

Preliminary evaluation of *Scenedesmus Obliquus* growth on organic solid waste digestate, with different mechanical pretreatment.

P. Scarponi^{1*}, A. Costa Devoti¹, A. Volpi Ghirardini¹, C. Cavinato¹

¹Department of Environmental Sciences, Informatics and Statistics, University Ca' Foscari of Venice, via Torino 155, 30170 Mestre Venice Italy

* Corresponding author: paolina.scarponi@unive.it , +390412348909

Abstract

Purpose: Anaerobic digestion (AD) is considered the most sustainable method to produce energy (biogas) treating organic waste, in particular the organic fraction of municipal solid waste (OFMSW) or industrial food waste. During last ten years several wastewater treatment plant implemented the organic waste treatment in order to increase both wastewater treatment efficiency and energy recovery, but the AD effluent had substantial nitrogen, phosphorus and other pollutants load which are usually recycled back into the wastewater plant. A kind of digestate post-treatment could be use as culture medium for microalgae growth; this biotechnology could even be associated with biogas up-grading for CO₂ sequestration and/or focused on high-value products recovery.

Methods: In this research, *Scenedesmus obliquus* growth was tested on digestate obtained from the OFMSW-AD. Digestate was diluted 1:10 with no pretreatments and after centrifugation (AC) and ultrafiltration (AUF), in order to evaluate microalgae limiting growth factors. Autotrophic, heterotrophic and mixotrophic conditions were applied and the capacity of microalgae to store chlorophyll and other compounds was evaluated.

Results: The use of centrifugation and filtration showed the best growth results for *S. obliquus* in batch mixotrophic condition, with 0.42 (\pm 0.08) and 0.5 (\pm 0.04) g/l biomass dry weight, in AC and AUF conditions respectively, compared to 0.145 g/l (\pm 0.002) in mixotrophic control (with 1 g/l glucose). The ammonia has been reduced of about 95%, but it will be investigated the correlation with air stripping.

Conclusions: Centrifugation will be considered in next study as the best pretreatment even from economic point of view. The process will be scaled up in continuous mode and with a lower digestate dilution.

Keywords: microalgae, solid waste, anaerobic digestion, digestate.

1. Introduction

Anaerobic digestion is a biogas production technology that constitute today the most sustainable way to use the energy present in biomass and organic wastes, with a simultaneous nutrient recovery and greenhouse gas emissions reduction[1]. Anaerobic digestion treatment of the Organic Fraction of Municipal Solid Waste (OFMSW) is a widespread technology, and there is an increasing interest on it especially considering the sustainability of closing the waste cycle, using upgraded biogas as fuel for collection vehicle, and recovering nutrient, bioplastics and other compounds from liquid digestate. Concerning this last aspect, recently research studies are focusing on the use of microalgae for nutrients removal and for a simultaneous biogas upgrading and production of a biomass that could be exploited for lipid or other compounds recovery[2].

There are several studies about microalgae used for biomass production: this strain in mixotrophic condition can growth on wastewater with simultaneous biomass production and substrate degradation. The focus of these research is a prospective of microalgae large scale production for biofuels, thanks to their high biomass productivity [3]. Nevertheless, microalgae needs large quantities of phosphate and nitrogen to growth and stock by-products, which is, from an economic and environmental point of view, unsustainable. A possible strategy is to recycle phosphorus and nitrogen in order to reduce the use of fertilizers; an example is the integration of AD

and microalgae treatment. In fact, the effluent, plenty of nitrogen and phosphorus, could be used as substrate for microalgae growth [4-5]. The use of digested effluent instead of water and its use as low-cost nutrient source for microalgae growth, could decrease the operating cost [2].

Moreover, there is the possibility to use microalgae organic residues (after extraction of by-products) back into anaerobic digestion. This could increase methane production and, at the same time, to obtain other nutrients from waste effluent that could be re-use for microalgae growth [4-5]

In this study, *S. obliquus* growth was preliminary evaluated using organic solid waste digestate as culture medium, testing AC and AUF like mechanical pre-treatments.

2. Materials and methods

2.1 OFMSW digestate characterization

The anaerobic digestate was collected in a wastewater treatment plant (WWTP) located in the north-east of Italy, in which the anaerobic co-digestion of the OFMSW has implemented with waste activated sludge (WAS). The digestate was characterized in terms of total and volatile solids (TS, TVS), pH, alkalinity, ammonia nitrogen, volatile fatty acids (VFA) and soluble chemical oxygen demand (sCOD) (Table 1). All analyses were performed considering APAT, IRSA-CNR and APHA, AWWA, WET methods [6].

Table 1: Anaerobic digestate characteristic

	Value
pH	7.6
Total alkalinity (mgCaCO ₃ /l)	1.69
Partial alkalinity (mgCaCO ₃ /l)	2.84
N-NH ₄ ⁺ (mgN/l)	573
VFA (mg/l)	80
sCOD (mg/l)	300

2.2 Microalgal strain, inoculum and media

The microalgae strain *S. obliquus* was tested in this study: the culture was maintained on ISO 8692 media and its growth was monitored considering dry weight, cellular count and optical density at OD₆₈₀ - OD₇₅₀ nm. The dry weight was measured filtering 10 ml on acetate cellulose filter with 0.45 µm pore and then dried at 105°C; cellular count was evaluated using Leika microscope equipped with Bürker chamber; the optical density was measured using a spectrophotometer Unicam, Helos Y.

2.3 Experiment set-up

In this study the effects on *S. obliquus* growth on digestate was tested after three types of pretreatment: i) straight digestate; ii) digestate after AC (5 minutes at 5000 rpm) and iii) digestate after AUF (0.45 µm acetate cellulose filter). The microalgae growth was tested in mixotrophic and heterotrophic conditions, the inoculum was diluted 1:10 and all flasks in batch condition was maintained at 20 °C, mixed and with bubbling and irradiation (2010 lux). All flasks were in duplicate. The dry weight, abs (680 and 750 nm) and cellular count were daily analyzed.

In addition, it was carried out a microbiological contamination with a qualitative measuring only on digestate after AC and AUF both in mixotrophic conditions with dry weight and cellular count. (value in pg cell⁻¹). This kind of analysis is made by studying the ratio D_w/CC where D_w is the Dry Weight of the cell suspension and CC is the Cellular Count. This ratio shows the theoretical single cell dry weight (for example in 1 ml). If a change in this ratio it's showed and no morphological modification of the strain are observed, a possible microbiological contamination (i.e. bacteria or other microorganisms) may have happened.

2.4 Ammonia and chlorophyll a, b and carotenoid analysis

During this experiment, in addition to growth monitoring in all test conditions, ammonia reduction and the chlorophyll a, b and carotenoid cellular accumulation were examined.

The ammonia analysis has been performed with ISE Ammonia probe (Hanna instrument). The amount of chlorophyll a, b and carotenoid, was made in according with Jalal et al. [7]. 1 ml of microalgae suspension has been centrifuged at 8000 rpm for 5 minutes and cleaned with Milli-Q water. After water removal, the microalgae pellet has been putted in a thermostat bath at 60 °C for 1 h adding 1 ml of methanol. After that, 1 ml of sample (after centrifugation) has been added with 2 ml of methanol to perform abs analysis (λ 666, 653 and 470 nm).

The amount of chlorophyll a, b (C_a , C_b) and carotenoid (C_{x+c}) measured as $\mu\text{g ml}^{-1} \text{ cell}^{-1}$ was obtained applying the following equations [8]:

$$C_a = 15.65 * A_{666} - 7.340 * A_{653}$$

$$C_b = 27.05 * A_{653} - 11.21 * A_{666}$$

$$C_{x+c} = 1000 * A_{470} - 2.860 C_a - 129.2 C_b / 245$$

3. Discussion

3.1 Biomass analysis

During the first part of the experimental test, cellular growth was monitored to understand if *S. obliquus* could growth on OFMSW digestate. Test duration was 8 days, based on the beginning of stationary phase using a maintenance medium in control conditions.

In Figure 1 is reported the cellular count in all tested conditions, for one week. It is possible to observe that cellular count in all mixotrophic conditions (light plus carbon source) results in a higher number of cells than controls and heterotrophic conditions. This means that *S. obliquus* could growth on OFMSW digestate, using nutrients and ammonia. Otherwise, the same nutrient conditions were not enough to have a growth in heterotrophic condition (without light). This result show that illumination is a fundamental factor for the growth of microalgae on this substrate.

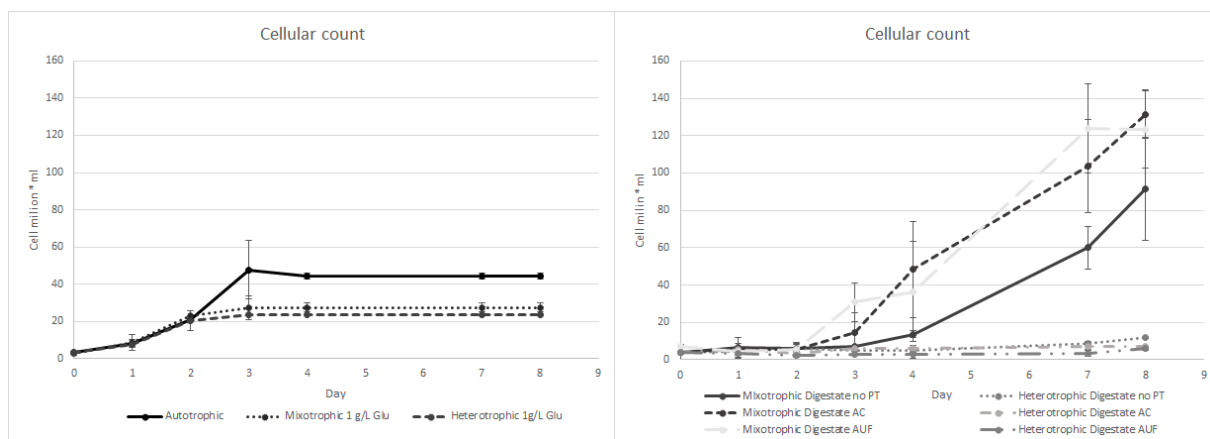


Figure 1: *S. obliquus* cellular count in all different experimental conditions with control phototrophic, mixotrophic with 1 g l⁻¹ glucose and heterotrophic with 1 g l⁻¹ glucose.

At the end of this test, cellular dry weight in mixotrophic conditions on digestate without pre-treatment (no PT), AC and AUF showed a dry weight of 0.3 g l⁻¹ (\pm 0.1) 0.42 g l⁻¹ (\pm 0.08) and 0.5 (\pm 0.04) respectively. The dry weight in control phototrophic, mixotrophic and heterotrophic conditions were 0.12 g l⁻¹ (\pm 0.01), 0.15 g

l^{-1} (± 0.002) and $0.08 \text{ g } l^{-1}$ (± 0.04) respectively. This low amount of biomass in control conditions could even be related to the low amount of nitrogen and other substance in the media used in these tests. Other authors, like Bhatnagar et al. [9], obtained the same results comparing dry weight obtained on synthetic medium and on wastewater mixed with synthetic medium ($0.248 \text{ g } l^{-1}$ and $0.487 \text{ g } l^{-1}$ production of biomass respectively). Moreover, Ji et al. [10] obtained the same results in mixotrophic condition of *S. obliquus* in municipal wastewater, with a dry weight of $0.44 \text{ g } l^{-1}$. In terms of growth rate, *S. obliquus* in mixotrophic conditions with OFMSW digestate (without pre-treatments, AC and AUF) showed $0.36 (\pm 0.2)$, $0.46 \text{ g } l^{-1} \text{ d}^{-1} (\pm 0.2)$ and $0.47 \text{ g } l^{-1} \text{ d}^{-1} (\pm 0.2)$ respectively whereas, in the mixotrophic control, was $1.27 \text{ g } l^{-1} \text{ d}^{-1} (\pm 0.1)$. This low value of growth rate versus control condition is a common result in this test conditions. In fact, in mixotrophic control condition the exponential phase was short with high biomass production. The exponential phase, in the other test conditions, has been longer, probably caused by complex substrate founded inside anaerobic digestate.

Comparing the results only for the mixotrophic test (Figure 2), it is possible to observe a better growth of microalgae with pre-treatments due to a lowered suspended solid; comparing AC and AUF the results were almost similar. We deduced aswell that just one AC cycle will be enough. In addition, considering Dw/CC rate, no biological contamination was observed. (data not reported).

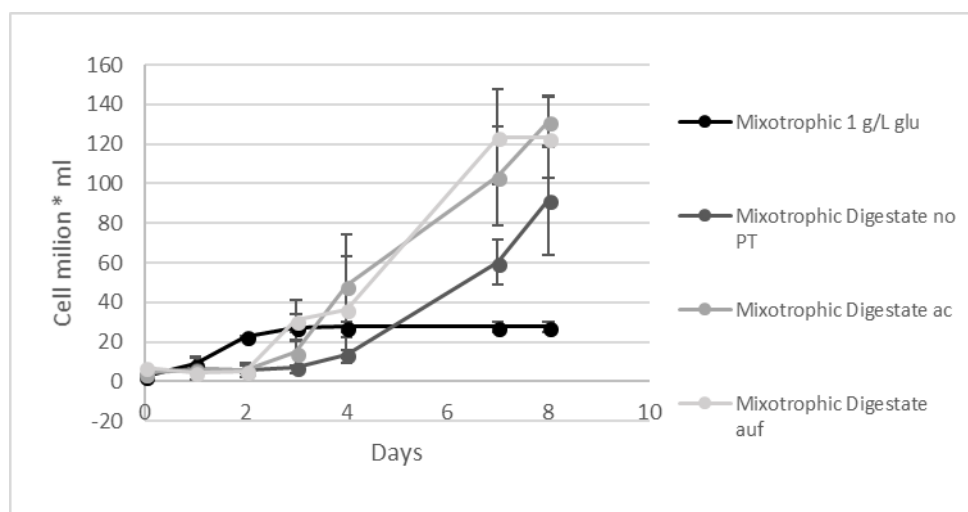


Figure 2: Cellular count of *S. obliquus* in mixotrophic conditions, with digestate and mixotrophic control with $1 \text{ g } l^{-1}$ glucose.

Table 2: Productivity ($\text{g } l^{-1} \text{ d}^{-1}$) of *S. obliquus* in stationary phase in all test conditions.

Productivity ($\text{g } l^{-1} \text{ d}^{-1}$)	Media	Dev.st.
Mixotrophic control 1 g/l glucose	0.018	± 0.00029
Mixotrophic on Digestate no PT	0.033	± 0.01649
Mixotrophic on Digestate AC	0.053	± 0.01001
Mixotrophic on Digestate AUF	0.063	± 0.00530

As shown in Table 2, the presence of digestate with pre-treatments exhibit the best results, probably thanks to the presence of complex substrate (different from a simple substrate like glucose), ammonia and phosphorus. Digestate with no pre-treatment showed a better productivity than control. On the other hand, this condition was not the higher, probably due to a problem with light penetration caused by particulate in the suspension that could limit biomass growth. The same results were obtained by Bhatnagar et al. [9]: authors compared microalgae growth in presence/absence of pre-treated wastewater. In fact, with no pre-treatment there was an higher cellular growth vs control condition using synthetic medium, that is because microalgae could use, with mixotrophic metabolism, both organic and inorganic carbon substrate. Those results are important because

it is highlighted that these microorganisms could growth on wastewater without a nitrogen supplementation in the medium, but could use nitrogen source directly from wastewater.

3.2 Ammonia reduction and chlorophyll a, b and carotenoids analysis

In the second part of the experiment, ammonia removal was evaluated in all test condition. Considering a digestate dilution of 1:10, the initial ammonia concentration was about 50 mg N l⁻¹. An ammonia reduction of 95.3% ± 3.5 was observed. Ji et al. [10] showed an ammonia reduction of 63-88% with *S. obliquus* in municipal wastewater with 21 mg l⁻¹. In our experimental condition, ammonia concentration was 50 mg l⁻¹ with no correlation between ammonia reduction made by microalgae and the same reduction made from stripping by air insufflation. In fact, Kim et al. and Ruiz-Martinez et al. [11], [12] showed that the effect of ammonia volatilization is correlated with air insufflation and pH condition. In their study, *S. obliquus* showed pH value near 8.5-9.5 determined by photosynthetic activity, in this condition, with also aeration and ammonia stripping increased. For these reasons, probably, the ammonia reduction was not correlated at microalgae growth at all, but the removal could be mainly caused by ammonia stripping during air bubbling. To confirm this, it will be necessary to test ammonia reduction with air insufflation and mechanical agitation without microalgae.

Chlorophyll a, b and carotenoid was carried out only in test conditions with cellular growth. Those values, on mixotrophic control, showed that during the steady state of growth (eighth day of test) were accumulated 6.11 µg ml⁻¹cell⁻¹ (± 1.8) and 7.4 µg ml⁻¹cell⁻¹ (± 2.4) respectively, while carotenoid was not detected. In mixotrophic test conditions, in stationary phase, (Figure 3) there wasn't an accumulation of chlorophyll a, b, but it was found a small storage of carotenoid if compared with control condition.

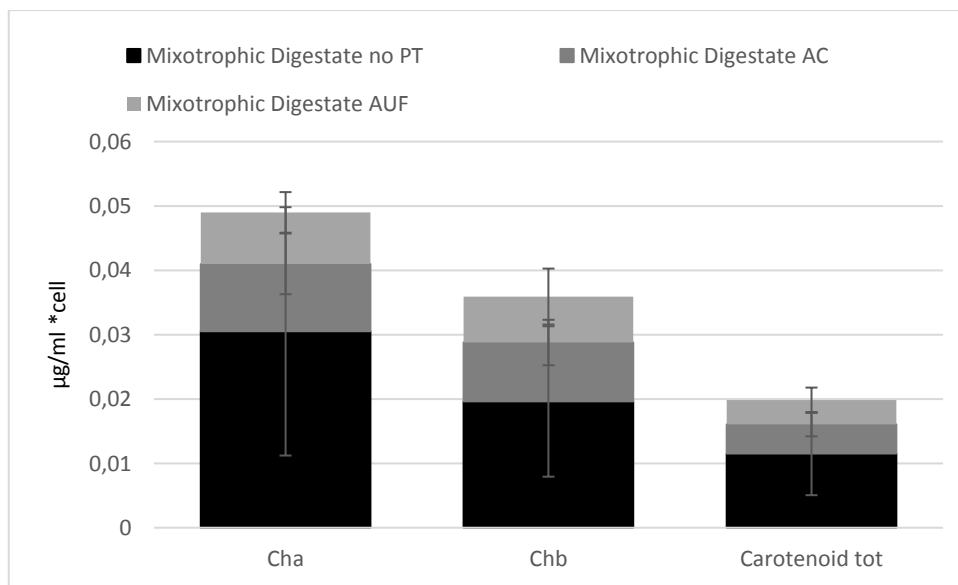


Figure 3: Chlorophyll a, b and total carotenoid accumulation in a single cell at the end of experiment in *Scenedesmus obliquus*.

This carotenoid accumulation in mixotrophic conditions could be explained with a light acclimation of this strain. This acclimation give normal physiological changes, focused to capture more light when there was a light limitation [13], [14]. The light deficit observed was probably determined by digestate with no pre-treatment and in presence of particulate, and by a high microalgae growth in the other conditions with pre-treatments. Considering the study carried out by Dubinsky et al. [13], it is possible to correlate the effect of light irradiation on chlorophyll content. Low light irradiation (determinate by little illumination or dark medium) increase also chlorophyll content in more microalgae species. This result wasn't confirmed in our work; if we compared control condition with experimental conditions, we found a decrease of 49.9%, 17.16% and 44.02% in chlorophyll a and 26.63%, 12.43% and 9.7% in chlorophyll b content in *S. obliquus* biomass on digestate with no PT, AC and AUF respectively. At the same time, these results on chlorophyll, decreased in stress condition,

are similar to the data reported by Spolaore et al. and Markou et al.[15], [16]. That stress condition increase carotenoid accumulation in opposite with the general behavior: chlorophylls are degrade under stress condition with a significant biomass reduction.

Conclusion

The use of pre-treatment like AC and AUF on digestate showed the best and similar growth results for *S. obliquus* in batch mixotrophic condition with a growth rate of $0.42 (\pm 0.08)$ and $0.5 (\pm 0.04)$ g l⁻¹ of biomass dry weight. Light radiation seems to be a fundamental factor. In fact, in heterotrophic condition, there wasn't cellular growth. Ammonia reduction of about 95% was detected, probably correlating with aeration stripping. Next studies will be focus on a scale up in continuous mixotrophic condition with a lower dilution and applying only AC as pre-treatment. It will be evaluated the nutrient removal capacity in a continuous system and the sustainability of the process in terms of lipids or other compounds accumulation and exhausted biomass recirculation in anaerobic digestion.

Acknowledgments

This study was supported by University Ca' Foscari of Venice (Italy) project "Energy recover and high value by-products from wastewater treatment: (Anaerobic Digestion and microalgae) biologicala-physical-chemistry processes integrated in a circular economy prospective".

References

- [1] R. Lora *et al.*, "Technology overview of biogas production in anaerobic digestion plants : A European evaluation of research and development," *Renew. Sustain. Energy Rev.*, vol. 80, no. May 2016, pp. 44–53, 2017.
- [2] A. Toledo-Cervantes, M. L. Serejo, S. Blanco, R. Pérez, R. Lebrero, and R. Muñoz, "Photosynthetic biogas upgrading to bio-methane: Boosting nutrient recovery via biomass productivity control," *Algal Res.*, vol. 17, pp. 46–52, 2016.
- [3] M. E. Huntley and D. G. Redalje, *CO2mitigation and renewable oil from photosynthetic microbes: A new appraisal*, vol. 12, no. 4. 2007.
- [4] S. M. Phang, M. S. Miah, B. G. Yeoh, and M. a Hashim, "Spirulina cultivation in digested sago starch factory wastewater," *J. Appl. Phycol.*, vol. 12, pp. 395–400, 2000.
- [5] H. E. Olguin EJ, Sánchez G, "The cleaner production strategy applied to animal production," *Environ. Biotechnol. an Clean. bioprocesses*, vol. London: Ta, pp. 277–43, 2000.
- [6] APAT-IRSA/CNR, "Metodologie analitiche per il controllo della qualità delle acque [Analytical methodologies for water quality management]," *Poligr. e Zecca dello Stato Roma, Ital.*, vol. 29, pp. 575–581, 2003.
- [7] N. Z. N. and M. R. K.C.A. Jalal, A.A. Shamsuddin, M.F. Rahman, "Growth and Total Carotenoid, Chlorophyll a and Chlorophyll b of Tropical Microalgae (*Isochrysis* sp.) in Laboratory Cultured Conditions.," *J. Biol. Sci.*, pp. 10–17, 2013.
- [8] Ş. Dere, T. Güneş, and R. Sivaci, "Spectrophotometric determination of chlorophyll - A, B and total caretenoid contents of some algae species using different solvents," *Turk. J. Botany*, vol. 22, pp. 13–16, 1998.
- [9] A. Bhatnagar, S. Chinnasamy, M. Singh, and K. C. Das, "Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters," *Appl. Energy*, vol. 88, no. 10, pp. 3425–3431, 2011.

- [10] M. K. Ji, H. S. Yun, Y. T. Park, A. N. Kabra, I. H. Oh, and J. Choi, "Mixotrophic cultivation of a microalga *Scenedesmus obliquus* in municipal wastewater supplemented with food wastewater and flue gas CO₂ for biomass production," *J. Environ. Manage.*, vol. 159, pp. 115–120, 2015.
- [11] G. Kim, G. Mujtaba, and K. Lee, "Effects of nitrogen sources on cell growth and biochemical composition of marine chlorophyte *tetraselmis* sp. For lipid production," *Algae*, vol. 31, no. 3, pp. 257–266, 2016.
- [12] A. Ruiz-Martinez, N. Martin Garcia, I. Romero, A. Seco, and J. Ferrer, "Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent," *Bioresour. Technol.*, vol. 126, pp. 247–253, 2012.
- [13] Z. Dubinsky and N. Stambler, "Photoacclimation processes in phytoplankton: Mechanisms, consequences, and applications," *Aquat. Microb. Ecol.*, vol. 56, no. 2–3, pp. 163–176, 2009.
- [14] J. C. Kromkamp *et al.*, "Short-term variations in photosynthetic parameters of *Nannochloropsis* cultures grown in two types of outdoor mass cultivation systems," *Aquat. Microb. Ecol.*, vol. 56, no. 2–3, pp. 309–322, 2009.
- [15] G. Markou and E. Nerantzis, "Microalgae for high-value compounds and biofuels production : A review with focus on cultivation under stress conditions," *Biotechnol. Adv.*, vol. 31, no. 8, pp. 1532–1542, 2013.
- [16] P. Spolaore, C. Joannis-cassan, E. Duran, A. Isambert, L. De Génie, and E. C. Paris, "Commercial Applications of Microalgae," vol. 101, no. 2, pp. 87–96, 2006.