ANAEROBIC DIGESTATE AS SUBSTRATE FOR MICROALGAE CULTURE: THE ROLE OF AMMONIUM CONCETRATION ON THE MICROLAGAE PRODUCTIVITY

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Abstract

In spite of the increasing interest received by microalgae as potential alternatives for biofuel production, the technology is still not industrially viable. The utilization of digestate as carbon and nutrients source can enhance microalgal growth reducing costs and environmental impacts. This work assesses microalgal growth utilizing the liquid phase of anaerobic digestate effluent as substrate. The effect of inoculum/substrate ratio on microalgal growth was studied in a laboratory batch experiment conduced in 0.5 L flasks. Results suggested that digestate may be an effective substrate for microalgal growth promoting biomass production up to 2.6 gTSS/L. Microalgal growth rate was negatively affected by a self-shading phenomenon, while biomass production was positively correlated with the inoculum and substrate concentrations. Thus, the increasing of both digestate and microalgal initial concentration may reduce the initial growth rate (μ from 0.9 to 0.04 d^{-1}) but significantly enhances biomass production (from 0.1 to 2.6 gTSS/L).

Keywords: High rate ponds, Wastewater, Anaerobic Digester, Biomass production, Nutrients.

1. Introduction

The depletion of petroleum resources together with the important rise of the global energy demand makes necessary the development of new renewable energy source. For this reason, microalgae have received an increasing interest over the last ten years as a potential alternative for biofuel production (Chisti, 2007). In spite of the attention received, microalgae cannot yet be considered as a commercially available option for biofuel production (Chiaramonti et al., 2013). Specific aspects need more research in order to enhance the industrial development of microalgae culture as a renewable biological resource.

First of all, the availability of water and nutrients to promote microalgal growth are determinant to the success of this biofuel source, both in terms of economic competitiveness and environmental impact (Jones and Mayfield, 2012). In fact, according to Pittman et al. (2011) and Lundquist et al. (2012), based on the current technology, microalgal cultivation for biofuel production is economically viable only if wastewater is used as source of water and nutrients. For this reason, coupling microalgae culture for biofuel production and wastewater treatment is nowadays seen as an appropriate and economic solution (Rawat et al., 2011; Olguin et al., 2012). The effectiveness of high rate ponds (HRPs) for microalgal production and nutrient removal has been largely demonstrated with urban wastewater (Garcia et al. 2006) and with different other effluents such as piggery wastewater (De Godos et al., 2009), dairy farm wastewater (Craggs et al., 2003) and olive-oil mill wastewater (Hodaifa et al., 2013).

In this context, digester effluents can be seen as a source of carbon and nutrients to enhance microalgal production with reduced costs, as suggested by Lundquist et al. (2012). In fact, it is generally recognized that the organic carbon is rapidly oxidized

biologically by bacteria, and then the CO_2 produced during the aerobic bacteria oxidation is used by microalgae for the photosynthesis (Oswald and Gotaas, 1957). In this way, biomass production would be coupled with anaerobic digestion of either microalgal biomass or residual biomass after fuel extraction. The energy generated from biogas can be used to offset the energy requirements for anaerobic digestion of microalgae during biogas production or to decrease the energetic needs of the cultivation and lipid extraction process for microalgae biodiesel. At the same time, part of the flue gas after cogeneration can be used to provide a CO_2 stream for microalgae growth, while the digester residuals are recycled to the microalgae production ponds.

In spite of the attractiveness of this solution, the effect of digestate properties on microalgal growth is still poorly studied. Nowadays, the few research works focusing on this topic (Bchir et al., 2011; Cho et al., 2013) show encouraging results. However, the liquid phase of digestate is often characterized by high turbidity and ammonia content, which is not reduced during anaerobic digestion (Noike et al., 2004). Such characteristics can be responsible for microalgal growth inhibition (Kallqvist and Svenson, 2003). Thus, its effects need to be further investigated in order to determine the suitability of digestate as medium for microalgal growth.

The aim of this research work was to assess microalgal growth by utilizing the liquid phase of anaerobic digester effluent as substrate. Specifically, the study focused on the effect of inoculum/substrate ratio on microalgal growth (initial growth rate and biomass production). A better understanding of microalgal growth response to digestate characteristics could extend the range of application of HRPs for microalgal production to a wider number of effluents. This would contribute to reduce nutrients requirements, costs and environmental impacts of microalgal production.

2. Materials and methods

2.1 Experimental set-up

The experiments were conducted in batch for 7 days at room temperature (30±4 °C) by using 500 mL flasks (15 cm height, 8 cm of diameter). To avoid microalgae sedimentation, flasks were continuously stirred by means of a stirring device (IKAMAG Waerke, RO 15 power) turning at 5000 rpm. During the whole experiment, light intensity of 80-90 µmol/m^2·s was continuously provided by 8 lamps (18W) and measured at the surface of the flasks by means of a PAR Quantum Sensor (SKP 215, Skyeinstruments, UK).

Mixed microalgal culture dominated by *Scenedesmus* sp. was used to inoculate the flasks, while the liquid phase of anaerobic digester effluent obtained from the wastewater treatment plant of Castres (France) was used as substrate for microalgal growth. Physico-chemical characteristics of inoculum and substrate are shown in Table 1.

Different volumes of microalgal culture (from 25 to 375 mL) and liquid digestate (from 25 to 200 mL) were properly mixed and, when necessary, diluted with tap water to attain 500 ml. Dilutions performed results in TSS concentrations ranging between 0.4 and 1.8 gTSS/L; while nutrients concentrations varied between 50 and 260 mgNH_4^+-N/l. Taking into account the maximal digestate volume (200 mL) and the total suspended solids (TSS) concentration (1.1g/L), the TSS generated from digestate account for up to 15% of the total TSS. Thus, bacteria from the digestate account for less that 15% of the microalgal TSS.

This study was performed without replicated since a previous experiment performed with 4 replicates showed a good repeatability of the results (standard deviation ±6.22% for absorbance and ±11.93 for TSS) (data not shown).

2.2 Analytical methods

Water temperature and pH were daily measured by a pH probe (InPro 426i, Mettler Toledo, CH). The absorbance of the sample at λ=680nm and λ=800nm were determined daily by optical spectrophotometry (Orion RS232, Thermo Fisher Scientific, USA). Samples of the mixed liquor were taken from each flask every two days, immediately filtered at 1.6 µm (Wathmann fiber glass filter 1820-047) and analyzed for TSS according to the Standard Method (APHA-AWWA-WPCF, 2001). In the same samples, ammoniacal-N (NH_4^+-N) nitrites (NO_2^--N) and nitrates (NO_3^--N) were analyzed with ion chromatograph (ICS 3000, Dionex, USA) equipped with pre-columns NGI 2mm and CG 11 2mm followed by separation columns CS 16 3mm and AG 15 2mm for cations and anions, respectively. The eluents used for this analysis were HMSA (25-40 mM) pumped at 0.3mL/min for cations and KOH (10-74 mM) pumped at 0.35mL/min for anions.

2.3 Calculations

The initial growth rate (μ_0) of the exponential phase was determined according to Eq.1.

$$\mu_0 = \frac{\ln(Abs_{exp}) - \ln(Abs_0)}{t_{exp} - t_0} \qquad \text{(Eq. 1)}$$

where Abs_0 corresponds to the absorbance (λ=680nm) at the beginning of the experiment ($t=t_0$) and Abs_{exp} corresponds to the absorbance at the end of the exponential phase ($t=t_{exp}$). The exponential phase of each sample was visually determined from the logarithmic growth curve (Figure 1).

The statistical significance of differences between results was evaluated by the ANOVA test.

In order to investigate the possible NH_3 inhibition on microalgal production, free ammonia concentration (NH_3) at the beginning of the experiment was calculated from the following formula (Hansen et al. 1997) (Eq. 2).

$$\frac{[NH_3]}{[NH_3]+[NH_4^+]} = \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018+\frac{2729.92}{273.2+T}\right)}}\right)^{-1} \quad \text{(Eq. 2)}$$

3. Results and discussion

3.1 Growth rate

The initial growth rate (μ_0) values were calculated for each sample at the end of the exponential phase, which normally correspond to the first 24 hours of the experiment (Figure 1). Values were highly variable and oscillated between 0.04 and 0.9 d^{-1} (Figure 2). ANOVA test shows significant differences between results ($p<0.05$). Literature values (Table 2) range between 0.2 and 1 d^{-1}. Excluding the case of higher initial nutrients concentration (260 mgNH_4^+-N/l), the other results from this work fall within the same range as previous laboratory studies carried out in small volumes (0.1-9 L) using wastewater as substrate. This fact indicated that digestate effluent does not prevent microalgal growth even when high nutrients concentrations were applied (up to 260 mgNH_4^+-N/L). Only Bouterfas et al. (2002) found growth rate values higher than 1 d^{-1} by testing a wide range of light intensities (30–456 µmol/m^2·s) and temperatures (15–35°C) in a mineral medium. According to these authors, the conditions able to maximize growth rate were 400-420 µmol/m^2·s and 35°C. The difference of light intensity between this and our study (400 vs. 90 µmol/m^2·s), together with the substrate medium and the microalgal

specie explains the variance of growth rate performances. In their study, Bouterfas et al. (2002) indeed highlighted the influence of light intensity on growth rate and their results showed an exponential increase of growth rate in correlation with the light intensity. This effect was more pronounced with the temperature increase.

In our experiment, μ_0 was inversely proportional to the absorbance measured at the beginning of the experiment (λ= 680 nm) which is an estimation the initial microalgal concentration (Figure 3). Initial growth rate decreased from 0.9-0.7 to 0.4-0.3 d^{-1} with the increase of absorbance. A previous study investigating the effects of microalgal inoculum concentrations on microalgal biomass generation with wastewater (Su et al., 2012) supports these findings. In fact, Su and coauthor's results showed that the increase of microalgal inoculum concentrations (from 0.2 to 0.8 gTSS/L) reduced the biomass generation rates (from 7.5 to 1.5 $gTSS/m^2 \cdot d$).

Our results suggest that the microalgae concentration in the medium, more that the digestate turbidity prevent light diffusion and consequently reduce the microalgal growth rate. In fact, growth rate were similar when the initial digestate concentration increased (50 and 185 $mgNH_4^+$-N/L). The effect of mutual shading in microalgal population was already mentioned by Guieysse et al. (2002) and these authors observed that the increase of microalgal population density improved the O_2 consumption due to algal dark respiration caused by the mutual shading.

In our study, for the highest initial microalgae concentrations (absorbance > 1), the initial growth rate ranged between 0.1 and 0.3 d^{-1}. This fact supports the hypothesis of the mutual shading; hence the abundant initial microalgae concentration limits the initial growth rate in all cases.

Another factor affecting the initial growth rate is the initial ammonia concentration (NH_3^{in}). As illustrated in Figure 2, the different concentrations of digestate applied in this experiment (from 50 to 260 $mgNH_4^+$-N/L) resulted in initial NH_3 concentrations ranging from 2 to 34 $mgNH_3$/L. When the initial ammonia concentration was increased from 2 to 9 $mgNH_3$/L, the growth rate decreased, on average, by 18%. Besides, the increasing from 9 to 34 $mgNH_3$/L was responsible for 77% reduction of the growth rate. Actually, it is well known that high ammonia concentrations (about 2.3µM) present in anaerobic digester effluents is often responsible of microalgal growth inhibition (Cho et al., 2013). Indeed, although ammonia is an excellent source of nitrogen for microalgal growth, free ammonia is toxic to most strains of microalgae due to its uncoupling effect on photosynthetic processes in isolated chloroplasts (Crofts, 1966). However, in order to control ammonia inhibition, ammonia content may be reduced by diluting digester effluents (e.g. with wastewater).

It should be noted that other compound of digestate listed in Table 1 might have an inhibitory effect on microalgae (i.e. calcium, magnesium, potassium, sodium), however the concentrations of such elements founded here are largely below the inhibition limits found in literature (Chen et al., 2008).

Summarizing, this study demonstrates 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. Moreover, as a certain ammonia inhibition was observed, its concentration should be monitored and eventually reduced by digestate dilution.

3.2 Biomass production

The biomass production was calculated by the difference of TSS_{end} corresponding to the total solids concentration at the end of the experiment and TSS_0 corresponding to the total solids concentration at the beginning of the experiment. The biomass content at the end of the experiment is represented versus the initial microalgal concentration in Figure 4.

Contrary to the initial growth rate, microalgae production was directly proportional to the initial microalgal concentration. In fact, when initial TSS concentration increased from 0.4 to 1.3 g/L, the difference of absorbance increased from 0.05 to 0.37, reaching a final TSS concentration of 0.5 gTSS/L (Figure 5). The effect was even more evident for the highest initial digestate concentrations (260 mgNH_4^+-N/L). In this case, the initial TSS concentrations varying from 1.3 to 1.8 gTSS/L corresponded to an absorbance increment from 0.4 to 1.0, reaching a final TSS concentration of 2.6 gTSS/L. This means that the more microalgae are concentrated at the beginning of the experiment, the more biomass is produced. Significant differences between results were statistically proved by the ANOVA test ($p<0.05$).

The explication to this phenomenon can be found in the pH, ammonia and nitrite patterns (Figure 6). Looking at the pH evolution along the experiment, it can be observed that, from an initial value around 8, in most cases, pH increased at the beginning of the experiment and it remained constant values around 9 or 10. The high pH variation is due to the alkalinity that is certainly proportional to the digestate concentration. In fact, for the lowest digestate concentrations (Figure 6a) the highest pH variability was recorded as a consequence of the scarce buffer capacity.

For the highest initial TSS concentrations (1.3 or 1.8 gTSS/L, depending on the initial digestate concentration) pH increased during the first days and then rapidly decreased to values near 7. A stop of NH_4^+-N consumption and a high NO_2^--N production were

observed in correspondence with the pH decrease. This fact is particularly evident in the case of 185 mgNH_4^+-N/L (Figures 6d, 6e, 6f). Here the pH decrease from 8 to less than 7 corresponds to a nitrite increase from 40 to 140 mgNO_2^--N/L. Nitrate increase was less important in the other cases (from 38 to 70 mgNO_2^--N/L and from 20 to 40 mgNO_2^--N/L), in correspondence with minor pH decrease (pH>7).

In such cases, neutral pH values were reached as a consequence of the nitrification process and the carbon dioxide production. Similar pattern was already observed by Gonzalez-Fernandez et al. (2011). These authors found that, when anaerobic digestate was tested as substrate for microalgae growth, pH was around 7-8 and nitrification process tooks place. In our case, the high microalgae concentration since the beginning of the experiment produced large quantity of oxygen stimulating ammonium oxidation by nitrifiers, which enhanced nitrite and nitrate production (Figure 6). Ammonium nitrification is indeed a common process taking place when high dissolved oxygen in present in the medium (Gonzalez-Fernandez et al., 2011).

As a consequence of the aerobic bacterial oxidation, CO_2 and ammonia were produced, responding to the microalgal photosynthesis requirements (Oswald and Gootas, 1957). Microalgae growth was thus enhanced by synthetizing the organic matter from carbon dioxide and ammonia produced by bacteria.

In our case, nitrogen and phosphorus were not the limiting factors. On the other hand, the pH increase recorded in almost every case suggests an inorganic carbon limitation due to the algal uptake of CO_2. The scarce carbon dioxide or inorganic carbon availability was already highlighted as a limiting factor to intensive algal culture by Talbot et al. (1991). Thus, in the case where higher microalgae biomass was present from the beginning of the

experiment; inorganic carbon was brought through microalgae respiration and bacteria activity and let to pH stabilization around 7.

This fact can support the hypothesis that microalgae growth could have been enhanced by synthetizing the organic matter from carbon dioxide and ammonia produced by bacteria. In fact, CO_2 and ammonia were produced as a consequence of the aerobic bacterial oxidation of the organic matter, responding to the microalgal photosynthesis requirements (Oswald and Gootas, 1957). However, it should be taken into account that results were obtained indoor during a relatively short laboratory experiment. More studies are required to confirm our findings and to transpose results to a full scale system.

The positive effect of pH regulation by means of CO_2 addition to microalgal culture was previously highlighted in several studies (Heubeck et al. 2007; Park and Craggs 2010; Park and Craggs 2011). These authors noted an increase in microalgal production due to the augmentation of daytime CO_2 availability. In our case, a kind of self pH regulation was taking place as a consequence of the high bacteria activity producing CO_2.

According to our results, the increase of the initial microalgal concentration increased oxygen availability, which stimulated bacteria activity. Bacteria activity supplied carbon to the culture and thus improved microalgae production.

4. Conclusions

This work assessed microalgal growth by utilizing anaerobic digestate effluent as substrate.

Digestate may be an effective substrate for microalgal growth with initial growth rate up to 0.9 d^{-1} and biomass production up to 2.6 gTSS/L.

Microalgal growth rate was negatively affected by a self-shading phenomenon depending on the microalgal substrate (ammonia) concentration.

On the contrary, microalgal biomass production was positively correlated with the inoculum and substrate concentrations.

Summarizing, the increasing of both digestate and microalgal initial concentration may reduce the initial growth rate (from 0.9 to 0.04 d^{-1}) but significantly enhances biomass production (from 0.1 to 2.6 gTSS/L).

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Tables and figures

Table 1. Physico-chemical characteristics of the microalgal/bacterial inoculum and the liquid phase of the anaerobic digester effluent from Castres' facility.

Parameter	Inoculum (microalgal/bacterial biomass)	Substrate (liquid phase of anaerobic digestate)
TSS (g/L)	2.00	1.13
Total COD (mg/L)	910	210
NH_4^+-N(mg/L)	30	950
PO_4^{3-}-P (mg/L)	17	415
Cl^- (mg/L)	280	160
SO_4^{2-} (mg/L)	140	43
Na^+ (mg/L)	52	126
K^+ (mg/L)	220	240
Mg^{++} (mg/L)	24	3
Ca^{++} (mg/L)	80	65

Table 2. Experimental growth rate values at laboratory scale found in literature and in this study.

Growth rate (d^{-1})	**Experimental volume (L)**	**Substrate**	**Dominant specie**	**Reference**
0.5-1	1	Secondary wastewater	*Scenedesmus obliquus*	Martinez et al., 2000
1.6-1.7	N.D.	Mineral medium	*Chlorophyceaen* sp.	Bouterfas et al., 2002
0.3-0.5	9	Settled sewage enriched with nutrients	N.D.	Kayombo et al., 2009
0.1-0.9	1	Synthetic wastewater	*Chlorella vulgaris*	Perez-Garcia et al., 2010
0.4-0.9	N.D.	Raw, primary, secondary wastewater and liquid phase of centrifuged sludge	*Chlorella* sp.	Wang et al., 2010
0.4	3	Synthetic wastewater	*Scenedesmus obliquus* and *Chlorella vulgaris*	Ruiz-Marin et al., 2010
0.2	0.5	Secondary wastewater	*Scenedesmus* sp.	Xin et al., 2010
0.5-0.7	0.1	Liquid phase of thickened activated sludge	Freshwater microalgal mixture	Li et al., 2011
0.04-0.9	0.5	Liquid phase of anaerobic digestate	Freshwater microalgal mixture dominated by *Scenedesmus* sp.	This study

386 N.D.: Not Defined

387

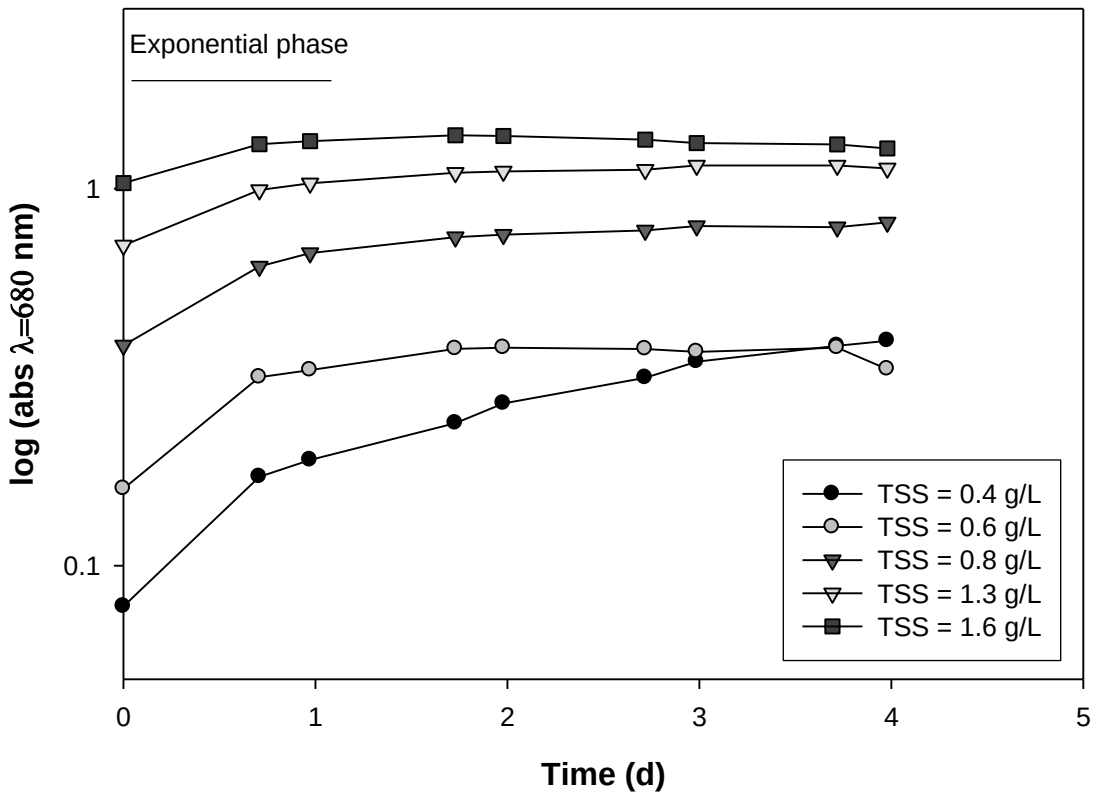


Figure 1. Growth rate curves of microalgae with digestate substrate (50 mgNH_4^+-N/l) for different values of initial total suspended solids (TSS).

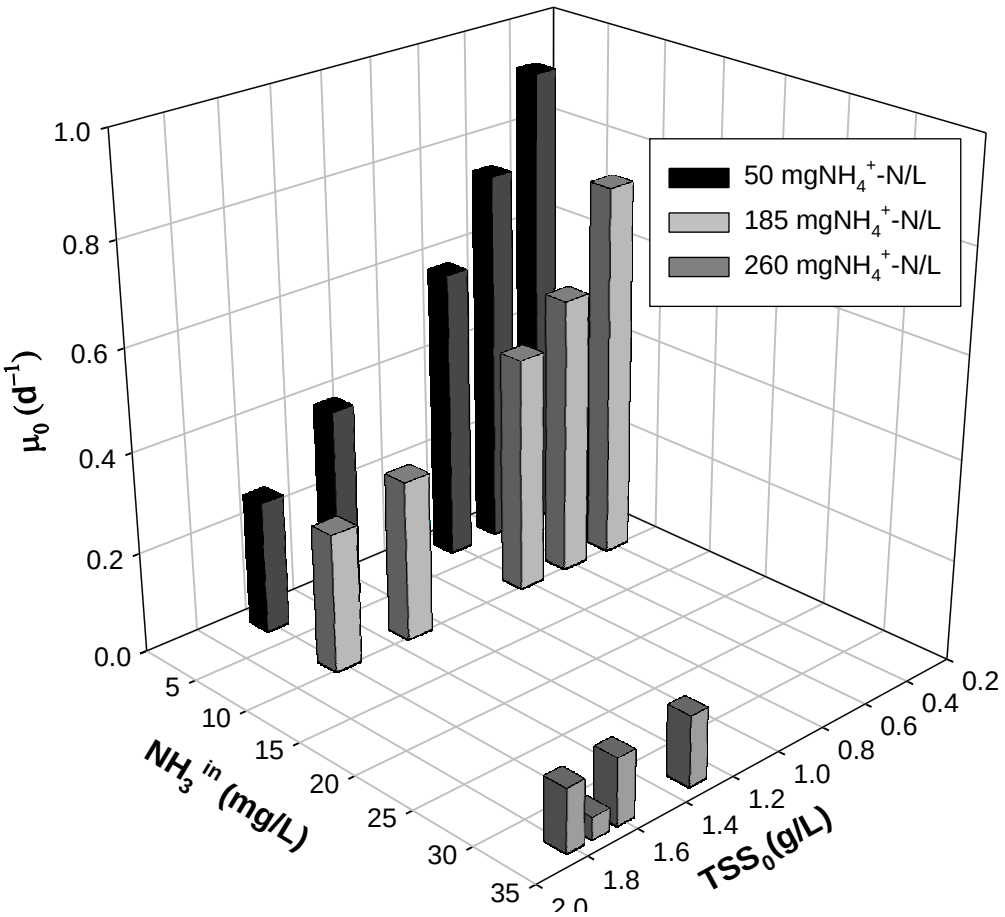


Figure 2. Initial growth rate (μ_0) versus initial microalgal concentration (TSS_0) and initial ammonia concentration (NH_3^{in}) for each initial substrate concentration ($mgNH_4^+$-N/l).

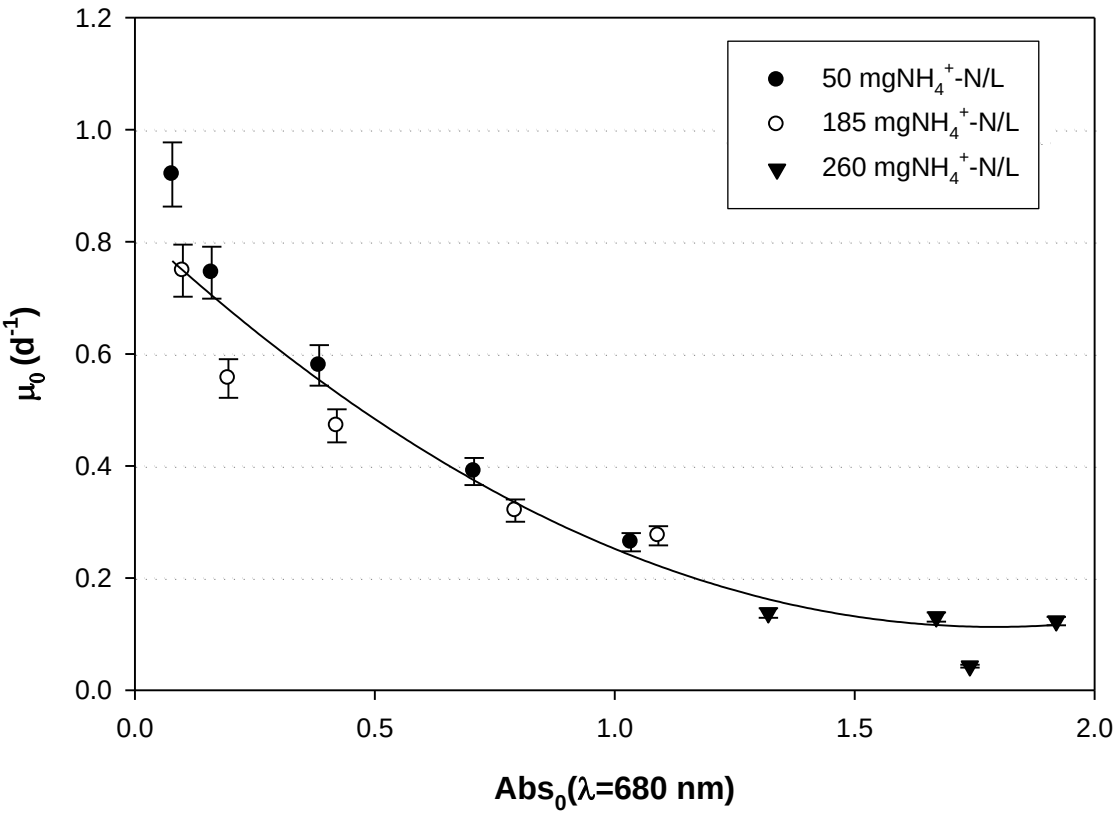


Figure 3. Correlation between growth rate (μ_0) and the initial absorbance (λ= 680 nm).

The standard deviation (±6.22%) was obtained in a previous study performed with 4 replicates.

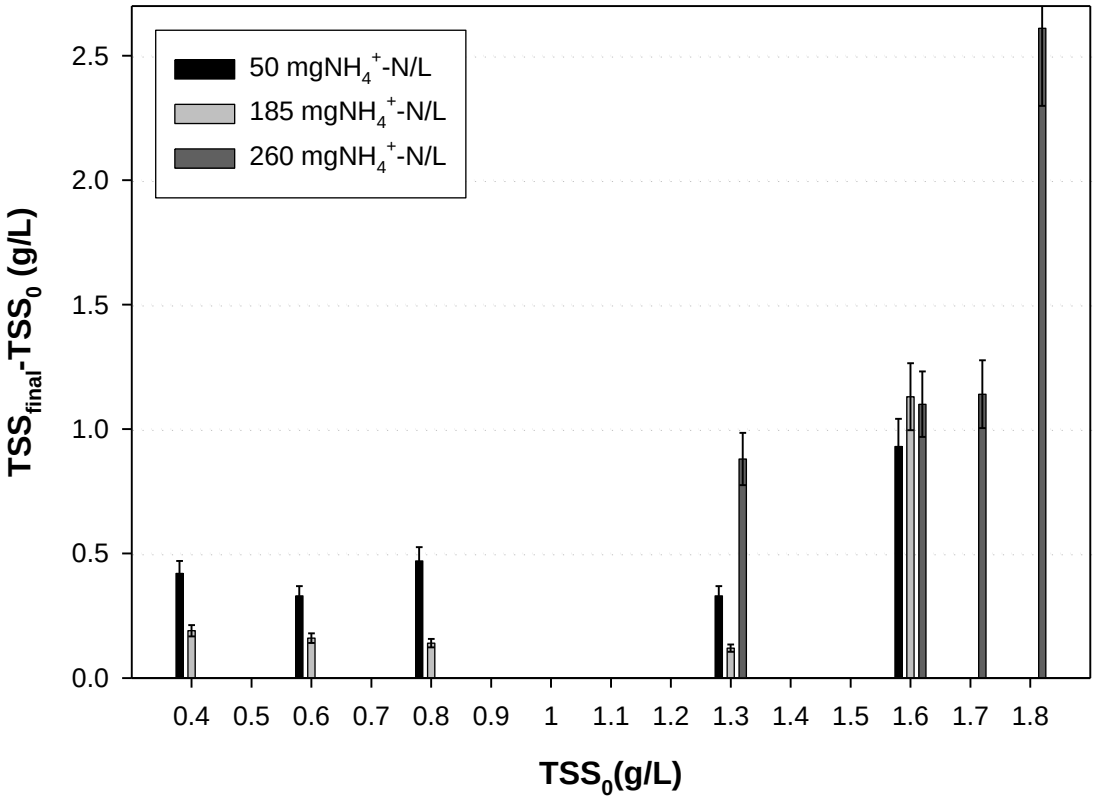
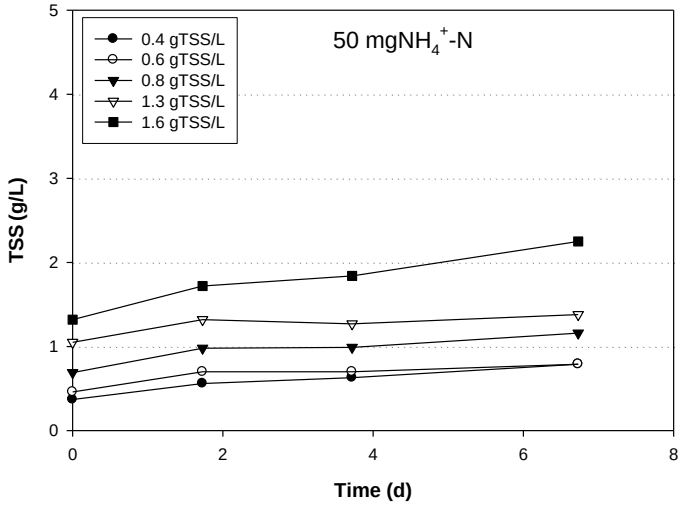
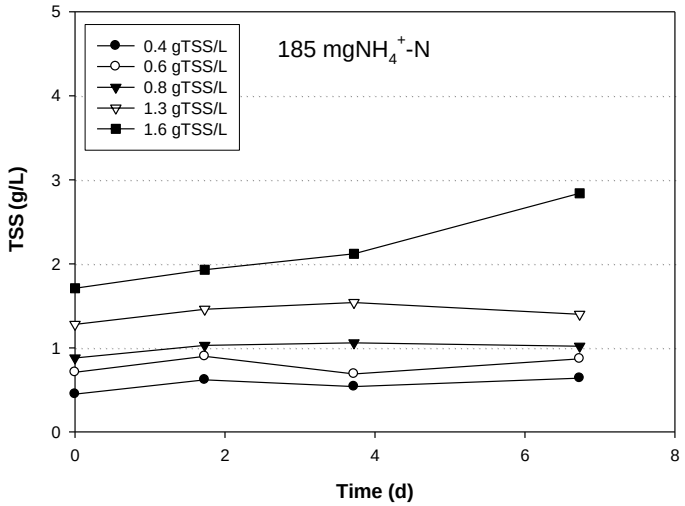


Figure 4. Microalgae biomass production (calculated as difference of total solids between the end and the beginning of the experiment) versus initial microalgal concentration (TSS_0). The standard deviation (±11.93%) was obtained in a previous study performed with 4 replicates.


0.4 gTSS/L
0.6 gTSS/L
0.8 gTSS/L
1.3 gTSS/L
1.6 gTSS/L
50 mgNH4+-N
TSS (g/L)
Time (d)


0.4 gTSS/L
0.6 gTSS/L
0.8 gTSS/L
1.3 gTSS/L
1.6 gTSS/L
185 mgNH4+-N
TSS (g/L)
Time (d)

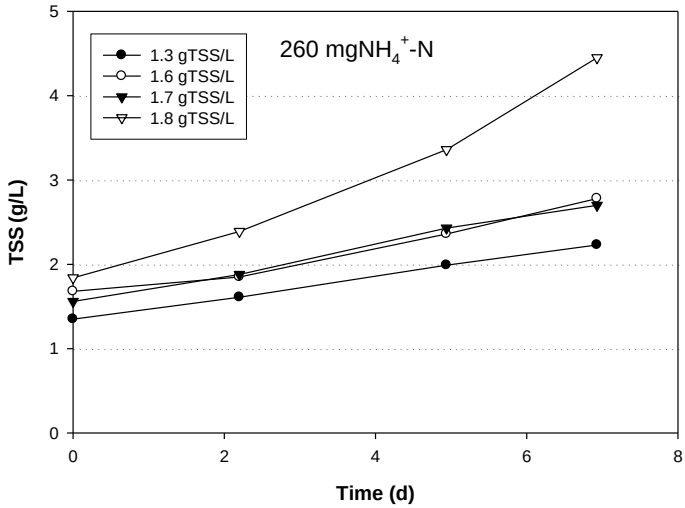
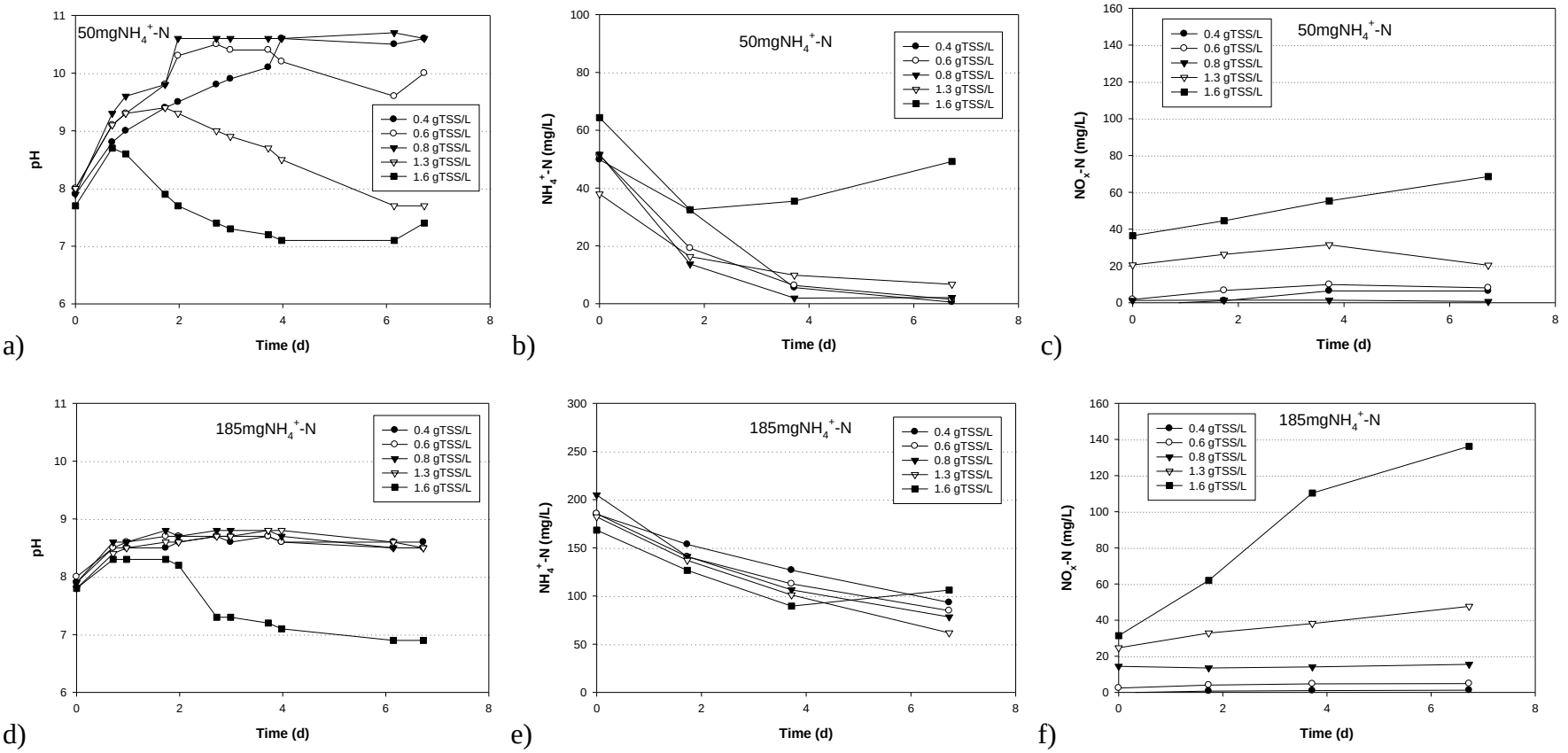
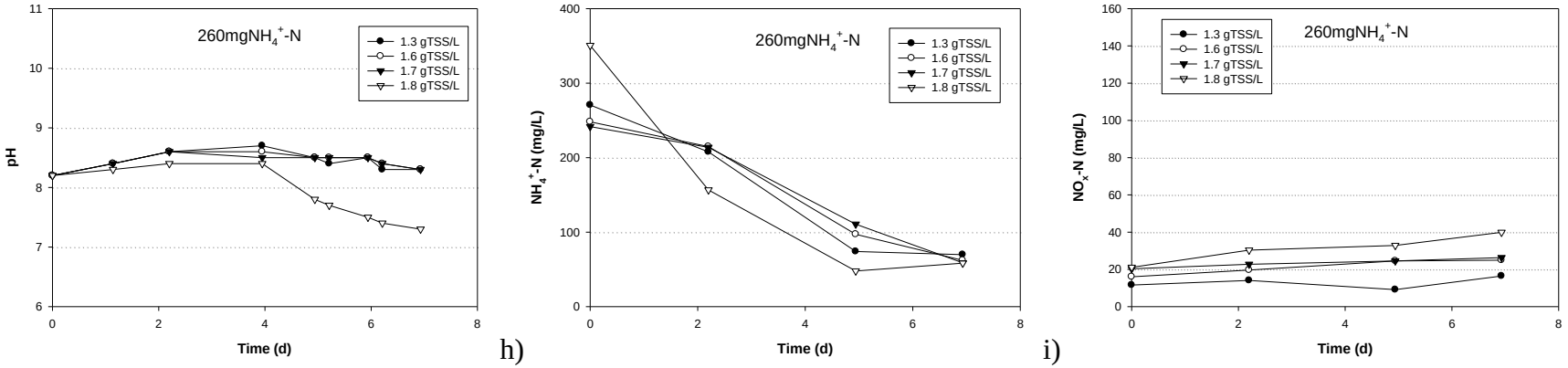

1.3 gTSS/L
1.6 gTSS/L
1.7 gTSS/L
1.8 gTSS/L
260 mgNH4+-N
TSS (g/L)
Time (d)

Figure 5. Microalgal concentration (TSS) along each experiment. The 3 graphs correspond to 3 initial digestate concentrations (50, 185 and 260 mgNH_4^+-N). Each graph shows the TSS evolution along the time for different initial microalgal (gTSS/L) concentrations.


50mgNH4+-N
pH
Time (d)
0.4 gTSS/L
0.6 gTSS/L
0.8 gTSS/L
1.3 gTSS/L
1.6 gTSS/L
a)
50mgNH4+-N
NH4+-N (mg/L)
Time (d)
b)
50mgNH4+-N
NOx-N (mg/L)
Time (d)
c)
185mgNH4+-N
pH
Time (d)
d)
185mgNH4+-N
NH4+-N (mg/L)
Time (d)
e)
185mgNH4+-N
NOx-N (mg/L)
Time (d)
f)



g) h) i)

Figure 6. pH values, ammonium and nitrite concentration along each experiment for different initial microalgal (gTSS/L) concentrations.

The graphs correspond to 3 initial digestate concentrations (50, 185 and 260 mgNH_4^+-N).