Effect of Macronutrients and of Anaerobic Digestrate on The Heterotrophic Cultivation of Chlorella Vulgaris Grown With Biodiesel By-Products

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Abstract

The aim of this work was to investigate the kinetics of the heterotrophic growth of *Chlorella vulgaris* as a means of producing bio-oil for biodiesel production. Glycerol was used as the sole organic carbon substrate. Anaerobic digestrate from a local plant was used to examine its effect on the kinetics and the protein and lipid content of the biomass. The effect of the initial carbon and nitrogen concentrations on the carbon uptake rate was studied independently. In one set of five experiments the organic carbon in the form of glycerol, varied from 0.27 g L^{-1} to 5.36 g L^{-1} while the concentration of atomic nitrogen was held constant and equal to 45.4 mg L^{-1}. The Co/No ratio varied from 6 to 118.1. In a second set, also of five experiments, the organic carbon was held constant and equal to 3.3 g L^{-1} and atomic nitrogen varied from 22.7 mg L^{-1} to 450 mg L^{-1}. The Co/No ratio varied from 7.3 to 145.4. In a third set of experiments anaerobic digestrate was added in increasing amounts into the culture media from 4% to 16%. It was found that the carbon uptake rate as well as the lipid and protein content depended on the Co/No ratio. Increasing ratios of Co/No lead to higher carbon uptake rates, higher lipid content and lower protein content. The initial nitrogen concentration was also found to affect the growth rate of *C. vulgaris.* The addition of anaerobic digestrate did not affect appreciably the protein and lipid content of the biomass while, addition of anaerobic digestrate up to 16% in the culture medium increased the carbon uptake rate up to about 24%.

1. Introduction

Microalgae can grow and produce biomass either autotrophically by using light and carbon dioxide, like plants or heterotrophically in the absence of light if an organic carbon source becomes available (Mata et al. 2010; Chojnacka and Marquez-Rocha 2004). Different sources of carbon can be used for microalgae growth, such as glucose, sucrose, fructose, lactose or galactose (Perez-Garcia et al. 2011).

The heterotrophic growth of microalgae offers some distinct advantages over the autotrophic cultivation such as faster growth rates, higher biomass and lipid productivities and no limitations imposed by sufficient light penetration into the growth media (Huang et al. 2010). Additionally, a better control over the process parameters like temperature, pH, oxygen levels and carbon source can be achieved. Disadvantage of the heterotrophic growth is the potential of contamination (Xiong et al. 2008), as well as the higher cost for equipment and installation.

Anaerobic digestrate (AD) is mainly used for field fertilization in various crops such as corn. When there are no fields in adequate hectares of cultivation land or the land is not flat nitrification of underground water and rivers or lakes may result as a result of excess biofertilizer being applied or because of run off. In these cases, biological treatment is required in order to reduce the organic carbon and nitrogen in the anaerobic digestrate. Nitrogen, in the form of ammonium ions, can be reduced by a combination of aerobic and anaerobic treatment. However, this procedure is an expensive preposition, and in most cases excess anaerobic digestrate is being applied to cultivation fields in excessive amounts, which leads to nitrification and other potential negative effects on underground and surface water as well as on the soil chemistry and aquatic life. Therefore, developing new applications for the efficient use of anaerobic digestrate is needed to utilize the macro and micro nutrient content of the anaerobic in the framework of circular economy. A good solution is the use of anaerobic digestrate for microalgae cultivation due to the fact that it is a good source of nitrogen, phosphorus and potassium, as well as a source of different micronutrients, such as cobalt, molybdenum and iron that are necessary for microalgae growth. Potassium and phosphorus availability is projected to decrease in the future.

It should be mentioned that different parameters affect the microalgae growth and biomass production, such as pH, temperature, aeration, as well as the composition of the culture medium (concentration of nitrogen, potassium, phosphorous and of micronutrients) (Dean et al. 2010). What is more, the composition of biomass produced by microalgae is rich in proteins, lipids and antioxidants, such as carotenoids and phenolic compounds. Depending on cultivation practice applied, microalgae can accumulate high levels of lipids or proteins or carbohydrates (Metsoviti et al. 2019).

Microalgae have different applications due to the high nutritional value of their biomass. The high lipid production of microalgae can be used in the fuel industry for the production of biodiesel, bioethanol and biomethane (Aramrueang et al. 2016). Antioxidants derived from microalgae can be used in cosmetic and pharmaceutical industry (Spolaore et al. 2006). The high protein production can be used in aquaculture for the replacement of fishmeal in fish feeds (Patil et al. 2005).

Most studies have focused on the heterotrophic cultivation of microalgae with the use of carbon sources, such as glucose, fructose, mannose, galactose, acetate and various industrial effluents and by-products. A number of review papers focus on the heterotrophic growth of several microalgal species and the trend is that heterotrophic growth enhances both the biomass and lipid productivity (Morales-Sanchez et al. 2015; Bumbak et al. 2011). Work on the cultivation of microalgae using glycerol is limited.

It should be noted that some species, such as *Prymnesium parvum* and *Dunaliella tertiolecta* are unable to grow when cultivated with glucose (Neilson and Lewin 1974). On the other hand, Kong et al. (2013) found that the growth rate of *C. vulgaris* as well as the biomass production of the species was enhanced when cultivated with a mixture of glycerol and glucose. The growth rate of heterotrophic cultivation of *Euglena*

gracilis was enhanced when cultivated with glucose in comparison with cultivation with ethanol (Ogbonna et al. 1998). Not only the source of carbon, but also the ratio of C/N plays a significant role in growth rate and lipid production of microalgae. The lipid content of *C. sorokiniana* increased at high C/N ratios (Chen and Johns, 1991), while the proportion of unsaturated fatty acids increased at low C/N ratios in the same species.

The present study was conducted in order to investigate the heterotrophic growth of *C. vulgaris* as a way of producing bio-oil for the production of biodiesel. In the light of sustainable biofuel production, low value industrial by-products such as glycerol from the biodiesel production were used as the sole organic carbon substrate. Also, in separate experiments, anaerobic digestrate from a local plant was used in the formulation of the growth medium in order to examine its effect on the kinetics and lipid productivity and utilize its valuable nutrients. Three sets of experiments were conducted. Each set was comprised of five experiments in which: a) the initial carbon concentration was varied while the initial nitrogen concentration was held constant (1st set), b) the initial carbon concentration was held constant while the initial nitrogen concentration was varied (2nd set) and c) both the initial carbon (from glycerol) and initial nitrogen concentrations were held constant and a varying amount of anaerobic digestrate (AD) (0%-control, 4%, 8%, 12% and 16%) was added to the culture medium. In terms of cyclic economy, the digestrate was used to examine its effect on the growth rates and compare the growth kinetics, biomass and lipid productivities with the respective ones when inorganic macro and micro-nutrients were used. The effect of the initial carbon and nitrogen concentrations as well as the percent AD used on the carbon uptake rate ($\Delta C/\Delta t$) in the exponential growth phase was also studied and compared. The specific growth rate coefficient μ_{exp} in the exponential growth phase was calculated and compared with the corresponding one, for the same strain, during the autotrophic growth cultivation.

2. Materials And Methods

2.1 Inoculum preparation

The microalgae *C. vulgaris* (211-11b) was obtained from the University of Goettingen in Germany (EPSAG). It was cultivated in Basal Medium (= ES "Erddekokt + Salze"). Each liter of the culture medium contained: 0.2 g KNO_3/L, 0.02 g K_2HPO_4/L, 0.02 g $MgSO_4.7H_2O$/L, 30 mL of soil extract/L and 5 ml/L, of a solution containing the following micronutrients: (1 mg $ZnSO_4.7H_2O$, 2 mg $MnSO_4.4H_2O$, 10 mg H_3BO_3, 1 mg $Co(NO_3)_2.6H_2O$, 1 mg $MoO_4.2H_2O$, 0.005 mg $CuSO_4.5H_2O$, 700 mg $FeSO_4.7H_2O$ and 800 mg EDTA)/L (SAG, 2007).

The culture medium was inoculated with a standard quantity (50 mL of *C. vulgaris* inoculum) which was prepared as follows: 1 L flask, containing the necessary culture medium, was inoculated with *C. vulgaris* culture directly obtained from EPSAG and cultivated in a sterile environment until it reached an absorbance reading of 0.5. The cultivation of the inoculum was done always under the same conditions namely, at a temperature of 25 °C, under natural illumination and by using an orbital shaker at 60 rpm in order to prevent sticking of algae to the surfaces of the flask.

2.2 Experiments

Three sets, each of five simultaneous experiments, were carried out. Each set was repeated three times. In the first set of experiments the effect of the initial carbon was studied independently while holding the initial nitrogen concentration constant. Organic carbon in the form of glycerol, with an 85% content in glycerine, varied from 0.27 g L^{-1} to 5.36 g L^{-1} while the concentration of atomic nitrogen was held constant and equal to 45.4 mg L^{-1}. Co/No ratios varied from 6 to 118.1. In the second set experiments (also of five experiments), the organic carbon was held constant and equal to 3.3 g L^{-1} and atomic nitrogen varied from 22.7 mg L^{-1} to 450 mg L^{-1}. The Co/No ratio varied from 7.3 to 145.4. In a third set of experiments anaerobic digestrate was added in increasing amounts into the culture media. The amount of organic carbon from glycerol was held constant and equal and atomic nitrogen wad also held constant and equal to 129.9 mg L^{-1}. The amount of anaerobic digestrate (AD, as % v/v) added to the five growth media was 0% (control), 4%, 8%, 12% and 16%. A small amount of organic carbon from the undigested organic material of the AD was not corrected. From separate experiments, it was determined that its rate of uptake is similar that that of organic carbon from glycerol and that about 75% of it is absorbed and utilized by the microalgae. This contribution from the undigested organic carbon shows up in the initial organic carbon concentration and, as the AD percentage increases from 0–16% it increases the Co/No rate by about 15%. The nitrogen contribution, in the form of ammonium nitrogen, from the AD was corrected in all five growth media. Table 1 shows all the relevant initial parameters of each of the three sets of experiments.

Table 1
The initial parameters of each set of experiments (initial carbon and nitrogen concentration).

EXPERIMENT	PARAMETER														
	Co (g L^{-1})					No (mg L^{-1})					Co/No				
	Bioreactor no.					Bioreactor no.					Bioreactor no.				
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	5th
Variable Co	0.27	0.59	1.15	2.79	5.36	45.4	45.4	45.4	45.4	45.4	6	13	25.3	61.5	118.1
Variable No	3.3	3.3	3.3	3.3	3.3	22.7	45.4	114	227	450	145.4	72.5	28.9	14.5	7.3
Variable AD	2.91	3.11	3.40	3.50	3.69	129.9	129.9	129.9	129.9	129.9	22.4	23.9	26.2	26.9	28.4

2.3 Raw materials

Crude glycerol was obtained from a local biodiesel manufacturing plant. Its composition was approximately 86% glycerine, 0.5% methanol, 4% free fatty acids and 7.5% H_2O. The carbon content was calculated according to this analysis so that the approximate C/N ratios could be estimated. The exact carbon content and the exact C/N ratios were determined by analytical measurement. Specifically, the method of Walkley-Black was used for the determination of organic carbon. The samples were first centrifuged and then filtered. According to this method organic carbon was oxidized by a mixture of $K_2Cr_2O_7$ and H_2SO_4 in a ratio of 1:2. The remaining $K_2Cr_2O_7$ was titrated with 0.5N $FeSO_4$ $7H_2O$.

The anaerobic digestrate was obtained from a local biogas plant. It was prepared by centrifugation at 4000 rpm for 10 minutes and subsequent heat treatment at 105 ^oC for 3 hours. After the heat treatment, analysis was performed for its macronutrient and major micronutrient content. The nitrogen content of the sterilized AD was 211 mg L^{-1}, all as NH_4^+.

2.4 Methods of analyses

The nutrient composition of the samples was determined according to AOAC (1995) methods. Organic carbon was analysed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$. Total nitrogen content in samples was measured with digestion using the Kjeldhal method. Digestion is accomplished by boiling of the samples in concentrated sulfuric acid with catalyst potassium sulphate, copper sulphate and selenium. After cooling, the digestion product was distilled with NaOH in a solution 2% H_3BO_3 in presence of indicator methyl red and was titrated with 0.1 N HCl. Total protein content of samples was calculated using a conversion factor of 6.25 (Biancarosa et al. 2017). The ammonium nitrogen content was measured by distillation in the presence of MgO and collection of the product in a solution 2% H_3BO_3 in presence of indicator methyl red, and subsequently titration of the product with 0.1 N HCl. The total lipid content was determined with extraction using co-solvents of n-hexane/isopropanol in the microalgal biomass according with the method of Bian et al. (2018). The solvent ratio of n-hexane to isopropanol was 3/2 (V/V), the ratio of co-solvents to microalgal dry biomass was 10/1 (V/W). Extraction was carried out in horizontal-circular movement "tehtnica Zelezniki EV-402" machine. Stirring speed was 300 rpm, and the extraction time was 48 hours. The total lipid extract was determined gravimetrically after filtration and evaporation of the solvents.

2.5 Bioreactors

The cultivations were carried out in glass cylindrical bioreactors each of 5L capacity that were filled up to 4.5L. Air was continuously passed through the solution at 300 L hr^{-1} through 2 mm glass tubing positioned at the tip of a magnetic bar and the air bubbles were dispersed with the magnetic bar at the bottom of the glass flasks at a rotational speed of 500 rpm. The bioreactors, the glass tubing and the culture medium were sterilized before use.

2.6 Measurements and Determination of kinetics

The organic carbon reduction kinetics (as rate of Carbon uptake) was determined from the slope of the experimental data of C(t) vs t, by plotting the C(t) vs t data and obtaining the average slope $\Delta C(t)/\Delta t$.

The specific growth rates in the exponential growth phase (μ_{exp}) were calculated from the equation:

$$\mu_{exp} = \frac{\ln \frac{a_2}{a_1}}{t_2 - t_1} \quad (1)$$

where, α_1 and α_2 are the absorbances at the beginning and the end of exponential growth phase at time t_1 and t_2, respectively.

2.7 Statistical Analysis

Comparison of means was performed by subjecting the data to one-way analysis of variance at a significance level of 0.05 using the IBM SPSS Statistics 24 statistical package. The significant differences between treatments were determined using Tukey's multiple comparison test.

3. Results And Discussion

Figure 1 shows the reduction in dissolved organic carbon with respect to cultivation time for five different initial carbon concentrations from 0.27 g L^{-1} up to 5.36 g L^{-1}. The initial nitrogen concentration is constant in all five runs and equal to 45.4 mg L^{-1}. The corresponding Co/No ratio varied from 6 up to 118.1. All carbon is utilized from 5 to 16 days. Figure 2 shows the reduction in dissolved organic carbon with respect to cultivation time for five different initial nitrogen concentrations from 22.7 mg L^{-1} up to 450 mg L^{-1}. The initial carbon concentration is constant in all five runs and equal to 3.3 g L^{-1}. The corresponding Co/No ratio varied from 7.3 up to 145.4. Low initial nitrogen concentrations limit the rate of carbon utilization (uptake) as nitrogen is needed in the exponential growth phase of the microalgae cells. At higher initial nitrogen concentrations carbon is fully utilized after about 8–9 days.

Figure 3 shows the rate of carbon utilization (uptake) as a function of the ratio Co/No for the above two cases where No is held constant and Co is varied and vice versa. The rate of carbon uptake varies by almost a factor 6, between 0.05 and 0.31 g/(L-d) as the Co/No ratio is increased from 6 to 118.1. When Co is varied, excess nitrogen and low carbon concentrations lead to low rates of carbon uptake but as the carbon concentration is increased and for a ratio Co/No above about 25 the rate of carbon uptake becomes constant. This is probably due to the fact that in the exponential growth phase the microalgal cells utilize both carbon and nitrogen for their growth so, at higher Co/No ratios the rate of carbon uptake is independent of the carbon concentration as nitrogen becomes the limiting nutrient during the exponential growth phase.

On the other hand, when initial nitrogen concentration is varied and carbon is held constant (upper curve), at low Co/No ratios, with an initial Co = 3300 mg L^{-1} and relatively high nitrogen concentrations, the rate of carbon uptake initially slightly increases from 0.48 to about 0.60 g/(L^{-d}). This is probably due to the inhibition of nitrogen at this relatively high nitrogen concentration, reported by Metsoviti et al. (2019) during the autotrophic growth of *C. vulgaris*. At the very high Co/No ratio and at No = 22.7 mg L^{-1}, nitrogen is again the limiting nutrient and as it is needed for cell growth, the rate of carbon uptake is low.

Figure 4 shows the culture media absorption versus the cultivation time. The biomass growth rate is slow at low initial nitrogen concentrations. Initial nitrogen concentrations equal to or higher than 45.5 mg L^{-1} appear to lead to similar growth rates in the exponential growth phase. At No = 45.5 mg L^{-1} the stationary phase is quickly reached apparently because nitrogen at this relatively low concentration is quickly utilized for biomass growth. Carbon is utilized both for biomass growth and lipid accumulation. At initial nitrogen concentration higher than 45.5 mg L^{-1} the stationary phase is reached later as absorption could not be measured accurately above about a value of 1.8.

From the data of Fig. 4 and Eq. 3 the specific growth coefficient in the exponential growth phase μ_{exp} is obtained and is shown in Fig. 5 versus the initial nitrogen concentration. Although the Co/No ratio varies from 7.3 to 145.4, the specific growth coefficient (μ_{exp}) is relatively independent of the initial nitrogen concentration above No = 45.5 mg L^{-1}, i.e. above a ratio Co/No of 72.

Table 2 shows the protein and lipid content of the biomass (on a dry basis). It is noted that as the Co/No ratio increases the protein content decreases and the lipid content increases. The same trend with respect to increasing the initial nitrogen concentration was found for autotrophic growth (Metsoviti et al. 2019). Carbon is utilized both for protein and lipid synthesis while nitrogen is used mainly for growth. Therefore, protein synthesis is limited by the availability of nitrogen while excess carbon promotes lipid synthesis.

Table 2
Lipid (C_l) and protein (C_p) content of the biomass of *Chlorella vulgaris* for initial Carbon and nitrogen concentrations as shown

Co (g/L)	3.30	3.30	3.30	3.30	3.30
No (mg/L)	450	227	114	45.5	22.7
Co/No	7.3	14.5	28.9	72.5	145.4
C_p (%)	63.3 ± 0.7^a	43.7 ± 0.5^b	36.6 ± 0.8^c	31.7 ± 0.7^d	22.8 ± 0.6^e
C_l (%)	9.9 ± 0.5^e	16.1 ± 0.4^d	29.2 ± 0.7^c	34.9 ± 0.4^b	46.7 ± 0.8^a

It is also interesting to compare biomass productivities with the respective ones for autotrophic growth. In these experiments and for initial nitrogen (No) concentrations from 450 to 22.7 mg L^{-1} the biomass productivities range from about 0.30 to about 0.16 g/(L-d). In the

autotrophic growth of *C. vulgaris*, the biomass productivities are 0.022 g $L^{-1}d^{-1}$ for cultivation during June and 0.018 g $L^{-1}d^{-1}$ during September for initial nitrogen concentration equal to 122.6 mg L^{-1} (Metsoviti et al. 2020). In the same microalgae species and again in autotrophic growth as initial nitrogen concentration was varied, the biomass productivities were equal to 0.017, 0.021 and 0.029 g $L^{-1}d^{-1}$ for initial nitrogen concentrations equal to 61.3, 122.6 and 300 mg L^{-1} respectively (Metsoviti et al. 2019). Thus, biomass productivities in the heterotrophic growth, for the same species and strain and for similar initial nitrogen concentration is about 15 times higher than the autotrophic growth.

It is also interesting to compare the specific growth rate coefficient obtained from this study of the heterotrophic growth of *C. vulgaris* with typical values obtained for the same strain which was cultivated autotrophically in full sun in a greenhouse environment during the June and September months respectively. Metsoviti et al. (2020), report μ_{exp} values of 0.33 d^{-1} and 0.29 d^{-1} for the autotrophic growth during June and September respectively. From Fig. 5 the specific growth coefficient in the exponential growth phase μ_{exp} obtained from this study for heterotrophic growth is about 1.2 d^{-1}, substantially higher than the autotrophic cultivation. Another substantial difference between autotrophic and heterotrophic growth is observed in the lag phase. The lag phase in heterotrophic growth is less than 1 day (Figs. 1, 2 and 4) while, the corresponding value for autotrophic growth range from 3 to 5 days (Metsoviti et al. 2020), substantially higher compared to the corresponding ones of heterotrophic growth.

Figure 6 shows the reduction in dissolved organic carbon with respect to cultivation time for the case where Anaerobic Digestrate (AD) was added to the culture medium at percentages 0, 4, 8, 12 and 16%. The initial concentration of nitrogen was the same and equal to 129.9 mg L^{-1} and the amount of glycerine added was the same as well and equal to 25 mL L^{-1}. Small differences in the initial carbon concentration are due to undigested or partly digested organic carbon present in the anaerobic digestrate.

It is noted that the addition of AD to the growth media has an effect on the average carbon uptake rate. Only a small addition of AD of 4% is needed to substantially increase the average rate of carbon uptake by about 14%. Higher AD concentrations also affect carbon rate uptake. This is evident from Fig. 7 where the average carbon uptake rate in g/(L-d) is depicted versus the % AD added to the culture medium. The carbon uptake rate remains unaffected up to 8% AD and it then increases by almost 32% when 16% AD is added compared to a growth medium without any AD. The nitrogen concentration of the AD was taken into account (210 mg L^{-1} as NH_4^+) and the equivalent amount, also in the form of NH_4^+, was reduced from the culture medium for each of the four culture media. Therefore, total initial nitrogen and both nitrate and ammonium nitrogen were kept the same in all five runs while the percentage of AD was varied from 0% to a maximum of 16%. Anaerobic digestrate is a very complex medium. It contains a great number of elements as well as various organic compounds. Micronutrients present in the AD, especially cobalt, molybdenum and iron, may be affecting carbon uptake kinetics and this is presently being investigated. Cobalt and especially molybdenum may be affecting nitrogen uptake by the microalgal cells which utilize these micronutrients for cell growth and therefore directly may be involved in carbon utilization process.

Table 3 shows the *C. vulgaris* biomass content in lipids (C_l) and protein (Cp). However, unlike the strong dependence of the lipid and protein content on the initial carbon and nitrogen concentrations (Co/No, Table 2), the protein and lipid contents of the *C. vulgaris* biomass are not significantly affected by the percentage of anaerobic digestrate added to the culture medium. The mean protein content of the five treatments is 38.4% and the highest and lowest values deviate by 2.6% and 3.5% from the mean value respectively. Also, the mean value of the lipid content of the five treatments is 27.2% and the highest and lowest values for the lipid content deviate by 1.7% and 1.8% from the mean value respectively. Therefore, the dependence of the protein content or the lipid content does not show a trend with respect to percentage digestrate in the growth medium.

Table 3
Effect of anaerobic digestrate on the lipid (C_l) and protein (C_p) content of the biomass of *C. vulgaris*.

% AD	0	4	8	12	16
Co/No	22.4	23.9	26.2	26.9	28.4
C_p(%)	36.7 ± 1.3[a]	40.2 ± 1.0[a]	34.9 ± 1.3[a]	38.4 ± 0.9[a]	41.7 ± 1.4[a]
C_l(%)	27.1 ± 0.9[a]	26.7 ± 0.8[a]	28.9 ± 1.1[a]	27.7 ± 1.0[a]	25.4 ± 1.2[a]

4. Conclusions

Both initial carbon and nitrogen concentration affect the rate of carbon utilization and the biomass growth rate. Low nitrogen concentration limit both the biomass growth rate as well as the carbon uptake rate. Carbon utilization is limited by nitrogen as well, as both nutrients are needed for biomass growth. Lipid content was found to be proportional to the Co/No ratio while the protein content is inversely proportional to

the Co/No ratio. The lag phase for autotrophic growth is very short, less than 24 hours while the specific growth coefficient is much higher than the corresponding one of the autotrophic growth. Biomass productivities in this study were found to be higher by at least 15 times when compared to those obtained in the autotrophic growth. Anaerobic digestrate was not found to affect substantially the carbon uptake rate up to 8% and an increase of about 32% is noted when 16% is added to the culture medium. The protein and lipid content were not found to be substantially affected by the amount of AD used in the growth media. It should be mentioned that anaerobic digestrate was added to the culture medium only up to 16%. Therefore, future research should be focused on the effect of addition of higher percentages of digestrate on the growth rate and lipid and protein productivities of *C. vulgaris* as well as investigate the use of AD in culture media of other microalgal species and also look into other combinations of bioreactor parameters and culture media formulations. However, it appears from these preliminary experiments that anaerobic digestrate may be of potential use in the formulation of bioreactor culture media thus, presenting an alternative use of AD for the preparation of bioreactor culture media and reducing the use of potassium, phosphorus and micronutrients from natural resources.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions: All authors red and approved the final manuscript and took part in the experiments and in the preparation of the growth media and the digestrate. However, each author contributed more heavily to the following: G. Papapolymerou was a major contributor in writing the manuscript and a general supervisor of all experimental set- up. A. Mpesios, M. N. Metsoviti and M.-E. Gregoriou contributed mostly in the experimental set up of bioreactors, the control of the parameters of the experiments and the processing of the digestrate. D. Kasiteropoulou analyzed and interpreted the data of the experiments and N. Gougoulias, performed all the chemical analyses of the experiments.

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Figures

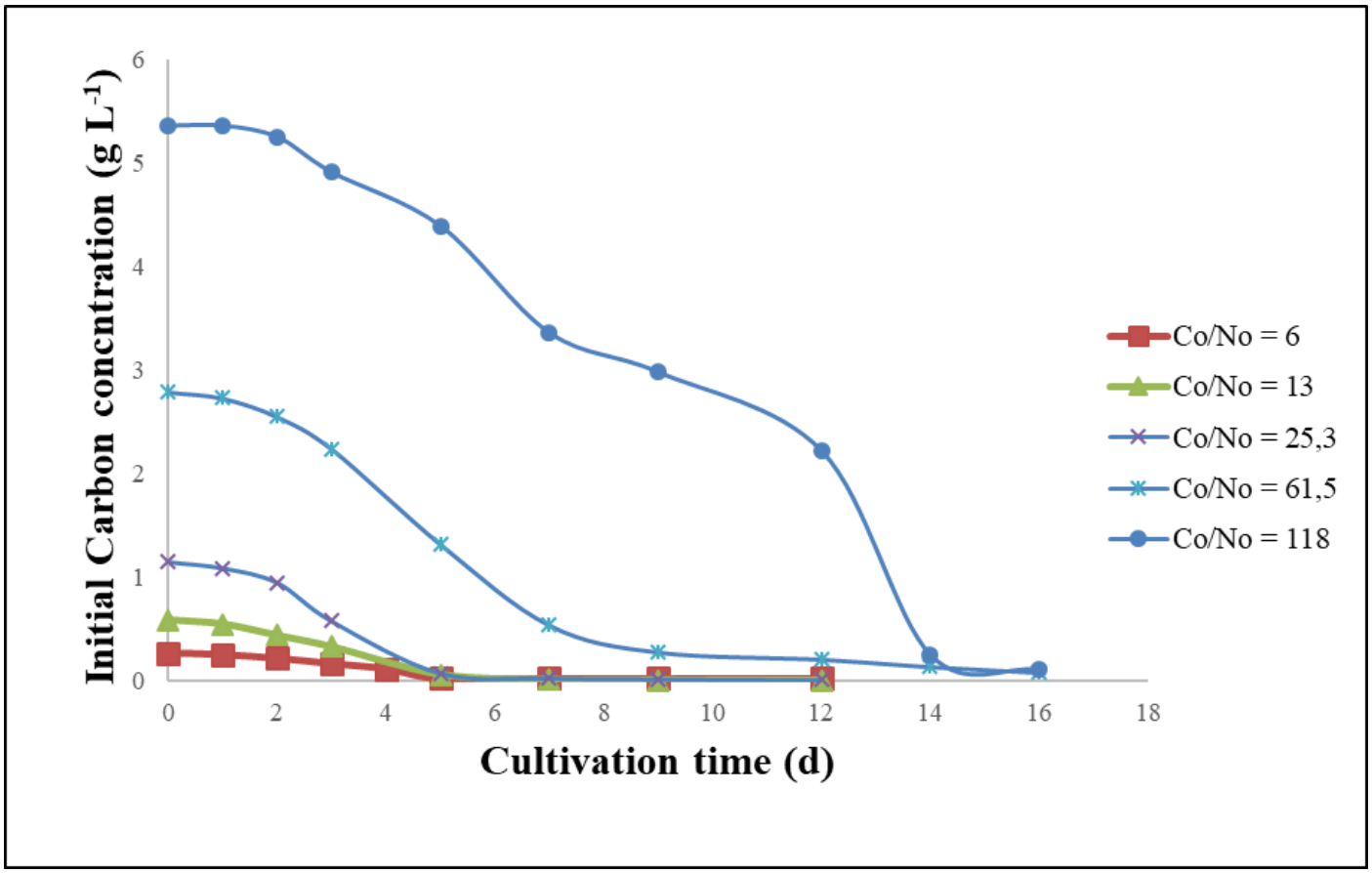

Figure 1

Reduction in organic carbon versus cultivation time for five different Co/No ratios at a constant initial nitrogen concentration (No) equal to 45.4 mg L-1. Curves are drawn between data point for clarification.

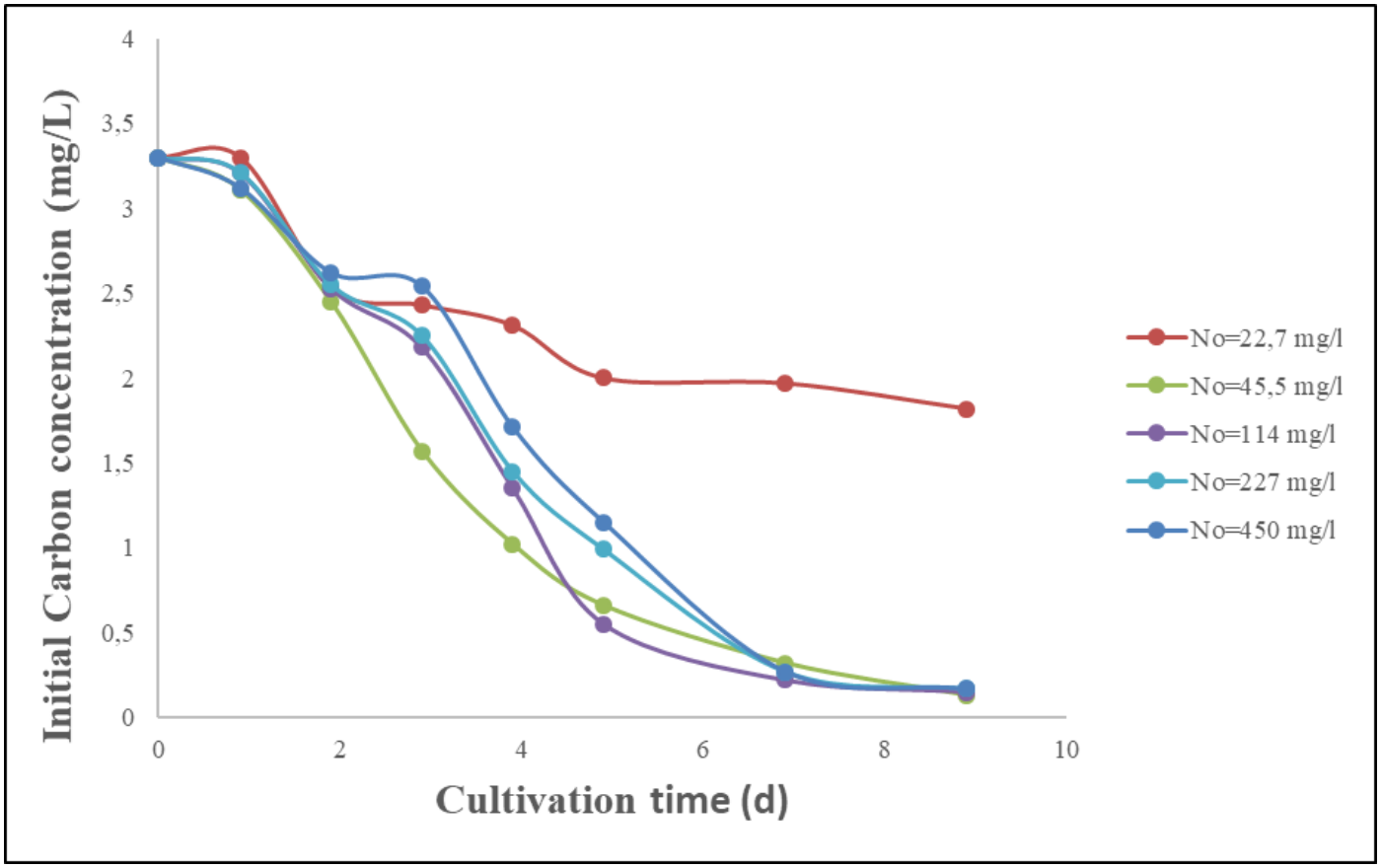

Figure 2

Reduction in organic carbon versus cultivation time for five different Co/No ratios at a constant Co. Co/No = 145.4, 72.5, 28.9, 14.5 and 7.3. Curves are drawn between data point for clarification.

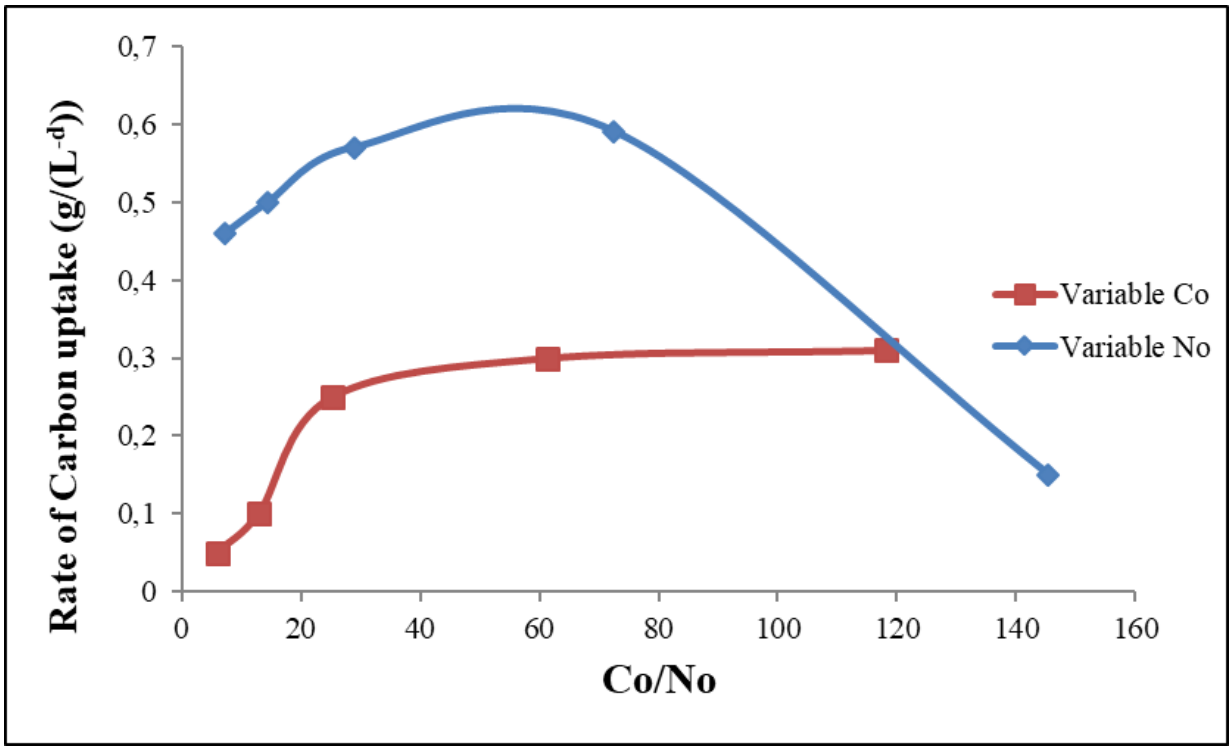

Figure 3

The rate of carbon uptake (g/(L-d)) versus the ratio Co/No. Curves are drawn between data point for clarification.

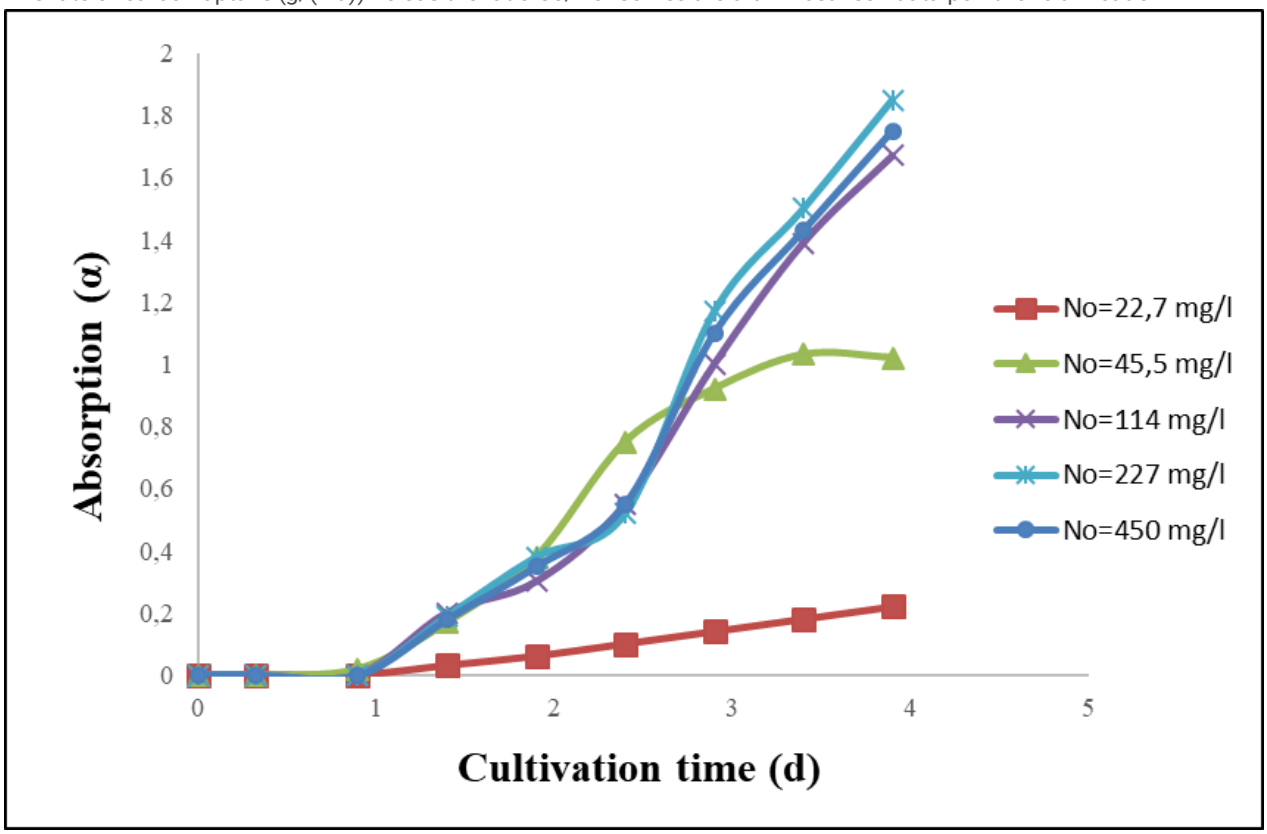

Figure 4

Absorption of the culture medium versus cultivation time for the initial nitrogen concentrations shown. Curves are drawn between data point for clarification.

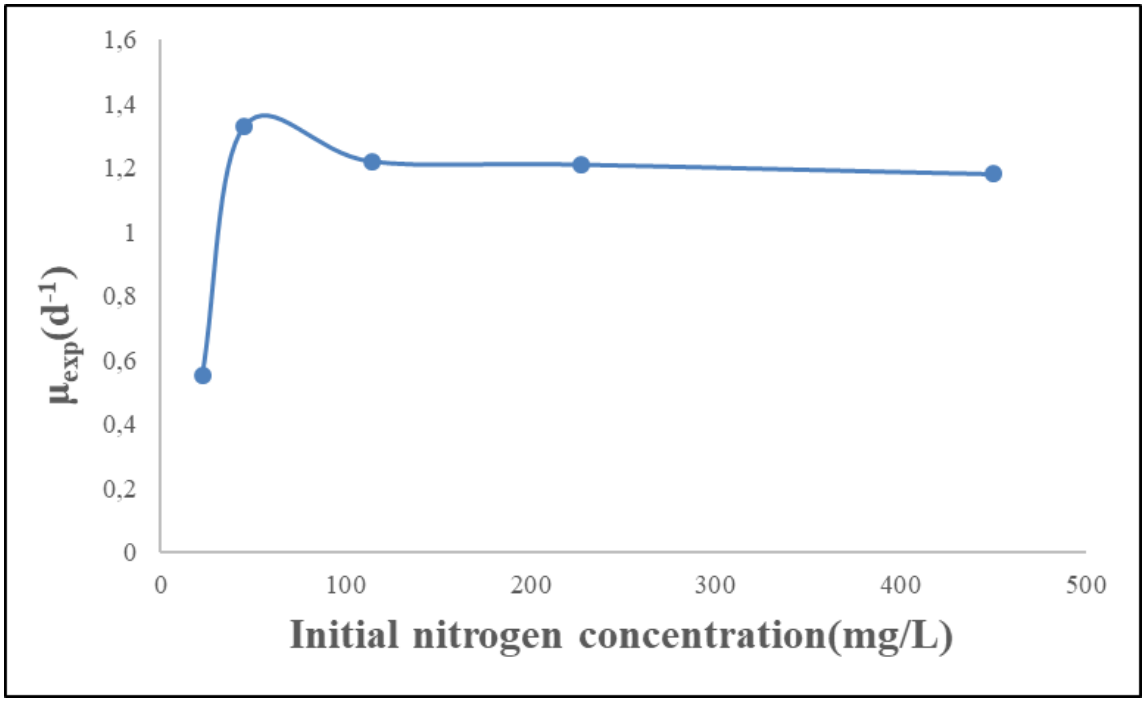

Figure 5

The specific growth coefficient versus the initial nitrogen concentration. Curves are drawn between data point for clarification.

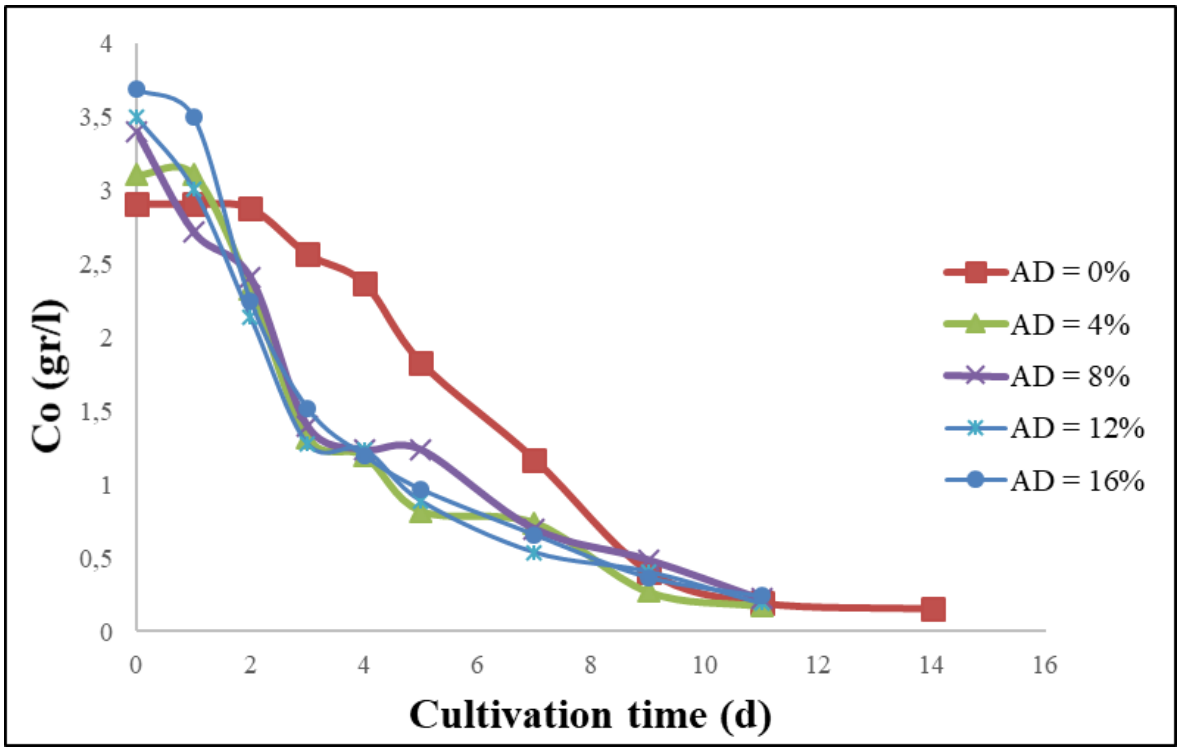

Figure 6

Reduction in organic carbon versus cultivation time for five different concentrations in organic digestrate and at an initial glycerol concentration equal to 25 ml L-1. The initial concentration of nitrogen is equal to 129.9 mg L-1. Curves are drawn between data point for clarification.

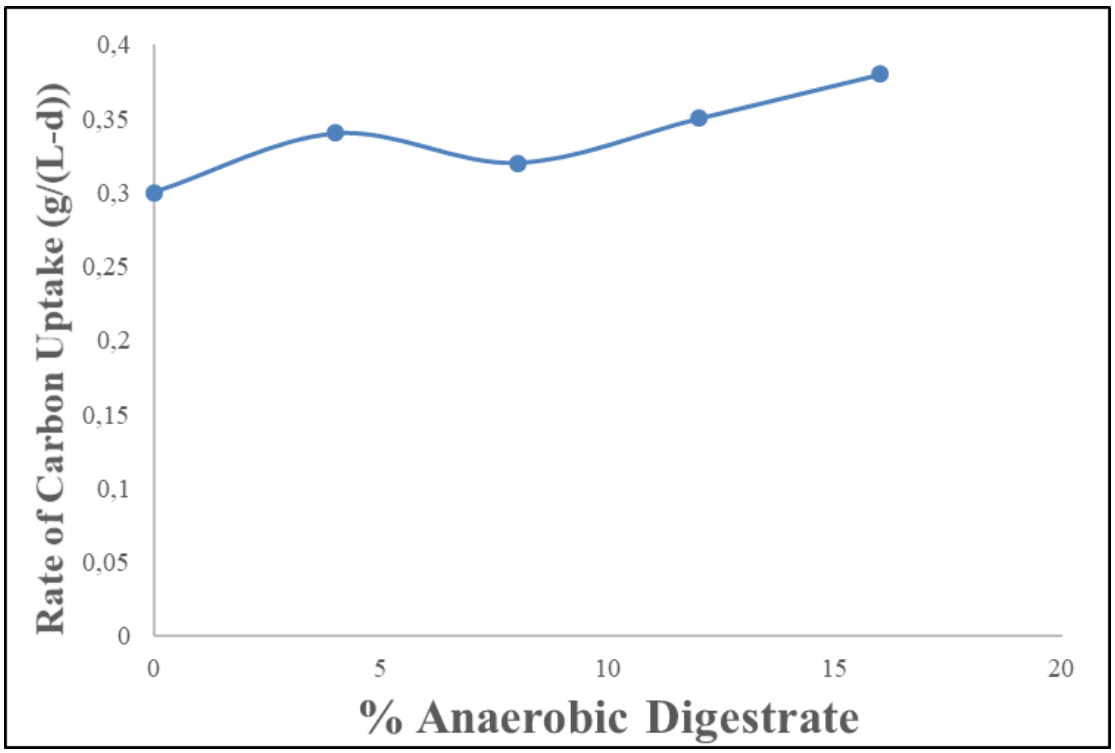

Figure 7

The average Carbon uptake rate versus the digestrate concentration.