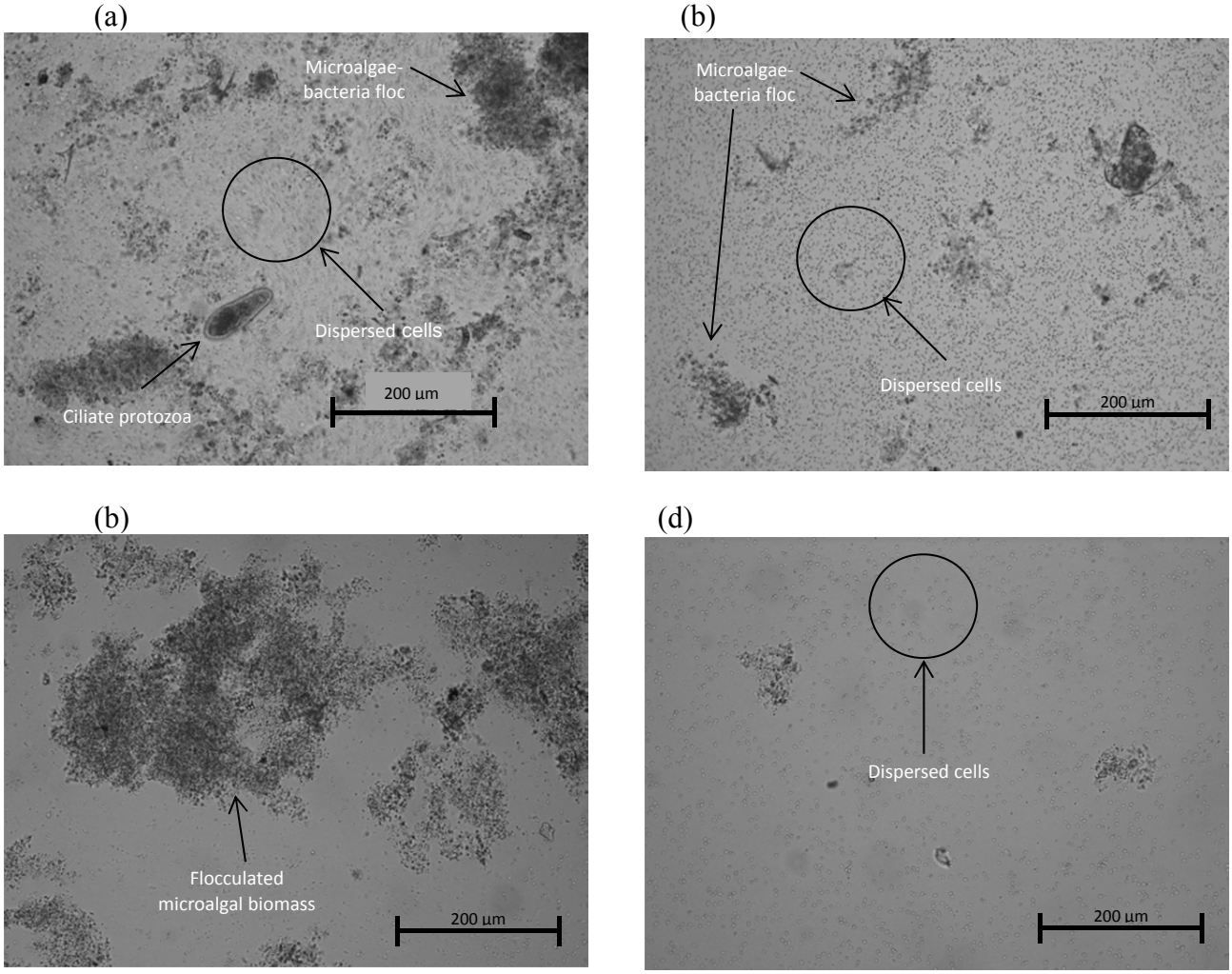


Fig. 6. Images of 8 days-HRAP mixed liquor samples before and after dynamic sedimentation tests. (a) Inlet tank sample without flocculant. (b) Outlet tank sample without flocculant. (c) Inlet tank sample with flocculant. (d) Outlet sample with flocculant.