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Effectiveness of starch on microalgal biomass recovery, settleability and biogas production

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Abstract

In the context of wastewater treatment with microalgal cultures, coagulation-flocculation followed by sedimentation is one of the suitable options for microalgal harvesting. This process is enabled by the addition of chemicals (e.g. iron). However, in a biorefinery perspective, it is important to avoid possible contamination of downstream products caused by chemicals addition. The aim of this study was to evaluate the effect of potato starch as flocculant for microalgal biomass coagulation-flocculation and sedimentation. The optimal flocculant dose (25mg/L) was determined with jar tests. Such a concentration led to more than 95% biomass recovery (turbidity<9NTU). The settleability of flocs was studied using an elutriation apparatus measuring settling velocities distribution. This test underlined the positive effect of starch on the biomass settling velocity, increasing to >70% the percentage of particles with settling velocities >6.5 m/h. Finally, biochemical methane potential tests showed that starch biodegradation increased the biogas production from harvested biomass.

Keywords Coagulant, Flocculant, Harvesting, High rate algal pond, Microalgae, Starch.

1. Introduction

During the last decade the potential of microalgae as biorefinery feedstock has been widely investigated (Uggetti et al., 2014). In spite of the promising results obtained, scaling-up the technology is hampered by the high costs of the process. In particular, the biomass harvesting step represents 20-30% of microalgal biomass production costs (Barros et al., 2015).

A number of solids separation techniques are currently available in the field of water treatment technology including centrifugation, flocculation and flotation (Uduman et al., 2010; Kurniawati et al., 2014) or membrane procedures such as magnetic, vibrating and rotating membranes. In general, for the production of low-value products such as biofuels, harvesting techniques should consist in low-cost and low-energy demand methods capable of processing a large volume of culture medium. Thus, coagulation-flocculation followed by sedimentation is among the most suitable options. This process is enhanced by the addition of chemicals such as salts of aluminum or iron. In a biorefinery context, it is important to ensure that downstream products are not contaminated by chemicals (Zheng et al, 2012). For this reason, the use of natural organic flocculants like tannin based polymers or modified starch are being increasingly investigated (Vandamme, 2013). Indeed, potato starch could be seen as a residue from the potato industry (e.g. starch contained in potatoes peel).

The aim of the present study was to evaluate the effect of potato starch on the coagulation-flocculation and sedimentation of microalgal biomass grown in a pilot high rate algal pond (HRAP) used for wastewater treatment. The optimal dose was determined with jar tests and the settleability of formed flocs was studied using an elutriation apparatus measuring the settling velocities distribution. Moreover, the effect of starch on biogas production was determined in biochemical methane potential (BMP) tests.

2. Materials and methods

2.1 Microalgal culture

Microalgal biomass used for this experiment was cultivated in a pilot plant located at the laboratory of the GEMMA research group (Universitat Politècnica de Catalunya, Barcelona, Spain). The pilot plant consists of a HRAP in the form of a raceway pond (0.5 m^3volume) fed with a continuous flow of 60 L/day of primary treated wastewater (24 g chemical oxygen demand (COD)/ $m^2 \cdot d$; 4 g ammonium nitrogen (NH_4^+-N)/ $m^2 \cdot d$). The system had been in operation for 4 years prior to the experiment, and was described in detail by Passos et al. (2013). At the time the experiments were conducted microalgal populations were mainly composed by *Chlorella* sp. with a total suspended solids (TSS) concentration about 200 mg/L.

2.2 Flocculant

Starch is a natural product having strong flocculating properties. Potato starch solution 1% ($C_6H_{10}O_5$) provided by Panreac (Spain) was used as flocculant. Starch addition did not modify significantly the pH of the system, which remained almost constant along the experiments (9.5±0.6). The zeta potential was determined for the selected starch concentrations and resulted in values of -35.8 mV (for 10 mg/L) and -19.4 mV (for 25 mg/L).

2.3 Jar tests

The optimal dose of flocculant was determined by means of jar tests performed following standard protocols (Metcalf and Eddy, 2003). Five jar tests were carried out during February and March 2014, with starch concentrations from 5 to 80 mg/L.

Turbidity and pH (measured with a HI93703 Hanna Instruments Turbidimeter and a Crison 506 pH-meter, respectively) were determined from fresh HRAP mixed liquor at the beginning of the experiments and from the supernatant liquid after the jar test. Then, biomass recovery (RE) was calculated based on initial (Ti) and final (Tf) turbidity measurements (Eq. 1). Recovery values of the five jar tests were then averaged.

$$RE\ (\%) = (Ti - Tf) / Ti * 100 \qquad (1)$$

2.4 Elutriation test

Elutriation is a water-current separation technique in which particles are washed out according to their weight, volume or form. This test can be used to assess the feasibility of separation treatment by settling (Krishnappan et al., 2004).

The water elutriation apparatus used in the present study was a modified version of a system proposed by Walling and Woodward (1993). The system (Figure 1) consisted of 3 cylindrical settling columns of different diameter (corresponding to different settling velocities) interconnected in series by glass and PVC tubing. The diameters of the columns were: 50 mm in the first column, 100 mm in the second and 200 mm in the third one. The sample entered the columns near the bottom and exited near the top, allowing the sediment flocs that had settling velocities higher than the upward suspension velocity to settle in the respective columns. Considering a flow rate of 0.21 L/min, the upward velocities generated in the three settling columns were 6.5 m/h, 1.6 m/h and 0.4 m/h, respectively. Thus, the first column collected biomass with settling velocities >6.5 m/h, second column collected biomass with settling velocities between 6.5 and 1.6 m/h, and the third column collected the biomass with settling velocities between 1.6 and 0.4 m/h. The outlet suspension contained the biomass fraction whose settling velocity was lower than the suspension velocity in the third column (0.4 m/h). All fractions of biomass retained in columns were collected and TSS analyzed according to Standard Methods (APHA AWWA-WPCF, 2001) for calculating the settling velocity distribution.

Samples collected from the mixed liquor HRAP were mixed with starch at 2 different concentrations (10 and 25 mg/L) and successively pumped through the series of settling columns by means of a peristaltic pump. A sample without starch was also tested as control. All tests were carried out in triplicate. In order to establish differences between samples, ANOVA test was performed with Excel.

2.5 Biochemical methane potential tests

Biochemical methane potential tests were used to compare microalgal anaerobic biodegradability and biogas production with and without flocculant addition. The BMP test was performed in triplicate in samples with and without flocculant addition. Digested sludge from a full-scale anaerobic reactor located in a municipal wastewater treatment plant was used as inoculum.

BMP tests were performed in 160 mL bottles, in which 60 mg of microalgal biomass (40.4 g VS/L) and 40 mg of digested sludge (30.4 g VS/L)were added, along with 2 different starch concentrations (10 and 25 mg/L)Three controls were also performed, namely microalgal biomass and the 2 selected starch doses. All bottles where flushed with Helium gas, sealed with butyl rubber stoppers and incubated at 35 ºC until biogas production ceased. Accumulated volumetric biogas production (mL) was calculated from the pressure increase (periodically measured with a Greisinger GMH 3151 manometer), expressed under standard conditions. The net values of biogas production and yield were obtained by subtracting the endogenous production of blank trials, containing only inoculum.

3. Results and discussion

3.1 Dose of flocculant

In the jar test, all tested starch doses led to high biomass recovery (RE>90%) (Figure 2). The optimal dose was 25 mg/L, which reduced turbidity from 151±14 to 6±2 NTU (biomass recovery of 95.7%). On the other hand, increasing the starch concentration further decreased biomass recovery (to 90.2% for a dose of 80 mg/L). The zeta potential of 25 mg/L of starch (-19.4 mV) indicated an incipient instability of the solution (from ±10 to ±30), with reduced electrical repulsion between particles. Jar test results indicate that high biomass recoveries (about 95%) were also attained with doses lower than the optimal. From a pragmatic point of view these doses would be preferred to avoid overcosts. Even if the zeta potential of 10 mg/L (-35.8 mV) indicated only moderate instability of the solution with still strong electrical repulsion between particles, the dose of 10 mg/L (94% of recovery and 9 NTU) was also selected for settling velocity distribution and biochemical methane potential experiments.

Considering that the initial biomass concentration was approximately 200 mg/L, from 0.05 to 0.15 g of starch per g of biomass were required to harvest more that 95% of the biomass. Table 1 reports optimal doses for different chemicals, note that results comparison among different studies has to be taken with caution since the efficiency of flocculants is dependent on the type of microalgae, their concentration and the medium conditions (Garde et al. 2014; Divakanar and Pillai, 2002).

According to Vandamme et al. (2010), increasing the initial biomass concentration from 75 to 300 mg/L, increased the cationic starch required from 5 to 7.5 g per g of biomass. Values higher than in this study corresponded to lower recovery efficiencies (85% vs. 95%). Higher recovery efficiencies (>95%) were obtained with cationic starch for the flocculation of *Scenedesmus dimorphus* in growth phases at the dosage of 10 mg/L (Hansel et al., 2014). In that study, the initial culture turbidity was 50 NTU, which corresponded to a biomass density of 0.12 g/L, lower than in this case (0.2 g/L).

When comparing doses it is important to consider the flocculants cost which can be significantly different depending on the product. For example, chitosan (10US$) is about 10 times more expensive than cationic starch (1-3 US$) (Vandamme et al., 2010).

3.2 Settling velocity distributions

The elutriation test was performed in order to determine the starch effect on the settling velocity distribution of microalgal biomass. Results shown in Figure 3 represent the percentage of biomass (expressed as TSS) retained in each column, which corresponds to different settling velocities. Considering the control, which is microalgal biomass without starch addition, 46% of the TSS were retained in the first column (settling velocities >6.5 m/h), while 37% of the TSS were retained in the second column (settling velocities between 1.6 and 6.5 m/h).

The effect of coagulation-flocculation on biomass settling velocities was very clear since TSS retention in the first column increased from 46 % (in the control) to 78 and 73% for starch concentrations of 10mg/L and 25mg/L, respectively. This means that starch raised settling velocities of biomass thanks to flocs formation. Results obtained with 10 and 25 mg/L of starch were not significantly different.

Settling velocities have a direct impact on the settler dimensioning. In fact, considering the results of this study, the addition of starch with the consequent increase in settling velocity, would reduce the settler volume by 4 times maintaining the biomass recovery efficiency > 70%.

3.3 Biochemical methane potential

Considering anaerobic digestion as downstream process, the flocculant should not have any inhibitory effect on biogas production. On the contrary, an organic biodegradable flocculant such as starch may even increase biogas production. For this reason, biochemical methane potential tests were carried out to compare the biogas production obtained from control samples (microalgal biomass, and starch at 10 and 25 mg/L) and from samples with microalgal biomass and selected starch concentrations (10 and 25 mg/L). The results obtained (Figure 4) indicate that the addition of starch increased the biogas production with respect to control microalgae biomass (503-536 vs. 467 mL biogas), even though the results are not significantly different ($p<0.05$). The comparison of microalgal biomass and starch codigestion (503-536 mL biogas) with the digestion of both substrates separately (467 mL biogas for microalgae, 10-11 mL biogas for starch) suggests certain synergic effect of the mixture which may be attributed to a more balanced C/N concentration, enhancing to some extent the biogas production.

4. Conclusions

The effectiveness of starch as flocculant was tested on microalgal biomass. Low starch doses (5-60 mg/L) allowed high biomass recovery >92%. This is a promising result, improving both microalgal biomass recovery and treated wastewater discharge. The most appropriate starch dose was 25mg/L, leading to >95% biomass recovery and an effluent turbidity <9 NTU. The elutriation test underlined the positive effect of starch addition on the biomass settling velocity, increasing to >70% the percentage of particles with settling velocities >6.5m/h. Finally, biochemical methane potential tests show that starch biodegradation increased the biogas production from harvested biomass.

5. Acknowledgements

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6. References

1. APHA-AWWA-WPCF Standard methods for the examination of water and wastewater (20th ed.) (2001) American Public Health Association, Washington DC.

2. Barros, A.I., Gonçalves, A.L., Simões, M., Pires, J.C.M. (2015) Harvesting techniques applied to microalgae: A review. Renew Sust Energ Rev 41, 1489-1500.

3. Divakaran, R., Pillai, V.N.S (2002) Flocculation of algae using chitosan. J Appl Phycol 14, 419–422.

4. Farid, M.S., Shariati, A., Badakhshan, A., Anvaripour, B. (2013). Using nano-chitosan for harvesting microalga Nannochloropsis sp. Bioresour Technol 131, 555-559.

5. Gerde, J.A., Yao, L., Lio, L. J., Wen, Z., Wang, T. (2014).Microalgae flocculation: Impact of flocculant type, algae species and cell concentration. Algal Res 3, 30–35.

6. Hansel, P.A., Riefler, R.G., Stuart, B.J. (2014). Efficient flocculation of microalgae for biomass production using cationic starch. Algal Res 5, 133-139.

7. Krishnappan, B.G., Marsalek, J., Exall, K., Stephens, R.P., Rochfort, Q., Seto, P. (2004) Measuring settling velocity distribution of suspended solids in combined sewer overflows. Water Qual Res J 39, 432–438.

8. Kryvoruchko, V.,Machmuller, A. Bodiroza, V. Amon, B,. Amon T. (2009) Anaerobic digestion of by-products of sugar beet and starch potato processing. Biomass Bioenerg 33, 620–627.

9. Kurniawati, H. A., Ismadji, S., Liu, J.C. (2014). Microalgae harvesting by flotation using natural saponin and chitosan. Bioresouce Technol. 166, 429–434.

10. Letelier-Gordo, C.O., Holdt, S.L., De Francisci, D.Karakashev, D. B., Angelidaki, I. (2014) Effective harvesting of the microalgae Chlorella protothecoides via bioflocculation with cationic starch. Bioresource Technol 167, 214–218.

11. Metcalf and Eddy. Wastewater Engineering. Treatment and Reuse (2003) .4 edn. McGraw-Hill, New York.

12. Passos, F., García, J., Ferrer, I. (2013a) Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment. Appl Energ 108, 168-175.

13. Pienkos, P.T. and Darzins, A. (2009) The promise and challenges ofmicroalgal-derived biofuels. Biofuels Bioprod Bior 3, 431–440.

14. Smith, B.T., Davis, R.H. (2012) Sedimentation of algae flocculated using naturally-available magnetic-based flocculants. Algal Res 1, 32-39.

15. Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A. (2010) Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. Renew Sust Energ 2, 012701-1. 012701-15.

16. Uggetti, E., Sialve, B., Steyer, J.P. (2014) Integrating microalgae production with anaerobic digestion: a biorefinery approach. Biofuels Bioprod Bior 8, 516-529.

17. Vandamme, D., Foubert, I., Meesschaert, B., Muylaert, K. (2010) Flocculation of microalgae using cationic starch. J Appl Phycol 22, 525-530.

18. Vandamme, D., Foubert, I., Muylaert, K. (2013). Flocculation as a low-costmethod for harvesting microalgae for bulk biomass production. Trends Biotechnol 31, 233-239**.**

19. Walling, D.E., Woodward, J.C.(1993). Use of a field-based water elutriation system for monitoring the in-situ particle size characteristics of fluvial suspended sediment. Water Res 27, 1413–1421.

20. Zheng, H., Gao, Z., Yin, J., Tang, X., Ji, X., Huang, H. (2012) Harvesting of microalgae by flocculation with poly. Bioresour. Technol 112, 212-220.

Tables and figures

Table 1. Literature results on microalgal biomass recovery using different chemicals.

Microalgal species	Microalgal concentration (g/L)	Chemical	Optimal dose (mg/L)	Biomass recovery (%)	pH	Reference
Schizochytrium limacinum	0.09 0.93 4.65	Aluminum sulfate	200	- 90 90		Gerde et al. 2014
	0.09 0.93 4.65	Cationic starch	10	37 80 80		
Chlamydomonas reinhardtii	0.03 0.31 1.06	Aluminum sulfate	250	90 90 60		
	0.03 0.31 1.06	Cationic starch	20	- 90 20		
Scenedesmus sp.	0.09 0.93 4.65	Aluminum sulfate	200	90 90 90		
	0.09 0.93 4.65	Cationic starch	40	70 90 90		
Chlorella protothecoides	0.44 0.56 0.77	Cationic starch	40	87 95 96	4 7.7 10	Letelier-Gordo et al. 2014
Scenedesmus dimorphus		Cationic starch	10-100	70-95		Hansel et al., (2014)
Parachlorella sp.	0.075 0.15 0.3	Cationic starch	20 25 25	90 90 85	5-10	Vandamme et al., 2010
Mixture of *Spirulina* sp., *Oscillatoria* sp., *Chlorella* sp., *Synechocystis* sp.	10 NTU 20 NTU 30 NTU 55 NTU	Chitosan	15 5 2 15	60 78 82 80	4-9	Divakanar and Pillai, 2002
Nannochloropsis sp.	665 × 10^6cell/mL	Chitosan	60	70-98	7-9	Farid et al., 2013
Mixture dominated by *Chlorella* sp.	0.2	Starch	25	95	8.9-10.1	This study

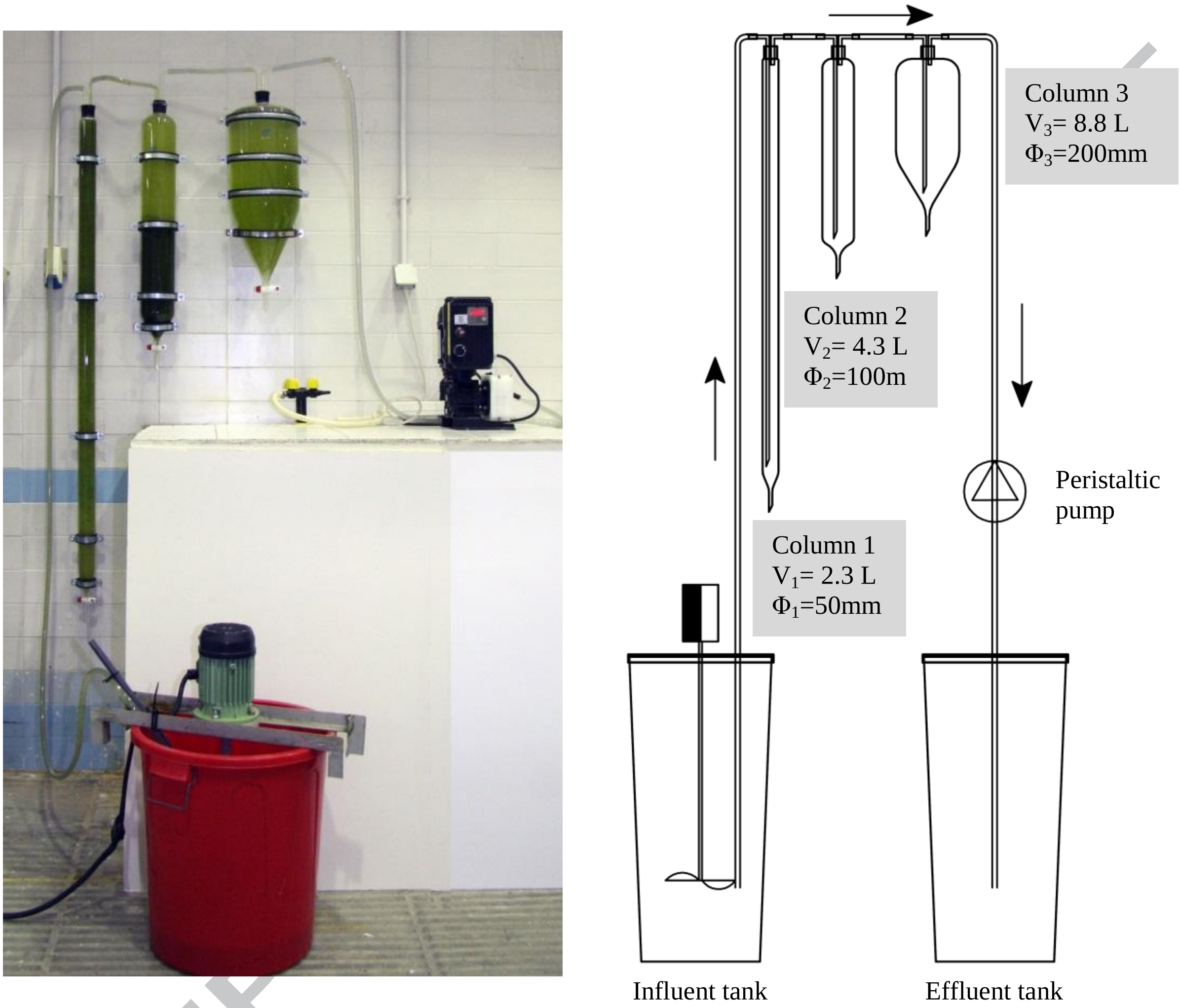

Figure 1. Image and schematic view of the experimental eluriation apparatus used for testing the settleability of microalgal biomass.

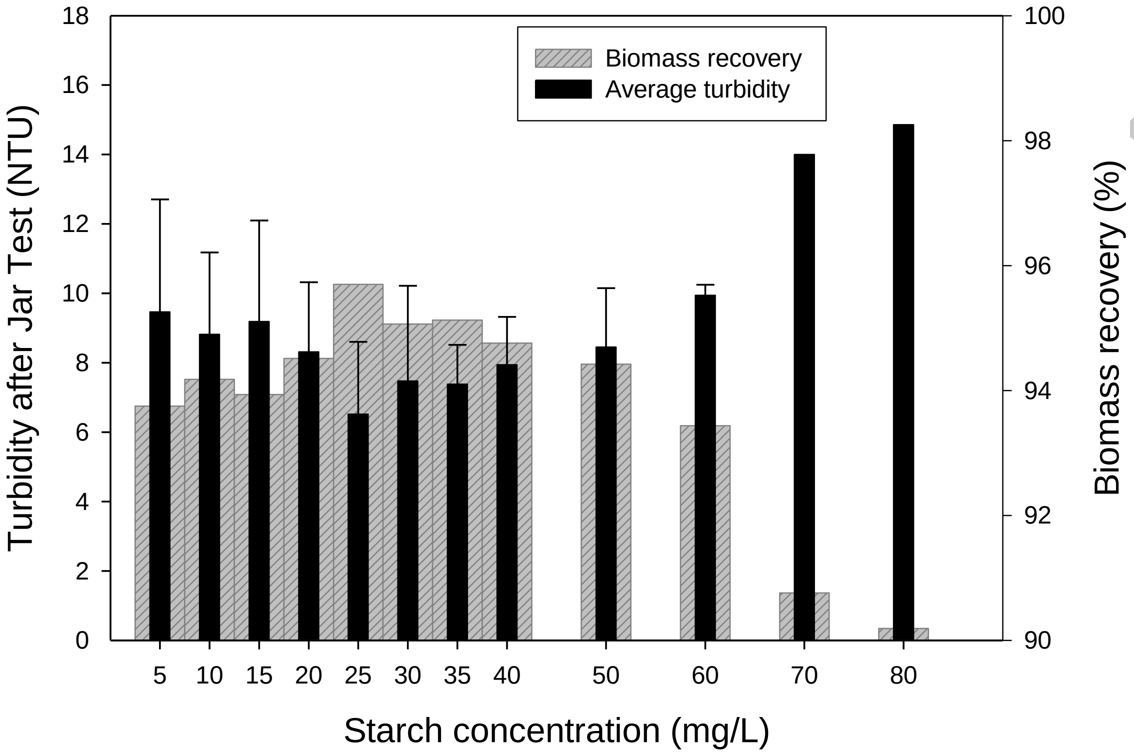

Figure 2. Jar test results expressed as final turbidity and biomass recovery. Biomass recovery was calculated from the initial turbidity (151±14) and final turbidities shown in the graph.

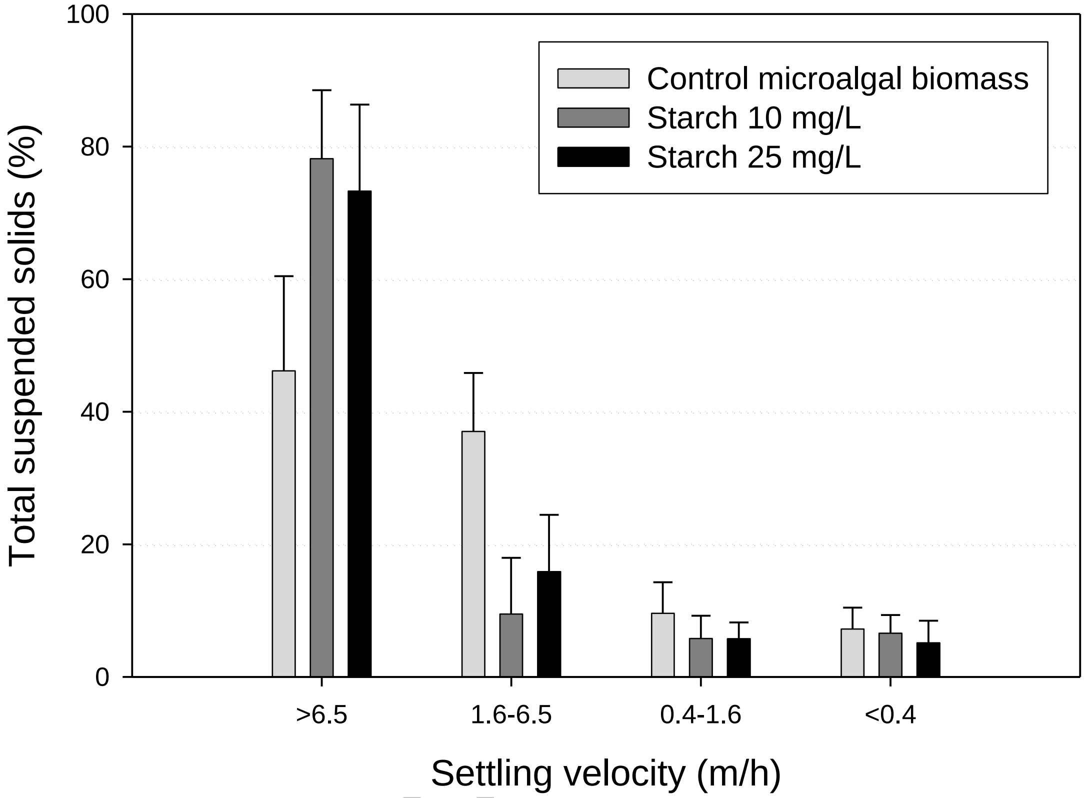

Figure 3. Elutriation test results expressed as percentage of total suspended solids corresponding to different settling velocities.

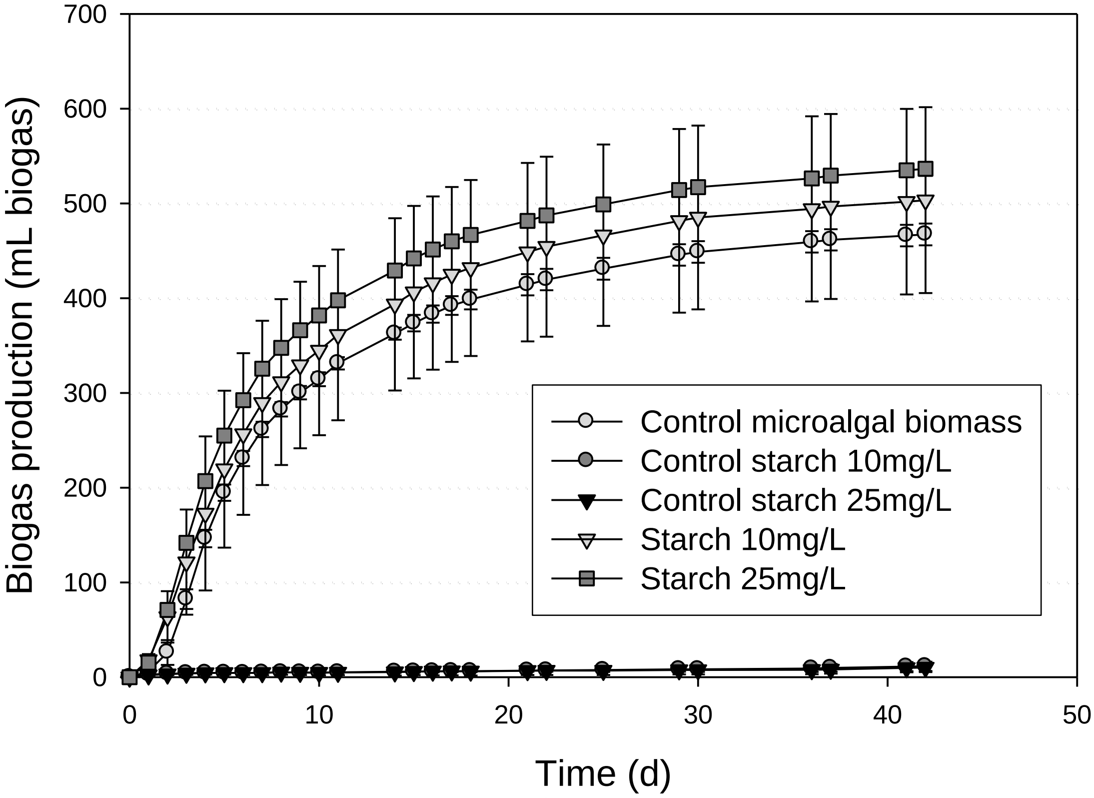

Figure 4. Biochemical methane potential for control (microalgal biomass, starch 10 mg/L and 25 mg/L) and microalgal biomass flocculated with starch doses of 10 and 25 mg/L.

This study aims at evaluating starch as flocculant for microalgal harvesting

The optimal flocculants dose (25 mg/L) leads to more than 95% biomass recovery

Settleability was studied in elutriation apparatus measuring velocities distribution

Flocculants increased by 30% the particles with settling velocity higher than 6.5 m/h

Biochemical methane potential tests results show a biogas yield increment of 8-15%

Figure 1. Image of the experimental settling columns used for testing the settleability of microalgal biomass.