Testing the waste based biorefinery concept: pilot scale cultivation of microalgal species on spent anaerobic digestate fluids

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Abstract:

Purpose: A waste based algal biorefinery approach has been tested.

Methods: This has been investigated by culturing in a 800L photobioreactor two autotrophic microalgae namely *Nannochloropsis oceanica* and *Scenedesmus quadricauda* utilising filtered spent anaerobic digestate fluids of N:P ration 14.22 as substrate.

Results: Significant rates of bioremediation simultaneously with biomass and associated end product formation were achieved. Nitrogen and phosphorus of waste based media was decreased up to 90%. The biomass biochemical analysis of the microalgae when grown on the waste based formulated media demonstrated the comparable content of lipids and proteins with the species grown on f/2 media.

Conclusions: Theoretical biomethane potential generation, should the algal cultures be placed in an anaerobic digester, was calculated at 0.58 L CH_4 g^{-1} VS for *N. oceanica* and 0.48 L CH_4 g^{-1} VS for *S. quadricauda* showing comparable results with other studies of different source of biomass.

Keywords: nitrogen; phosphorus; bioremediation; algal pilot cultivation; biomass; energy production; biomethane potential

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Introduction

Recycling of waste derived nutrients such as nitrogen and phosphorus has become a contemporary global priority. Especially in the case of phosphorus, a non-renewable natural resource of great importance due to its multiple uses in products such as fertilisers, food additives and drinking water treatment , the need for recycling becomes apparent [1,2]. Although its production is carbon neutral, the mining of phosphate is increasingly expensive as there are supply risks related to environmental and socio-political issues [3]. It has been calculated that by 2035 the demand for phosphorus will outpace its supply as this finite resource economic value rises exponentially (800% rise between 2006 (US$50) and 2008 (US$400), current value over US$500/tonne). At the same time phosphorus removal percentages from wastewater must be improved as water discharge international and national standards become more stringent, constituting water treatment more costly [4,5].

In addition ,ammonia and phosphate rich waste disposal by landspreading and landfilling is generating serious environmental constraints such as gases (NO_X) while nutrients dissolved in water (NH_3 and PO_4) can lead in progressive degradation of air and water resources. These phenomena cause soil acidification and there upon eutrophication of rivers and estuaries [6]. These challenges both environmental and economical could be addressed effectively using algal biotechnology [7,8]. Photosynthetic microorganisms including algae have the ability of biological nitrogen and phosphorus fixation with the simultaneous production of oxygen and high value end products such as oils, lipids or cellular biomass [9]. Algae have been previously successfully grown on anaerobically digested waste streams within the scope of bioremediation and the production of valuable chemicals [10,11].

Among algal species, microalgae have attracted much interest for the biological treatment and recovery of nutrients from various types of wastewater, due to their central role in CO_2 fixation and their effective uptake of nitrogen and phosphorus. Therefore, microalgae could serve as a “natural bioreactor” in an integrated waste based biorefinery approach (Fig.1.) [7-9] simultaneously with wastewater treatment and biobased waste remediation. Such an approach would generate large amounts of biomass , that could be used itself as anaerobic digestion feed or as a substrate for development of valuable commercial products. Scaling up technology for the mass cultivation of microalgae is available in numerous mechanical arrangements including photobioreactors, ponds and rotating disk reactors. Large scale microalgal cultivation requires the availability of primary nutrients namely carbon, nitrogen, phosphorus and other elements including minerals [4]. These substances can be highly expensive when used in powdered form suitable for nutrient media formulation. On the other hand, the use of wastewater streams as inexpensive growth substrates taking advantage of microalgae ability to accumulate inorganic nutrients is an attractive option [12,13].

Algae grown on waste streams can yield biomass that can be processed to recover other intracellular products or to be used itself as a source of energy by methane generation, through anaerobic digestion [14].Thus, growing microalgae on waste streams has numerous benefits. The use of wastewater can significantly reduce the cost of algal propagation, by minimising the cost of nutrients used as substrate. Conversely, algal wastewater treatment that generates high value end products can be commercially viable, offsetting high costs related with the current methods of waste treatment [15]. Regardless of microalgae’s great ability to uptake nutrients from waste water, and the economic and ecological advantages of the use of waste sources, the complexity of its physicochemical properties such as high viscosity, colour, metal ions [16,17] can be proven highly toxic for microalgae

growth and represent an engineering challenge. This adversity could be resolved using a pressure driven membrane process namely membrane filtration. It has been successfully applied [18] in the case of spent agriculture digestates to formulate nutrient media with suitable N:P ratios, since biotechnological applications of algal biomass strongly depends on the cell composition and growth potential of the algal strain as well as the propagation medium composition and the cultivation conditions [19].

Therefore, this work reports on a pilot scale waste based biorefinery approach, utilising formulated digested agricultural streams as growth media, suitable for mass cultivation of industrially significant microorganisms, associated with chemicals and fuels production. Within this concept and to test its usefulness, two well known [20,21] autotrophic microalgae namely *Nannochloropsis oceanica* and *Scenedesmus quadricuada* were grown on treated digested agricultural wastewater of specific N:P ratio on a 800 L photobioreactor (PBR). The effluents were physico-chemically characterised and treated through membrane filtration to produce a balanced composition of nitrogen and phosphate effluent suitable for autotrophic algal cultivation. Comparative studies were done using common standardised growth media (f/2 medium) and the anaerobically digested (AD) filtrates. The growth of microorganisms was evaluated in term of growth rates and biomass productivities. The content of algal biomass in lipids, carbohydrates and proteins was quantified in the varying culturing conditions with regards to the reduction of ammonia and phosphorus media content, investigating the potential of the microorganisms as bioremediation agents. The calorific value, energy and biomethane production potential were also analysed in order to evaluate the value of the produced biomass.

Materials and Methods

Materials

Inoculum source

N. oceanica Suda & Miyashita, (Eustigmatophyceae), marine algae and *S. quadricauda* (Turpin) Brébisson fresh water algae were provided from the culture collection of the Centre for Sustainable Aquatic Research (CSAR) Swansea University, being originally outsourced in 2011 by the Hellenic Centre for Marine Research (HCMR) in Greece and Ingrepro Renewables B.V. in the Netherlands. The inocula for both species were maintained at constant room temperature (18°C) with light of 100 µmol photons m^{-2} s^{-1} light: dark cycle 16:8 hours. The f/2 standardised nutrient medium [22], (deionised water, $NaNO_3$ 0.075 g L^{-1}, $NaH_2PO_4.2H_2O$ 0.00565 g L^{-1}, trace elements stock solution 1 ml L^{-1}, vitamin mix stock solution 1 ml L^{-1}, with the trace elements solution comprised of Na_2EDTA 4.16 g L^{-1}, $FeCl_3.6H_2O$ 3.15 g L^{-1}, $CuSO_4.5H_2O$ 0.01 g L^{-1}, $ZnSO_4.7H_2O$ 0.022 g L^{-1}, $CoCl_2.6H_2O$ 0.01 g L^{-1}, $MnCl_2.4H_2O$ 0.18 g L^{-1}, $NaMoO_4.2H_2O$ 0.18 g L^{-1}, and vitamin mix stock solution made of cyanocobalamin (vitamin B_{12}) 0.0005 g L^{-1}, thiamine HCl (vitamin B_1) 0.1 g L^{-1}, biotin 0.0005 g L^{-1}),was supplied in readily mixed powder by Varicon Aqua Solution Ltd (Cell-Hi F2P all -in-one powder) [23] Conical flasks of 250 mL volume, was utilised on a weekly basis to maintain the master culture of this species. Both microalgae were grown for at least 10 generations on f/2 medium (10 weekly re-inoculations), offering optimised results regarding growth, and demonstrating adaptation of algae on this media.

Waste Effluents

Waste effluent streams (agricultural wastewater derived from spent agricultural digested sludge namely mixed waste of cattle slurry, vegetable waste and silage), taken off the output line of a 700 m^3d^{-1} anaerobic digester (AD) used for manure and methane production (80 kWd^{-1}) but before passing through the automatic coarse particle separator (>5mm), were collected off Farm Renewable Environmental Energy Limited (Fre), Wrexham, United Kingdom (http://www.fre-energy.co.uk/). These samples were pre-treated using dilution, mixing, sedimentation and sieving in a 200 L capacity stainless steel vessel. The resulting effluents were then microfiltered through a pilot scale unit equipped with a ceramic membrane (pore size <0.2µm) [18]. The resulting particle free, effluents were used as nutrient media in this study.

Methods

AD Effluent Sludge to Nutrient Formulations Processing Scheme

The effluents were formulated as follows; they were microfiltered through a pilot scale unit equipped with a ceramic membrane (Pall Membralox, 3.70 mm channels, 0.22 m^2 area with nominal pore size <0.2µm) [19]. The design of the unit has been described in extensive detail elsewhere [19]. To achieve the desired concentrations of nutrients, a scheme combining diafiltration with pre-acidification was applied [19]. The batch process involved 2 sequential leaching steps which consisted of first concentration, and then dilution of the sludge with fresh tap water. The permeate flow rate was manually recorded using a graduated vessel where the permeate fluid was collected. The difference in volume was recorded per minute using a stopwatch (Casio electronics, UK); on a two decimal point precision electronic scale (OHAUS I-10). The resulting, particle free effluents of the desired N:P ratio, were stored at 4°C and used as nutrient media in this study. The scheme has been extensively described elsewhere [24,35].

Physicochemical characterisation of the treated agricultural wastewater

Total solids (TS, gL^{-1}), total suspended solids (TSS, gL^{-1}), total dissolved solids (TDS), alkalinity, optical density, nitrogen measured as ammonia (NH_3–N) and phosphorous (PO_4–P) using the phenate and vanadomolybdo-phosphoric acid colorimetric methods were determined according to Standard Methods for the Examination of Water and Wastewater published by APHA, AWWA and WPCF 20th Edition, 1998. VFA were determined using headspace gas chromatography, offering highly significant results. Particle size distribution (PSD) of the sludge samples was determined by light scattering technique using Mastersizer 2000 (Malvern, UK), the zeta potential was determined by the Zetasizer (Malvern, UK),the conductivity and salinity of the samples were measured used a conductivity meter (Russell systems, UK) calibrated with a standard solution of 0.1M of KCl.

Inoculum preparation and maintenance of *N. oceanica* and *S. quadricauda*

The two algal species were cultured separately, in fresh and seawater conditions with addition of f/2 standardised medium (pH 8.0). Separate inocula *N. oceanica* and *S. quadricauda* were prepared according to the following procedure. The experiment was set-up at 18.0 ± 3 °C (controlled temperature room) on bench scale at 20L plastic carboys externally illuminated on one side by a twin florescent tube (natural daylight Osram tube, Munich, Germany), at an 18:6 light cycle, at 200 µmol photons m^2 s^{-1}. Each carboy was sealed using a nitrile rubber stopper and two separate ports created,

with two glass tubes inserted; for aeration and sampling and exhausting gas. The carboys were continuously sparged with ambient air at 0.1 VVM with the addition of 0.03% v/v CO_2 during the light cycle. During the growth of the culture the pH was maintained by the addition of 10 mg L^{-1} sodium bicarbonate and after a few days of growth the algal culture provided buffering capacity [25].

Horizontal Tubular Pilot Scale Algal Culturing System (Photobioreactor, PBR)

Two horizontal tubular PBRs (Biofence model) (Fig.2) of up to 800L capacity each purchased from Varicon Aqua Solution Ltd. located at the Biosciences Department, Swansea University was used. These systems were designed to effectively utilise algal photosynthetic capacity and was held in a transparent glass greenhouse (location 51°36'29.1"N 3°58'53.1"W, Swansea UK). Temperature and pH were monitored using probes installed in each system. CO_2 injection was regulated with an automated pH sensor; that was injecting CO_2 in the system when the pH rose above 8.1, maintaining the pH between 7.5 to 8.0 across the system. Temperature was controlled and maintained between 18-24 °C.

The system was arranged as to have a 400 L sunlight exposed part and a 400 L dark part. It was injected with inoculum volume in exponential growth (10% v/v, 80 L) had a final concentration of 5 million cell per mL for each specie. Then the inoculum culture was transferred between the light and dark part every 3 to 4 minutes. The treated and reformulated digestate was diluted 10 times (10% v/v injection of the total culturing capacity) with treated seawater to become suitable for the algal growth. Comparative growth studies were performed with the f/2 media (where five times concentrated f/2 media were used). The experimental trials duration was 16 days for both algal species. Samples for the biological assessment of cultures and the determination of physicochemical parameters were taken daily.

Sample Analysis

Measurement of cellular growth and biomass

Cell concentration and biovolume was measured on a 24h basis, by Coulter counter (Multisizer 4, Beckman, USA). Algal culture growth rate was calculated according to the following formula [8].

$$\mu = \frac{\ln\left(\frac{N_2}{N_1}\right)}{t_{2-}\, t_1} \qquad (1)$$

where N_1 and N_2 are the biovolume (measured by Coulter counter) at time 1 (t_1) and time 2 (t_2), respectively.Dry cell weight (g L^{-1} as DCW) measurements were made directly by collecting 10 ml cultured broths, filtering the sample through Whatman No. 2 filter paper (pre-weighted), rinsing thrice with distilled water to remove salts and drying the papers for 4 h at 105 °C. DCW was determined by a weight difference (Sartorius, CP4202S, JENCONS-PLS, Germany) between the blank filter paper and the dried sample) [26] .

Bioremediation, nutrients uptake measurements and biochemical composition of algal biomass

Samples for measurements of total nitrogen and phosphorus analyses in the culture media were taken every 24 h and centrifuged for 5 min under 8000 rpm to obtain the supernatant. These supernatant samples were suspended and washed twice in deionised water. Then they were frozen at −80 °C

overnight. The samples were then analysed for total nitrogen (TN), ammonium (N) and phosphorus (P) using an automated segmented flow analyser (Model SEAL AA3, Bran Luebbe, Germany).

The analysis of algal biomass for carbon (C) and nitrogen (N) content was done using the SerCon GSL elemental analyser (1000 °C combustion temperature). The protein content of the biomass was determined by multiplying the nitrogen content of dried biomass previously measured by a factor of 6.28 [27]. This approach is considered being analogous to that based upon Kjeldahl digestion [27].

Total lipids were determined according to the following method, the culture liquid was removed from the samples by centrifugation and then the pellets were washed with distilled water twice. Washed pellets were dried at 105 °C for 4 h. Lipids were extracted from dried biomass with a 2:1 chloroform: methanol mixture until the colour of the solvent layer disappeared. The amounts of lipids (w/w%) produced were determined by the weight difference between the blank flask and the flask containing the extracted oil after the solvent evaporation under vacuum [28] .

Total carbohydrate content of lyophilised algal biomass was determined by the colorimetric Dubois method [29] using phenol and sulphuric acid. Quantification was performed using a glucose known concentration solutions standard curve at 485 nm.

Gross energy content and calorific value (KJ g^{-1} and Kcal g^{-1}) was measured by combustion of freeze dried biomass of algae using an oxygen bomb calorimeter (Model 1341 Plain Jacket Calorimeter, Parr Instrument Company, US) using benzoic acid as the standard [14]. The biomass was freeze dried using shelf freeze dryer (Edwards Modulyo 304 Stainless Steel Freeze Dryer).

Statistical analysis

All the experiments were performed in triplicate. The standard deviation and means were analysed for significance using biostatistics software Excel through one way ANOVA. The Duncan multiple range test was used to compare the significance of difference among tested algae at *P* values of <0.05. Results are reported as either mean ± SD or error bars.

Theoretical

Nutrient uptake rate calculation

To compare the kinetics for nitrogen and phosphorus removal, a calculation has been made of the time required to reach 10 mg L^{-1} (Total N, ΣN) and 1 mg L^{-1} ($P - PO_4^{3-}$) (these concentrations derive from the most restrictive concentrations in European Union Directive 98/15/CE [30] concerning requirements of N and P in the effluents permitted from urban wastewater treatment). Nutrient uptake rate was calculated based on equation [31]:

$$\mathrm{R}\ (mg\ L^{-1}) = \left(\frac{R_t - R_0}{\mathrm{X}}\right) \quad (2)$$

where, R is nutrient removal efficiency, X is the biomass concentration at stationary phase and R_t and R_0 are the nutrient concentration at day t and day 0, respectively.

Estimation of Methane production potential

After the biochemical and elemental composition analyses were completed, the maximum possible methane potential of selected algal biomass was estimated. The elemental composition of the algal biomass was utilized. Based on the elemental composition, it was assumed that the algal lipid, protein and carbohydrate chemical formulae were $C_{57}H_{104}O_6$, $C_6H_{13.1}O_1N_{0.6}$ and $(C_6H_{10}O_5)_n$, respectively [32] and the specific methane yield for these three organic components of algal biomass were used for methane production potential estimation. The calculation for theoretical methane potential (TMP) was done through the following equation.

$$BMP = \frac{1}{100}(A \times C_L + B \times C_P + C \times C_C) \quad (3)$$

where *A* is the specific methane yield of lipids (1.014 CH_4 g^{-1} volatile solids (VS)); *B* is the specific methane yield of protein (0.851 CH_4 g^{-1} VS); *C* the specific methane yield of carbohydrates (0.415 CH_4 g^{-1} VS) and C_L,C_P, C_C are the % (on TS basis) of lipid, protein and carbohydrates, respectively, in algal biomass [33,34].

Results and Discussion

Physicochemical Composition of Treated Agricultural Waste Effluent Streams

The idea of utilising waste effluents for biotechnological production of chemicals and other substances has been positively viewed over the last decade [9,10]. This strategy can be beneficial in certain cases since the waste effluents might help with for example , the growth of algal species , while in other microorganisms may hinder intensive growth due to toxicity. For bacteria, algae or fungi, with metabolic products such as acids or biofuels like ethanol, biodiesel or lipids, these effluents can be used safely. After 24 h sedimentation, the spent agricultural effluent was filtered , using a diafiltration with acidification scheme, through the ceramic cross-flow microfiltration unit at a TMP of 10 psi. The flux of the fluid was 87.48 L m^{-2} h^{-1}. Filtration ensures the removal of any larger than the pore size suspended material including bacterial load, while recovering important nutrients in the supernatant fluid that are normally loosely associated with the solids. The ecotoxicity aspects and environmental risk of the use of waste as nutrient media will need to be constantly evaluated and addressed. Engineering methodologies such as dilution, sedimentation, screening and filtration are offering tangible and effective solutions for these challenges [35]. In anaerobic digester plants producing biogas, several cubic meters of spend digestate are produced per day [36] with high content of nitrogen and phosphorus. Membrane treatment of the spent waste effluents would have removed bacterial and algal grazers' contaminants. Their treatment with dilution to such a pilot scale could be feasible, economically viable and environmentally friendly if greywater or industrial grade water is to be used, potential costs of this process has been discussed elsewhere [10]. Therefore, algal mass cultivation for commercial purposes in PBRs or in raceway ponds using the waste based media can be applied, while continuous cultivation processes would be practically applicable.

The purpose of diafiltration was to investigate the effects of removing the soluble components of the sludge, such as inorganic ions like calcium, phosphorus and metals. Phosphorus is often found in AD effluents in the form of struvite which is a phosphate mineral comprising of ammonia, phosphate and magnesium, therefore acidification and leaching are of great importance to dissolve these substances

into the resulting solutions. During filtration, the majority of solids and insoluble organic matter were retained by the membrane filter. Interestingly, the cross flow arrangement of the filtration unit allowed the continuous circulation of the processing fluid in the system. This enabled the continuous disengagement of nutrients retained in a compressible permeable cake, formed by the deposition of solids in the membrane channels [18]. The nutrients found to the permeate, allowed the formulation of the complex but large particle free solutions to a molar N: P ratio of 14.22 (Table 1). The resulting solution had nutritive content including metal ions that can be used as growth stimulants (nitrogen, carbon and hydrogen intake). This is expected to generate different responses from the microalgal species when compared with the *in vitro* media, favouring either biomass or lipids formation. The solid matter content, the conductivity and the ions (Table 1) related indicate a solution rich in mineral salts that may be taken up during the microbial metabolism. In addition, sequestration of carbon and nitrogen is being tested, aiming to develop a low cost, two step remediation process of AD effluents, combining mechanical (membrane processing) and biological treatment (algal growth in which N are P are accumulated in potentially useful products). This strategy is scalable and can be applicable to an on-site or to a centralised processing setting.

Growth performance, biomass productivity and nutrient uptake

The two autotrophic algae, a marine and a freshwater specie, namely *N. oceanica* and *S. quadricuada* were grown successfully on pilot scale on waste formulated nutrient media , offering comparable growth with the *in vitro* standardised media for algal culturing (Table 2, Fig. 3). Growth rates were good for both species on waste based media, showing adaptability, regardless of the high content in ammonia [37,38]. However, for *S. quadricuada* the growth on waste based media is significantly lower compared to the f/2 media, with the growth slowing down significantly by day 8 (Fig. 3). Both species' demonstrated an adaptation phase, up to 2 days for *N. oceanica* and *S. quadricuada*. The exponential growth was observed, starting at the 2nd day , reaching stationary phase at the 10th day of growth for *N. oceanica* , while for *S. quadricuada* this stage was reached at day 6 for waste based conditions (Fig.3). *N. oceanica* when grown on f/2 achieved a growth rate of 0.51 d^{-1}, while on the formulated waste media achieved 0.50 d^{-1}, on the other hand *S. quadricauda* had similar growth rate, *in vitro* media was 0.48 d^{-1} and in waste based media was 0.50 d^{-1} (Table 2, Fig. 3) . For both species, biomass production rate and biomass productivity were higher on the *in vitro* standardised media. However, the results are comparable with the waste formulate media, consisting them a suitable option for the mass production of algal biomass. Biomass productivity rate is not significantly different for both species when grown on the *in vitro* media and the waste based media, demonstrating the high potential of the virtually inexhaustible and inexpensive waste effluents to produce streams of agricultural origin to be used as source of growth for algal biomass. Within this context, the spent anaerobically digested effluents are not a waste product but represent a valuable by-product of significant economic importance [39]. Algal biotechnology applications using this waste material show an extensible and safe application [40], especially produced algal biomass could be used for high value end products.

Total nitrogen concentration in both the waste based and the *in vitro* media was high, as the nutritional dependence of algae on nitrogen is well documented [41]. *N. oceanica* , demonstrated a higher depletion rate of nitrogen on waste based media (Fig. 4), than f/2 media. By the 4th day of growth TN was uptaken by 90% for *N. oceanica* and completely depleted by day 10 when grown on the waste based media, while for *S. quadricauda* consumed TN by 70% by the 8th day of culturing

when grown on the waste based media. The average N uptake rate is around 7 mg (N) L^{-1} for both species, slightly higher than the rate observed in the readymade media, with *N. oceanica* showing a better performance (Fig.4). In the case of phosphorus consumption, relatively quick depletion was observed by both species when grown on waste formulated media, with *N. oceanica* demonstrating a better performance, having consumed most of the phosphorus by day 4 of the culturing. On the other hand, *S. quadricuada* accumulated phosphate relatively slower, with a significant reduction being observed at day 6 (Fig.4).

These results indicate another benefit of this approach, the remediation of waste, reaching the complete removal of nitrogen and ammonia by both Chlorophyta species by the 12th day of culturing. Algae have been proven capable to adapt to a different environment and successfully uptake nutrients such nitrogen and phosphorus. The accumulation of nitrogen in high concentration by algae has been previously demonstrated [34,42], confirming this study's findings. Several researchers have been previously grown algal species on digestate of different composition [42-46] achieving varying remediation results and generation of biogas , confirming that the utilisation of waste as a substrate for algal growth in a biorefinery concept is a viable option.

Consequently, the waste based biorefinery approach utilising algae is an integrated process combining high value end products production, nutrient recovery and waste remediation. In further detail, *N. oceanica* and *S. quadricauda*, have been previously grown successfully on spent digestate [49,50] within the scope of bioremediation. *Scenedesmus* sp. have also been used as a bioremediation agent of municipal wastewater effluents. Other studies have shown *Scenedesmus* species being highly effective to a rate of 70% to 80%, in remediating brewery and aquaculture waste depending on the dilution rate [19].

Biochemical composition and energy potential of algal biomass

The biochemical composition of both species grown of the waste formulated media and the *in vitro* media was presented on Fig. 6,7. These analyses are reflecting the data of various nutrient conditions influence on the biochemical composition of the produced biomass. In the case of *N. oceanica* when grown on f/2 media (Fig. 6a), protein content varied between 20.42% (day 1) to 30.78% (day 12) of culturing, lipids ranged from 20.37% (day 1) to 39.04% (day 12) and carbohydrates from 17.34% (day 1) to 29.48% at the 12th day of culturing. When the specie was grown on the waste based formulated media protein content varied between 11.60% (day 1) to 29.57% (day 12) of culturing, lipids ranged from 24.59% (day 1) to 46.74 % (day 12) and carbohydrates from 8.82% (day 1) to 19.38 % at the 12th day of culturing. Lipids are predominantly present when grown on waste based media ($F^{6,28}$= 95.588, P<0.05), while carbohydrate and protein content was lower (Fig. 6b) when compared with the growth on the *in vitro* media (Fig. 6a). Lipids accumulation was higher, at the expense of protein carbohydrate content of *N. oceanica* ($F^{6, 28}$= 26.266, P<0.05) was significantly lower (Fig.6b) on waste based media in comparison with the control conditions. High content of lipids could be a result of the rapid nutrients uptake [51] while the time of cultivation did not have a significant effect on the biomass compositions on either growth conditions. On the other hand, protein content ($F^{4, 19}$= 83.111, P<0.05) was higher on the *in vitro* standardised media.

In the case of *S. quadricuada*, when grown on f/2 media (Fig. 7a), protein content varied between 17.6% (day 1) to 33.49% (day 12) of culturing, lipids ranged from 12.60% (day 1) to 21.50% (day 12) and carbohydrates from 9.78% (day 1) to 30.77% at the 12th day of culturing. When the specie was

grown on the waste based formulated media protein content varied between 15.06% (day 1) to 37.00% (day 12) of culturing, lipids ranged from 13.28% (day 1) to 26.73 % (day 12) and carbohydrates from 9.47 % (day 1) to 27.78 % at the 12th day of culturing.*S. quadricauda*, major biomass chemical components were proteins and carbohydrates, with the growth of waste media favouring the proteins ($F^{7,\ 31}$= 60.439, P<0.05) (Fig. 6 a,b) content while carbohydrates ($F^{7,31}$= 47.568, P<0.05) were predominant in *in vitro* media (Fig. 6 a), a phenomenon previously described [37]. Overall, the biomass compositions on both nutrient regimes were relatively similar in the case of *S. quadricuada*.

The biosynthetic pathway used for biomass development by each species was different. This was reflected in the varying concentration of storage compounds (carbohydrates, lipids), indicating nutrient depletion towards the end of the project trials [26,37]. This approach could be utilised to develop a semi-continuous mode of cultivation [40] with waste based nutrient media tailored to the enhancement of energy and biomethane production potential of the algal biomass [39].The varying biochemical composition of biomass was being reflected to the calorific value, energy and biomethane production potential (BMP) (Table 3). For *N.oceanica* the calorific value was higher when grown on the waste based media 7496 kcal g^{-1} versus 5151 kcal g^{-1} in f/2 media, while BMP values for both species, were higher when grown on the waste based media. This could be attributed to the high content of lipids and carbohydrates [40,41] in the algal biomass (Fig 6, 7). The use of algal biomass as an energy generation source through anaerobic digestion has been previously proposed [42,43]. The significant calorific value of algal biomass based in the content of lipids , proteins and carbohydrates suggests the good potential of algae to be used as biomethane production substrate , an important step of closing the loops in contemporary circular economy.

In this study, the algal biomass of *N. oceanica* with high lipid content provided a higher methane potential, 0.58 CH_4 g^{-1} VS than the less lipids-rich biomass of *S. quadricuada* (BMP 0.48 CH_4 g^{-1} VS) . These theoretical values were obtained using the method developed by Prajapati et al, 2014 and demonstrated comparable results with *Chlorella* and other species [42-46]. These values are demonstrating the potentiality of *Nannochloropsis oceanica* and *Scenedesmus quadricauda* to effectively support as a substrate biogas generation. Therefore, the produced algal biomass can be harvested and concentrated by the use of filtration and reused as anaerobic digestion substrate closing effectively the loop, enhancing sustainability and valorising waste, by remediation . The production of methane–enriched biogas can reduce the cost of algal downstream processing [47] as well as biomass fractionation through membrane technology. Although algal biomass can be used if fractionated for high value food and feed products development with possibly numerous benefits, its growth on waste based media for such applications , is not currently being promoted due to substances in the waste that are considered contaminants such as heavy metals or pharmaceutical and endocrine disrupting pollutants.

Conclusions

In this current study these species have shown a high nutrient uptake rate (above 90% at the day 4) and productive cultivation in large scale (pilot, semi-industrial 800 L) results that can lead to a sustainable way of waste remediation since efficient nutrient's uptake is being observed simultaneously with valuable biomass production. The results further demonstrate the potential for semi-continuous or continuous cultivation in pilot scale with continuous biomass production [48-52].

Summarising

- Formulated waste based nutrient media of anaerobically digested sludge can be used successfully in pilot scale culturing of microalgae, being an economically viable option to synthetic media for *N. oceanica* and *S. quadricauda.*
- Microalgae rapidly adapted to the formulated waste based media demonstrating a short lag phase
- Algae nutrients uptake rate was high, demonstrating a good bioremediation potential.
- The produced algal biomass could be applied for the biomethane production or fractionated into high value products.

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References

1. Morel, F. M. M. (1987). Kinetics Of Nutrient Uptake And Growth In Phytoplankton 1. *Journal of Phycology, 23*(2), 137-150. doi:10.1111/j.1529-8817.1987.tb04436.x

2. Scholz, R. W., Ulrich, A. E., Eilittä, M., & Roy, A. (2013). Sustainable use of phosphorus: A finite resource. *Science of The Total Environment, 461–462*, 799-803. doi:http://dx.doi.org/10.1016/j.scitotenv.2013.05.043

3. de Ridder, M., de Jong,S., Polchar, J., Lingeman, S., Risks and Opportunities in the Global Phosphate Rock Market The Hague Centre for Strategic Studies (HCSS) Rapport No 17 | 12 | 12

4. Slade, R., & Bauen, A. (2013). Micro-algae cultivation for biofuels: Cost, energy balance, environmental impacts and future prospects. *Biomass and Bioenergy, 53* (Supplement C), 29-38. doi:https://doi.org/10.1016/j.biombioe.2012.12.019

5. Shilton, A. N., Powell, N., & Guieysse, B. (2012). Plant based phosphorus recovery from wastewater via algae and macrophytes. *Current Opinion in Biotechnology, 23*(6), 884-889. doi:http://dx.doi.org/10.1016/j.copbio.2012.07.002

6. Tigini, V., Franchino, M., Bona, F., & Varese, G. C. (2016). Is digestate safe? A study on its ecotoxicity and environmental risk on a pig manure. *Science of The Total Environment, 551-552* (Supplement C), 127-132. doi:https://doi.org/10.1016/j.scitotenv.2016.02.004

7. Pittman, J. K., Dean, A. P., & Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology, 102*(1), 17-25. doi:http://dx.doi.org/10.1016/j.biortech.2010.06.035

8. Christenson, L., & Sims, R. (2011). Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances, 29*(6), 686-702. doi:https://doi.org/10.1016/j.biotechadv.2011.05.015

9. Morales-Amaral, M. d. M., Gómez-Serrano, C., Acién, F. G., Fernández-Sevilla, J. M., & Molina-Grima, E. (2015). Production of microalgae using centrate from anaerobic digestion as the nutrient source. *Algal Research, 9*, 297-305. doi:http://dx.doi.org/10.1016/j.algal.2015.03.018

10. Silkina, A., Zacharof, M.-P., Hery, G., Nouvel, T., & Lovitt, R. W. (2017a). Formulation and utilisation of spent anaerobic digestate fluids for the growth and product formation of single cell algal cultures in heterotrophic and autotrophic conditions. Bioresource Technology, 244(Part 2), 1445-1455. doi:https://doi.org/10.1016/j.biortech.2017.05.133

11. Stiles, WAV., Styles, D., Chapman, SP., Esteves, S., Bywater, A., Melville, L., et al. (2018) Using microalgae in the circular economy to valorise anaerobic digestate: challenges and opportunities Biores. Tech. 267 (2018) 732-742, 2018

12 . Gonçalves, A. L., Pires, J. C.M., Simões, M.(2017). A review on the use of microalgal consortia for wastewater treatment. *Algal Research 24*, 403-415 doi:https://doi.org/10.1016/j.algal.2016.11.008.

13. Prajapati, S. K., Kaushik,P., Malik, A., Vijay, V. K. (2013). Phycoremediation coupled production of algal biomass, harvesting and anaerobic digestion: Possibilities and challenges,. *Biotechnology Advances 31*, 1408-1425 . doi:https://doi.org/10.1016/j.biotechadv.2013.06.005

14. Sakarika,M., Kornaros, M. (2019). Chlorella vulgaris as a green biofuel factory: Comparison between biodiesel, biogas and combustible biomass production. *Bioresource Technology 273*, 237-243 . doi:https://doi.org/10.1016/j.biortech.2018.11.017

15. Mayers, J. J., Flynn, K. J., & Shields, R. J. (2013). Rapid determination of bulk microalgal biochemical composition by Fourier-Transform Infrared spectroscopy. *Bioresource Technology, 148*, 215-220. doi:http://dx.doi.org/10.1016/j.biortech.2013.08.133

16. McGinn, P. J., Dickinson, K. E., Park, K. C., Whitney, C. G., MacQuarrie, S. P., Black, F. J.,O'Leary, S. J. B. (2012). Assessment of the bioenergy and bioremediation potentials of the microalga Scenedesmus sp. AMDD cultivated in municipal wastewater effluent in batch and continuous mode. *Algal Research, 1*(2), 155-165. doi:http://dx.doi.org/10.1016/j.algal.2012.05.001

17. Dickinson, K. E., Whitney, C. G., & McGinn, P. J. (2013). Nutrient remediation rates in municipal wastewater and their effect on biochemical composition of the microalga Scenedesmus sp. AMDD. *Algal Research, 2(*2), 127-134. doi:http://dx.doi.org/10.1016/j.algal.2013.01.009

18. Zacharof, M.-P., Lovitt. R.W. (2014 b). The filtration characteristics of anaerobic digester effluents employing cross flow ceramic membrane microfiltration for nutrient recovery. *Desalination,* 341, 27-37.

19. Chen, C.-Y., Yeh, K.-L., Su, H.-M., Lo, Y.-C., Chen, W.-M., & Chang, J.-S. (2010). Strategies to enhance cell growth and achieve high-level oil production of a Chlorella vulgaris isolate. *Biotechnology Progress, 26*, 679-686. doi:10.1002/btpr.381

20. Xiao, Y., Zhang, J., Cui, J., Feng, Y., & Cui, Q. (2013). Metabolic profiles of *Nannochloropsis oceanica* IMET1 under nitrogen-deficiency stress. *Bioresource Technology, 130*, 731-738. doi:http://dx.doi.org/10.1016/j.biortech.2012.11.116

21. Mayers, J. J., Ekman Nilsson, A., Albers, E., & Flynn, K. J. (2017). Nutrients from anaerobic digestion effluents for cultivation of the microalga Nannochloropsis sp. — Impact on growth, biochemical composition and the potential for cost and environmental impact savings. *Algal Research, 26* (Supplement C), 275-286. doi:https://doi.org/10.1016/j.algal.2017.08.007

22. Guillard, R. R. L., & Ryther, J. H. (1962). Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Canadian Journal of Microbiology, 8*, 229-239.

23. http://www.variconaqua.com/products-services/algal-nutrients-and-culture-media/ last accessed 19.03.2019

24. Zacharof, M.P., Vouzelaud, C., Mandale, S.J., Lovitt, R.W. (2015). Valorization of spent anaerobic digester effluents through production of platform chemicals using *Clostridium butyricum*. *Biomass & Bioenergy 81*, 294-303

25. Silkina, A., Nelson, G.D., Bayliss, C.E. et al., (2017 b). Bioremediation efficacy—comparison of nutrient removal from an anaerobic digest waste-based medium by an algal consortium before and after cryopreservation. *Journal of Applied Phycology 29*, 1331-1341 doi:https://doi.org/10.1007/s10811-017-1066-x

26. Stephenson, A. L., Dennis, J. S., Howe, C. J., Scott, S. A., & Smith, A. G. (2010). Influence of nitrogen-limitation regime on the production by *Chlorella vulgaris* of lipids for biodiesel feedstocks. *Biofuels, 1*(1), 47-58. doi:10.4155/bfs.09.1

27. Safi, C., Charton, M., Pignolet, O., Silvestre, F., Vaca-Garcia, C., & Pontalier, P. Y. (2013). Influence of microalgae cell wall characteristics on protein extractability and determination of nitrogen-to-protein conversion factors. *Journal of Applied Phycology, 25*(2), 523-529.

28. Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of biological chemistry, 226*(1), 497-509.

29. Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry, 28*(3), 350-356.

30. European commission directive. In 98/15/EC, Official J. Eur. Commun. : 1998.

31. Bougaran, G., Bernard, O., & Sciandra, A. (2010). Modeling continuous cultures of microalgae colimited by nitrogen and phosphorus. *Journal of Theoretical Biology, 265*(3), 443-454. doi:http://dx.doi.org/10.1016/j.jtbi.2010.04.018

32. Gerardo, M. L., Oatley-Radcliffe, D. L., & Lovitt, R. W. (2014). Integration of membrane technology in microalgae biorefineries. *Journal of Membrane Science, 464*, 86-99. doi:http://dx.doi.org/10.1016/j.memsci.2014.04.010

33. Prajapati, S. K., Choudhary, P., Malik, A., & Vijay, V. K. (2014a). Algae mediated treatment and bioenergy generation process for handling liquid and solid waste from dairy cattle farm. *Bioresource Technology, 167*(Supplement C), 260-268. doi:https://doi.org/10.1016/j.biortech.2014.06.038

34. Prajapati, S. K., Malik, A., & Vijay, V. K. (2014b). Comparative evaluation of biomass production and bioenergy generation potential of Chlorella spp. through anaerobic digestion. *Applied Energy, 114(0)*, 790-797. doi:http://dx.doi.org/10.1016/j.apenergy.2013.08.021

35. Gerardo, M. L., Zacharof, M. P., & Lovitt, R. W. (2013). Strategies for the recovery of nutrients and metals from anaerobically digested dairy farm sludge using cross-flow microfiltration. *Water Research, 47*(14), 4833-4842. doi:http://dx.doi.org/10.1016/j.watres.2013.04.019

36. Koszel, M., & Lorencowicz, E. (2015). Agricultural Use of Biogas Digestate as a Replacement Fertilizers. *Agriculture and Agricultural Science Procedia, 7*, 119-124. doi:https://doi.org/10.1016/j.aaspro.2015.12.004

37. Samorì, G., Samorì, C., Guerrini, F., & Pistocchi, R. (2013). Growth and nitrogen removal capacity of *Desmodesmus communis* and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I. *Water Research, 47*(2), 791-801. doi:http://dx.doi.org/10.1016/j.watres.2012.11.006

38. Oldroyd, G. E. D., Dixon, R. (2014). Biotechnological solutions to the nitrogen problem. *Current Opinion in Biotechnology, 26*(Supplement C), 19-24. doi:https://doi.org/10.1016/j.copbio.2013.08.006

39. Collos, Y., & Harrison, P. J. (2014). Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Marine Pollution Bulletin, 80*(1–2), 8-23. doi:http://dx.doi.org/10.1016/j.marpolbul.2014.01.006

40. Ji, F., Zhou, Y., Pang, A., Ning, L., Rodgers, K., Liu, Y., & Dong, R. (2015). Fed-batch cultivation of Desmodesmus sp. in anaerobic digestion wastewater for improved nutrient removal and biodiesel production. *Bioresource Technology, 184*(0), 116-122. doi:http://dx.doi.org/10.1016/j.biortech.2014.09.144

41. Pires, J. C. M. (2017). COP21: The algae opportunity? *Renewable and Sustainable Energy Reviews, 79*(Supplement C), 867-877. doi:https://doi.org/10.1016/j.rser.2017.05.197

42. Ayre, J. M., Moheimani, N. R. , Borowitzka, M.A.: Growth of microalgae on undiluted anaerobic digestate of piggery effluent with high ammonium concentrations. *Algal Research 24*, 218-226 (2017). doi:https://doi.org/10.1016/j.algal.2017.03.023.

43. Prajapati, S.K.,Kumar,P., Malik, A, Vijay, V.K.(2014c) Bioconversion of algae to methane and subsequent utilization of digestate for algae cultivation: A closed loop bioenergy generation process. *Bioresource Technology 158*, 174-180 (2014). doi:https://doi.org/10.1016/j.biortech.2014.02.023.

44. Pivato, A., Vanin, S., Raga, R., Lavagnolo, M. C., Barausse, A., Rieple, A., Cossu, R. (2016). Use of digestate from a decentralized on-farm biogas plant as fertilizer in soils: An ecotoxicological study for future indicators in risk and life cycle assessment. *Waste Management*, 49(Supplement C), 378-389. doi:https://doi.org/10.1016/j.wasman.2015.12.009

45. Prajapati, S.K.,Kumar,P.,. Malik, A, Vijay, V.K (2017) Cultivation of native algal consortium in semi-continuous pilot scale raceway pond for greywater treatment coupled with potential methane production,. *Journal of Environmental Chemical Engineering 5*, 5581-5587. doi:https://doi.org/10.1016/j.jece.2017.10.044.

46. P. Kumar, S.K. Prajapati, A. Malik, et al., (2019) Evaluation of biomethane potential of waste algal biomass collected from eutrophied lake: effect of source of inocula, co-substrate, and VS loading. *Journal of Applied Phycology 31*, 533-545 . doi:https://doi.org/10.1007/s10811-018-1585-0

47. Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., & Tredici, M. R. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering, 102*(1), 100-112. doi:10.1002/bit.22033

48. Uggetti,E., Sialve,B.,Latrille,E., Steyer, J.-P.: Anaerobic digestate as substrate for microalgae culture: The role of ammonium concentration on the microalgae productivity. Bioresource Technology 152437-443 (2014). doi:https://doi.org/10.1016/j.biortech.2013.11.036

49. Zhou WenGuang, R. R. (2014). Biological mitigation of carbon dioxide via microalgae : Recent development and future direction. *Scientia Sinica Chimica, 44*(1), 63-78. doi:10.1360/032013-256

50. Van Den Hende, S., Carré, E., Cocaud, E., Beelen, V., Boon, N., & Vervaeren, H. (2014). Treatment of industrial wastewaters by microalgal bacterial flocs in sequencing batch reactors. *Bioresource Technology, 161*(0), 245-254. doi:http://dx.doi.org/10.1016/j.biortech.2014.03.057

51. Rothlisberger-Lewis, K. L., Foster, J. L., & Hons, F. M. (2016). Soil carbon and nitrogen dynamics as affected by lipid-extracted algae application. *Geoderma, 262* (Supplement C), 140-146. doi:https://doi.org/10.1016/j.geoderma.2015.08.018

52. Mayhead, E., Silkina, A., Llewellyn, C.A., Fuentes-Grünewald, C. (2018) Comparing Nutrient Removal from Membrane Filtered and Unfiltered Domestic Wastewater Using *Chlorella vulgaris*. *Biology , 7,* 12 https://doi.org/10.3390/biology7010012

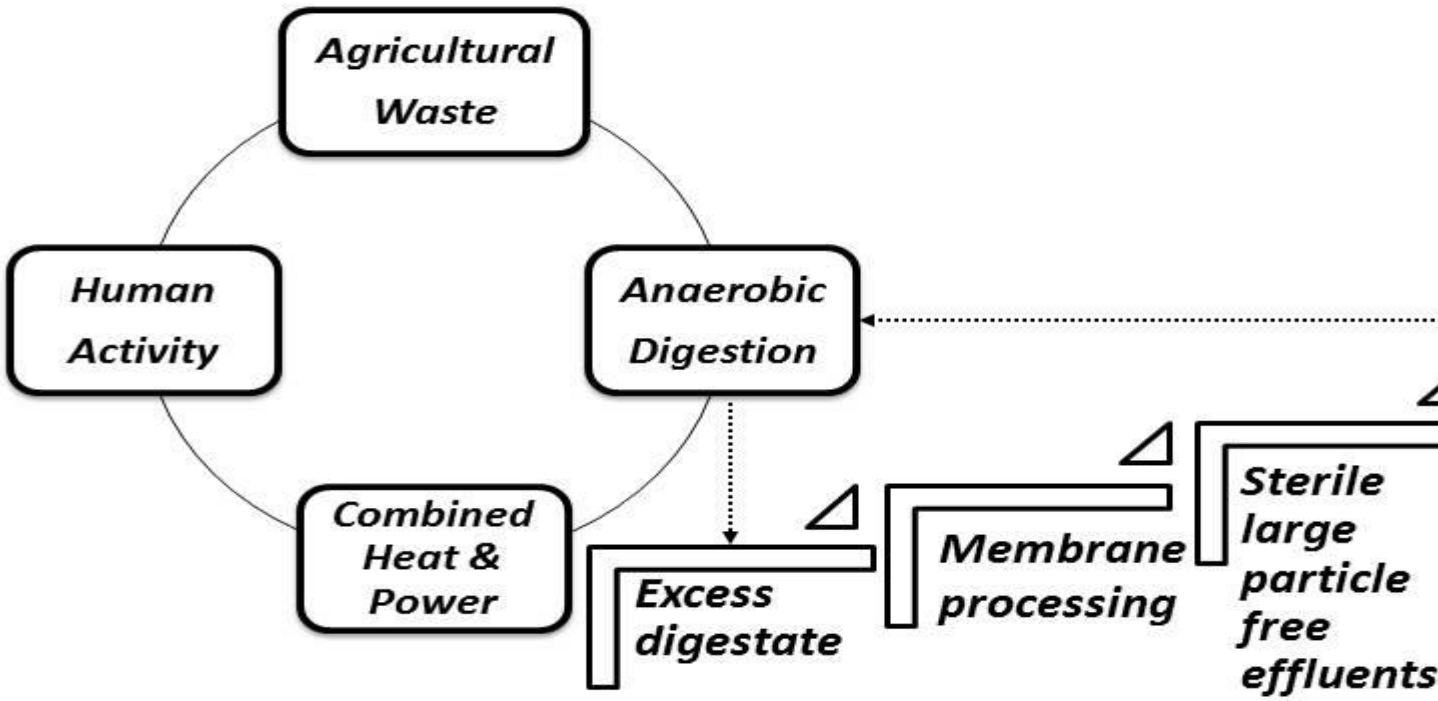

Fig.1.Waste based biorefinery process integration with current systems of waste treatment i.e. anaerobic digestion

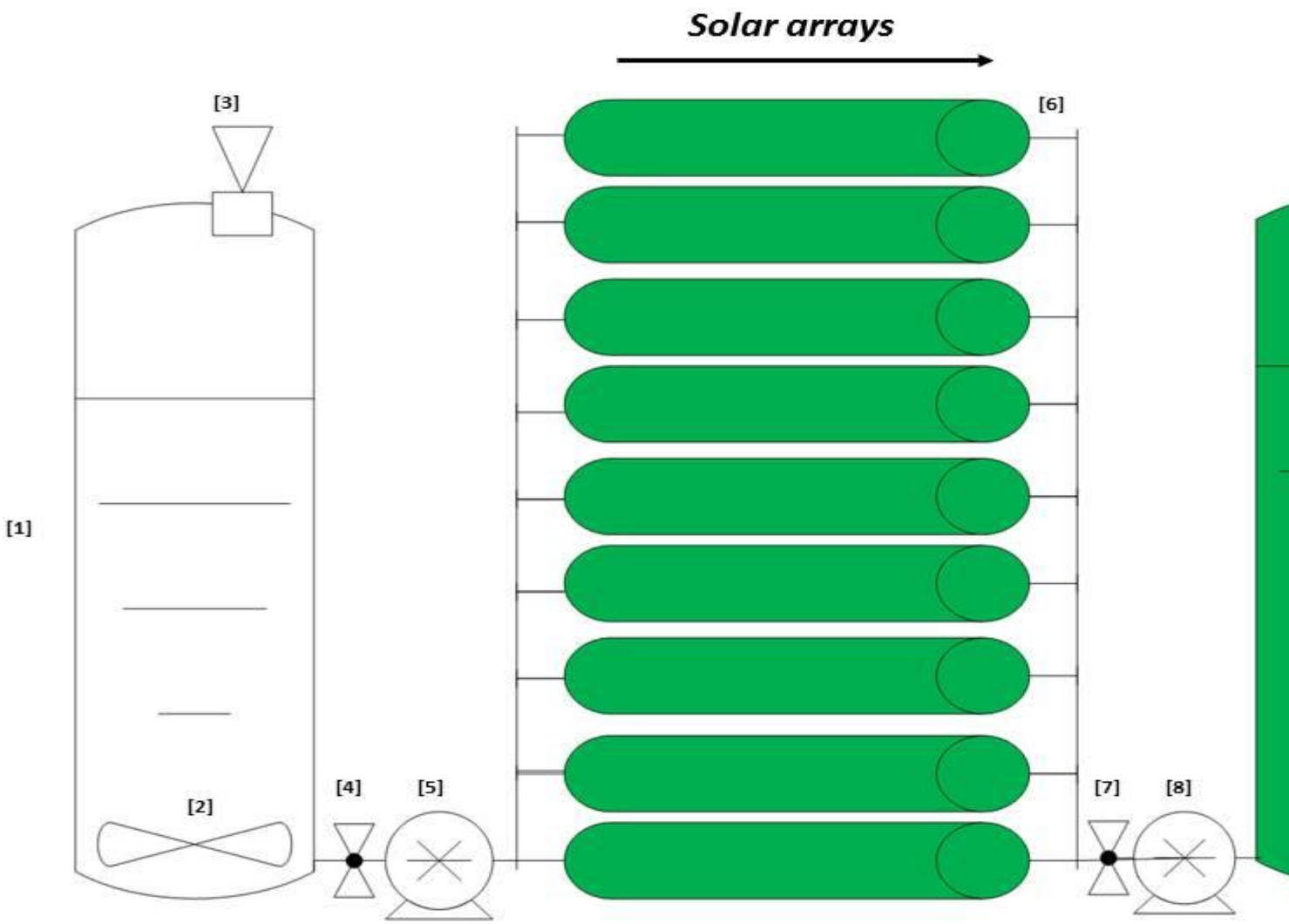
Solar arrays
[1]
[2]
[3]
[4]
[5]
[6]
[7]
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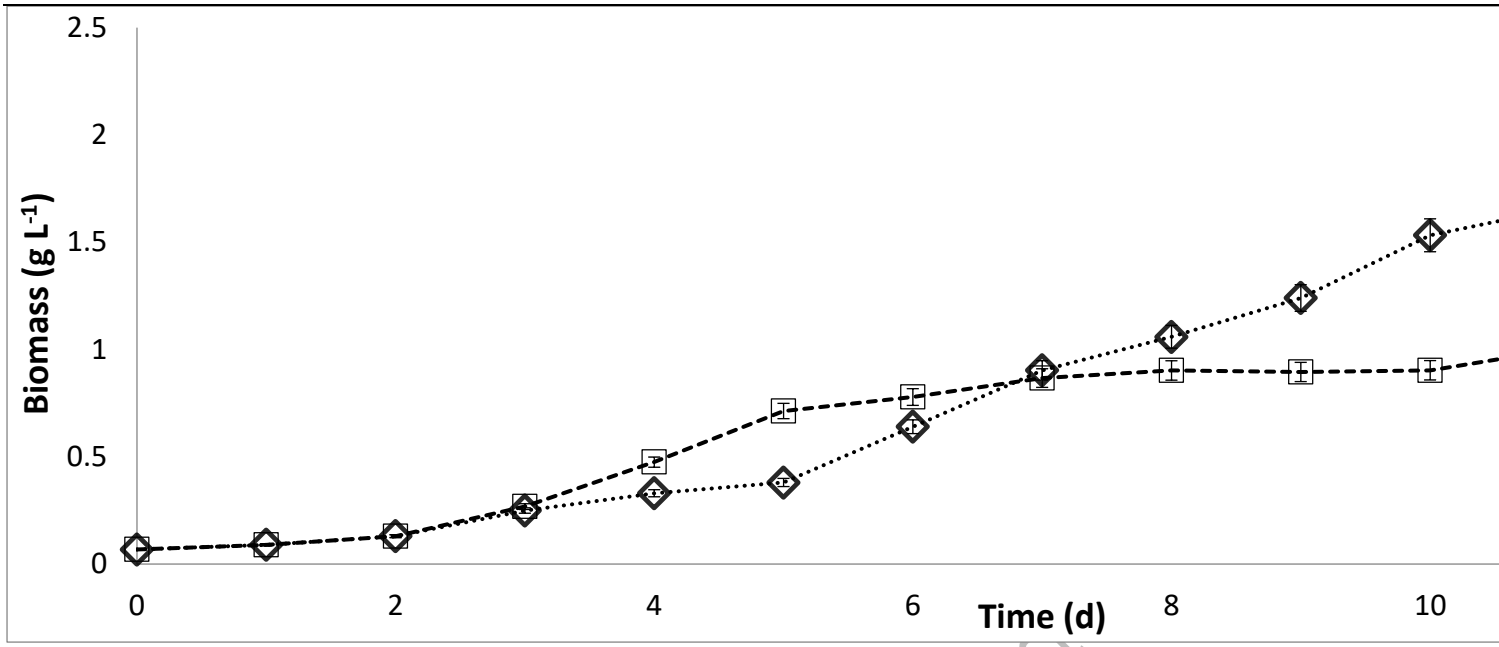
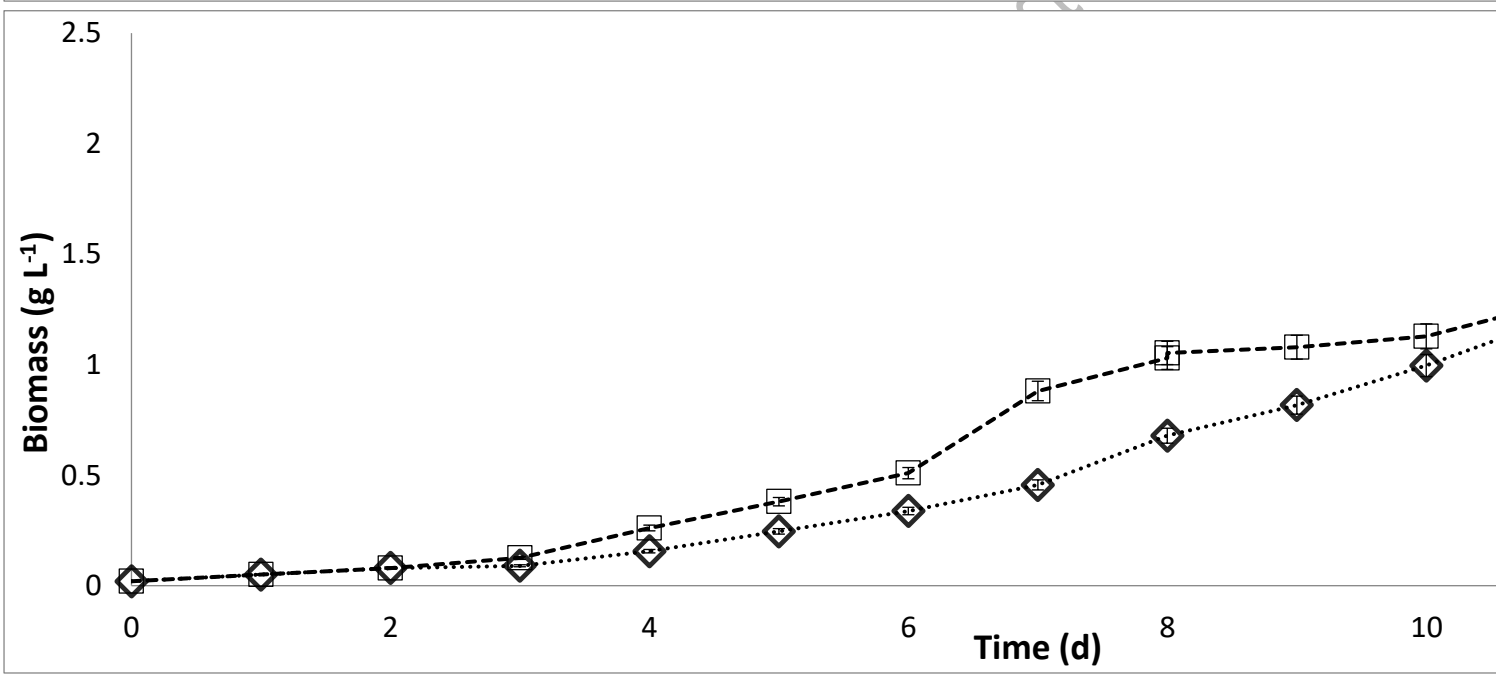
Fig.2. Simplified design of the photobioreactor (PHB) algal culturing system [1] Feed vessel [2] mixing blades [3] propagation p
displacement pump [6] culturing glass cylinders [7] diaphragm valve [8] positive displacement pump [9] harvesting vessel [10]
inlet (Biofence from Varicon Aqua Solution Ltd, http://www.variconaqua.com/)


2.5
2
1.5
1
0.5
0
Biomass (g L-1)
0
2
4
6
8
10
Time (d)


2.5
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Biomass (g L-1)
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Time (d)

Fig. 3. Growth of *N.oceanica* (A) and *S. quadricuada* (B) on in vitro standardised liquid media (f/2) (◇). treated digested agricu

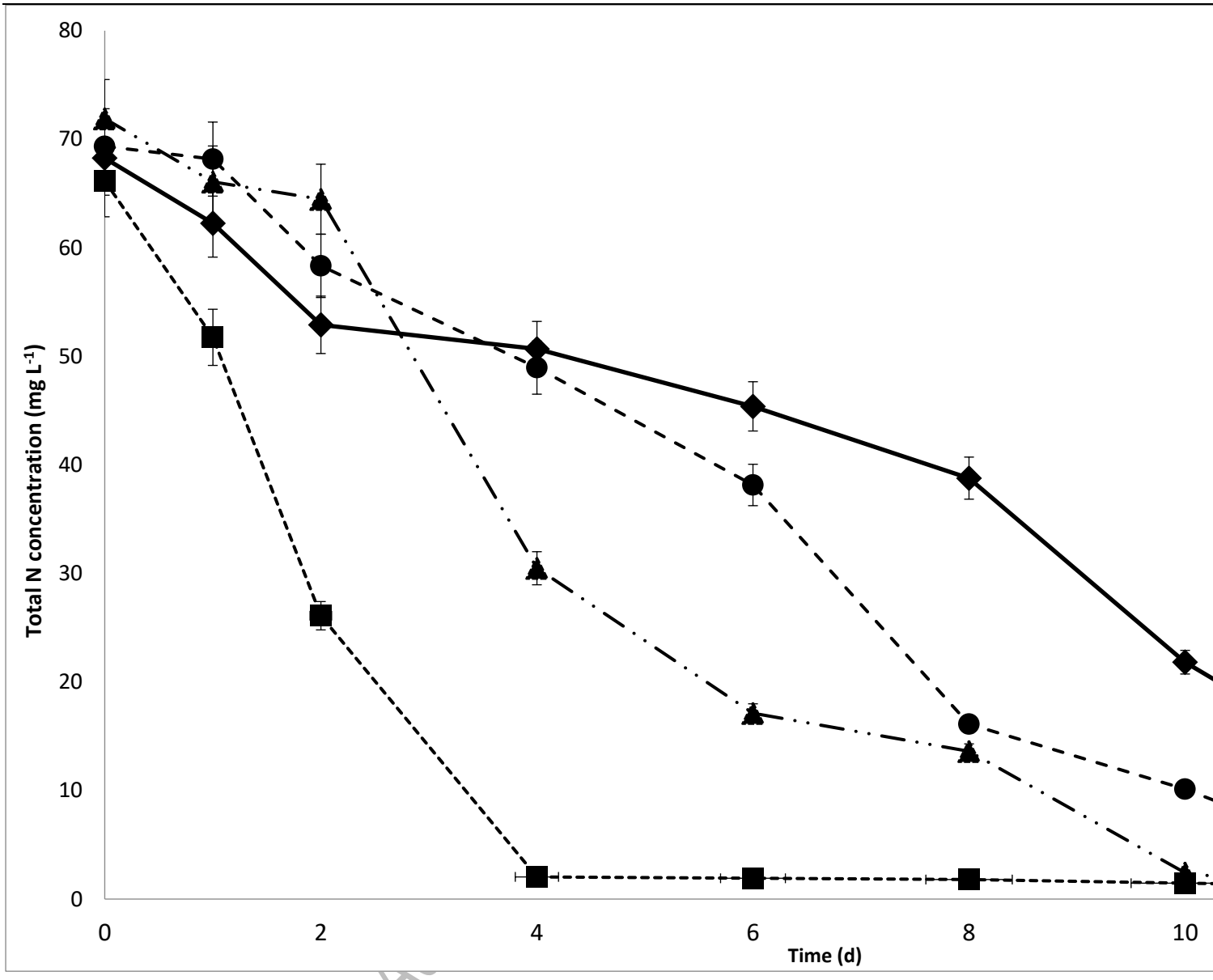


Fig. 4. Total N (TDN, TON and Nitrates) consumption by *Nannochloropsis oceanica* in vitro standardised liquid media (f/2) (◆) N:P 14.22 (balanced) (■), and *S. quadricuada* grown in vitro standardised liquid media (f/2) (▲) and on treated digested agric

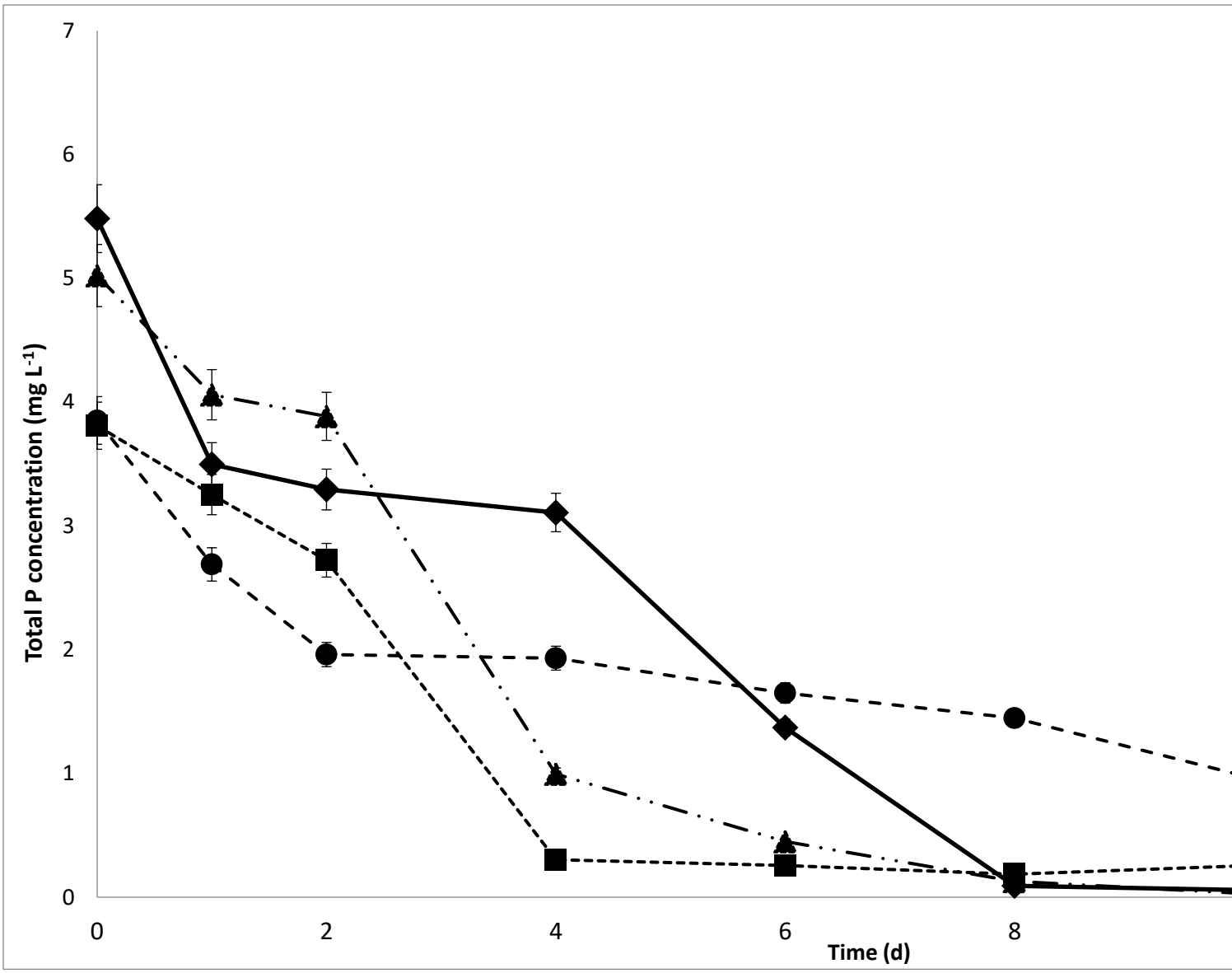
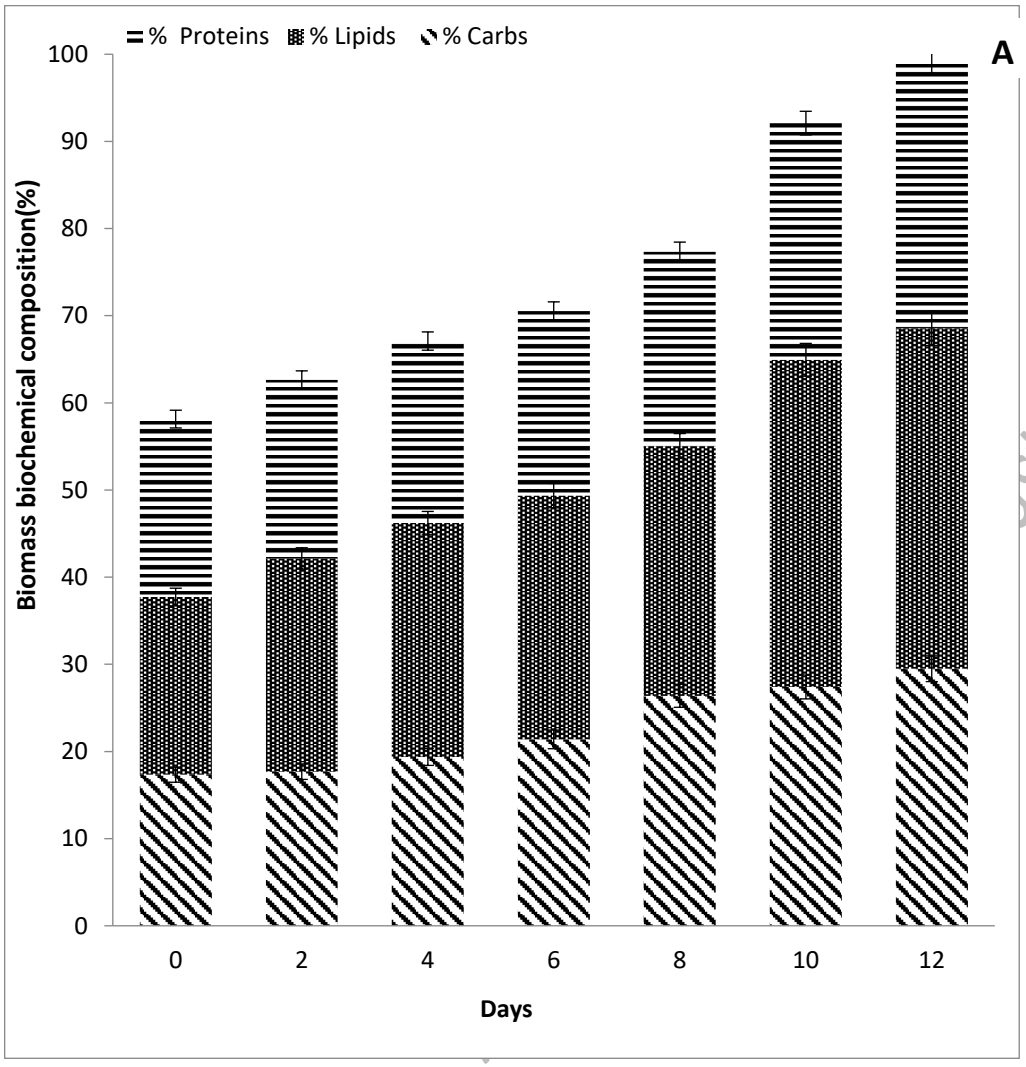

Total P concentration (mg L-1)
7
6
5
4
3
2
1
0
0
2
4
6
8
Time (d)

Fig. 5 Total P consumption by *Nannochloropsis oceanica* in vitro standardised liquid media (f/2) (◆) & on treated digested agr
(■), and *S. quadricuada* grown in vitro standardised liquid media (f/2) (▲) and on treated digested agricultural wastewater N



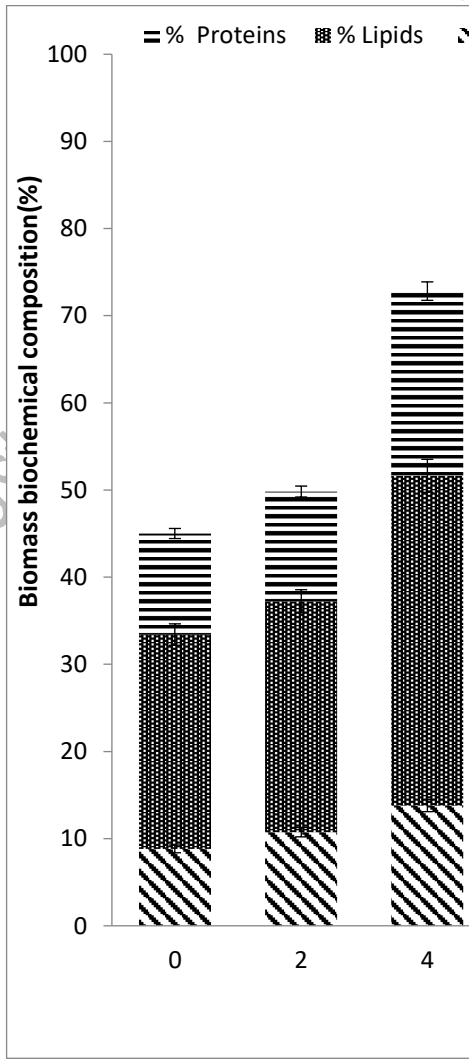
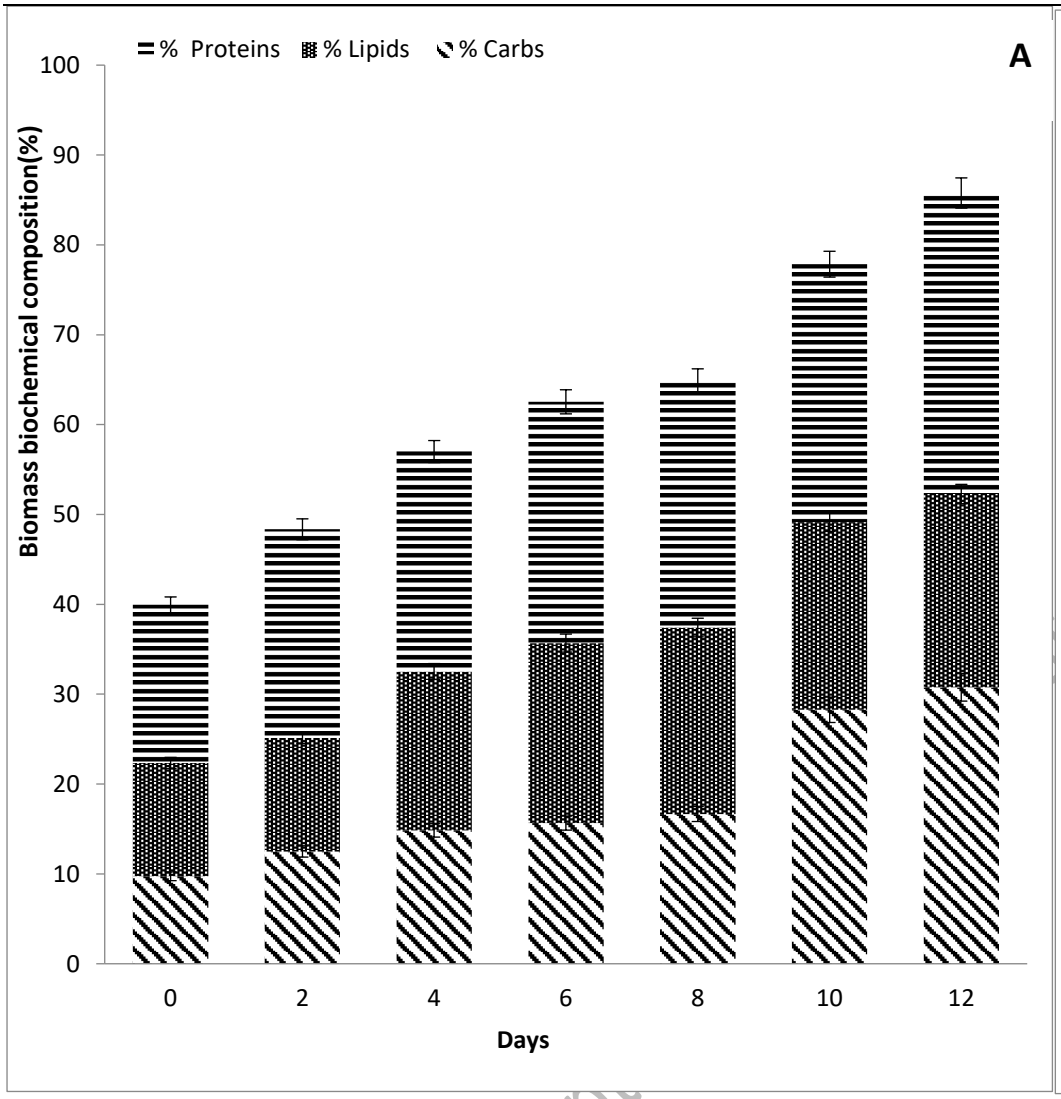


Fig.6. Biochemical composition of *Nannochloropsis oceanica* biomass in vitro standardised liquid media (f/2) (A) & on treated
(balanced) (B)



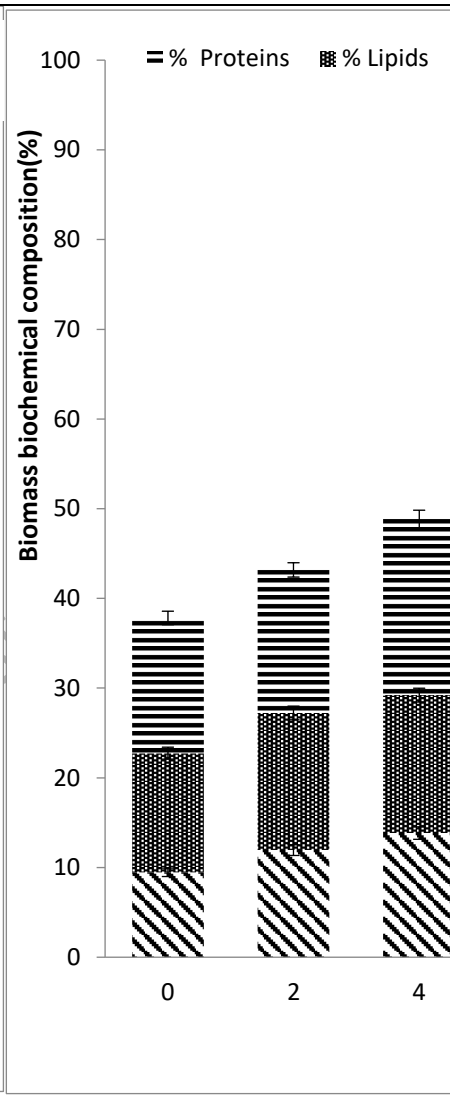


Fig.7 Biochemical composition of *S. quadricuada* biomass in vitro standardised liquid media (f/2) (A) & on treated digested ag

Parameters	**Treated digested agricultu** **(0.2 µm) permeate used as**

	N:P 14.22
Total Solids (TS, g L^{-1})	10.2
Total Suspended Solids (TSS, g L^{-1})	0.21
Total Dissolved Solids (TDS, g L^{-1})	4.38
Conductivity (mS cm^{-1})	5.16
Alkalinity (equivalent to mg $CaCO_3$ L^{-1})	2500
Optical Density (580 nm)	0.11
pH	8.50
Zeta potential (mV)	-29.60
Sizing (µm)	5.70
	Concentration g L^{-1}
Acetic Acid	0.64
Butyric Acid	0.70
Metal ions (Ca,Cu, Co,Fe, Pb, Mg, Mn, Zn,K, As)	0.59
Ammonia	0.36
Phosphate	0.03

Table 1: Physical characteristics and chemical composition of the pretreated anaerobically digested agricultural slu

Algal strain	Growth media	Dry weight ($g\ L^{-1}$)	Growth rate (μ_{max}, D^{-1})	Biomass production rate ($g\ L^{-1}D^{-1}$)	Algal Carbon (C, DWC $g\ L^{-1}$)	Algal Nitrogen (N, DWC g ^{1})
	Standardised growth media (f/2)	1.73	0.51	0.12	0.31	0.04
	***Treated digested agricultural wastewater* N:P 14.22**	1.56	0.50	0.11	0.34	0.02
N. oceanica						
	Standardised growth media (f/2)	2.03	0.48	0.11	0.28	0.04
S. quadricauda	***Treated digested agricultural wastewater* N:P 14.22**	1.13	0.53	0.09	0.18	0.04

Table 2: Comparison of the effect on *N.oceanica* and *S. quadricauda* growth, biomass composition, nutrients uptak
media and on standardised media

Algal strain	Growth media	Calorific value	Energy
		Kcal g^{-1}	**kJ g^{-1}**
	Standardised growth media (f/2)	5151	21.56
N. oceanica	***Treated digested agricultural wastewater 14.22***	7496	31.38
	Standardised growth media (f/2)	-	-
S.quadricauda	***Treated digested agricultural wastewater N:P 14.22***	5013	20.98

Table 3: Practical results and theoritical calculations of energy potential and biomethane production