UNIVERSIDAD DE VALLADOLID

ESCUELA DE INGENIERIAS INDUSTRIALES

Grado en Ingeniería Química

Integration of Microalgae in a Wastewater Treatment Plant

Autor:

Rodríguez Herrero, Ana Elena

Tutor:

D. Ficara, Elena
Proff. of Environmental engineering,
Politecnico di Milano

Milano, July 2016

Agradecimientos

En primer lugar quiero agradecer a mi tutora, Elena Ficara su buena acogida su total involucración con la realización de este proyecto. Gracias a su tiempo, consejos y correcciones he podido llevar a cabo este trabajo satisfactoriamente.

Asimismo quiero agradecer a todas las personas que ha hecho posible la realización de este trabajo.

A mis padres, por haberme apoyado siempre y haberme dado fuerzas en los malos y en los buenos momentos, sin su apoyo esto habría sido imposible.

Gracias a quien tengo la inmensa suerte de poder llamar amigos, a Jesús y a Cristina, por su tiempo, su paciencia y estar siempre a mi lado.

A la gran familia que tengo.

A mi abuela gracias por tu amor incondicional, tu paciencia, tu cariño, tus consejos, por tu apoyo, por creer en mí, por protegerme, por tu infinita bondad, por tu risa, por enseñarme el compromiso absoluto por lo que uno hace. Por ser mi ejemplo.
Gracias por tu ilusión por la vida. Gracias por haberme regalado 22 años de absoluta felicidad. Esto es gracias a ti. Te quiero Lula, sé que siempre me cuidarás.

Abstract

The aim of this study is evaluated the employment of a digestate of a conventional wastewater treatment plant us the only source of nutrient in the cultivation of microalgae culture of *Scenedesmus* and *Chlorella* in an open pond photobioreactor.
Firstly it was studied batch operation mode to understand the behavior of the culture.
After that the reactor started to operate in continuous. The percentage of ammonium removal was 97 %.
The percentage of that ammonium which was destined to microalgae growth was 58.1 %.
22 % of this NH4 was stripped to the atmosphere.
Indeed the use of digestate for growth microalgae with light sun and aerea to provide oxygen is viable.

Table of contents

Abstract ....

1. Introduction 1

2. Literature review 3

2.1 Microalgae: metabolism and factors of influence 3

2.1.1 Metabolism 3

2.1.2 Factors of influence.. 4

2.2 Integration of Microalgae in wastewater treatment plants 8

2.2.1 Growth of microalgae on digestate 8

2.2.3 Integration of microalgae in the secondary treatment 21

2.3 Heavy metals in urban wastewater. 24

2.4 Microalgae harvesting.. 26

2.4.1 Thickening (concentration) 27

2.4.1.1Coagulation /flocculation.. 27

2.4.1.2 Gravity sedimentation 30

2.4.1.3 Flotation 32

2.4.1.4 Electrical methods... 35

2.4.2 Dewatering.. 36

2.4.2.1 Filtration.. 36

2.4.2.2 Centrifugation 38

2.4.2.3 New technologies.. 39

3. Materials and methods 42

3.1 Reactors and equipment.. 42

3.2 Analytical methods 43

3.3 Biomass harvesting and solid/liquid separation tests 43

3.4 Operation Condition 44

3.4.1 Batch operation mode 44

3.4.2 Continuous operation mode 48
3.5 Harvesting of microalgae 49
4. Results and discussion 50
4.1 Batch operation 50
4.2 Continuous operation 58
4.3 Harvesting microalgae 67
5. Conclusions 68
6. Bibliographic references 69
Annex I 74
Annex II 76
Annex II 78

1. Introduction

Large amounts of water used for agricultural, municipal and industrial purposes results issues due to the large volumes of wastewater generated. This poorly treated wastewater contains excessive nutrients, such as nitrogen and phosphorus, may cause eutrophication of the receiving waters and negatively impact on its aesthetic, biological, recreational and economic values. (Cai et al. 2013).

A major requirement in wastewater treatment is the removal of nutrients and toxic metals to acceptable limits prior to discharge and reuse.

Urban wastewater (UWW) treatment plants in current use have been designed and operated solely for the purpose of meeting the mandatory discharge regulations to protect receiving waters and public health. While this treatment plants have generally performed well in terms of wastewater organic solids removal (BOD removal), the removal of nutrients and disinfection are highly inconsistent and therefore unlikely to meet more stringent regulations starting to be applied to discharge consents (Craggs et al. 2014).
Technologies deployed in these wastewater treatment plants consume significant electrical energy and dissipate valuable carbon- and nutrient-content of the wastewater into the environment (Henkanatte-Gedera et al. 2015). The progressive application of European Directives is limiting the discharge of total nitrogen to 15 mg N/L and phosphorous to 2 mg P/L (Council Directive 91/271/EEC) and urging industrial sectors to follow Integrated Pollution Prevention and Control practices (Council Directive 96/61/EC) by integrating Best Available Techniques (BAT) in both production and wastewater treatment lines. (Velasquez-Orta et al. 2014).
However in the lasts years the treatment of livestock effluents is receiving an increasing attention in Europe

Microalgae are able to combined wastewater treatment with biofuel production, however increased microalgal productivity and nutrient removal together with reduced capital costs are needed before it can be commercially viable (Shuterland et al. 2015).

Early studies demonstrated that the application of micro-algae systems for the treatment of livestock effluents offers the possibility of in-situ oxygen production via photosynthesis and nutrients removal, thus reducing the number of treatment steps (Garrett and Allen, 1976; Fallowfield and Garrett 1985).

Algal-bacterial consortia are able to establish a cycle of O_2 production and usage thereof, the so-called photosynthetic oxygenation. (Muñoz 2005). In the same step, nutrients like ammonium and phosphorous can be eliminated by microbial

assimilation due to micro-algae's utilization of nitrogen for protein formation and phosphorous for nucleic acid and phospholipids synthesis.
CO_2 assimilation by micro-algae contributes to a higher nutrients uptake, which improves nutrients removal by assimilation when compared to mechanically aerated bacterial systems. (Gonzalez Fernandez et al. 2008). In addition, pH can mediate an enhanced removal of ammonium and phosphorous. Indeed, photosynthesis provokes a raise in pH which triggers phosphorous precipitation and, in open systems, ammonia stripping (Garrett and Allen, 1976). Consequently, the number of necessary treatment steps can be reduced to only two, a primary step for the removal of larger particles and a second step combining both secondary and tertiary treatment steps.

Microalgae have been proven to be efficient in removing nutrients like nitrogen and phosphorous and use these nutrients to produced biomass (Sturm et al. 2011; Zhou et al. 2012).
Microalgae have the potential to be an environmentally friendly biofuel feedstock. They have the following advantages: Microalgae do not compete with crops for arable land and freshwater because they can be cultivated in brackish water and on non-arable land; they can grow rapidly and have high oil contents of 20-50 % on dry weigh basis; microalgae have the ability to fix carbon dioxide, thus reducing greenhouse gas emissions and improving air quality; microalgae can utilize nutrients form most wastewaters; and byoproducts of microalgae cultivation after lipid extraction can be used as nitrogen source, such as a protein-rich animal feed or fertilizer for crops.

2. Literature review

2.1 Microalgae: metabolism and factors of influence

2.1.1 Metabolism

The main objective of the implementation of microalgae in the wastewater treatment is the nutrient removal through biomass growth, which is subsequently transformed into methane, source of electric power.

Microalgae are able to photosynthetically convert carbon dioxide into potential biofuel feedstocks. Besides light, microalgae need nutrients (mainly N and P) and CO_2 to grow.
Microalgae, unlike other oil crops, are able to grow extremely fast and can double their biomass within a period of 24 h. Microalgae grow throughout the year, regardless of the season and land fertility, condition that eliminates the need of herbicides and pesticides, for that reasons microalgae can be harvested continuously on insignificant land.
Microalgae require less water and they are flexible to the type and quality of it so they can grow without problem in domestic wastewater, where nutrients are contained in excess.
Also they have higher photosynthetic efficiency and superior efficacy in nutrient uptake.
And additional advantage is the possibility of obtaining subproducts like proteins, biopolymers or biogas, among other options, from residual biomass of microalgae once lipids have been extracted.
It is noteworthy that the most important competitive advantage of biodiesel from microalgae consists of lipid yields per unit area considerably higher than those obtained with oil plants.

The oil content and profile of lipid composition of microalgae can be controlled as a function of culture conditions, mainly by limiting nutrients.
Wastewater treatment with microalgae can be coupled to recycling of CO_2 released on industrial emissions.

Although studies have proved that microalgae have definite advantages over conventional biofuel sources, broad implementation of microalgae in biofuel production has not been developed due to high costs of operation during processing. The main cost is dewatering of microalgae, which requires a big amount of energy.

2.1.2 Factors of influence

By manipulating temperature and chemical composition of the culture medium, it is possible to increase the production of lipids.
One study showed that N deficiency increased the lipid content of a culture of *Chlorella* by 63% (Illman et al. 2000).
P limitation also appears to stimulate lipid accumulation, found by Rhee (1978). The lipid content was higher when P was unavailable. However, most studies have found that N deficiency produces a higher percentage of lipid than P deficiency (Illman et al. 2000, Mandal and Mallick 2009, Feng et al. 2012). With respect to temperature, one study determined that raising the temperature from 30°C to 42°C increased the lipid content of the bluegreen alga *Spirulina maxima* (Paoletti et al. 1980).

The following conditions are the principal factors which influences on microalgae growth.

Light

Light intensity is one of the main parameters to be considered in microalgae culture (Contreras-Flores et al. 2003).
In the absence of nutrient limitation, photosynthesis increases when increasing light intensity until the maximum specific growth rate for each species when the light saturation point is reached (Park et al. 2011a).
After that point, photoinhibition point is reached, with harmful results for the cell implying loss of photosynthetic efficiency and crop productivity.
In that case an outdoor cultivation is studied photoinhibition in the main hours of the day due to the high light intensity can be an issue (Martinez 2008).
An author Ying-Hu et al. 2013, studies microalgae growth with different light intensity

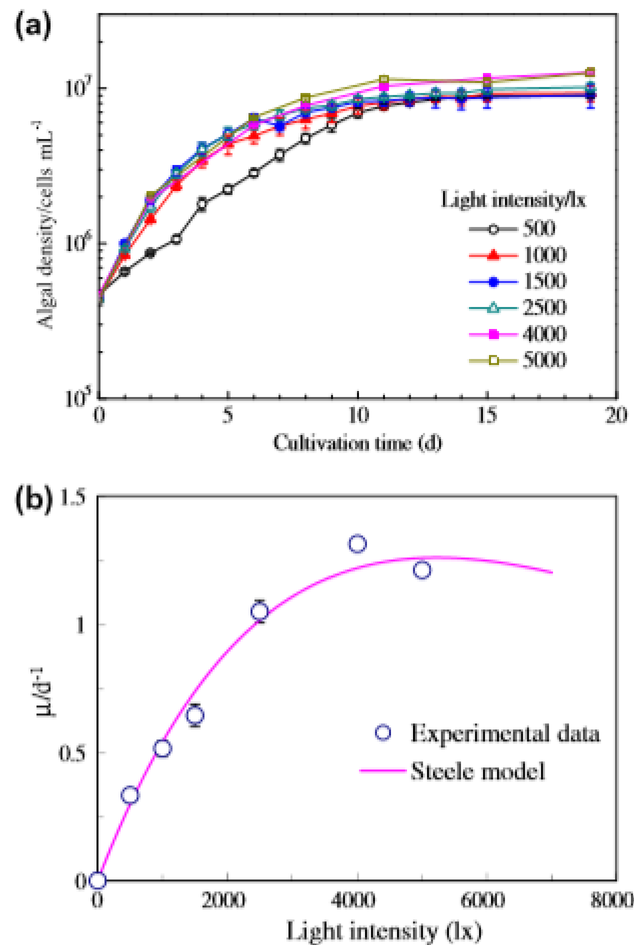


Figure 1 - The growth curves of *Scenedesmus sp* (a) and the specific growth rate of *Scenedesmus sp*. (b) under different light intensity

It can be observed when light intensity increases, microalgae growth is higher.

Temperature

Algal production increases proportionally with the temperature until achieving the optimum temperature for each species. Above this temperature photorespiration increases reducing overall productivity.

The optimal temperature is different among the species of microalgae, but in general it is about 28 ºC and 35 ºC (Park et al. 2011a). For *Clhorella Vulgaris* and *Scenedesmus* the optimal temperature is 30 ºC, at which growth of 0.704 and 0.673 d^{-1} respectively is obtained. (Devgaswami et all. 2006).

For *Schenedesmus* culture at 35 ºC the cells results broken (Martínez et al.)

However, optimal temperature varies when nutrient or light conditions are limiting, and growth often declines when algae are subjected to a sudden

temperature change, for example, exposure at a high temperature of algal strain adapted to 10 ºC resulted in a 50% reduction in chlorophyll in just 15 h (Harris, 1978).
Temperature can also alter the water ionic equilibrium, pH, and gas (oxygen and CO_2) solubility.

Nutrients
Nitrogen is the most important nutrient for microalgae (behind carbon) and is incorporated as ammonium (NH_4^+) or nitrate (NO_3^-). It is also critical for regulating the lipid content of microalgae.
Goldman et al. have identified nitrogen as a growth-limiting nutrient in wastewaters and coastal marine waters through continuous culture algal assays**.**
Typically, microalgae have lipid content about 20%, but when the nitrogen becomes the limiting factor for growth, the accumulation of lipid levels increased over 40 %. (ATS ingeniería 2013)
However, using nitrogen limitation to stimulate lipid accumulation in algal cells often reduces the production of algae, suggesting that high productivity can be mutually exclusive. (ATS ingeniería 2013)
The principal processes followed by nitrogen in microalgae cultures are:

- nitrification/denitrification

$$NH_4^+ \rightarrow NO_2^- \quad (1)$$

$$NO_2^- \rightarrow NO_3^- \quad (2)$$

- Stripping where the ammonia contained in the liquid of the reactor pass to the air.

$$NO_{3,l} \rightarrow NO_{3,g} \quad (3)$$

- The rest of the nitrogen present in the digestate is employed to biomass growth.

This is an important aspect of microalgae-based wastewater treatment and although many works concluded that microalgae, in batch operation mode, are able to reduce almost 100% of nitrogen in wastewater. Martinez et. Al. 2000 studied an ammonium removal in a *Scenedesmus* culture of 100%.
For continuous operation, in a *Chorella vulgaris* culture, this percentage is a little fewer: 98 %. (Ledda et al. 2015).

As for nitrogen content in ammonium form, note that ammonium is toxic for microalgae cells, then excess of its concentration can reduce the performance of the cultures.

The other principal nutrient that microalgae need for their correctly growth is phosphorous.

Phosphorus is critical in many cellular processes such as the formation of nucleic acids and energy transfer. Although the phosphorus content of microalgae is less than 1 %, its deficiency in the culture medium is one of the major constraints to growth. (Martinez et al. 2010).

In culture media usually incorporated as HPO_4^{2-} or HPO_4^{-} .

The highest percentage of phosphorus removal which is possible to reach in a *Schenedesmus* culture is 98%, (Martinez et. al. 2000). With *C. Vulgaris* this value is 94 % (Yujie and Zhanget al- 2011), in continuous operation.

Martinez et al. 2010 have studied a *Schenedesmus sp* culture; for the temperatures studied stirring was not necessary to provide the highest percentage of P elimination ($\%P_{max}$), but did reduce the time needed to reach that percentage (t_{max}).

It was found that it was not possible to simultaneously remove all nitrogen and phosphorus from the wastewater, because of the N:P ratio in the wastewater (Boelee et a.2012).

The optimal N:P ratio for a *Scenedesmus* culture is 12.9 (Martinez et. Al. 2000)

The optimal N:P ratio for *Chlorella vulgaris* is 8 (Kapson et al.2000), but other author Raddfield affirms that this parameter is 16.

Previous studies have shown that microalgal biofilms systems can achieve good removal of N and P from wastewater.

Always the removal efficiency of nutrient achieved higher level during the growth phase, due to the higher cell density and vigorous growth (Yujie and Zhanget al-2011).

Dissolved oxygen

The intense photosynthesis performed during the day in cropping systems can increase levels of dissolved oxygen > 200 % of saturation.

It is believed that a high saturation could affect the productivity of algae, but it is not yet demonstrated.

In 2001 Molina et al. determined that 200 % saturation there is a 17% reduction in productivity, while 300 % saturation reduces by the 25%

Pond water pH and CO2 availability

The pH of the pond water affects many of the bio-chemical processes associated with algal growth and metabolism, including the bio-availability of CO_2 for photosynthesis and the availability and uptake of nutrient ions. Pond water pH is in turn a function of algal productivity, algal/bacterial respiration, the alkalinity and ionic composition of the culture medium, autotrophic and heterotrophic microbial activity (eg. nitrification and denitrification) and the efficiency of the CO_2 addition system (ATS ingeniería 2013).

The high pH values can act to enhance ammoniacal-N removal from the pond liquid via ammonia striping and phosphorus removal through phosphate precipitation with uncharted ferric iron, calcium and magnesium.

Moreover, the equilibrium shift to free ammonia at high pH can significantly inhibit algae growth. (magrama.es)

For the algae species *Chlorella* and *Scenedesmus* the optimal pH it is between 9 and 11 (Devwasgami et al. 2011). This is according to (Kong et al., 2010) who affirms that the optimal pH of many freshwater algae is about 8 A pH above or below 8 decreases productivity,

Referring to CO_2 concentration, the optimal for *Chlorella* is 1191 ppm, and for *Scenedesmus* it is 714 ppm (Devwaswami et al. 2011).

2.2 Integration of Microalgae in wastewater treatment plants

Wastewater treatment by microalgae, known as the algal wastewater treatment, was proposed by Oswald and Golueke (1960) through an implementation study for the treatment of domestic wastewater using open ponds in California in the mid 1950's.

Algal wastewater treatment is regarded to have economic and environmental potentials for producing useful biomass while abating organic nutrients (Nurdogan and Oswald, 1995) and contamination sources in wastewaters (Pittman et al., 2011; Samorı` et al., 2013).

These advantages have enabled the open algal wastewater treatment to be easily incorporated into the advanced wastewater treatment process for nitrogen and phosphorus removals without additional carbon sources.

2.2.1 Growth of microalgae on digestate

Anaerobic digestion (AD) is a mature technology which uses microorganisms to decompose organic waste and produce biogas.
Most of the digestate, the effluent after AD, is separated by a dewatering system into liquid and solid fractions.
Centrate, the liquid fraction of digestate, has relatively lower carbon levels because microbial activity during the digestion converts the carbon to methane. The nitrogen in cetrate is mainly in form of ammonium. Dilution of centrate is usually needed before feeding to algae in order to avoid the potential inhibition of algal growth due to high ammonium concentration and turbidity.

Centrate is a nutrient-rich effluent that can be used as a nutrient source to produce microalgae biomass for energy purposes. Centrate contains not only nitrogen but

also other major nutrients as phosphorous, calcium and potassium, among others, thus it represents a complete culture medium for microalgae. Because the nitrogen content of centrate usually exceeds that required for microalgae production, it is necessary to dilute the centrate with water to prepare an adequate culture medium. (Uggetti et al. 2013). Moreover, as the nitrogen is in the ammonium form, the dilution of centrate is often mandatory: this is due to the fact that, although microalgae assimilate ammonium more easily than nitrate, as its uptake is thermodynamically more favorable.

A key factor in the successful development of this process is the N and P concentration, in addition to the N/P ratio into the centrate. This ratio should be closed to the optimum nitrogen-to-phosphorus stoichiometry characterizing phytoplankton cells (Ledda et al. 2015).

The feasibility of the system is limited by the tolerance of selected microalga to ammonium as the nitrogen source and by the biomass productivity achievable into the photobioreactor.

The utilization of closed tubular photobioreactors allows obtaining higher biomass productivities and at the same time removing more nitrogen from the culture medium, thus achieving higher nitrogen depuration rates. However, including on these conditions a fraction of nitrogen is lost to the atmosphere to stripping phenomena, caused by mixing and aeration, and favored by alkaline pH values of the culture medium and to the increase of non-ionized ammonia concentration (Ledda et al. 2015). This point is crucial as the loss of ammonia to the atmosphere is not environmentally acceptable as it may promote environmental problems such as the formation of particulate matter (PM), water acidification and eutrophication processes

It is known that the most biomass is produced at the beginning of the experiment.
The explication to this phenomenon can be found in the pH, ammonia and nitrite patterns.
For the highest initial TSS concentrations pH is higher in the first days and then rapidly decreased to values near 7. (Uggetti et al. 2013).
The high pH variation is due to the alkalinity that is certainly proportional to the digestate concentration. In fact, for the lowest digestate concentrations, the highest pH variability was recorded as a consequence of the scarce buffer capacity.

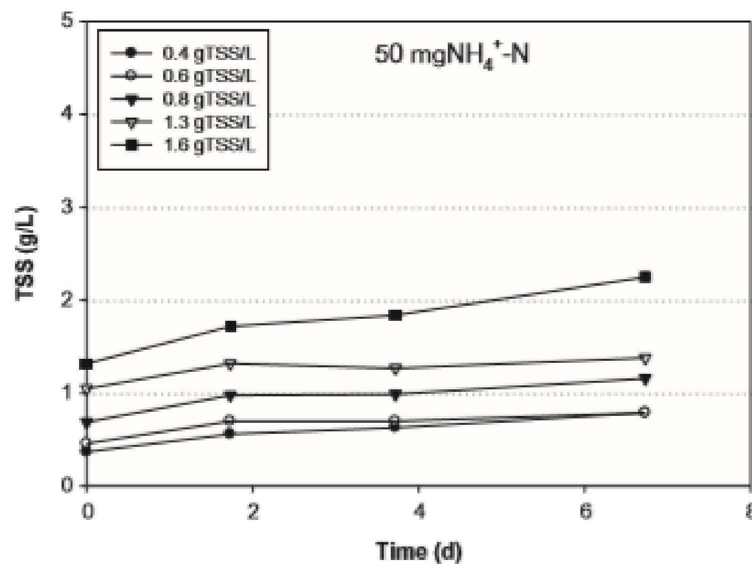
Bibliographic experiments

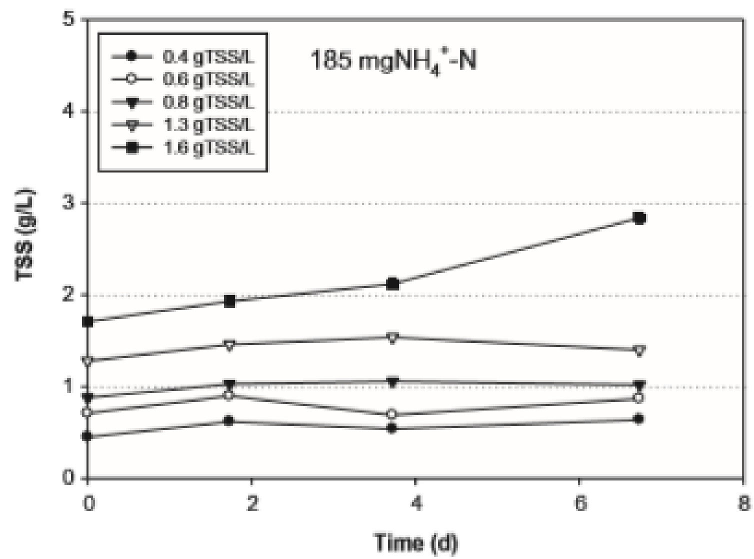
Uggetti et al. 2013

Effluent origin: Liquid phase of an anaerobic digester effluent from a wastewater treatment plant.
Pretreatment Effluent: No
Algae specie: Mixed cultured dominated by *Scenedesmus sp*
Scale: small-scale
Operation mode: Batch
Light: 80 -90 $\frac{\mu mol}{m^2 s}$
Initial conditions:
$N - NH_4^+{}_0$ = Experiment 1 50 $\frac{mg}{L}$; experiment 2 185 $\frac{mg}{L}$; experiment 3 260 $\frac{mg}{L}$
TSS_0 = sample 1 →0.2$\frac{g}{L}$; sample 2 →0.6$\frac{g}{L}$; sample 3→ 0.8 $\frac{g}{L}$; sample 4→ 1.5 $\frac{g}{L}$;sample 5 →1.6 $\frac{g}{L}$
The initial growth rate μ_0 it is observed at the end of the exponential phase, in all experiments it is between 0.4; 0.6; 0.8; 1.3; 1.6; 1.8 $\frac{g}{L}$

Results:

Biomass Production





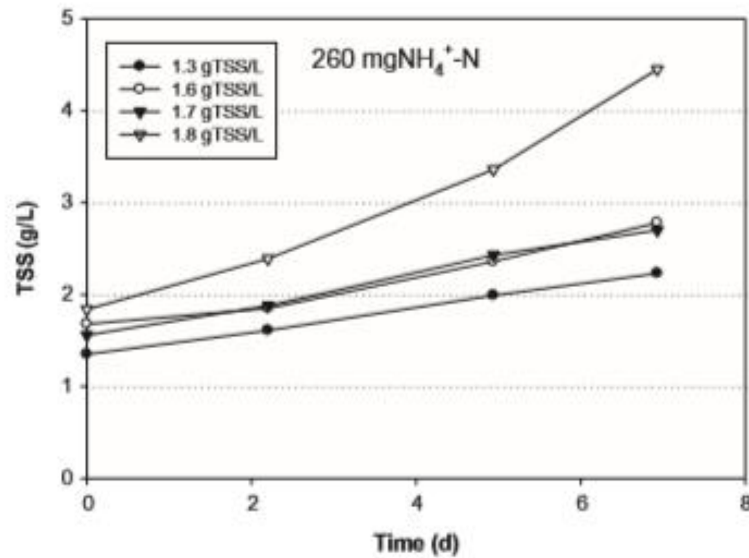


Figure 2 - TSS concentrations along the time, in different ammonium concentrations, in a *Scenedesmus* culture

Clonclusion**:** Digestate may be an effective substrate for microalgal growth.

Marcihac et al. (2015)

Effluent origin: diluted anaerobic digester and minerals effluent from a wastewater treatment plant.

Pretreatment Effluent: Dilution

Algae specie: Mixed cultured dominated by *Schenedesmus sp* and *Chlorella sp*.

Scale: small-scale

Operation mode: Batch
Light: 240 $\frac{\mu mol}{m^2 s}$
T = 25 ºC
pH = 6.95 – 7.05
Dilution 1/10
Initial conditions:
$N - NH_4^+{}_0$ = 190 $\pm$ 9 $\frac{mg}{L}$
4 cases study
N/P = 3 → HPC
N/P = 9 → HPC1
N/P = 26 → HPC2
N/P = 76 → LPC

<u>Results:</u>
Final microalgal concentration $1\ x\ 10^{\ 7}\ \frac{cells}{mL}$.

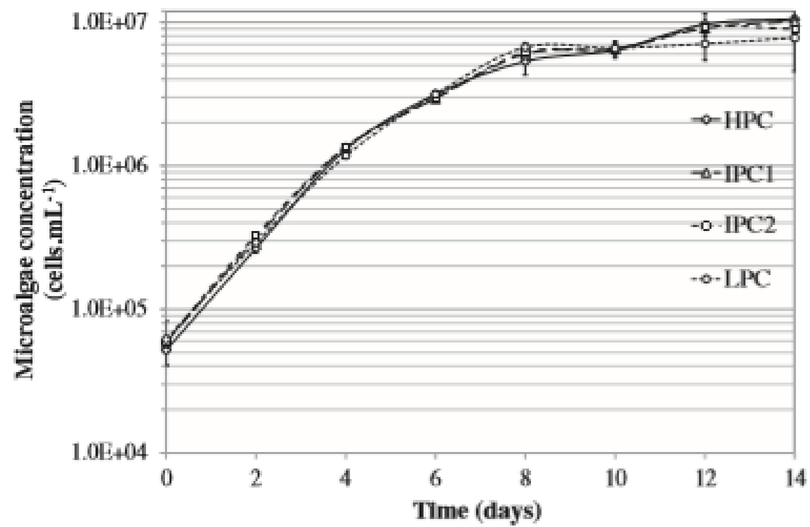


Figure 3 - Evolution of microalgae growth on 14-days for 4 study cases in a *Chlorella sp* and *Scenedesmus sp* culture.

Nutrients behavior

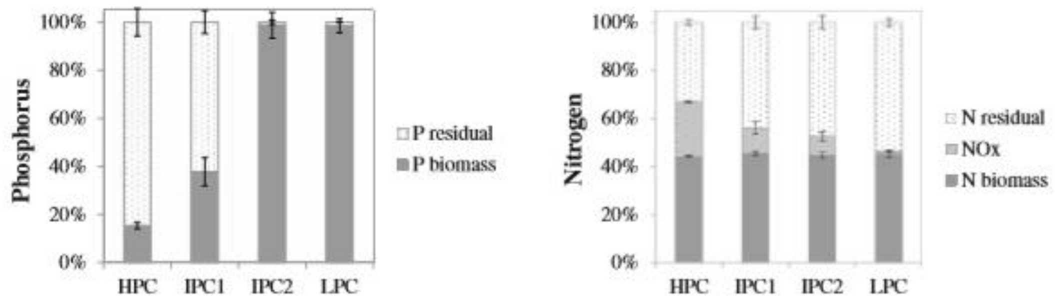


Figure 4 - Phosphorous and nitrogen distribution at the end of the Marcihac et al. (2015) experiment.

Conclusion: The phosphorous concentration in batch experiments was found to have no impact on microalgal growth which kept on growing even after P was depleted. This observation increased the phosphorous storage capacity of microalgae.
Nitrogen removal was not much affected by phosphorous concentration either, whereas phosphorous removal increased with rising P concentration. Also it is seen that *Chlorella* is more P-limited than *Scenedesmus*. Nitrification was limited by phosphorous, the final NOxbeing then dependent on initial P.

Prandini et al. 2015

Effluent origin: diluted anaerobic digester and minerals effluent from a wastewater treatment plant.
Pretreatment Effluent: Dilution
Algae specie: *Scenedesmus sp.*
Scale: small-scale
Operation mode: Batch
T = 25 ºC
pH = 7.9
Light: 148.5 $\frac{\mu mol}{m^2 s}$
Biomass initial 70 $\frac{mg}{L}$
Initial conditions:
2 cases study

A- Mixotrophic conditions (12 h; 12h; light; dark)
B- Autotrophic conditions (24 h; light)

Results:

Microalgal growth

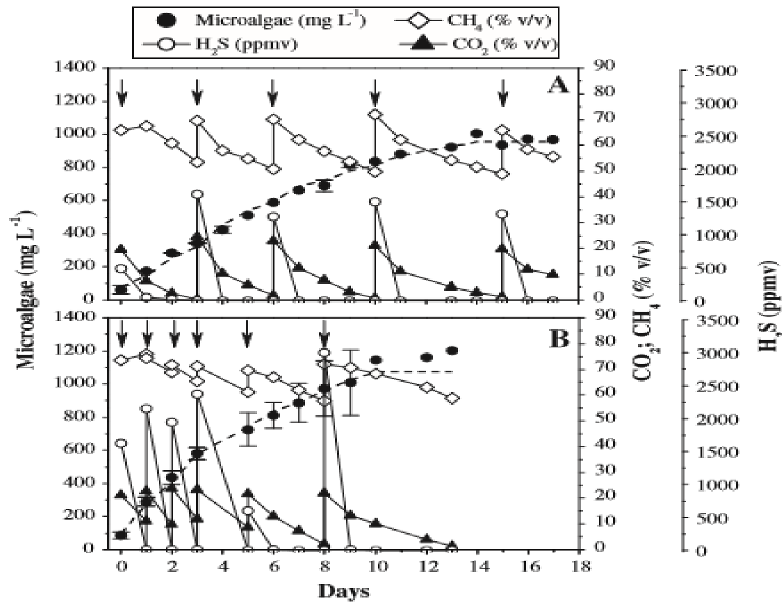


Fig. 5 CO2, CH4, H2S and microalgae biomass concentration profiles in the mixotrophic (A) and autotrophic (B) photobioreactors over time. Arrows indicates biogas reinjections. Dashed line shows the microalgae biomass model data fit. Bars depict standard deviation from the mean (n=2).

		$N\text{-}NH_2^+$ removal $(\frac{mg}{Ld})$	NH_2^+/microalgae $(\frac{mg}{mg})$	r^2
Biogas	Autotrophic	21.2 ± 1.2	0.14 ± 0.01	0.99 ± 0.01
	Mixotrophic	14.1 ± 1.2	0.15 ± 0.03	0.98 ± 0.01
Air	Autotrophic	12.9 ± 2.0	0.16 ± 0.02	0.96 ± 0.01
	Mixotrophic	11.5 ± 1.3	0.19 ± 0.07	0.96 ± 0.04

Table 1. Ammonia removal rates by microalgal cultivated in the presence and abence of biogas under different photoperiods. Different lettes indicates statistically significant differences (n = 2, ANOVA, p<0.05)

Maximun biomass reached (dry weight DW) =1.1 ± 0.2 $\frac{g}{L}$ en B.

Microalgal growth rates are higher in Autotrophic conditions than in Mixotrophic conditions: 141.8 ± 3.5 > 89.4 $\frac{mg}{Ld}$
Nutrients behavior

Free ammonia
Mixotrophic + biogas = 18.1 ± 3.3 $\frac{mg}{L}$
Autotrophic + biogas = 2.2 ± 0.9 $\frac{mg}{L}$
Mixotriphic + air = 36.7 ± 8 $\frac{mg}{L}$
Auttotrophic + air = 34 ± 17.6 $\frac{mg}{L}$

Conclusions: Increased microalgae yields were obtained under autotrophic conditions and biogas. N removal rate was significantly faster in the presence of biogas and autotrophic conditions. Biogas buffered pH and minimized N volatilization. The commercial applicability of purified biogas is critically dependent on system capacity to remove O_2 as well to minimize CH_4 losses.

Ledda et al. 2015

Effluent origin: Liquid phase of a biogas plan equipped with a full-sale digestate treatment unit.
Pretreatment Effluent: 3 cases study:
DIG: Digestate, effluent of th biogass plant, without pretreatment
CLF: Centrifugated liquid fraction
ULF: Ultrafiltered liquid fraction
Algae specie: *Chlorella sp.*
Scale: small-scale
Operation mode: Semi-continious
T = 25 ºC
pH = DIG →7.97 ± 0.13; CLF → 8.06 ± 0.08; ULF →8.61 ± 0.12
Initial total solids TS_0 =
Light: 150 $\frac{\mu mol}{m^2 s}$
Biomass initial 70 $\frac{mg}{L}$
Initial conditions:
Total solids TS_0 = DIG → 39 ± 2; CLF → 12 ± 1; ULF →9.5 ± 0.5 $\frac{g}{kg}$
$N-NH_4^+{}_0$ = DIG → 60± 0.61; CLF → 124.03 ± 0.68; ULF →124.00 ± 0.80 $\frac{mg}{L}$
COD = DIG → 1099.07 ± 6.83; CLF → 1092.00 ± 11.49; ULF →2.44 ± 1.05 $\frac{mg\ O_2}{L}$

P = DIG → 18.11 ± 0.06; CLF → 3.26 ± 0.6; ULF →181 ± 13 $\frac{mg}{L}$
N:P = DIG → 3; CLF → 38; ULF →51
$N_0 = 124 \frac{mg}{L}$

Results:
Microalgal growth

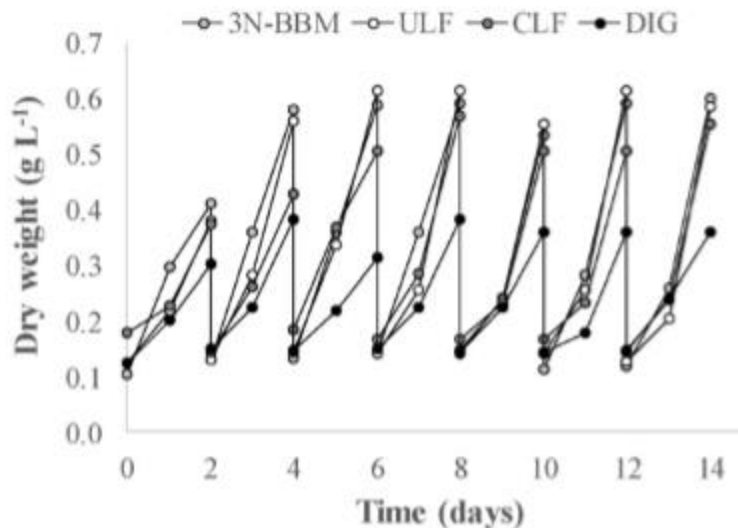


Figure 6 - *Chlorella sp* growth on 3 study cases: digestate (DIG), centrifuge liquid fraction (CLF) and ultrafiltration liquid fraction (ULF).

Maximum growth:
DIG → 0.39 ± 0.03; CLF → 0.52 ± 0.05; ULF →0.65 ± 0.03
$N - NH_4^+{}_0$ = DIG → 2.85± 0.05; CLF → 2.84 ± 0.1; ULF →124 ± 0.80 $\frac{mg}{L}$
Reduction (%)DIG → 95; CLF → 98; ULF →98
P = DIG → 2.72 ± 0.63; CLF → 0.08 ± 0.02; ULF →0.02 ± 0.01 $\frac{mg}{L}$
Reduction (%)DIG → 85; CLF → 97; ULF →99
COD = DIG → 296.75 ± 8.2; CLF → 349.44 ± 8.2; ULF →71 ± 4$\frac{mg\ O_2}{L}$
Reduction (%)DIG → 73; CLF → 68; ULF →61

Conclusion: Chlorella sp was capable of fast growth following the fraction of digestate, with the same rate as that achieved on synthetic media. Algal growth was however limited by media turbidity that depends of the COD of the liquid streams. Therefore ultrafiltered digestate worked better than untreated and centrifuged digestate. Chlorella growth resulted in the almost complete depuration of the substrates above all for both macro and micro nutrients.

One notable disadvantage of the process was the large amount of nitrogen released to the atmosphere during the experiments; in fact only 30 % of the removed nitrogen could be fixed into the microalgal biomass.

Indeed, all of these studies demonstrate that microalgae can grow in anaerobic digestate by attainting the same growth rate as in wastewater. However, microalgal concentration may inhibit growth rate by reducing the light availability. Also the concentration of bacteria and protozoa normally is high, for that reason the digestate has to be centrifuged or filtrated. Moreover, as a certain ammonia inhibition was observed, its concentration should be monitored and eventually reduced by digestate dilution.

It can be concluded in view of all experiments that the utilization of digestate as carbon and nutrients source can enhance microalgal growth reducing costs and environmental impacts.
This effluent may be diluted to avoid problems with ammonia inhibition.

2.2.2 Growth of microalgae on secondary effluents

Secondary effluent of domestic wastewater treatment plants contains low levels of nitrogen and phosphorus, but enough to growth microalgae. These inorganic nutrients need to be removed, and they are suitable and cost-effective for microalgal. By this way the eutrophication risk of the secondary effluent is decreased.

The nutrient composition in an effluent from an aeration tank of municipal WWTPs is generally stable and less-toxic for other organisms compared with an influent.
In the effluent that passed through an aeration tank in the conventional WWTP, the predominant forms of nitrogen are usually ammonia and nitrate. Both of them are easily utilized by microalgal cells, nitrate in greater amount, and nitrate is usually utilized after in vivo transformation to nitrite or ammonia by microalgae through an assimilation process (Perez-Garcia et al., 2011).

In this kind of water bacteria and protozoa are observed also, which may exert a negative impact on the growth of microalgae. To reduce the effect, filtration or UV-radiation are applied on the effluent water as pre-treatment methods. Of all the pretreatment options tested in the following experiments, the filtration resulted in the highest biomass and lipid productivity.
Meanwhile, the highest biomass production happened not in the culture with the autoclaved effluent but in the culture filtered. It demonstrated that the control still

contained some amount of suspended solid, which can cause a problem of light utilization in photosynthesis. (Cho et al. 2010).
The highest efficiencies in T-N and T-P removal were achieved from the culture with the effluent water filtered. (Cho et al. 2010).

But this way is not very cost-effective; the best option is growth microalgae without any pretreatment in the secondary effluent.

Indeed secondary effluent can be used as a source of nutrients to cultivate microalgae.

Bibliographic experiments

Cho et al. 2010

Effluent origin: Effluent after secondary treatment (aeration tank) at the Su-young Municipal Wastewater Treatment Facility, in Busan, Korea
Pretreatment Effluent: Filtration by 0.20, 0.45 pore and UV-B radiation, by 270, 540, and 1620 $\frac{mJ}{cm^2}$at 10 cmof distance.
Algae specie: *Clhorella sp.*
Scale: small-scale
Operation mode: Batch
T = 25 ºC
Light: 60 $\frac{\mu mol}{m^2 s}$

Initial conditions

Total suspended solids TSS_0 = 5 $\frac{mg}{L}$
Biomass initial = $1.3x10^6$ $\frac{cells}{mL}$
Initial conditions:
Total Nitrogen, TN = 18.9 ± 4.1 $\frac{mg}{L}$
N-NH_4 = 10.0 ± 7.1$\frac{mg}{L}$
N-NO_3 =6.6 ± = 4 $\frac{mg}{L}$
Total Phosporus, TP = 1.7 ± 0.3 $\frac{mg}{L}$
pH = 7.2 ± 0.1

Initial Biomass = $3x10^5$ $\frac{cells}{mL}$

The secondary effluent was treated in two different ways for remove the microorganism and the suspended solids.

A → 0.2 µm pore filtration

B → UV-B radiation 270 $\frac{mS}{cm^2}$

Results

Maximum microalgae growth

A = 0.074 $\frac{g}{Ld}$

B = 0.024 $\frac{g}{Ld}$

Nutrients removal

A → TN removal = 92 %

TP removal = 86 %

For B conditions this efficiencies were less (no data)

Conclusions. The secondary effluent of municipal WWTPs can be used for mass cultivation of microalgae for saving the unit cost of production by removing additionals nutrients supply.

Xin et l. 2010

Effluent origin: Domestic wastewater plant located in Beijing
Pretreatment Effluent: Filtered by a 0.45 µm membrane and sterilized.
Microalgae specie: Microalgae mixture, principally: *Chlorella Vulgaris, Chlorella Sorokiniana* and *Scenedesmus sp.*
Scale: small-scale
T = 25 ºC
Light: 55 - 60 $\frac{\mu mol}{m^2 s}$

Initial conditions:
COD = 24 ± 1 $\frac{mg}{L}$
Total Nitrogen, TN = 15.5 ± 1.1 $\frac{mg}{L}$
N-NH_4 = 2.5 ± 0.01$\frac{mg}{L}$
Total Phosphorus, TP = 0.5 ± 0.01 $\frac{mg}{L}$
pH = 7.7 ± 0.2

The secondary effluent was filtered by a 0.45 µm membrane and sterilized later.

<u>Results</u>

Microalgae growth

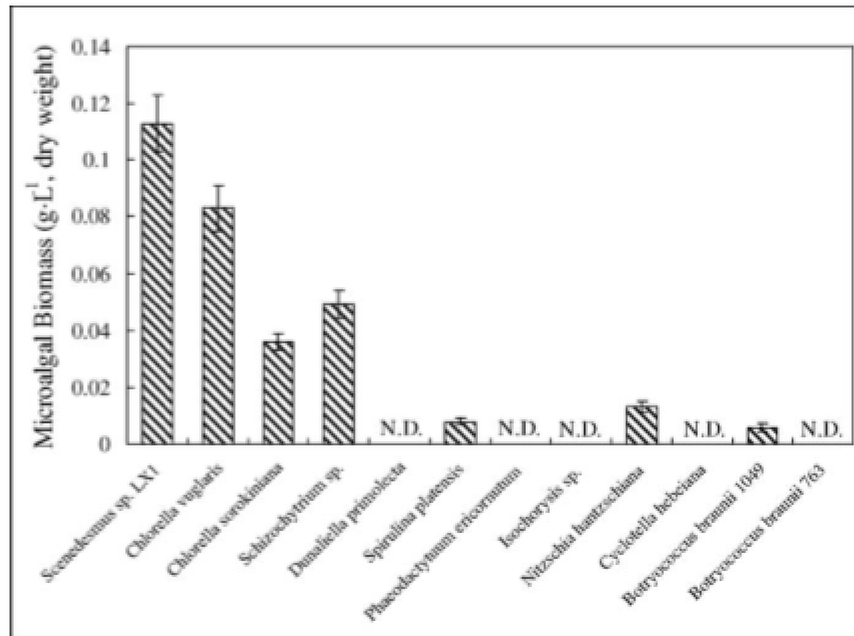


Figure 7 - Comparison of microalgal biomass biomass after 15 days of cultivation in secondary effluent.

Maximum microalgae growth is reached by *Scenedesmus sp*. 0.11 $\frac{g}{L}$

Nutrients removal

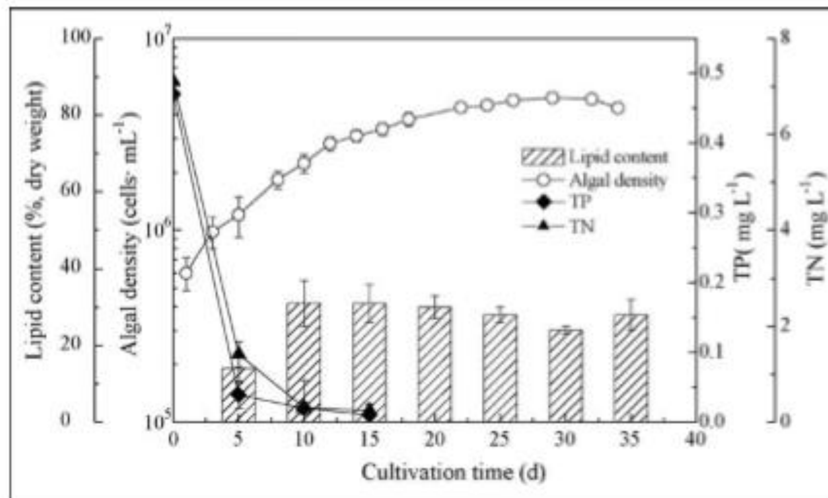


Figure 8 - *Scenedesmus sp* growth curve, nutrinent removal and lipid accumulation in the secondary effluent of domestic wastewater.

TN = 0.24 $\frac{mg}{L}$, elimination efficiency 98.5 %
TP = 0.01 $\frac{mg}{L}$, elimination efficiency 98 %

Conclusions. *Scenedemus sp* is the best microalgae to growth in secondary effluent. It can remove inorganic nutrients efficiently from secondary effluent.

These findings suggest that the secondary effluents of municipal WWTPs can be used for mass-cultivation of microalgae for saving the unit cost of production by removing additional nutrients supply. However, a proper pre-treatment method to remove algae-feeding microorganisms and competing microorganisms for nutrient should be applied for effective algae biomass production.

2.2.3 Integration of microalgae in the secondary treatment

Microalgae play an important role during the tertiary treatment of domestic wastewater in maturation ponds or the treatment of small–middle-scale municipal wastewater in facultative or aerobic ponds (Aziz and Ng, 1993; Abeliovich, 1986; Mara and Pearson, 1986; Oswald, 1988, 1995).

Microalgae enhance the removal of nutrients, heavy metals and pathogens and furnish O_2 to heterotrophic aerobic bacteria to mineralize organic pollutants, using in turn the CO_2 released from bacterial respiration. (Muñoz et al.2006).

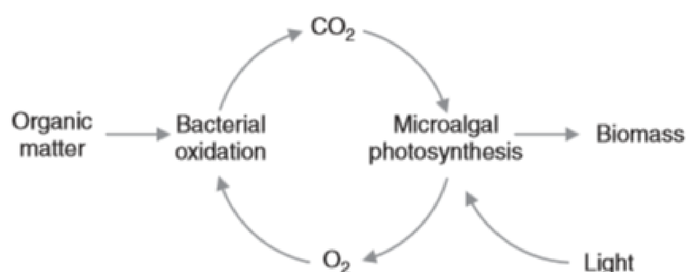


Figure 9 - Principle of photosynthetic oxygenation in BOD removal processes

Photosynthetic aeration is therefore especially interesting to reduce operation costs and limit the risks for pollutant volatilization under mechanical aeration and recent studies have shown that microalgae can indeed support the aerobic degradation of various hazardous contaminants (Muñoz et al. 2014).

The idea is developed a single-step process based on mixotrophic metabolism for simultaneous removal of carbon and nutrients from UWWs.

A central design advantage of the mixotrophic system over traditional WWT systems stems from the fact that stoichiometric carbon-to-nitrogen (C:N) ratio in UWW is closer to that of algal biomass composition than to that of heterotrophic bacteria. Even more important is that CO_2 capture via photosynthesis corrects the stoichiometric imbalance between C:N:P ratios in WW relative to either type of biomass to afford single-step biological treatment that can simultaneously achieve discharge standards for all three components. This offers a significant advantage over the traditional practice that necessitates a two-step process including energy-intensive aeration: aerobic oxidation for BOD removal followed by nitrification/denitrification for N removal with external carbon supply to bridge the C:N imbalance. (Henkanatte-Gedera et al. 2015).

Mechanical aeration accounts for more than 50% of the total energy consumption of typical aerobic wastewater treatments (Tchobanoglous et al., 2003): Hence, microalgae can improve the energy-efficiency of BOD removal from domestic wastewater by providing O_2 to the heterotrophic aerobic bacteria (Muñoz et al. 2006).

This synergistic relationship can also be used for the economical treatment of hazardous contaminants, which is also safer as there is less risk of pollutant or aerosol release than during intensive mechanical aeration (Brandi et al., 2000; Hamoda, 2006).

An energetic comparison of the wastewater-to-biomass-to-methane conversion pathways has shown that the mixotrophic pathway can yield more than double the net electrical energy than the traditional pathway (Selvaratnam et al., 2014b). Sturm and Lamer (2011) have reported similar advantage of algal-based UWW treatment systems.

It is also demonstrated the sensitivity of nutrient removal rates to changes in BOD:N:P ratios. Optimizing these ratios is critical to minimizing hydraulic residence times and plant costs.

Since mixotrophic metabolism does not require energy for oxygenation, it can conserve the energy currently consumed for aerobic BOD removal. By converting most of the carbon in the wastewater to biomass, it enables higher energy recovery than by current practice. Mixotrophic approach has the potential for energy-positive wastewater treatment. (Henkanatte-Gedera et al. 2015).

Unfortunately, microalgae are usually quite sensitive towards the hazardous compounds (Aksmann and Tukaj, 2004; Borde et al., 2003) and special care must be taken to improve microbial activity.
Heavy metals are particularly strong inhibitors of microbial photosynthesis (Clijsters and Vanassche, 1985). However, the system was efficiently protected by pre-treating the effluent with the algal–bacterial biomass generated during salicylate degradation (Muñoz et al.2006 a)
Microalgae are also sensitive to organic pollutants as Chen and Lin (2006) showed that in an air-tight environment
Microalgae are also sensitive to the combined effect of high NH_3 concentrations and high pH values because NH_3 uncouples the electron transport in photosystem II and competes with H_2O in the oxidation reactions leading to O_2 generation (Azov and Goldman, 1982).

The symbiotic microalgal–bacterial relationship is clear when microalgae provided the O_2 necessary for aerobic bacteria to biodegrade organic pollutants, consuming in turn the CO_2 released from bacterial respiration (Fig. 1). However, microalgae and bacteria do not limit their interactions to a simple CO_2/O_2 exchange (Fig. 2). Microalgae can have a detrimental effect on bacterial activity by increasing the pH, the dissolved oxygen concentration (DOC) or the

temperature of the cultivation broth, or by excreting inhibitory metabolites (Oswald, 2003; Schumacher et al., 2003)
Concluding, algal–bacterial systems are efficient for the treatment of hazardous pollutants but remains limited by the difficulty of harvesting the biomass formed, the high land requirement of open systems, or the high construction costs of enclosed photobioreactors.
Hence, suitable applications will be found when the effluents to be treated contain hazardous volatile pollutants, where combined removal capacities (organic pollutants/nutrients/heavy metals) are desired, or when the biomass produced can be commercialized. In such cases, the additional costs brought about by land use, reactor construction and biomass harvesting will be justified by the gains in safety and energy savings achieved.
Before algal–bacterial processes can widely be implemented for the treatment of industrial wastes, more research is still needed to: (1) select ‘‘extreme’’ algal strains capable to grow under wider and more extreme conditions of light, pH, pollutant concentrations, etc.; (2) understand and control the mechanisms of autoflocculation and bioflocculation to improve harvesting and biomass control; (3) scale-up and model photobioreactors to provide better design guidelines; and (4) develop new treatment methods such as membrane photobioreactors or combined physical–biological processes to improve biomass control and protect algae against inhibitory effects.

2.3 Heavy metals in urban wastewater.

Bioremoval, the use of biological systems for the removal of metal ions from polluted waters, has the potential to achieve greater performance at lower cost than conventional wastewater treatment technologies for metal removal.
This technique is especially attractive in applications where extremely low levels of residual metal ions are desired. Now that the traditional technologies for the removal of heavy metals, such as ion exchange or lime precipitation, are often ineffective and/or very expensive when used for the reduction of heavy metal ions to very low concentrations (Wilde et al. 1993).

Microalgae are known to sequester heavy metals (Rai et al.,1981), the bioremoval processes are conceptually simple. A suitable microalgae culture, immobilized in many occasions, is contacted with aqueous solution containing a metal ion. The contacting process is allowed to proceed for a sufficient time for the biomass to sequester the metal ions after which the biomass is separated from the liquid phase. The liquid phase is then discharged and the metal-containing biomass is either regenerated (by eluting the metal as a concentrated solution) or disposed of in an environmentally acceptable manner.

The principal advantages that microalgae present for removal heavy metals are:

- Use of naturally abundant renewable biomaterials that can be cheaply produced
- Ability to treat large volumes of wastewater due to rapid kinetics
- High selectivity in terms of removal and recovery of specific heavy metals
- Ability to handle multiple heavy metals and mixed waste
- High affinity, reducing residual metals to below i ppb in many cases
- Less need for additional expensive process reagents which typically cause disposal and space problems
- Operation over a wide range of physicochemical conditions including temperature, pH, and presence of other ions (including Ca_2^+ and Mg_2^+)
- Greatly improved recovery of bound heavy metals from the biomass
- Greatly reduced volume of hazardous waste produced.

On the other hand, one of the major problems in bioremoval research is the difficulty in developing generic technologies. A large number of variables are involved in selecting the biomass, processing methods, contacting environments, and waste compositions.

There are also, significant limitations to such an approach: The microbial biomass that is commercially available is not produced for bioremoval applications, and thus may not exhibit optimal performance. Furthermore, the use of dead, usually dried, biomass neglects the bioremoval capacities of living cultures, particularly when dealing with low concentrations of heavy metals. Perhaps most importantly, the currently available methods of immobilization have not proven to be satisfactory in large-scale applications. These issues are reviewed below as they relate to the bioremoval of toxic metal ions by microalgae.

Although the use of live algae offers many advantages, in practice, where typically the algal biomass is either purchased (as a dried powder) or cultivated in a separate operation prior to use, the method of choice has been to immobilize the biomass by some type of chemical or physical process. The advantages of such immobilization processes are clear-cut: they allow high cell densities and column operations. The major disadvantage is the diffusion limitations created (Radovich, 1991), which result in many of the surface sites on the biomass available only slowly to the metal ions.

It can be concluded that bioremoval is a technically efficient and economically feasible technology for removing and recovering metals from solutions. However these technologies are still being developed and much more work is required. Some practical applications have been achieved, and the fundamentals look promising: microalgae have the potential to remove metal ions to very low concentrations, to grow on light energy, and to accumulate large amounts of specific toxic elements. They appear to function well even in the presence of ions, in particular Ca and Mg, and organics.

2.4 Microalgae harvesting

The technology employed for the recovery of microalgae is considered to have the most influential effect on the economy of microalgae production. The harvesting techniques can generally be broken down into technologies that are used in a one or two stage process.

An ideal harvesting process should be effective for the majority of microalgal strains and should allow the achievement of high biomass concentrations, while requiring moderate costs of operation, energy and maintenance.
It is also desirable that the selected harvesting method allows the recycling of the culture medium. (Barros et al. 2014).

Microalgal harvesting currently involves mechanical, chemical, biological and, to a lesser extent, electrical based methods. It is very common to combine two or more of these methods to obtain a greater separation rate at lower costs.
Mechanical methods are the most reliable and there for the most commonly used to harvest microalgal biomass. However, these methods are often preceded by a chemical or biological coagulation/flocculation thickening stage to improve effectiveness and to reduce operation and maintenance costs.
Biological approaches are emerging techniques that can lead to further reduction of operational costs.

During the primary or bulk harvesting the biomass is concentrated to 2–7% total suspended solids (TSS), is generally costly and determination for the following downstream processing. Selection of an appropriate harvesting method depends on the end product, namely its value and properties. This can be achieved using flocculation, flotation and/or sedimentation. This is followed by a secondary dewatering or thickening step, which produces an algal cake with 15–25% TSS, this is achieved with filtration or centrifugation, and is often more energy intensive than primary harvesting. (Gerardo et al. 2015).

2.4.1 Thickening (concentration)

To increase solid concentration of microalgal suspension and to reduce the volume to be processed, thickening methods must be applied. Typically, thickening processes consist in

- Coagulation/Flocculation (both chemical and biologically based)
- Gravity sedimentation
- Flotation
- Electrical methods

2.4.1.1Coagulation /flocculation

Coagulation–flocculation is the process of aggregating single cells to larger flocs, thus overcoming the hurdle of repulsion with equicharged particles.
This harvesting step is used to concentrate the suspension 20–100 times. It increases the effective particle size, prior to dewatering, thus significantly reducing it energy demand.
For low cost harvesting of microalgae, coagulation/flocculation is generally followed by gravity sedimentation.
Ideally, chemical coagulation/flocculation should: (1) result in no biomass contamination; (2) lead to subsequent high efficiency biomass settling; (3) allow the reuse of the culture medium; (4) consider environmental impact; and (5) be cheap and non-toxic when applied in large scale.

Coagulation is a process which can increase the tendency of particles added to each other, to form larger particles and thus precipitate more rapidly.
Coagulation process can be induced by adding coagulating metal salts that ionize in the liquid and neutralize the surface charge of the algae. At a high pH, metal hydroxides are formed, which tend to precipitate on the flocs and cause physical linkages between algae, thus increasing the density of the biomass.

A wide variety of salts has been tested as coagulants. Multivalent metal salts, such as $FeCl_3$, $Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$, have been effectively tested (Gerardo et al. 2014). Dissociation of these salts in the culture medium lowers electrostatic repulsion between the negatively charged cell surfaces, enabling cell aggregates formation.
Solubility is also a key factor: salts with lower solubility are more effective. Finally, pH has to be low enough to form cationic hydrolysis products, which are crucial in coagulation, since this method functions by charge neutralization.

As to all harvesting methods, selection of the appropriate coagulant is determined by the target of the subsequent processes.
Despite being easily flocculated by metal coagulants, such as alum and iron chloride, large amounts of these salts are required, making it a very expensive option (Barros et al.2014).

Although coagulation/flocculation followed by gravity sedimentation is a cheap approach for microalgal harvesting, coagulant costs represent a significant portion of the overall process (4–7%) (Barros et al. 2014. Therefore, the use of naturally available coagulants such as phosphates, carbonates, calcium and magnesium ions, in wastewater frequently found in considered.
The relationship between coagulant dose and microalgal cell concentration is not clear, as it has been reported in the literature, as being linear, as well as proportional to the cell concentration logarithm. Nonetheless, high density cultures require almost 10-fold less coagulant addition than expected. This might be due to the presence of less charged material on the surface of cell walls or to the shorter distance between cells that leads to higher collision rates. (Barros et al. 2014).

Flocculation can be defined as the coalescence of finely divided particles in suspension on to larger aggregates followed by the agglomeration of these into larger flocs that settle to the bottom of the vessel, leaving a clear supernatant.

The most important ways of flocculation are: (1) chemical flocculation, (2) autofloculation, (3) biofloculation, (4) electrolytic flocculation, (5) polyelectolytic flocculants.

<u>Chemical flocculation</u> is carried out by the use of chemical substances from two different natures: inorganic or organic. The majority of inorganic chemical flocculants are based on multivalent cations such as aluminum sulfate, ferric chloride and ferric sulfate.

It can be induced by different ways:

A- *Electrostatic patch (or patching),* which occurs when a charged polymer (mostly thus polyacrylamides and polyamines) binds to an opposite charged particle, locally reversing that charge and creating a patch that will connect with opposite charged patches.
   These polymers can be cationic or anionic

B- Bridging, Specialized polymers work in a similar way, stabilizing the algal cells' electronegative charge due to the polymer adsorbing onto the surface of cell walls which links and binds cells together, forming a bridge between them.

C- Sweep flocculation, which occurs when particles are entrapped in a massive mineral precipitation.

The main disadvantage related to the use of chemical flocculants is that efficacy can be significantly impacted by the pH, the micro-organisms characteristics, water salinity, dose applied, and biomass concentration. Many of these variables are not static and tend to fluctuate during the algal growth cycle, therefore it is advantageous to consider the stationary phase to harvest microalgal biomass. In this phase, microalgae have lower metabolic activity and cell mobility, presenting higher intercellular interactions, as the zeta potential is lower.
For these reasons the optimum flocculant dosage can be difficult to achieve. For example if dosage is too high bridging potential can be reduced due to electrostatic/static hindering.

Alternatively, autoflocculation is an attractive alternative, as it is low cost, low energy, non-toxic to microalgae and does not require the use of flocculants, enabling simple medium reuse. It can be achieved without the addition of supplementary chemicals.
Autoflocculation is induced at a high pH, typically above pH 9. An increase of pH causes super-saturation of calcium and phosphate ions, resulting in a positively charged calcium phosphate precipitate which will result in a neutralization of the negatively charged algae cells. An increase in pH can be induced by stopping the air or the CO_2 supply, which could provide a cost-effective harvesting method. Although often demonstrated on a lab scale, autoflocculation still needs to be demonstrated at a significant scale and a greater understanding of its mechanisms and how to control them is required.

Bioflocculation is another option which relates to microalgal flocculation caused by secreted biopolymers. Flocculants produced by bacteria can be an important economical step towards sustain able microalgal based biofuel production. Bioflocculation eliminates the need for chemical flocculants, which represent an expensive, non-feasible and toxic alternative. However, co-culture of microalgae with bacteria, fungi or flocculating microalgae results in microbiological contamination, interfering with food or feed applications of microalgal biomass. In the case of biofuel production, the added microorganisms may even contribute to the increase in lipid yields.

Electrolytic flocculation is a physical–chemical technique where by sacrificial electrodes such as iron or aluminum are used. These electrodes release metal cations that induce coagulation. Nonetheless, electroflocculation may leave residual metals in the algal concentrate and thus further investigation is needed to establish the by-products that could be gained from using this harvesting method.

The disadvantages to this process include: cathode fouling and maintenance, temperature increase of the medium (b1.5 °C), influence of mixing (that is power induced), changes in pH, and the research gap relating to electrode design and arrangement.
There is very limited research regarding the application of this technology, however, Poelman et al. demonstrate one of the few applications of electrolytic flocculation, achieving up to 96% separation of cells while only consuming 0.3 $\frac{kWh}{m^3}$ in a 75 min time period.

Polyelectrolyte flocculants are natural or synthetic polymers of ionic or non-ionic species. The use of polymeric materials allows the reduction required dose by increasing their molecular weight. Nevertheless, the presence of some chemical substances and pH of the medium are crucial to effective flocculation. These flocculants can either be cationic, anionic or non-ionic. However, due to the negative netcharge of microalgal cells, anionic or non-ionic polymers have no effect on their flocculation.
Some cationic polymers, such as chitosan, cationic polyacrylamides, cellulose, surfactants and other man-made fibers proved to have successful flocculating activity towards microalgal cultures. Cationic polymers reduce microalgal cell surface electronegativity and bridge them to one another. Chitosan has been effectively used in the harvesting of both fresh and seawater microalgae and does not contaminate microalgal biomass; however it is too expensive for large scale applications.

2.4.1.2 Gravity sedimentation

Solid–liquid partitioning by sedimentation is one of the simplest ways to harvest microalgae. It entails the separation of the suspended algal cells that have a cell density greater than water by gravitational settling. This separation method works for various types of microalgae and is highly energy efficient.
The economical bulk removal of particles is critical. In water treatment the particles are left to settle according to Stokes Law (Sheleft et al.1984). Accordingly, the cells rapidly reach terminal falling velocity when the frictional force has become equal to the netgravitational force. The sinking velocity decreases by increasing the growth medium viscosity or by decreasing the algal cell diameter. (Barros et al. 2013).
For a spherical shaped algae such as *Chlorella*, the theoretical settling velocity of one single cell was calculated to be 0.1 $\frac{m}{d}$. Stokes Law only applies to spherical shapes, while most microalgae are morphologically more complex, therefore actual sedimentation rates vary between 0.4 and 2.2 $\frac{m}{d}$ (Gerardo et al. 2015). However other authors like Barros et al. have found that sedimentation rates for

most species of microalgae varies between 0.024 – 2.6 $\frac{m}{d}$. This results a very slow sedimentation process that leads to the deterioration of most of the biomass during the settling time, limiting the application of this method for routine harvesting.

Capital and operating costs are low, but land area requirement for settling ponds and tanks is relatively high. The local environment must also be taken into consideration, as it has been found that in high temperature environments much of the biomass produced will deteriorate during the harvesting process as a result of the lengthy harvesting process. Conventional sedimentation systems (e.g. clarification tank or lamella type sedimentation tanks) can achieve a final slurry concentration of between 1 and 3 % TSS, using less than 0.1$\frac{kWh}{m^3}$.This fact is attributable to microalgal autoflocculation.
Another Important disadvantage of this technique is that only relatively large microalgae (> 100 nm diameter) (Montes, 2009) can be settled and that it is a slow process (Pahl et al., 2013; Uduman et al., 2010b).
On the other hand compared to other harvesting systems, the absence of turbulent flows or high pressures guarantees the integrity of the microalgae structure.

When higher solid concentrations are required, sedimentation can be adopted as a pre-concentration step combined with other technologies.

Chlorella sp. con with *Moringa oleifera* (MO) seed were used to floculation, then the 95 % of particles sedimented in 20 minutes. (Bolad et al. 2014).

Gutierrez et al. 2016 carried out two experiments in a Chlorella vulgaris culture. Microalgal biomass was obtained from two experimental wastewater treatment high rate algal ponds (HRAPs) operated with 4 and 8 days of hydraulic retention time.

In the first set, most of the biomass of the 8 days-HRAP (63%) had settling velocities between 16.5 and 4 $\frac{\mathrm{m}}{\mathrm{h}}$, while most of the biomass of the 4 days-HRAP (65%) had settling velocities between 16.5 and 1 $\frac{\mathrm{m}}{\mathrm{h}}$,.

In the second set when a flocculant was applied, 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 $\frac{\mathrm{m}}{\mathrm{h}}$,. In this second set, settling velocities of <0.4 $\frac{\mathrm{m}}{\mathrm{h}}$, 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 $\frac{\mathrm{mg}}{\mathrm{L}}$ had impressive effects on the settling velocity distribution in this second set. 70% and

84% of biomass reached velocities of > 6.5 $\frac{m}{h}$, compared to 10% and 14% of microalgal biomass without flocculant for the 8 days-operation 4 days-operation, respectively.

With flocculant, a very small amount of biomass (3% for the 4 days-operation and 8% for the 8 days-operation) had settling velocities of <0.4 $\frac{m}{h}$.

According to these results, a settler designed with a critical settling velocity of 1 $\frac{m}{h}$ would reach biomass recoveries as high as 90-94% with flocculant compared to 77-88% without flocculant.

Other study, Escapa 2015, determined that *Chlorella sorokiniana* achieved the best flocculation results with $AlCl_3$ (95.23 % with 200 $\frac{mg}{g}$, 1 min incubation time.

2.4.1.3 Flotation

Flotation is often defined as "inverted" sedimentation where gas bubbles fed to the broth provide the lifting force needed for particle transport and separation and is often preceded by coagulation/flocculation.
Flotation has been successfully applied in the separation of freshwater microalgae, such as *Chlorella vulgaris*, and it is a promising low cost large scale harvesting method.

Microalgal removal depends on recycling rate, air tank pressure, hydraulic retention time and particle floating rate, while the concentration of the produced slurry depends on skimmer velocity and relative positions towards the surface of the water.

Given microalgal low density and self-float characteristics, flotation is more effective and beneficial in microalgae removal than in sedimentation.
The major advantage of flotation is that it has been proved at large scale although it generally requires the use of flocculants. Further advantages of flotation are low space requirements, relatively short operation times and high flexibility with lower initial equipment costs.

Some microalgae naturally float due to the presence of gas vesicles. However, for the majority of algal species, air, supersaturated water, or ozone is used. Bubbles are introduced at the bottom of the liquid, where the algae are collected from the liquid suspension and carried to the surface where it can be removed.

The success of flotation can be described as a product of two probabilities: (1) bubble-particle collision; and (2) bubble-particle adhesion after a collision has

occurred. In this way, it depends on the instability of the suspended particles, lower instability will result in higher air-particle contact, and on particle size, the smaller they are, the more likely they are to be levitated by the bubbles (Uduman et al.2010). However, the decrease in particle size also decreases the probability of the cells colliding with the bubble.
Particles in suspension must be hydrophobic, in order to attach to gas bubbles (Hanotu et al.2012), this can be achieved through the addition of surfactants (sometimes referred to as collectors) or coagulants.
The addition of surfactants improves particle separation by increasing the size of the algal aggregates, therefore increasing the likelihood of collision between the bubbles and cells. However, combining flocculation and flotation can be problematic. If the flocs produced become too large they are more likely to detach requiring multiple bubble attachments to reduce the increase in density caused by flocculation (Barros et al. 2014).

Presently, there are four main flotation techniques: (1) Dissolved air flotation (DAF – bubble diameter o100 mm); (2) Microflotation; (3) Dispersed air flotation (DiAF – bubble diameter 100–1000mm) and (4) Ozonation-dispersed flotation (ODF).

Dissolved air flotation (DAF) occurs in several stages (Fig. 10). The first stages occur in the saturator where a compressor is used to supersaturate the water with air (25–90 psi), for about 0.5–3.0 min in a pressure tank. This water is then released into a flotation tank at atmospheric pressure. The dissolved air precipitates out of the water forming small bubbles (10–100µm), which add here to the suspended matter carrying them to the surface, given the lower combined specific gravity than that of water. The biomass forms a layer at the top of the flotation tank which is continuously skimmed into a collection tank. The equation that governs the separation rate is also Stoke Law.

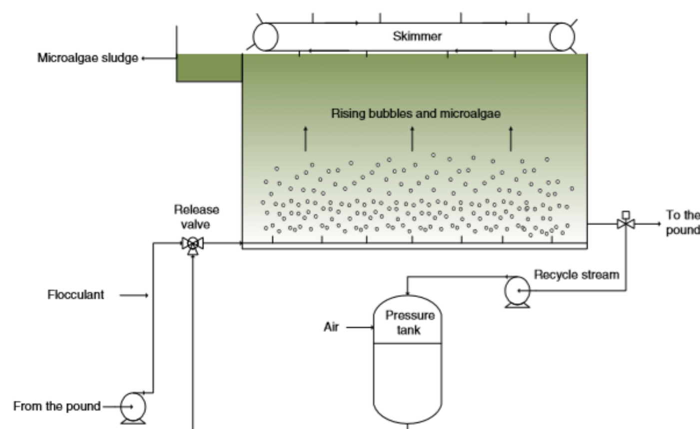


Figure 10 - Schematic diagram of a combined flocculation and DAF system microalgae harvesting.

Energy requirement associated with DAF has been reported to be high at around 7.6 $\frac{kWh}{m^3}$ mostly because of the high pressures required to supersaturate flotation water with air.

Thus, other methodologies for creating micron-sized bubbles have been exploited such as dispersed air, vacuum gas, microflotation and froth flotation. The main differences between these methodologies center on the way in which the bubbles are created in the bulk liquid.

Microflotation uses fluidic oscillation at a specific frequency which facilitates bubble detachment from the exiting pores in the diffuser. The work by Hanotu et al. 2012 showed that frequencies of 70–200 kHz produced bubbles radius sizes of 34–100 μmat11.6 psi. However, no energy consumption or biomass concentration in the floated material was determined, yet up to 99 % separation efficiency was reported.

Dispersed air flotation uses a technique similar to DAF to harvest biomass; however it eliminates the need for an expensive energy intensive compressor by generating bubbles and foam with the addition of a surfactant and a low pressure sparger.
Dispersed air flotation was reported to operate at 15 psi and energy consumption was reported to be 3 $\frac{kWh}{m^3}$. Coward et al. 2014 reported a flotation device which combines dispersed air flotation with foam fraction at ion to allow harvesting, concentration and physical separation of algal biomass.
A 10.2 L dispersed air flotation–foam fractionation was reported to achieve maximum biomass concentration of 14–24 g DCW/L with an energy consumption of 0.015 $\frac{kWh}{m^3}$, using a limewood sparger. When combined with fluidic oscillation, the maximum biomass concentration increased to 28 g DCW/L, and the energy consumption was estimated to be 0.105 $\frac{kWh}{m^3}$.

Velasquez-Orta et al. 2014 reported that the amount of lipid extracted from the biomass recovered by **ozono-flotation** doubled when compared to biomass harvested by centrifugation. In theory, flotation can be used to harvest and as a cell disruption method, for the improved extraction of lipids.
However, this work is still in the early stages of development and there is little data.

2.4.1.4 Electrical methods

Electrical approaches to microalgal harvesting are not largely disseminated. But they are environmentally friendly (they do not require the addition of chemicals) (Barros et al.2014).
As microalgal cells are negatively charged, when an electrical field is applied to the culture broth, the cells can be separated (Uduman et al. 2010). They can form precipitates on the electrodes (electrophoresis), as well as accumulate on the bottom of the vessel (electro-flocculation).

One of the most employed methods is **Zeta Potential:** Zeta (ζ) potential is the potential generated by the formation of an electrical double layer and it is responsible of the electrokinetic phenomena of colloid's stability. Electrical double layer is depicted in Figure 11

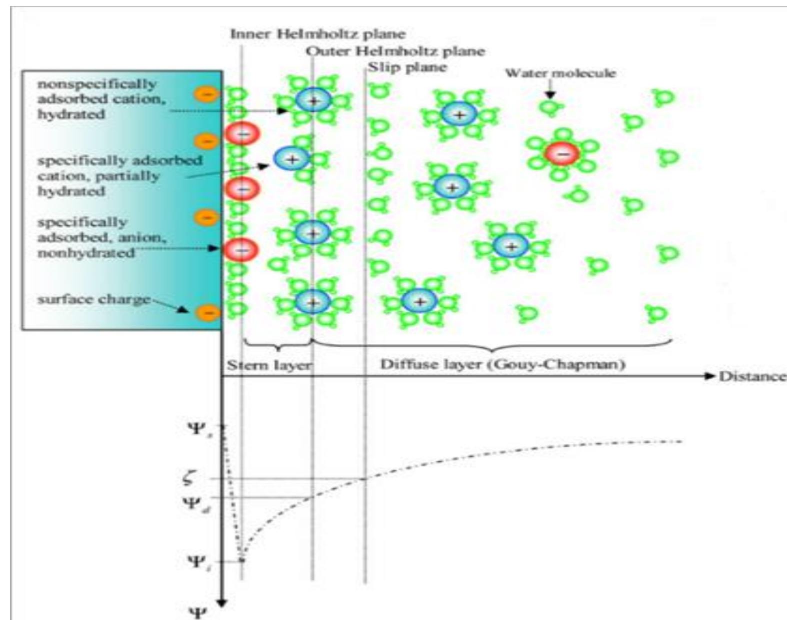


Figure 11- Structure of electrical double layer, with the corresponding potential distribution with distance from a charged wall.

As shown in the picture, the first layer is at the inner Helmholtz plane and bears the potential ψ_i, where co-ions and counter ions are not hydrated and are specifically adsorbed to the surface. The second layer is defined by the outer Helmholtz plane with potential ψ_d, consisting of a layer of bound, hydrated, and partially hydrated counter ions. The outermost and third layer is the diffuse layer, composed of mobile co-ions and counter ions, in which resides the slip plane bearing the zeta potential, ζ. In most cases, the outer Helmholtz plane and the slip plane are situated close to each other, allowing the approximation of ψ_d with the ζ potential for practical purposes. The slip plane, or shear surface, is an imaginary

plane separating ions that are immobile at the surface from those that are mobile in solution.
According to the Helmholtz-Smoluchowski theory, the electro-osmotic velocity, needed to compute zeta potential, can be derived based on the balance of the electrical and frictional forces between water and the wall of the capillary.
It is described by the following equation:

$$v_{\varepsilon o} = \frac{\varepsilon\zeta}{\eta}\frac{\Delta V}{\Delta L}$$

Where:
$v_{\varepsilon o}$ [m/s]: electro-osmotic velocity
ζ [V]: zeta potential
ε [F/m]: dielectric permittivity of the liquid
η [kg/ms]: viscosity of the liquid medium
ΔV [V]: electric potential
ΔL [m]: length of the capillary between the electrodes

The magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion: a high zeta potential will confer stability and resistance to aggregation, while when the potential is small, attractive forces may exceed the repulsion and the dispersion may break and flocculate.

2.4.2 Dewatering

2.4.2.1 Filtration

This method is based on a solid-liquid separation where a semi-permeable filter acts as a barrier. This barrier contains pores smaller than the cells being retained permitting a selective passage of water, salts and other soluble substances. It is normally applied following coagulation/flocculation to improve harvesting efficiency. Its application requires the maintenance of a pressure drop across the system to force fluid flow through a membrane (Gerardo et al. 2014).

Two simple main flow configurations of membrane filtration processes are possible: dead-end and tangential flow.

In **dead-end** filtration, the direction of the flow is directed perpendicular to the membrane surface. This is usually a batch process. This harvesting method is effective in the recovery of large microalgal cells (diameter over 70 mm) (Rawat et al. 2011, Molina Grima et al. 2003).

As the name suggests, in **tangential filtration** the flow is tangential to the membrane surface, retentate water is removed from the same side further downstream, whereas the permeate flow is tracked on the other side. Tangential flow filtration (TFF) was developed in order to improve filtration times by minimizing the buildup of the cake layer and consequent fouling. This is considered more appropriate for the harvesting of smaller suspended algae due to minor fouling problems.

Depending on the pore size of the membrane, the filtration is classified as microfiltration, ultrafiltration, nanofiltration and reverse/forward osmosis.

The performance of membrane filtration processes may be described by the Darcy's equation:

$$J = \frac{\Delta P - \Delta\pi}{(R_m + R_c)\mu}$$

Where:

J is the membrane flux ($\frac{m^3}{m^2 s}$)
ΔP is the transmembrane pressure (Pa)
$\Delta\pi$ is the osmotic pressure (Pa)
R_m is the intrinsic membrane resistance (m−1)
R_c is the cake resistance owing to fouling (m−1)
μ is the viscosity of the microalgae suspension (Pa·s).

Filtration is only sustainable for harvesting long length microalgae or those forming large colonies (Zhou W. et al. 2013). Despite microalgal cells of very low densities can be harvested by this method (a major advantage), membrane filtration is not commonly applied in large scale processes.

The use of membrane filtration for microalgae harvesting is most commonly reported across the ultrafiltration-microfiltration range (Gerardo et al. 2014). However, forward osmosis membranes have also been reported for the recovery of microalgae from dilute broths in an attempt to reduce power consumption (Zou et al. 2013). Throughout the literature, the influence of the membrane pore size on the harvesting efficiency is no clear. Indeed there is no conformity in terms of pore size for the general harvesting of microalgae.

A variety of membranes has been investigated for a wide-range of microalgae species. A common rule of thumb in membrane filtration is to choose a pore size between 10–20 times smaller than the cells that are to be retained. Studies have demonstrated that at steady-state permeance, ultrafiltration membranes have slightly better performances than those of microfiltration membranes, even though the intrinsic membrane resistance was higher.

Filtration major costs are related with membrane replacement and pumping; thus, it is cost-effective only for small volumes.
In fact, microfiltration can be more cost-effective than centrifugation when the volume to be processed is less than 2 For volumes greater than 20 $\frac{m^3}{d}$, centrifugation may be more economic.

2.4.2.2 Centrifugation

The use of centrifuges for the recovery of microalgae biomass offers many advantages when compare to other methods. The recovered biomass is free from flocculants or chemicals, it can be applied to all strains of microalgae, and high recovery rate and concentrate are easily, predicatively and quickly achieved.
Centrifugation is the fastest harvesting method, but also the most expensive due to its high energy consumption, which limits its application to high-valued products.
To achieve high harvesting efficiencies, longer retention times in the bowl are needed to enable their sedimentation, due to the small size of these cells. (Xu et al. 2011).

The energy consumption estimates for harvesting microalgae by centrifugation is considered energy intensive at 8 $\frac{kWh}{m^3}$ of microalgae suspension at feed rate of 1 $\frac{L}{min}$. However it has been demonstrated that flow rates of 18 $\frac{L}{min}$ can signifivantly reduce cost 10-fold in exchange for lower harvesting efficiency (Dassey et al. 2013)

Centrifuges are able to harvest the great majority of microalgae (Rawat et al. 2014). Some are even efficient as one-step separation process, while others require a pre-concentrated algal slurry. However, there are evidences that the exposure of microalgal cells to high gravitational and shear forces results in cell structure damage (Griffiths et al. 2011).

Molina Grima et al. 2013 have studied microalgae harvesting by centrifugation, the results are these:

Equipment	Operation mode	Steps number	Energy compsuption	Resulting TTS (%)
Self-cleaning, disk-stack centrifuge, westalia	Continious	One	$1\ \frac{kWh}{m^3}$	12 Concentration factor = 120
Nozzle discharge centrifuge Westalia	Continious		$0.72\ \frac{kWh}{m^3}$	2-15 Cocentration factor= 20 - 150

Table 2. Microalgal biomass recovery by centrifugation

2.4.2.3 New technologies

In recent years, a number of novel technologies have been investigated in an attempt to harvest microalgae at lower energy consumptions.
Limited to lab-scale process, Bosma et al. 2003 reported the use of ultrasound as an effective technology for microalgae harvesting by exploiting the dielectric properties of the microalgae cells. The ultrasounds force the cells to move towards the nodules of the standing waves, flocculate and sediment.
Separation efficiencies up to 92% have been reported although only at lab-scale. These authors reported a concentration factor up to 11 at flow rates of 4 to 6 $\frac{L}{d}$. Such processing capacity is extremely low due to the limitations in scaling up and thus unavailable at larger scales (Bosma et al. 2003).
However, the benefits of this technology are that it is multi-purpose, as it can be used not only to harvest by creating standing waves, but also to lyse the cells at lower frequencies and higher pressure amplitudes to initiate cavitation, and consequently separate the components based on density.
Other more recent developments make use of the magnetophoretic properties of the microalgae cells. Driven by an external magnetic field, ferric(Fe_2O_3) magnetic nanoparticles induce the attachment of the cells to the particles which can be easily removed from the broth. Harvesting efficiencies up to 95% have been reported using Fe_3O_4 nanoparticles with a maximum adsorption capacity of 5.83g DCW/g particles for *Chlorella ellipsoidea* at pH 7 (Xu et al. 2011).
Approximately 85–95% of the magnetic material can be recovered by using a rotating magnetic drum and can be recycled and reused in the process. However, the use of magnetic flocculants has shown to be energy intensive due to the additional mixing and the need to use compressors, and shearmills to separate the nanoparticles from the flocs. Another disadvantage is that separation efficiencies can only be maintained at flow rates less than 0.6 $\frac{L}{h}$ (Hu et al. 2014).

It is showed a comparative table of all technologies

Harvesting method	Advantages	Disadvantages
Coagulation/Flocculation	Simple and fast No energy requirements	Influence of pH and solubility of the coagulant Difficult to achieve the optimal dosage Chemicals flocculants may be expensive and toxic to microalgae flocculants Recycling of culture medium is limitant
Autoflocculation and bioflocculation	Inexpensive Allows culture medium recycling Non-toxic to microalgae biomass	Only for small-scale Changes in cellular composition Possibility of microbiological contamination
Electrolytic flocculation	Low energy requirements Low time High efficiency	Residua metals on the algae Cathode fouling and maintenance Increase temperature on the medium Influence of mixing
Polyelectrolite flocculnts	Low dose of flocculants	Use of chemicals Influence of the pH
Gravity sedimentation	High energy efficiency Integrity of the microalgae structure Simple Inexpensive	Only for large microalgae Local environment affects High land area requirement Time-operation high Possibility of biomass deterioration Low concentration of the algal cake

Flotation	Feasible for large scale applications Low cost Low space requirements Short operation times	Influence of air tank pressure, HRT and article floating rate Generally requires the use of chemicals flocculants Unfeasible for marine microalgae harvesting
Electrical methods	Applicable to a wide variety of microalgae species Do not require the addition of chemicals flocculants	Poorly disseminated High energy and equipment costs
Filtration	For microalgae cells of very low density	Small-scale Only for large microalgae
Centrifugation	Simple Fast Do not require the addition of chemicals flocculants High recovery efficiencies Suitable for almost microalgae species	Expensive High energy requirements Suitable only for the recovery of high-value producs Possibility of cells damage

Table 3. Harvesting methods.

3. Materials and methods

3.1 Reactors and equipment

Microalgae were grown in a pilot scale photobioreactor (figure12) located in a large WWTP in Milano, Italy. The pilot plant was made of a 90 L, outdoor plexiglas column (150 cm height, 29.2 cm internal diameter); the column was connected to a feeding tank (150 L approx.) through a variable-flow peristaltic pump (Qmax 115 mL min-1). The produced suspension was sent to a storage tank (150 L approx.) by a gravity driven overflow. The gas employed to mix the system and provide oxygen was air.

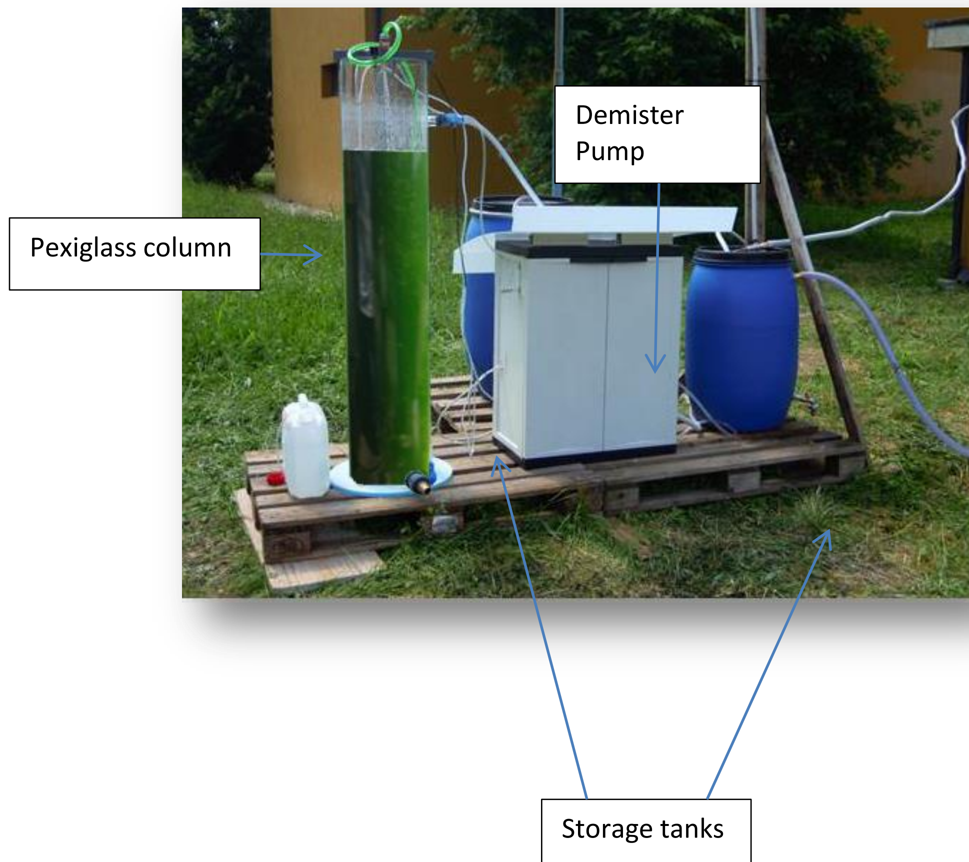


Figure 12. Pilot scale of microalgae photobioreactor in Bresso, Milano, Italy

The system started to operate in batch mode during 55 days, (in the annex I it is showed the experimental procedure).

Firstly the column is filled with the microalgae inoculum, then is added the digestate.

The main monitoring parameters were the total and volatile solid concentrations, nitrogen contents in form of NH_4, NO_2 and NO_3, phosphorous content, COD,

temperature, pH, conductivity, turbidity and absorbance, when the microalgae presents peaks in the absorbance spectrum. Sampling and analyses were performed 2-4 times a week within the period from 12nd April to 20th July. The hydraulic retention time is 10 days. *Chlorella sp*. and *Scenedesmus sp*. were the main algal strain in the pilot plant.

The characteristics of the digestate are shown in the following table (3).

TSS (mg TSS/L)	35955
TS (mg TS/L)	884
COD (mg O2/L)	135,2
COD (mg O2/L) *	109
P-PO4 (mg P/L) *	7.2
N-NH4 (mg N/L) *	280
N-NO3 (mg N/L) *	1.82
ABS (ƛ 680 nm)	0.011
ABS (ƛ 420 nm)	0.036
Turbidity (NTU)	24.9
Conductivity (mS)	1.62
pH	8.48
Temperature (ºC)	24.1

Table 4. Characteristics of digestate for batch operation.

* These parameters have been analyzed after 0.45 µm filtration.

3.2 Analytical methods

Analyses were carried out according to the Standard Methods for ammonia, nitrate nitrite, total phosphorus, COD and Total Suspended Solids (TSS).
Temperature, pH, conductivity and absorbance were measured directly with a pH-meter: Instruments model PC 210, TecnoVetro XS.

3.3 Biomass harvesting and solid/liquid separation tests

Microalgal biomass was processed by four solid/liquid separation mechanisms.

Capillary suction time: Determines the rate of release of water from the algae suspension.
It is performed placing a sludge sample in a small steel cylinder on a sheet of chromatography paper (pore diameter of 8 µm), which extracts water by capillary

suction. The time required to move in a specified distance is monitored measuring the conductivity change between two points appropriately spaced and in contact with the paper, thanks to the presence of electrode sensors across the top plate, which are connected to a timer.
It is a rapid and simple measurement, but it is unrealistic because of no pressure application.
(Repeated 3 times).

Time to filter: A wastewater treated with microalgae sample is placed in a Buchner funnel with a paper support filter (pore diameter of 20μm) and vacuum is applied (-50 kPa), the funnel is connected to a graduated cylinder and the amount of filtrate (100 ml) is measured as a function of time.

Centrifugation: Add in the centrifuge 50 mL of sample, 4000 rpm X 5 min. Compute sludge dry solid amount in the cake separate from the supernatant.

Zeta potential: Because microalgae particles size must be lower than 100 μm to be measured by a zeta-meter, samples have to be treated before the analysis. Therefore, the sludge samples are first centrifuged to remove the supernatants at 4000 rpm for 5 min.

The samples are placed in the zeta-meter viewing chamber where an electric field is activated. This causes the colloids to move with a velocity that is proportional to their zeta potential, and their direction indicates whether their charge is positive or negative.

3.4 Operation Condition

3.4.1 Batch operation mode

Firstly the reactor was operating in batch system for determinate the characteristics of the wastewater and the microalgae culture.
In batch operation there are not inlet and outlet flow, the reactor is filled once and the culturing was analysed during 55 days, from 12nd April to 6th June of 2016.
The aim objective is study how are employed the nutrients presents on the digestate to microalgae growth. Also it is interesting observe how the meteorological conditions, especially light availability, affects to microalgae growth.

To determinate microalgae growth in batch operation, have been carried out the following performances:

Turbidity, in this method a light beam is transmitted through the bacterial suspension to a light-sensitive detector. While the number of microalgae increases, the light captured by the detector will be reduced. Tortora, G. J (2004). So it is proportional to the quantity and size of particles.
This method is very fast and easy, it is only needed add a sample (or a diluted sample if the result of it is out of the measure range) in the spectrophotometer and the equipment shows the results in NTU.
However to know the concentration ($\frac{mg}{L}$) of microalgae present in the reactor it is necessary analyze by other way TSS and correlated it with a graphic.
Neither it is a specific analysis method, meaning, the spectrophotometer do not distinguish between solids.

Absorbance, consists on the measure of the amount of radiant energy absorbed a chemical system on function of a specific wavelength.
Beer-Lambert law affirms that the absorbance of a sample at a certain wavelength depends on the amount of absorbing species with which the light passing through the sample is. So if it is known the specific wavelength that microalgae absorb maximum light, it is possible to determine the amount of cells that are presented in our culture system.
This method also is fast and easy, but it requires to know which is the specific wavelength (ƛ) where microalgae species behave better.
On the other hand for correlated the value with the concentration, it is necessary make a calibration curve with a solution with a known concentration.

Cell Counting, on a sample of known volume is counted under a microscope the number of cells present in it.
It is a very reliable method to determine the number of microalgae that there are in the reactor.
However when the amount of microalgae is large and colonies are formed these procedure can not be used.
This method requires more time than the previous. Also the species founded in the counting must be corroborated in the bibliography for identify them, which can be hard work if appear strange species.

Total suspended solids (TTS), this parameter shows the concentration of suspended solids contained in the water. This analysis procedure requires more experimental time than the others (at least 3 hours), but gives directly the concentration of the solids in the study liquid.
However it can be impossible distinguish by this procedure which kind of solids it is being measured, considering that in the wastewater are presented other solids apart of microalgae. So it is important to know how much of the TSS corresponding with microalgae.

Nutrients consumption, another factor that is going to be used for analyze microalgae growth is the consumption of NH_4. It has been explained in the test that microalgae use nitrogen to incorporate grow. The mayor form of nitrogen existed in the digestate appears like ammonium. So if it is studied the amount of nitrogen in form of ammonium at the feed, (digestate), and the amount in the outlet (column), the difference between them will have been employed in the growth of biomass. Nevertheless, not all this nitrogen is incorporated in the biomass. Some is employed by nitrificant batteries in the process of nitrification/denitrification eq(1,2). Also as our reactor operates in an open pond system, a percentage of ammonium nitrogen go to the air like NH_3. This process is known like stripping, eq (3).

For study this phenomenon are being developed mass balances to the system.

Summarizing, this way to analyze microalgae growth is easy and quickly in the experimental phase, but it requires subsequent work with the mass balances to analyze all mentioned factors. The other methods are more direct.

COD consumption, this element allows us to know how many substances presented in the wastewater are able to oxidize, or which is the same, how many substances in the rector consume oxygen.

This method, like nutrients consumption analysis, is easy and quickly but it is impossible to know by this way, if only the microalgae are consuming the oxygen or there are other different microorganisms that are using it.

In this study, it is going to be evaluated the behavior of the factors mentioned before along the time. Not is going to be correlated with the concentration.

Another factor important to study is the extinction or generation rate of the different parameters followed in the reactor. They can be estimated by mass balances.

<u>Batch Mass Balances</u>

Defining

X = TSS

It is defined

$$TSS\ accumulation = TSS\ inlet - TSS\ outlet + TSS\ generation/(-)eliminatio$$

$$\frac{dVX}{dt} = \dot{Q}_{in}X_{in} - \dot{Q}_{out}X_{out} + r_{TSS}V \quad (4)$$

There are not inlet and oulet flow:

$$X\frac{dV}{dt} + V\frac{dX}{dt} = r_{TSS}V \quad (5)$$

$$r_{TSS} = V\frac{dX}{dt} + X\frac{dV}{dt} \quad (6)$$

X= it is known by the analytic methods carried out at the samples from the column.

It is used the medium value along the time. $X_{out} = \frac{X_{t+1}-X_t}{2}$

$\frac{dX}{dt}$ = Can be discretized; $\frac{\Delta X}{\Delta t} = \frac{X_{t+1}-X_t}{t_{t+1}-t_t}$.

$\frac{dV}{dt}$ = Can be discretized; $\frac{\Delta V}{\Delta t} = \frac{V_{t+1}-V_t}{t_{t+1}-t_t}$.

Variations in the reactor volume are caused by evaporation, rain and dosed samples. $V = \frac{V_{t+1}-V_t}{2}$

For calculate how much nitrogen there are on microalgae it has been proceeded by the following way:

In terms of general composition, Grobbelar et al. 2004 has proposed an equivalent molecular formula for microalgae: $C_{106}H_{181}O_{45}N_{16}P$.

The molecular mass of each component is:

C = $12\frac{g}{mol}$
H = $1\frac{g}{mol}$
O = $16\frac{g}{mol}$
N = $14\frac{g}{mol}$
P = $31\frac{g}{mol}$

If it is multiplied the number of moles of each element by its molecular weight, it is obtained the percentage of nitrogen contained in microalgae

C → $12\frac{g}{mol}\ x\ 106\ mol = 1272\ g$

H → $1\frac{g}{mol}\ x\ 181\ mol = 181\ g$

O → $16\frac{g}{mol}\ x\ 45\ mol = 720\ g$

N → $14\frac{g}{mol}\ x\ 16\ mol = 224\ g$

P → $31\frac{g}{mol}\ x\ 1\ mol = 31\ g$

The percentage in mass of nitrogen on microalgae is around 10 %

The rate of generation of nitrogen in mass is $r_{N,TSS} = 0.1\ x\ r_{TSS}$

The mass balance for the others controlled parameters are equal.
Where X = NH_4^+, NO_2, NO_3, P, COD.

3.4.2 Continuous operation mode

Once the culture medium is characterized by the batch test, the system was adjusted for operate in continuous mode. For this purpose the pump which impulses the inlet flow to the reactor was started on, providing a flow rate of 6.25 $\frac{mL}{min}$.
The outlet flow was working by overflow.
Once per week new digestate is added to the storage feed tank.
Similarly to the batch case, collection of information was performed 2-3 times a week, considering the same monitoring parameters as in the first case. This set of experiments was carried out within the period from 7th June to 31st July 2014.

As it has been said previously, once the last batch test was concluded, the system was adjusted for changing the configuration to a continuous operation.

The principal objective of this operation mode is study the nutrients consumption by the microalgae to growth, using a digestate of a wastewater treatment plant in Bresso, Milano.

The extinction or generation rate of the different parameters followed in the reactor can be estimated by mass balances.

Defining
X= TSS
In= Digestate
Out = Column. It is assumed that the reactor operates in a perfect mix model, where the composition of the out flow is the same that in all reactor.

It is defined

$$TSS\ accumulation = TSS\ inlet - TSS\ outlet + TSS\ generation/(-)elimination$$

$$\frac{dVX}{dt} = \dot{Q}_{in}X_{in} - \dot{Q}_{out}X_{out} + r_{TSS}V \quad (7)$$

$$X\frac{dV}{dt} + V\frac{dX}{dt} = \dot{Q}_{in}X_{in} - \dot{Q}_{out}X_{out} + r_{TSS}V \quad (8)$$

$$r_{TSS} = \frac{dX}{dt} + \frac{X}{V}\frac{dV}{dt} + \frac{\dot{Q}_{out}}{V}X_{out} - \frac{\dot{Q}_{in}}{V}X_{in} \quad (9)$$

$\dot{Q}_{out}$ = it is known adjusting the pump which drives the inlet flow.
X_{out} = it is known by the analytic methods carried out at the samples from the column.
It has been taken the average the two values in the considered period time
$X_{out} = \frac{X_{t+1}-X_t}{t_{t+1}-t_t}$
Also for the volume has been taken the average the two values in the considered period time
$V_{out} = \frac{V_{t+1}-V_t}{t_{t+1}-t_t}$
X_{in} = it is known by the analytic methods carried out at the samples from the digestate.
$\frac{dX}{dt}$ = Can be discretized; $\frac{\Delta X}{\Delta T} = \frac{X_{t+1}-X_t}{t_{t+1}-t_t}$.
$\frac{dV}{dt}$ = Can be discretized; $\frac{\Delta V}{\Delta t} = \frac{V_{t+1}-V_t}{t_{t+1}-t_t}$.

For calculate how much nitrogen there are on microalgae it has been proceeded like in batch operation.

So the percentage in mass of nitrogen on microalgae is around 10 %

The rate of generation of nitrogen in mass is $r_{N,TSS} = 0.1\ x\ r_{Tss}$

Mass balances for the others controlled parameters are the calculated by the same way same.
Where X = NH_4^+, NO_2, NO_3, P, COD.

3.5 Harvesting of microalgae

Has been carried out the proves described in the previous section.

4. Results and discussion

4.1 Batch operation

Microalgae growth

During this first period the culture system was exposed to different climate conditions. They are showed in the figure 13.

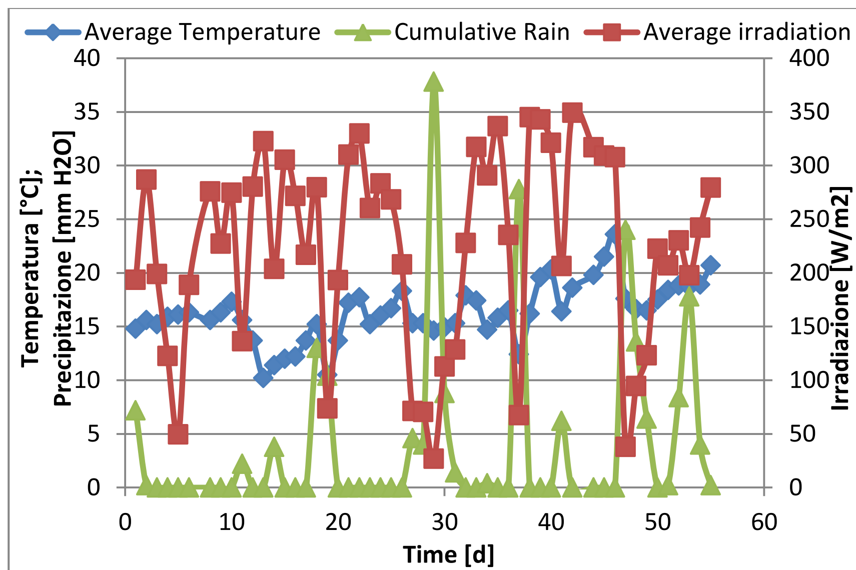


Figure 13 meteorological conditions during batch operation.

And now it is going to be analysed the influence of that meteorological conditions in the culture medium.

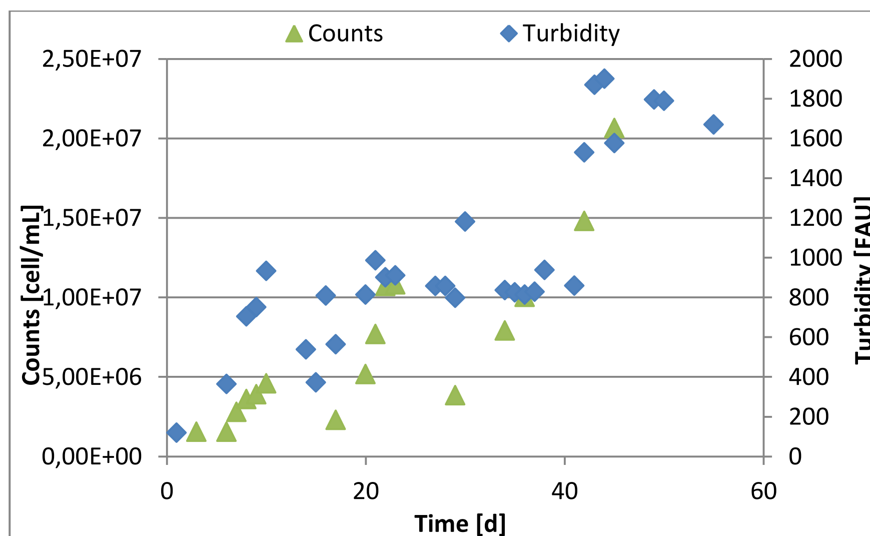


Figure 14. Cells counting and turbidity on batch operation.

It is observed when the turbidity of the culture is increased, the amount of cells it is become higher.

In these two graphics can be observed how the environmental conditions affect to the development of the microalgae.

When precipitation is high, decreased the irradiation and microalgae do not get enough light for grow. These phenomena can be appreciated during the days 26 – 31 of the experiment.

During this period of time, also the temperature of the culture decreased, and as it has been demonstrated in the bibliographic experiments, microalgae grow exponentially with the temperature, and so also for this fact microalgae reduced their growth.

Another factor that denotes microalgae growth in the medium is the absorbance.

This behaviour can be observed at the figure 14.

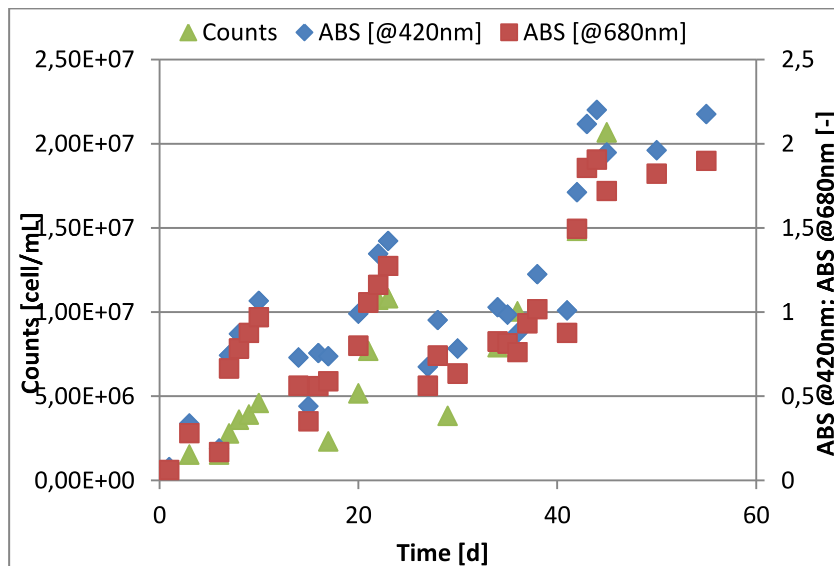


Figure 15. Absorbance and cells counted in batch operation

It has been mentioned, if the absorbance increased it is because there are more suspended solids in the culture medium. So this study parameter confirms that microalgae are been growing during the batch period. Because along the time, the absorbance is higher.

As it has been exposed at the beginning of the thesis, for the microalgae growth is not only necessary light also it is essential nutrients consumption. So it is

necessary analyse the behaviour of the nitrogen at the phosphorous present on the digestate.

The following figures show these behaviours.

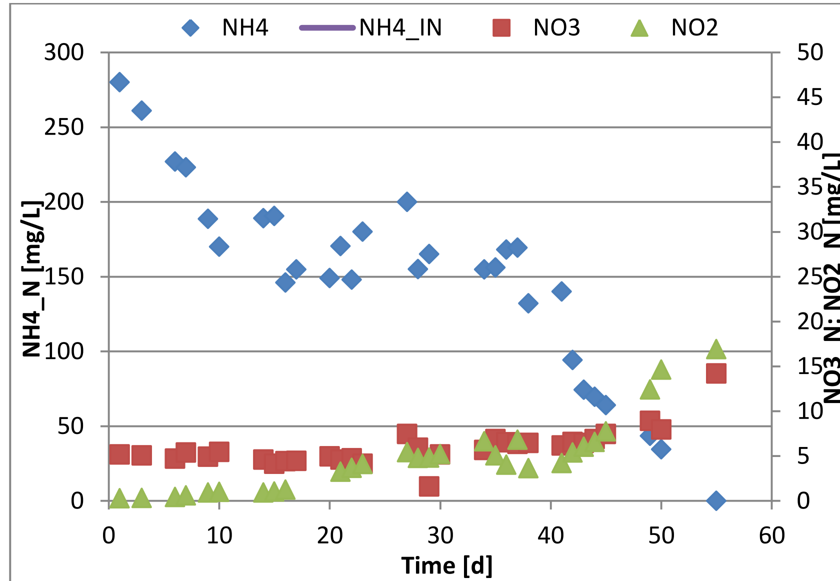


Figure 16. Nitrogen behaviour in batch operation.

In this graphic it is appreciated how decreased the amount of ammonium.
Since the first day of the experiment until 14 the consumption rate of ammonium increases with the time.

Based on the ammonium consumption it was found a consistent reduction of it during this period; this means a high consumption of the substrate. Furthermore, this reduction has to be correlated to the biomass production. Because as has been said before, not all nitrogen of the ammonia is employed to growth biomass, part of it is uses by nitrificant bacteria; another amount goes to the atmosphere by stripping.
Moreover, after the day 10, exhaustion of this substrate reaches around the 50% of the initial content.
Another fact to stand out it is that during the days 17 to 31 coinciding with the rain time, as it has been note before microalgae were not growing up and it can be observed that the N-NH_4 consumption was reduced even almost interrupted.
On day 36 until the end of the experiment it is observed that the amount of ammonium in the column is again decreasing.
It can be note that microalgae grow more slowly than in the previous days.
It could be because there are more microalgae on the reactor which need substrate to grow up and the substrate also is lower than during the days before

Also for this population increment the caption of light is more difficult and the microalgae growth slowly, so the consumption of nutrients is less.

In the figure 16, can also be observed, that NO_3 and NO_2 concentration in the reactor are mainly constant. Since the day 50 of the experiment the amount of this element increases so the generation rate becomes a little higher.
During the first days the nitrificant bacteria had not got time to develop the nitrification/denitrification. So the NO_2 rate of generation/elimination is zero. It can be observed in the figure 16 the amount of NO_3 is increasing so the amount of NO_2 increases to.

The percentage of NH_4 removal during batch operation mode was 87.68 %.
The percentage NH_4 converted to NO_3 was 5.10 %.
The percentage NH_4 converted to NO_2 was 6.04 %.
The percentage NH_4 converted to biomass was 36.89 %.
The NH4, which was not be converted to NO_3, NO_2, biomass or held in the column, was converted to NH_3, and go to the atmosphere by stripping
The percentage NH_4 stripped was 39%.

Figure 17 shows the velocity of generation/consumption of this nutrients.

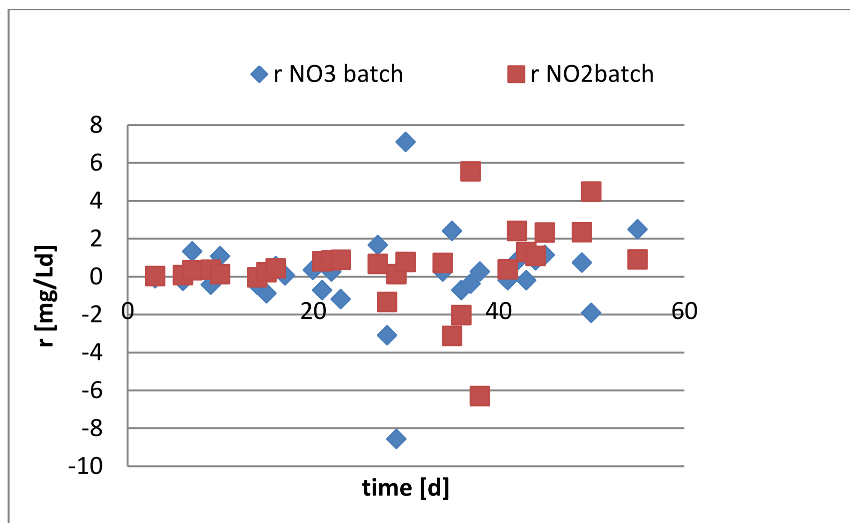


Figure 17. NO_2 and NO_3 rate in batch operation.

This rate is sometimes positive and other negative because is an intermediate product, as is has been explained in the equations 2 and 3, sometimes the elimination is high of the generation.

The other principal nutrient that microalgae need to growth is phosphorous. As has been said previously it is mostly incorporated like PO_4.

As in the case of the ammonium the amount of phosphorous along the batch experiment is reduced, which indicates that nutrients are being consume with the objective to growth biomass.

This behaviour can be observed in the figure 18.

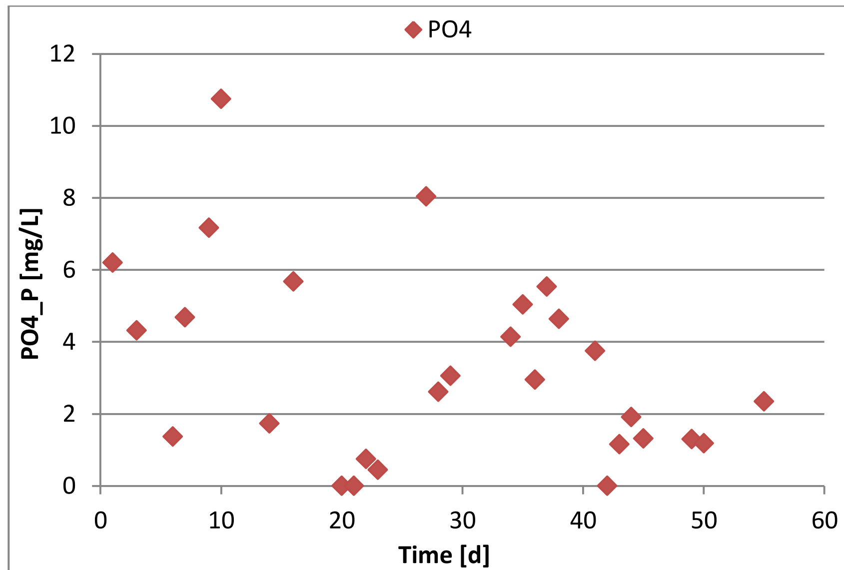


Figure 18. Phosphorous behaviour in batch operation.

In the previous part of the thesis it has been said that there are an optimal N/P ratio for the correct growth of the microalgae, and that depends on the specie of them. The optimal N:P ratio for a *Scenedesmus* culture is 12.9 (Martinez et. Al. 2000), and for *Chlorella vulgaris* is 8 (Kapson et al.2000), but other author Raddfield affirms that this parameter is 16.

In the figure 19 it can be studied the optimal N/P rates of this experimental case.

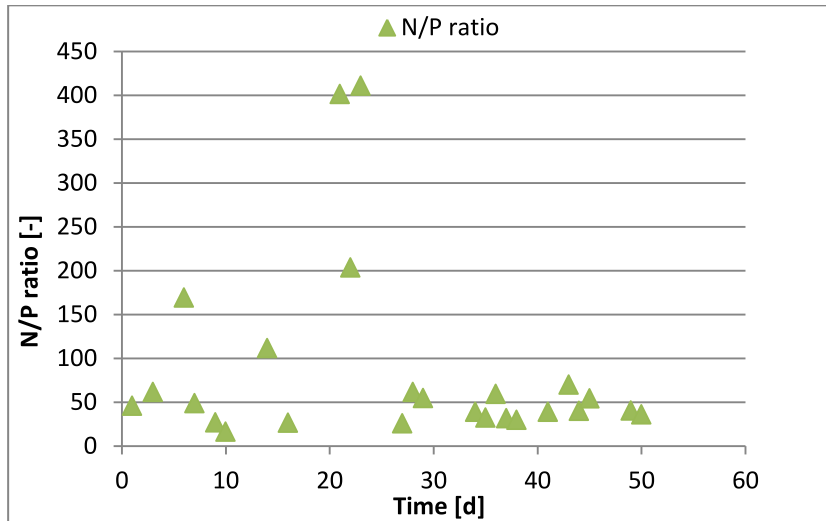


Figure 19. N/P ratio in batch operation mode.

In our microalgae culture, this rate is around 19, most of the time.
The explication of that could be that there are more than one microalgae specie growing in the culture, so the N/P total different to the optimal ratio that has been found in the literature for each species individually.

As well it is important study how pH and conductivity affect to the culture system.

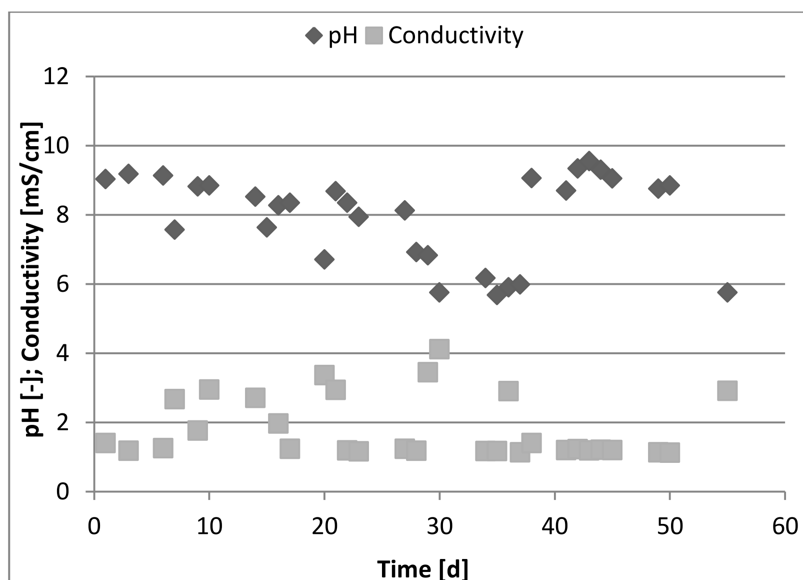


Figure 20. pH and conductivity behaviour in batch operation mode.

It can be observed in the figure 20. That from day 27 of the experiment the pH decreased until the day 37, in that time pH is going up.

A possible explanation of this fact could be in nitrification denitrification, when NO_2 and NO_3 increased in the medium, the pH is lower.
The pH along all experiments suffers big changes; it is around 5.7 – 9.

Viewing the information it is clear when the concentration of NO_2 and NO_3 increase the conductivity, opposite than pH.

Other interesting factor that has been followed to measure the biomass presented in the PBR is the COD (Chemical oxygen demand). This element allows us to know how many substances presented in the wastewater are able to oxidize, or what is the same, how much organic matter is contained in the column.

The figure 21 shows the COD behaviour along the experimental time:

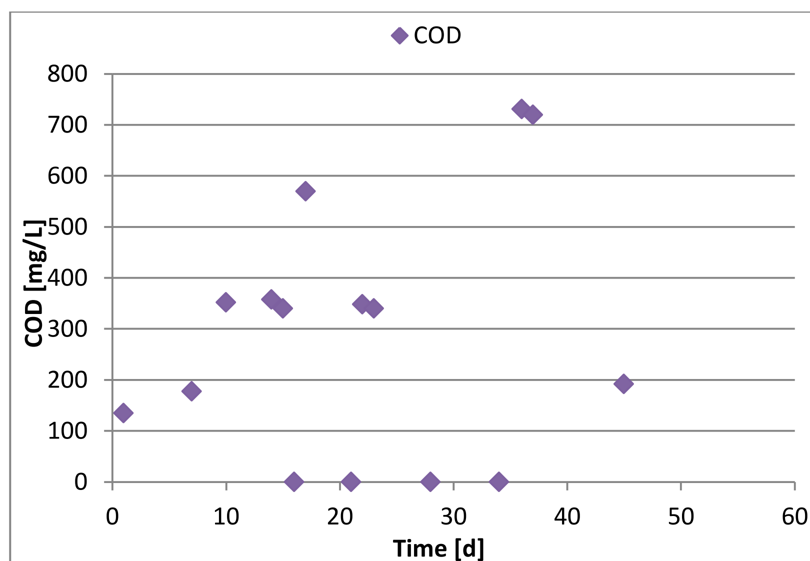


Figure 21.COD behaviour in

It can be seen that the concentration of COD is higher in the time, which indicates that in the PBR exists biomass.
This fact corroborates which previous data expounded on this document had affirmed.

Total Suspended Solids (TSS) are being followed during this batch operation time. In the figure 22 it can be see the results.

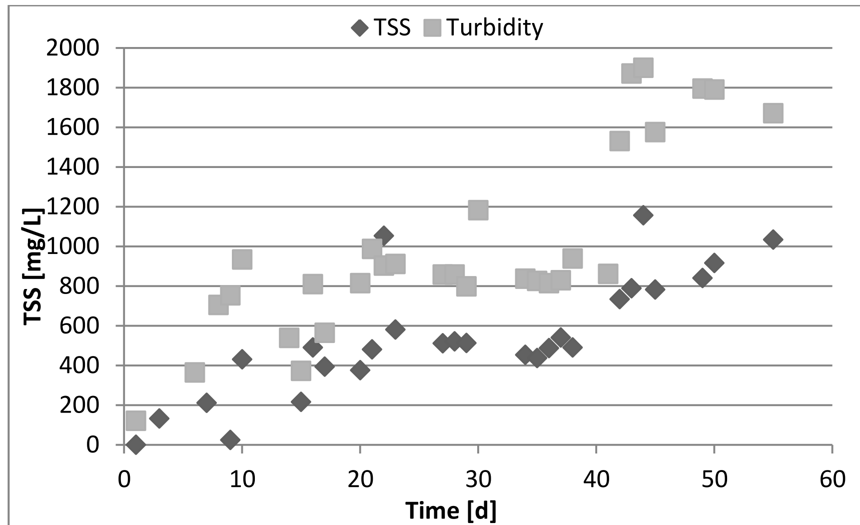


Figure 22. TTS and turbidity in batch operation.

During the experimental time had problems with the procedure to measure TSS concentration so the data of them are not reliable. In the figure 23 it can be seen that the turbidity increases but the TSS not, or they increases in less proportion, which is no real.
For that reason has been made a correlation between the TTS and the absorbance measured.
In the figure 23 are shown these results.

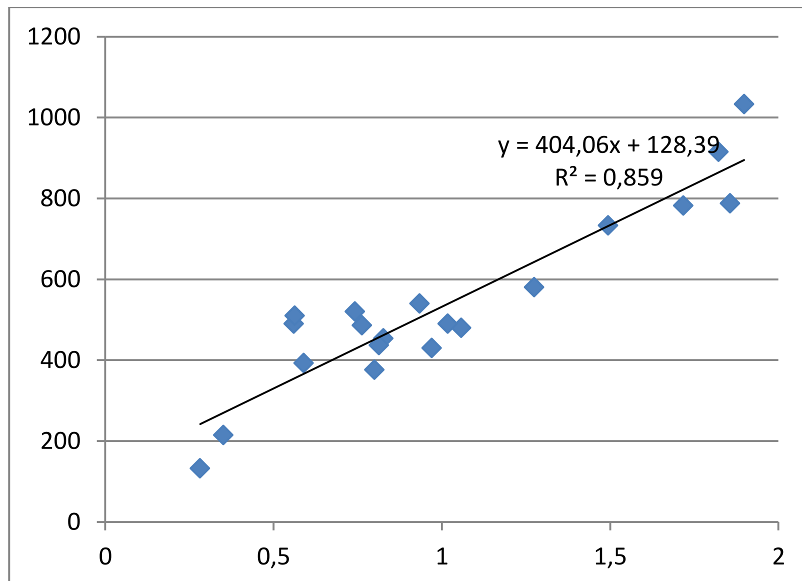


Figure 23. Correlation between TSS-Adsorbance.

For all calculations in bath and in operation mode it is going to be employed the concentration of TSS calculated with the absorbance.

4.2 Continuous operation

Microalgae growth

The principal aim of continuous operation mode is visualize how the nutrients presented on digestate, together with light, will be transformed in biomass.

The average temperature during this period is showed in the figure 24

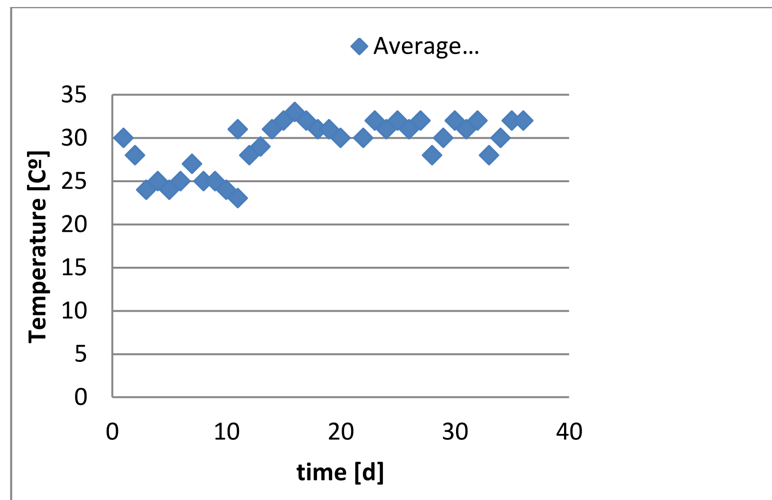


Figure 24. Average temperature in continuous operation.

The behavior of pH during continuous operation mode was that:

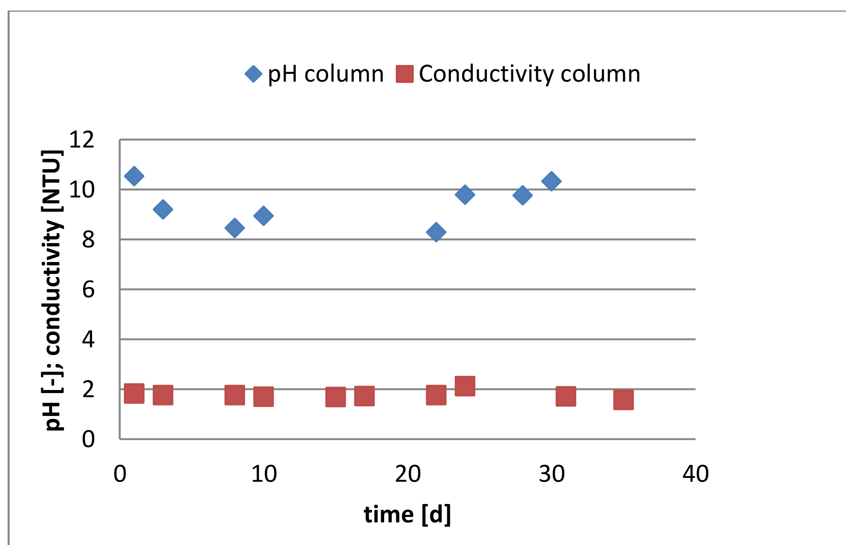


Figure 25. pH and conductivity in continuous operation.

It can be noted that the pH in the column was high, between 8 and 10.

As the digestate is added to the feed storage tank once per week, the characteristic of that feed could change a few, because has been treated in different days.
So are going to be reflected also the characteristics of the digestate.

Firstly it is going to be analyzed the turbidity of the medium.

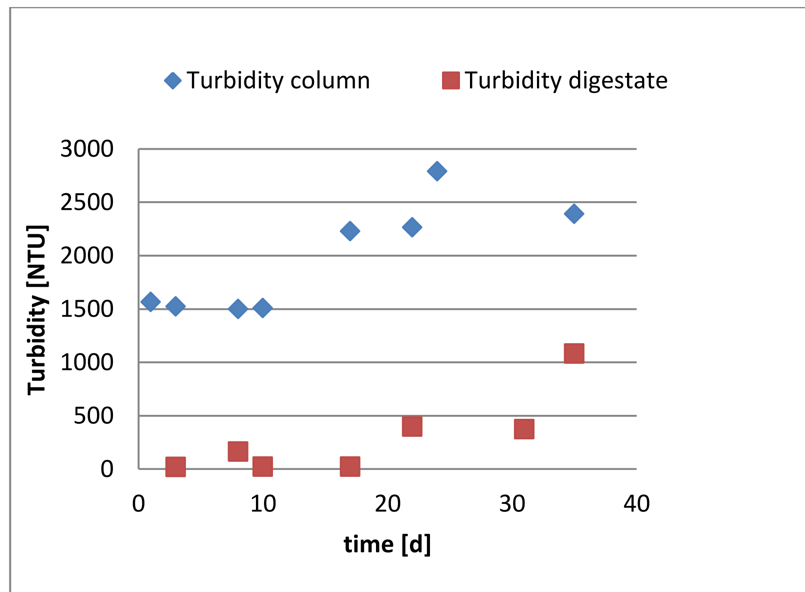


Figure 26. Turbidity in continiuos operation.

It is clear that the amount of solids in the reactor is becoming higher with the time, so microalgae were being growing.

On digestate effluent there are not microalgae, but exists some particles (suspended solids) like organic matter diluted presents on the wastewater, which after the anaerobic digestion has been transformed in suspended solids.

Between days 10 to 17 of the experimental case, turbidity in the reactor became higher quickly, which means that there has been produced more microalgae.

For visualize the microalgae growth, it also can be studied, like in batch operation mode, the amount off total suspended solids (TSS) in the reactor.

In the Figure 27 it can be observed the results.

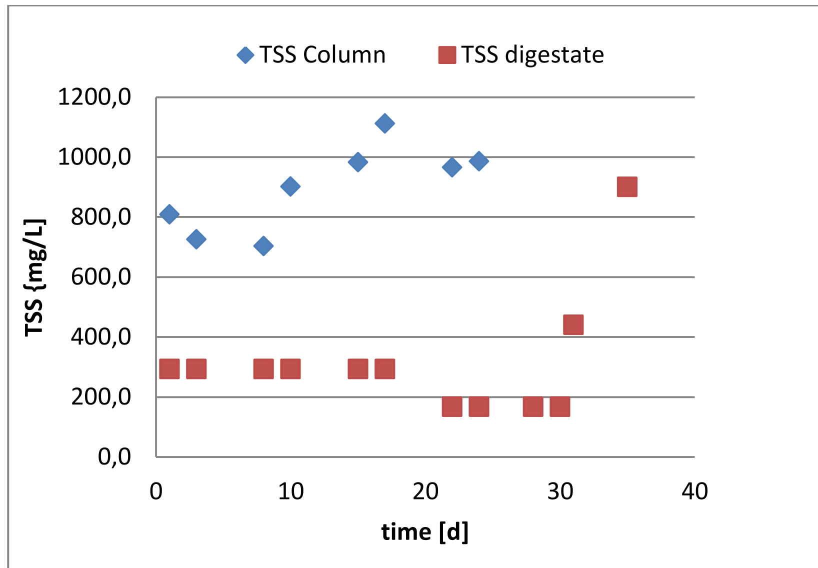


Figure 27. Total suspended solids (TSS) in continuous operation.

How it was expected this factor corroborated that microalgae were growing in the PBR, the amount of suspended solids.

The amount of TTS has been showed in the figure 27. It has been supposed that all TSS in the column are biomass, and the amount on nitrogen on it is 10 %.

The concentration of nitrogen in TSS of the column is reflected in the figure 28:

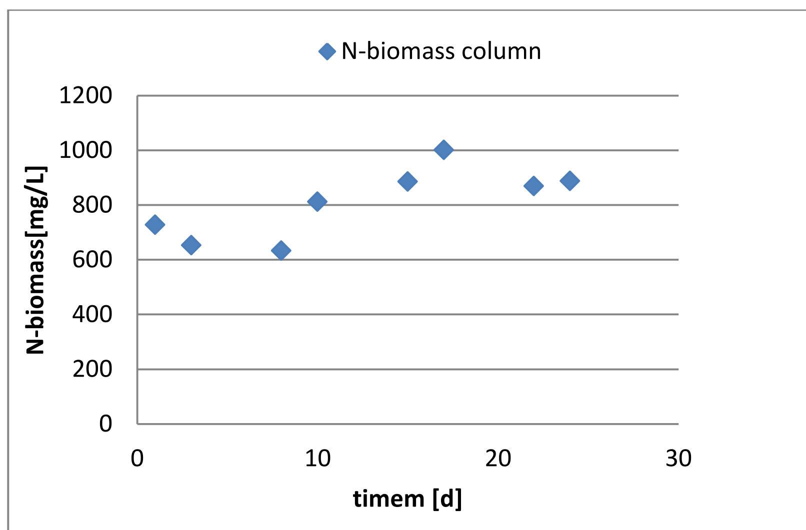


Figure 28. N-biomass in continuous operation.

The generation rate of TSS is showed in the following figure (30).

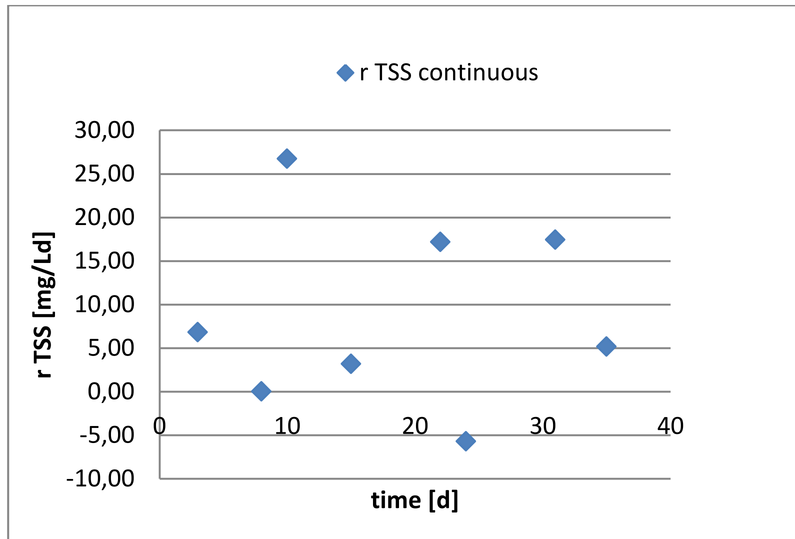


Figure 29. Generation rate of TSS in continuous operation.

The percentage of NH_4 transformed to N-biomass is:

Day	% NH_4 to N-biomass
1	60,4
3	54,2
8	58,5
10	73,7
15	55,5
17	56,2
22	55,5
24	56,7
31	51,3
35	58,9

Table 5. Percentage of NH4 transformed to N-biomass in continuous operation mode.

As has been explained in the section of batch operation mode absorbance while Beer-Lambert law.

The results are showed on figure 30.

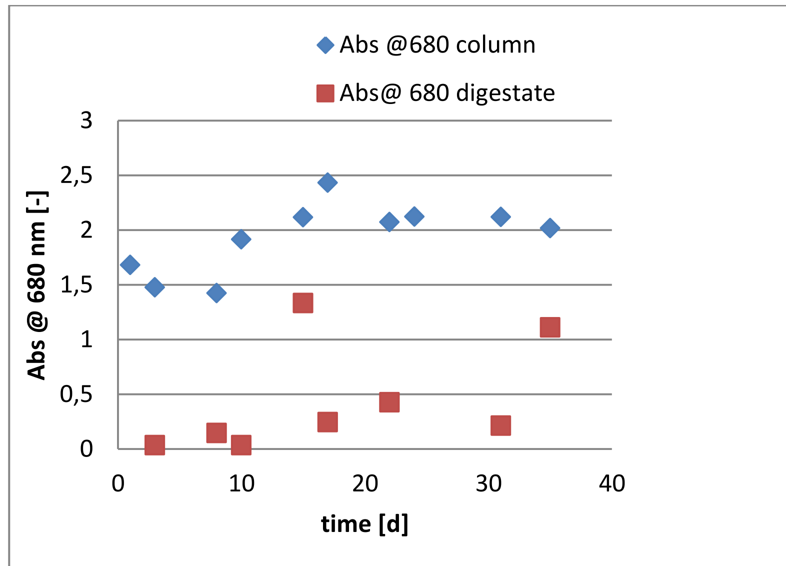


Figure 30. Absorbance in the column ƛ = 680 nm.

It is observed, like in batch operation mode, along the time the absorbance is higher which indicates that there are more microalgae cells presents in the culture medium, how is was expected.

The absorbance for the digestate also affirms that since day 15 of continuous operation the concentration of suspended solids increased.

Nutrients consumption

It has been explained along this article microalgae consume nutrients, principally nitrogen and phosphorous and with light help, convert it in biomass.
The principal way that nitrogen is content on digestate is like ammonium N-NH_4.
Ammonium is thought to be the preferred form of nitrogen because a redox reaction is not involved in its assimilation; thus, it requires less energy. Several studies have shown that, in general, algae tend to prefer ammonium over nitrate, and nitrate consumption does not occur until the ammonium is almost completely consumed.

The figure 32 signs the evolution of total NH_4 in the digestate and in the reactor.

On the digestate total nitrogen is presented by NH_4 and NO_3, while in the culture system, nitrogen is contained like NH_4, NO_3, NO_2 and biomass.

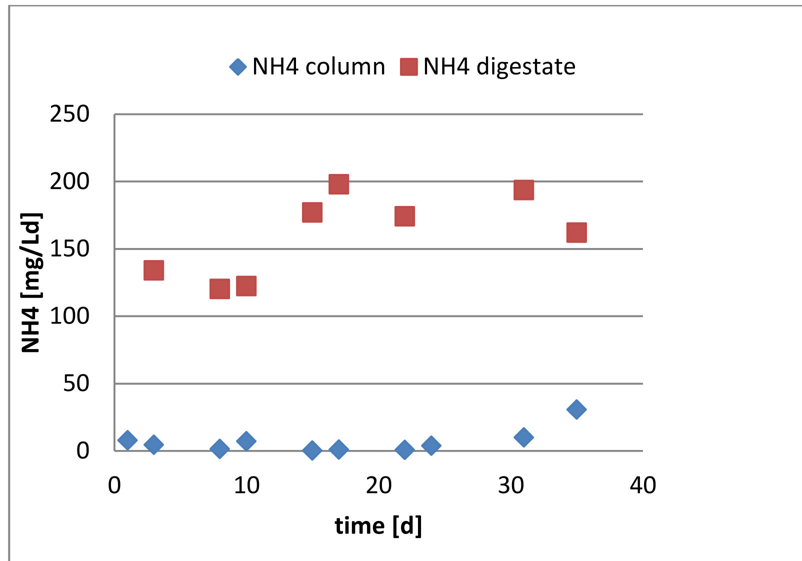


Figure 31. NH_4 in continuous operation.

Figure 31 shows that practically all ammonium contained in the digestate is consumed in continuous operation, since in the column the amount of it is very low.

The percentage of NH_4 removal is:

Day	% NH_4 removal
1	94,3
3	96,7
8	98,9
10	94,2
15	100,0
17	99,6
22	99,6
24	97,8
31	94,8
35	81,1

Table 6. Percentage of NH_4 removal in continuous operation mode.

This ammonium, as has been explained before has been transformed in biomass, nitrification/denitrification and NH_3 by stripping.

NO_3 and NO_2 behavior is the next:

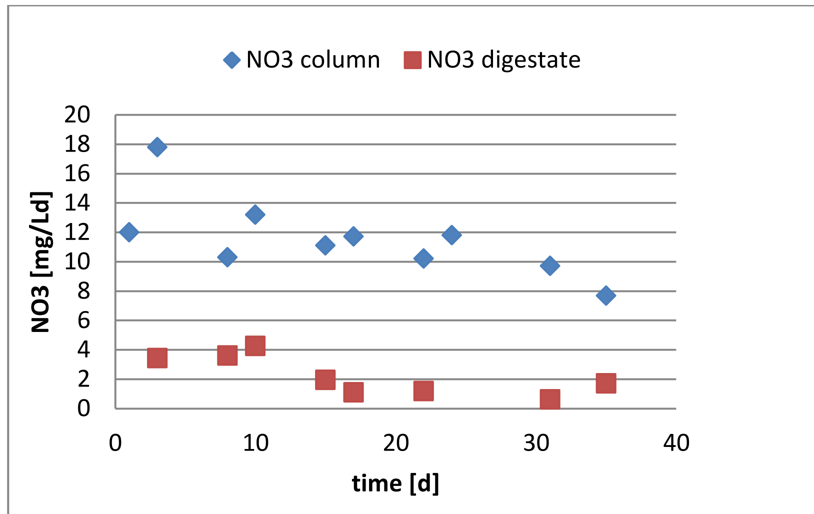


Figure 32. NO_3 in continuous operation.

It can be observed that compared with the concentration of microalgae the amount of NO_3 is low.
Viewing the graphic it is clear that nitrification was producing, because the concentration of NO_3 in the column is higher than in the digestate, so nitrificant bacteria are converting NH_4 in NO_3.

Generation rate of NO_3 is showed in the figure 33.

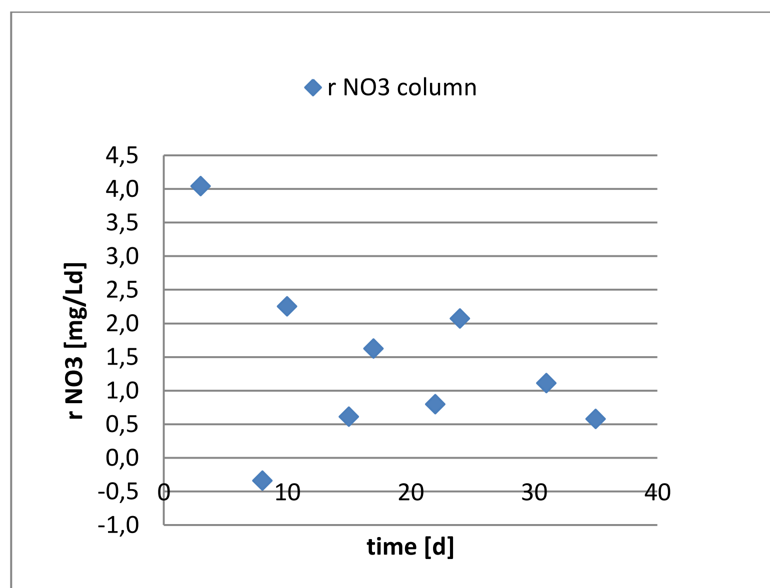


Figure 33. Generation rate of NO_3

This NO_3 generation rate it is more or less constant along the time.

The percentage of NH_4 transformed to NO_3 is:

Day	% NH_4 to NO_3
1	9,0
3	13,3
8	8,6
10	10,8
15	6,3
17	5,9
22	5,9
24	6,8
31	51,3
35	58,9

Table 7. Percentage of NH4 converted to NO3 in continuous operation mode.

Regarding NO_2 amount, the figure 35 explained its behavior.

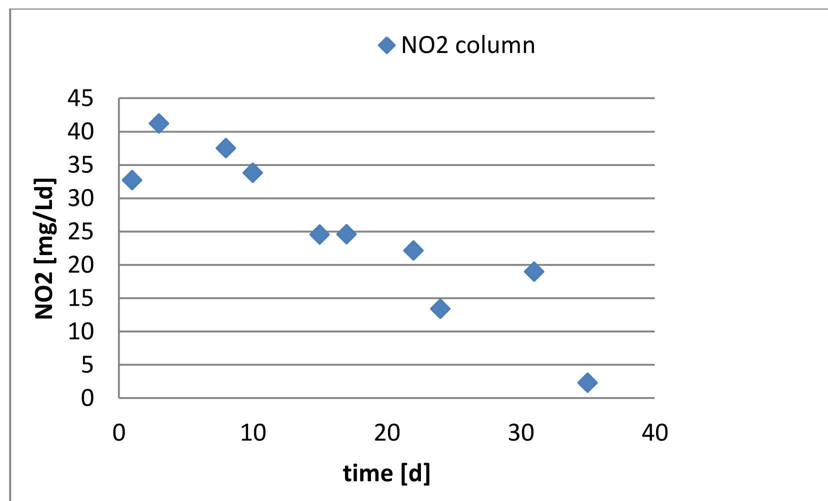


Figure 34. NO_2 in continuous operation

In the first days of the experiment there were more NO_3, so were producing more NO_2, causing by the process described by equations 2 and 3.
The following days the concentrtion of NO_2 was decreasing.

The rate of that generation/disappearing is shown in the figure 35:

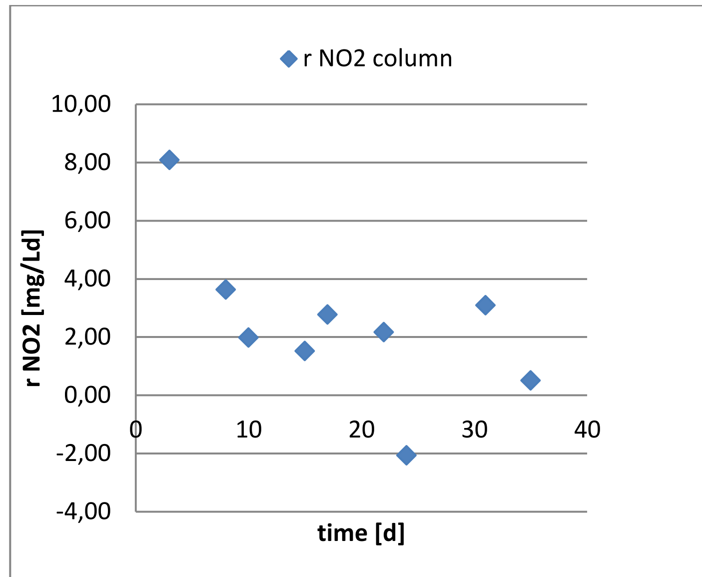


Figure 35. Generation rate of NO_2 in continuous operation.

The percentage of NH_4 transformed to NO_2 is:

Day	% NH_4 to NO_2
1	24,4
3	30,8
8	31,2
10	27,6
15	13,9
17	12,4
22	12,7
24	7,7
31	9,8
35	1,4

Table 8. percentage of NH_4 transformed to NO_2

As in batch operation mode the amount of NH_4, that was introduced in the column and has not been transformed in NO_2, NO_3 and biomass and is not in the column, has gone to the atmosphere by stripping.

The percentage of stripping along experimental time is:

Day	% NH_4 stripped
1	6,2
3	1,7
8	1,8
10	-12,2
15	24,3
17	25,5
22	25,9
24	28,9
31	33,8
35	35,0

Table 9. Percentage of stripping in continuous operation.

It is observed that the percentage of stripping in the first days was 2% and it increased until 35 %. It can be because the temperature in the column is higher and also the pH.

On day 10 of continuous operation mode the percentage of stripping is not correct.

Negative sign it is means that there are more NH_4 converted into NO_2, NO_3 or biomass that it was in the inlet, which is impossible. It could be because the TTS concentration must be overestimated.

There are not enough experimental data for the behavior of phosphorous and COD in along the experimental time.

4.3 Harvesting microalgae

The average harvesting results for the photobioreactor studied were the followings.

Filtration Suction time = 4.44 $\frac{s}{ml}$

Capillary Suction time = 7.4 s

Zeta potential = -11.9 mV

These methods have not been much investigated and could not be possible find previous experiments to compare.

However the results suggest that for small-scale harvesting of microalgae is feasible with these procedures.

5. Conclusions

The results indicate that the digestate of a conventional wastewater treatment plant can be treated in a photobioreactor with the microalgae *Scenedesmus* and *Chlorella* to remove Nitrogen and Phosphorous.

An open pond system with only sun light, area to providing oxygen and mix the system and the digestate as the only source f nutrients is perfectly viable to reach a high percentage of ammonia removal (around 97%).

In addition a high amount of biomass is produced, which can be employed in the production of renewable energy.
However harvesting of microalgae has associated high cost and must be investigated in depth.
Microalgae production is was demonstrated to be proportional to the amount of nitrogen removed.

Also the amount of NH_4 which is converted to NH_3 and go to the atmosphere must be reduce for avoid environmental contamination.

6. Bibliographic references

Aguirre, A. Study of high pressure steaming on lipid recovery from microalgae. Western University. 2014.

Ashauer, R. Post-ozonatio in a municipal wastewater treatment plant improves water quality in the receiving stream. Ashauer Environ Sci Eur (2016) pp. 1 -28.

Barros, A. et al. 2014. Harvesting techniques and methids applied to microalgae: A review Renewable and sustainable energy reviews. 41 (2015) pp. 1489-1500.

Beuckels, A, et al. 2015. Nitrogen availability influences phosphorous removal in microalgae-based eastewater treatment. Water research 77 (2015) pp 98-106.

Bilad, M.R. et al. 2014. Membrane technologie in microalgae cultivation and havesting: A review. Biotechnologi Advances 32 (2014) pp. 1283-1300.

Billuri, M. Impact of natural cationic polumers on charge and clarification of microalgae suspensions. Environmental engeniering science 3 (2015). Pp. 212-221.

Bohnenberger, J. et al. 2013. Influence of temperature and nutrient content of lipid production in freshwater microalgae cultures. Anais da Academia Brasileira de Ciencias (2014) 86(3): pp. 1239-1248.

Bohutsky, P. Bioporpecting of microalgae for integrated bimass prodution and phytoremediation of unsterilized wastewater and anaerobic digestion centrate. Appl Microbiol Biotechnol (2015) 99:6139-6154.

Cai, T. et al. 2013. Renewable and Sustainable Energy Reviews 19 (2013) pp. 360–369.

Charsungnoen, T. Chisti, Y. Continuous flocculation-sedimentation for harvesting Nannochloripsis salina biomass. Journal of Biotechnology 222 (2016) pp. 94-103.

Chatsungnonoen, T. et al. 2015. Harvesting microalgae by flocculation-sedimentation. Algal Research 13 (2016) pp. 271-283.

Chen, C. et al.2010. Cultivation, photobioreactor design and harvesting of icroalgae for biodiesel production: A critical rewiev. Bioresurce Technology 102 (2011), pp.71-81.

Cho, S. et al. 2010. Reuse of effluent water from a municipal wastewater treatment plant in microalgae cultivation for biofuel producton. Ioresource Technology 102 (2011) pp. 8639-8645.

Choi, H. et al. 2014. Effect of the N/P ratio of biomass productivity and nutrient removal from municipal wastewater. Biopreocess Biosyst Eng (2015) 38: pp. 761-766

Chu,H. et al 2014. Continious cultivation of Chlorella Pryrenoidosa using anaerobic digester starch processing wastewater in outdoors. Bioresource Technologie 185 (2015) pp 40-48.

Coward, T. eta al. 2014. Harvesting microalgae by CTAB-aided foam flotation increaes lipid recovery and improves fatty acid methyl ester characteristics. Biomass Bioenergy 67 (2014. Pp. 354-362.
Culebro Cmacho, J. Cosechado de microalgas cultivadas en lagunas de alta carga para el tratamiento de aguas residuales: efecto del almidón sobre la floculación y la producción de biogás. 2015.
Dassey, A.J., Theegala, CS. Harvesting economics and strategies using centrigugation for cost effective separation of microalgae cells for biodiesed
L applications. Biouresource Technology 2013; pp. 128-241.
Deconinck, N. Innovative steps in harvesting (Separation and dewatering) microalgae biomass for the production of biofuels. 2015.
Devgoswami, CH.R. et al. 2011. Studes on the growth behavior of Chlorella Haematococccus and Scenedesmus sp. In clture media with different concentrations of sodium bicarbonate and carbon dioxide gas. African Journal of Biotechnology Vol. 10(61) pp. 13128-13138.
Erkeens, M. et al. 2014 Microalgae digestate effluent as a gowth medium for Tetraselmis sp. The production of biofuels. Bioresource Technologie 167 (2014) pp. 81-86.
Farooq, W. et al. 2015. Energy efficient process for microalgae cell disruption for iol recovery using triidide resin. Algal Researg 13 (2016) pp. 102-108.
Feng, Y. et al. 2010. Lipid production of Chlorella vulgaris cultured in artificial wastewater medium. Bioresource Technology 102 (2011) pp. 101-105.
Ficara, E. Growth of microalgal biomass on supernatant from biosolid dewatering. Iwa Publishing 2014.
Gani, K. M. Kazmi, A.A. Evaluation of three full scale sewage treatment plants for occurrence and removal efficacy of priority phthalates. Journal and Environmental Chemical Engineering 4 (2016) pp. 2628-2636.
Gao, F. et al. 2014. A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent. Bioresource Technology 179 (2015) pp. 8-12.
Gerardo, M. et al. 2015. Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. Alga Research 11 (2015) pp. 248-262.
Gutiérrez, R. et al. 2015. Harvesting microalgae from wastewater treatment system with natural flocculants: Effect on biomass settling and biogas production. Algal Reseach 9 (2015) pp. 204-2011.
Gutiérrez R. et al.2014. Influence of starch on microalgal biomass recovery, settleability and biogass production. Bioresource Technologie 185 (2015) pp. 341-345.
Gutiérrez, R. et al. 2014. Harvesting microalgae from wastewater treatment systems with natural flocculants: Effect in biomass settling and biogas production. Algal research 9 (2015). Pp. 204-211.

Hamid, S. et al. 2014. Harvesting microalgae, Chlorella sp. By bio-flocculation of Moringa olifeira seed derivates from aquaculture wastewater phytoremediation. International Biodeterioration & Biodegradation 95 (2014) pp. 270-275.
Hanotu J. et al. 2012. Microoflotation performance for algal separation. Biotechnol Bioeng 2012; 109 pp. 1663-1673.
Hernández Sánchez, P. Evaluación de un sistema de microalgas y bacterias ara la eliminación de nutrientes de las aguas residuales domésticas. 2004.
Hidaka, T. et al. 2014. Growth and anaerobic digestion characteristics of microalgae cultivated using various types of sewage. Bioresource Technology 170 (2014) pp. 83-89.
Knopp, G. et al. 2016. Elimination of micropollutants and transformation products from a wastewatertreatment plan effluent throughpilot scale zonation followed by various activated carbon and biological filters. Water Research 100(2016) pp. 580-592.
Hom-Diaz, A. et al. 2015. Microalgae cultivaoin on eastewater digestate: beta-estradiol and 17 alpha-ethynylestradiol degradation and transformation products identification. Journal of Environment Management 155 (2015) pp. 106-113.
Junping, L.V. et al. 2015. A comparative study on flocculating ability and growth potential of two microalgae in simulated secondary effluent. Bioresource Technology 205 (2016) pp. 111-117.
Kruglova, A. et al. 2016. Comparative study of emerging micropollutants removal by aerobic activatedsludge of large laboratory-scale membrane bioreactors and sequencing batch reactors under low-temperature conditions. Bioresource Technology 214 (2016) pp. 81-88.
Kurman Prajapati, S. et al. 2013. Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: A closed loop bioenergy generation precess. Bioresource Technology 158 (2014) pp. 174-180.
Hwang, T. et al. 2014m Microalgae recovery by ultrafiltration using novel fouling-resistant PVDF membranes with in situ PEGylated polyethylenimine particles. Water research 73 (2015) pp. 181-192.
Ledda, C. et al. 2015. Production of wild Chlorella sp. Cultivated in digested and membrane-pretreated swine manure derived from full-scale operation plant. Algal Research 12 (2015) pp. 68-73.
Ledda, C. et al. 2015. Utilization of centrate from wastewater treatment for the outdoor production of Nannochloropsis gaditana biomass at pilot-scale. Algal Research 12 (2015) pp. 17-25.Lee, C. et al. 2015. Effects of photoperiod on nutrient removal, biomass production, and algal-bacterial population dynamics in lab-scale photobioreactors treating municipal wastewater. Water research 68 (2015) pp 680-691.
Liao, J. et al. 2016. Fuelling the future: microbial engineering for the production os sustainable biofuels. Mac Milan Publisher.

Marchello, A. et al. 2014. Microalgae population dynamics in photobioreactors with secondary sewage effluent as culture medium. Brazilian Journal of Microbioligy 46, 1, (2015) pp. 75-84.
Mazzari, F. Biogas production florm microalgae grown on supernatant biosolids dewatering.
Mazzari, F. Using microalgae for removing nitrogen from supernatant from biosolids dewatering.
Mazzuca Sobzuk, T. et al. 2015. Forward osmosis with glicerol for concentrating microalgae slurries. Algal Research (2015) pp. 168-173.
Melvin, S. D. Leusch, F.D.L. Removal of trace organic contaminants from domestic aastewater: A meta-analysis comariosion of sewage treatment technologies. Environmental International 92-93 (2016) pp. 183-188.
Mezzanote, V. Ficaa, E. Integration of algal production in WWTP sludge line. IWA Specialzated International Conference. 2014.
Miazek, k. et al.2015. Effect of metals, metalloids and metallic nanoparticles on microalgae growth and industrial prduct biosynthesis: a review. International Journal of Mlecular Sciences 2015, 16, 23929-23969; doi10.3390/ijms161023929.
Misra, R. et al. 2015. Evaluation of operating conditions for sustainable harvesting of microalgal biomass applying electrochemical method using non sacrificial electrodes. Bioresource Technologi 176 (2015) pp. 1-7.
Molina Grima, E. et al. 2003. Recovery of microalgal biomass and metabolites: proess optionas and económicas. Biotechnologie Advances, 20 (2003), pp.491-5015.
Morales-Amaral, M. et al. 2015. Outdoor production of Scenedesmus sp. In thin-layer and raceway reactors using centrate from anaerobic digestion as the sole nutrient source. Algal Research 12 (2015) pp. 99-108.
Msrcilhac, C. et al. 2014. Control of nitrogen behavior by using phosphate concentration during microalgal-bacterial cultivation using digestate. Bioresource and Technology 175 (2015) pp. 224-230.
Pérez, R. Eliminación de nutrientes para el tratamiento biológico de agua residual usando un sistema inmovilizado microalga-bacteria en crecimiento autótrofo, heterótrofo y mixotrófico. 2009.
Prandini, J.M. et al. 2015. Enhancement of nutrient emoval from swine wastewater digestate coupled to bioas purification by microalgae Scenedesmus spp. –bioresource and Technology 202 (2016) pp. 67-75.
Prirwitz, K. et al. 2015. Compariosion of flocculation methods for harvesting Dunaliella. Bioresource Thechnologie 196 (2015) pp. 145-152.
Rawat, I. et al. 2011. Dual role of microalgae: phycorremediation of domesticwastewatr and biomass production for sustainaboe biofuels producion. Appl Energy 2011; 88. Pp. 3411-3424.
Salazar, M. Aplicaci´n e importancia de las microalgas en el tratamiento de aguas residuales. 2006.

Sandip, A. et al. 2015. An experimental investigation of microalgal dewatering efficiency of belt filter system. Energy Reports 1 (2015) pp. 169-174.
Shi, J. et al. 2013. Applcation of a prototype-scale Twin- layer photobioreactor for effective N and P removal from different process stages of municipal wastewater by immobilized microalgae. Bioresource Technology 154 (2014) pp. 260-266.
Sirin, S. et al. 2015. Cultivating and harvesting of marine alga Nannochloropsis oculata in local municipal wastewater for biodiesel. Bioresource Technologie 191 (2015) pp. 79-89.
Takabe, Y. et al.2015. Effects of hydraulic retention time on cultivation of indigenous microalgae as a renewable energy source using secondary effluent. Bioresource Technology 207 (2016) 399-408.
Tortora, G. J. (2004). *Microbiology "An Introduction"* (8va edición). Pearson Prentice Hall
Uduman N. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. Journal of renewable and sustainable energy 2, 0102701 (2010).
Uggetti, E. et al. 2013. Anaerobic digestate as substrate for microalgae culture: The role of ammonium concentration on the microalgae productivity. Bioresource and Technology. 152 (2014) pp. 437-443.
Velasquez-Orta, S.B. et al. 2014. Microalgae harvesting using ozoflotation that utilizes a chemically modified buble surface. Biomass Bioenergy 70 (2014) pp. 356-363.
Wang, B. Lan, C. Biomass production and nitrogen and phosphorus removal by green alga Neochloris oleoabundans in simulated wastewater and secondary muncial wastewater effluent. Bioresource Technology 102 (2011) pp. 5639-5644.
Wang, S. et al. 2015. Hrvesting of microalgae by magnetic separation: A review. Algal research 9 (2015) pp. 178-185.
Wu, J. et al. 2015. Evaluation of several flocculants for flocculationg microalgae. Bioresource Technologie 197 (2015) pp. 495-501.
Xin, L. et al. 2010. Lipid accumulation and nutrient removal propierties of newly isolated freshwater microalga, Scenedesmus sp. LX1, growing in secondary effluent. New Biotechnology. Volume 27, Number 1(2010). Pp. 59-63.
Yin-Hu, W. An et al. 2013. An integrated microalgal rowth model and its application to optimize the biomass production of Scenedesmus sp. LX1 in open ond under the nutrient level of domestic secondary effluent. Bioresource Technology 144 (2013) pp. 445-451.
Yin, Y. et al. 2014. The bioavailability of the soluble algal products of different micrialgal strains and its influence on microalgal growth in unsterilized domestic secondary effluent. Bioresource Technology 180 (2015) pp. 352-355.

Annex I

Batch operation

• Let empty the column and remove any residual by washing it with abundant water.

• Adjust the bubbling system and put the exit of the gas tube in the bottom of the column assuring a good distribution of the bubbles.

• By using a bucket fill the column at 90% of its capacity with fresh digestate solution. Be sure of proceed with caution and using suitable protection element (Wear labcoat, glasses, and gloves).

• Proceeding in a similar way fill the remaining 10% of the column volume with fresh microalgae solution.

• Open the valve that controls the gas flow for supplying the column and adjust the volumetric flow rate of gas in the desired value.

• After a short period of time, enough for a suitable mixing, take a 500 mL sample of the mixture for its characterization in terms of T, TS, TSS, TN, $N\text{-}NH_4$, $N\text{-}NO_3$, TP, Conductivity, turbidity, pH, COD and absorbance. This would represent conditions at time zero (t=0).

• Cover the top of the column for isolating it from the surroundings

Procedure to analyse the control parameters:

• Retire the covers on the top of the column carefully mix the water and take a 500 mL sample for subsequent characterization.

• If necessary perform a cleaning of the column taking care of avoiding any mass losses.

• Cover the top of the column for isolating it from the surroundings with a plastic wrap and complete the operation putting a protective mesh above the plastic cover.

- If any characteristic is strange or out of ordinary, note that taking a picture if it is convenient.

• Verify the volumetric flow rate of gas and if necessary adjust according to the defined value.

- During all operating time be sure of proceed with caution and using suitable protection element (Wear labcoat, glasses, and gloves).

Continuous operation

• Take off the cover of the top.

• Add more digestate if it is necessary for reach the operative volume.

• Mix this new digestate with the rest of the wastewater of the column.

• Put the gas out stream of digester into the column and fix it always at the same depth cylinder.

• Define the HRT (days).

• Establish the effective feed rate Q_{in} (L/day).

Connect the pump and feed the reactor with the inlet flow

Connect the outlet flow with the exit storage tank.

Take note of the cylinder volume V.

Take a sample according to the established procedure.

• Determine when it is necessary add digestate to the feed storage tank.

Annex II

List of figures.

Figure 1 - The growth curves of *Scenedesmus sp* (a) and the specific growth rate of *Scenedesmus sp*. (b) under different light intensity.
Figure 2 - TSS concentrations along the time, in different ammonium concentrations, in a *Scenedesmus* culture.
Figure 3 - Evolution of microalgae growth on 14-days for 4 study cases in a *Chlorella sp* and *Scenedesmus sp* culture.
Figure 4 - Phosphorous and nitrogen distribution at the end of the Marcihac et al. (2015) experiment.
Fig. 5 CO_2, CH_4, H_2S and microalgae biomass concentration profiles in the mixotrophic (A) and autotrophic (B) photobioreactors over time. Arrows indicates biogas reinjections. Dashed line shows the microalgae biomass model data fit. Bars depict standard deviation from the mean (n=2).
Figure 6 - *Chlorella sp* growth on 3 study cases: digestate (DIG), centrifuge liquid fraction (CLF) and ultrafiltration liquid fraction (ULF).
Figure 7 - Comparison of microalgal biomass biomass after 15 days of cultivation in secondary effluent.
Figure 8 - *Scenedesmus sp* growth curve, nutrinent removal and lipid accumulation in the secondary effluent of domestic wastewater.
Figure 9 - Principle of photosynthetic oxygenation in BOD removal processes
Figure 10 - Schematic diagram of a combined flocculation and DAF system microalgae harvesting.
Figure 11- Structure of electrical double layer, with the corresponding potential distribution with distance from a charged wall.
Figure 12. Pilot scale of microalgae photobioreactor in Bresso, Milano, Italy
Figure 13 meteorological conditions during batch operation.
Figure 14. Cells counting and turbidity on batch operation.
Figure 15. Absorbance and cells counted in batch operation
Figure 16. Nitrogen behaviour in batch operation.
Figure 17. NO_2 and NO_3 rate in batch operation.
Figure 18. Phosphorous behaviour in batch operation.
Figure 19. N/P ratio in batch operation mode.
Figure 20. pH and conductivity behaviour in batch operation mode.
Figure 21.COD behaviour in
Figure 22. TTS and turbidity in batch operation.
Figure 23. Correlation between TSS-Adsorbance.
Figure 24. Average temperature in continuous operation.
Figure 25. pH and conductivity in continuous operation.
Figure 26. Turbidity in continiuos operation.

Figure 27. Total suspended solids (TSS) in continuous operation.
Figure 28. N-biomass in continuous operation.
Figure 29. Generation rate of TSS in continuous operation.
Figure 30. Absorbance in the column λ = 680 nm.
Figure 31. NH_4 in continuous operation.
Figure 32. NO_3 in continuous operation.
Figure 33. Generation rate of NO_3.
Figure 34. NO_2 in continuous operation.
Figure 35. Generation rate of NO_2 in continuous operation.

List of tables

Table 1. Ammonia removal rates by microalgal cultivated in the presence and absence of biogas under different photoperiods. Different lettes indicates statistically significant differences (n = 2, ANOVA, $p<0.05$).
Table 2. Microalgal biomass recovery by centrifugation.
Table 3. Harvesting methods.
Table 4. Characteristics of digestate for batch operation.
Table 5. Percentage of NH4 transformed to N-biomass in continuous operation mode.
Table 6. Percentage of NH_4 removal in continuous operation mode.
Table 7. Percentage of NH4 converted to NO3 in continuous operation mode.
Table 8. percentage of NH_4 transformed to NO_2
Table 9 . Percentage of stripping in continuous operation.

Annex II

List of abbreviations

Abbreviation	Definition
Anaerobic Digestion	AD
BOD	Biological Oxygen Demand
CLF	Centrifuge Liquid Fraction
COD	Chemical Oxygen Demand
CST	Capillary Suction Time
DAF	Disolved Air Flotation
DCW	Dry Cell Weigh
DiAF	Dispersed air flotation
DIG	Digestate
FST	Filtration Suction Time
HRAPs	High Rate Algal Ponds
Max	Maximum
Min	Minimum
MO	*Moringa oleifera*
ODF	Ozonation-dispersed flotation
PBT	Photobioreactor
TFF	Tangential Flow Filtration
TP	Total Suspended Solids
TSS	Total Phosphorous
TN	Total Nitrogen
ULF	Ultrafiltration Liquid Fraction
UWWs	Urban Wastewaters
WWTP	Wastewater Treatment Plant

List of symbols

Symbol	Name	Unit
t	Time	d
R_c	Resistance	kg/m^3
	cake	kg/m^3
R_m	Resistance owing to	kg/dm^3
	fouling	m^3/(m^2 s)
Q_{in}	Flow inlet	Pa
Q_{out}	Flow outlet	Pa
r	Rate	-
J	Membrane flux	
ΔP	Transmembrane pressure	-
$\Delta \pi$	Osmotic pressure	
	intrinsic membrane	Pa·s
μ	Viscosity of the	
	microalgae suspension	