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Investigation of *Desmodesmus* sp. Growth in Photobioreactor using Vinasse as a Carbon Source

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Renewable source biofuels are a promising alternative to fossil fuel and can be economic and environmentally viable when associated with effluent gas and wastewater treatment. This work proposes the study of lipid accumulation and microalgae cultivation using vinasse which is a residue from sugarcane bioethanol production. Besides being a largely produced effluent that requires bioremediation, vinasse is a carbon and nutrient rich source that could be advantageous as microalgae cultivation medium. Therefore, the objective of this research is to cultivate the microalgae strain *Desmodesmus* sp. in different types of undiluted vinasse medium (raw, raw recycled and biodigested) in a photobioreactor system. Cultivation was performed under different conditions varying: I) bubble agitation; II) temperature; III) light incidence; IV) flux of atmospheric air supplemented with CO_2. Biomass concentration was measured by cell density, lipid accumulation was measured gravimetrically after solvent extraction, and vinasse biochemical composition was determined using TOC-VCSN Shimadzu® analyzer. The levels of dissolved O_2 and CO_2 were monitored using O_2 and CO_2 electrodes, respectively. Raw vinasse with relative biomass concentration of 1.5 g L^{-1} showed to be preferred medium for high lipid accumulations and carbon consumption. The maximum lipid content of 24.48% was obtained under heterotrophic, aerobic (0.05 VVM) growth conditions, at a temperature of 26 ˚C, without magnetic agitation and CO_2 presence. However, for effluent treatment, better results were obtained under mixotrophic, aerobic (0.05 VVM) and bubble agitated growth conditions, at 26 ˚C of temperature. Recorded carbon reduction was 46.35%. As vinasse consists of an organic carbon and nutrient source, cultivation cultures are susceptible to contamination by other microorganisms. Therefore, further study is necessary to the use of *Desmodesmus* sp. microalga for either biodiesel production or bioremediation.

1. Introduction

Microalgae have been considered as a promising feedstock for biodiesel production due to several advantages comparing to conventional crops such as soybean and palm oil (Georgianna and Mayfield 2012). They have higher growth rate, can accumulate fuel precursor's oils of good quality, do not require extensive areas and arable land for cultivation, are efficient CO_2 sequesters, among others (Katiyar et al. 2017). In addition to bioenergy, microalgal biomass is also applicable in food and pharmaceutical industries once it allows to obtain proteins, carbohydrates, and lipids that may be feedstock for such industries. To produce large-scale sustainable microalgae products, however, it is still necessary to develop technologies that guarantee its economic viability (Moheimani et al. 2015). Because growth systems require large quantities of water, one alternative for sustainable microalgae cultivation is to use an industrial or domestic residue as a culture media, such as wastewater. Several microalgae species have been efficiently applied in wastewater and effluent treatment, once they assimilate nutrients from the medium (Samorì et al. 2013).

A potential effluent that is both a source of nutrients (nitrogen, phosphorous, and potassium) and organic matter is vinasse. It is a residue generated after the fractional distillation of the wine from sugarcane juice fermentation, in the sugar and ethanol industry. For each litter of ethanol, 10 to 15 litters of vinasse are produced (Cortes-Rodríguez et al. 2018), which is mainly applied to fertirrigation of sugarcane crops. However, studies suggest that this application poses adverse impacts due to possible soil and subterranean

water pollution. Soil salinization was associated with vinasse probably due to high levels of total dissolved solids and conductivity (Fuess, Rodrigues, and Garcia 2017). Currently, the primary treatment for vinasse is biodigestion, a process that can aggregate value by producing biogas. Despite that, it does not eliminate the environmental impact obstacle, once the effluent still contains high quantity of inorganic nutrients (Candido and Lombardi, 2017). Therefore, microalgae are a possible and interesting solution as they could uptake nutrients and organic matter, potentially reducing oxygen biochemical and chemical demands, odor, conductivity, among other harmful parameters for soils and water environments.

Different microalgae species have been tested for cultivation in vinasse, showing biomass production improvements. Santana et al. (2017) obtained higher biomass productivities using clarified vinasse (164.44 and 222.22 mg L^{-1} d^{-1}) or 50% diluted vinasse (177.78 and 182.22 mg L^{-1} d^{-1}) in comparison with Bold's Basal Medium (BBM) (101.11 and 132.22 mg L^{-1} d^{-1}), for *Micractinium* sp. and *Chlamydomonas biconvexa*, respectively, cultivated in 15 L airlift flat plate photobioreactors. Candido and Lombardi (2017) showed that *Chlorella vulgaris* could grow in 60% conventional and 80% biodigested vinasse solutions in 250-mL flasks, resulting in high specific growth rate (1.2 g day^{-1}), competitively with the synthetic medium. Mattos and Bastos (2016) evaluated *Desmodesmus* sp. growth in sugarcane vinasse, obtaining 52.1% nitrogen removal and 36.2% chemical oxygen demand reduction. Other studies have approached microalgal growth in vinasse (Altenhofen da Silva et al. 2017; dos Santos et al. 2016; Olguín et al. 2015; Ramirez et al. 2014), thus confirming this is a potentially sustainable medium. Amongst those, Ramirez et al. (2014) were able to cultivate microalga *Scenedesmus* sp. at concentrations up to 40% vinasse in culture medium. Olguín et al. (2015) cultivated *Neochloris oleoabundans* in anaerobically digested vinasse (AEV), obtaining 62% cell density increase and 85.2% ammonium-nitrogen removal using 6% AEV and sodium bicarbonate, compared to BBM.

In this context, this work proposes the use of vinasse (raw and biodigested) as a carbon concentration culture medium, for microalgae *Desmodesmus* sp. cultivation in a closed system, aiming lipid accumulation for biodiesel production.

2. Methodology

2.1 Microalgae inoculum

Desmodesmus sp. strain was donated by the Aquatic Organisms Research Laboratory (LAPOA) from the Integrated Aquiculture and Ambiental Studies Group (GIA) at the Federal University of Paraná (UFPR) in Curitiba/PR. It was maintained in 100 mL tubes with BG-11 medium (Rippka et al., 1979), under continuous light (approximately 62 µmol $m^{-2}s^{-1}$). For the photobioreactor cultivation, the inoculum was previously transferred to a 250 mL Erlenmeyer and agitated through an orbital shaker (SOLAB SL-180/A) continuously operated for 2-3 weeks. All media for inoculum were sterilized in an autoclave (Phoenix Luferco® AV-50 Plus) and culture manipulation before inculcation in photobioreactor was performed in a laminar flux chamber (Pachane® Pa50) to avoid contamination.

2.2 Culture medium: vinasse

Vinasse (both raw and biodigested) was donated by the Brazilian Bioethanol Science and Technology Laboratory (CTBE) and maintained refrigerated between 4 and 8 °C. The medium pretreatment consisted in to remove suspended solid particles by centrifugation (Eppendorf 5810R), for 20 min, 18 °C, and 5000 rpm. For all cultivations, pH was adjusted to 7.5 with a NaOH 6.5 M solution. When air and/or CO_2 were added to the system, pH was placed between 7.5 and 8.0; and between 7.0 and 7.5 without CO_2 addition to account for CO_2 acidity in an aqueous medium.

2.3 Cultivation in flat-plate photobioreactor

Cultivation was performed in a flat-plate vertical photobioreactor (FMT-150/1000 Photons Systems Instruments®), varying injection of CO_2 and atmospheric air. For a total volume of 800 mL, cultures containing 20% inoculum in vinasse were added to the photobioreactor, with a headspace volume of 400 mL. All experiments (Table 1) were conducted from 4 to 11 days, varying parameters such as light, temperature, agitation, air injection, and vinasse type.

During the nine cultivations, dissolved gases, temperature, pH, cell density, dry biomass, and nutrients (carbon and nitrogen) present in the substrate were monitored. A rotameter controlled the gas injection. Aliquots were taken daily to evaluate biomass growth, monitoring cell density, using an optical microscope (Olympus® CX21) and Neubauer chamber. Also, total carbon (TC) and total nitrogen (TN) concentrations were determined using a total organic carbon analyzer (TOC-VCSN Shimadzu®). After cultivation, total lipid was quantified by Bligh and Dyer (1959) method.

Table 1: Growth conditions of Desmodesmus sp. *cultivation in a flat-plate photobioreactor.*

Parameters/ Cultivation	Period (days)	Vinasse	Light	Temperature (°C)	Agitation	Contamination	Air flow (vvm)	CO_2 flow (%/vvm)
A	11	Raw	No	26	No	Bacteria	0.5	0
B	10	Raw	Yes	25	Yes (bubbling)	Fungi	0.5	0
C	4	Recicled (B)	No	25	Yes (bubbling)	Bacteria	0.5	0
D	7	Recicled (C)	No	25	Yes (bubbling)	Bacteria	0.5	0
E	7	Recicled (D) 50% diluted	Yes	25	No	Fungi	0.5	0
F	7	Raw	No	20	No	Bacteria	0.5	0
G	10	Raw	Yes	21	Yes (bubbling)	Fungi	0.25	10
H	9	Recicled (F) + 1.28 g K_2HPO_4	Yes 12:12h light:dark	21	Yes (bubbling)	Bacteria	1	5
I	10	Biodigested	No	21	Yes (bubbling)	Fungi	0.5	0

3. Results and discussion

During all cultivations the pH showed no significant changes, remaining at approximately 8.0 and between 6.0 and 8.0 without and with the CO_2 injection, respectively. These values indicate the buffer characteristic of vinasse, which interfered in the dissolution of CO_2 since HCO_3 ions are generated (Nguyen and Rittmann, 2016). In turn, this was not observed during the injection of air, once the dissolution is not governed by a chemical reaction, but by mass transfer and oxygen solubility in water (Lee, 2017). The *Desmodesmus* sp. strain was cultivated in various vinasse types under different conditions (Table 1) to evaluate the effect of cultivation conditions upon microalgae growth (Figure 1), nutrients consumption (Figure 2) and lipid content (Figure 3). As can be seen from Table 1 cultivations were performed under mixotrophic (B, E, G, and H) or heterotrophic (A, C, D, F, and I) conditions. At this point is worthwhile mentioning that microalgae growth, presented as algal cell density, was periodically measured up to some interference such as contamination appeared. It was observed the presence of fungi and high concentration of bacteria, which compromised the further analysis of the growth curves.

Comparing to heterotrophic, mixotrophic conditions using raw vinasse show to be a more favorable way of *Desmodesmus* sp. cultivation (Figure 1). While heterotrophic cultures (A and F) showed practically constant cell density throughout the cultivations, mixotrophic cultures (B and G) presented an increase in cell density. Further, increasing temperature and air injection did not lead to significant accumulation of biomass (cell density of B cultures was higher compared to G). This means that low temperature kept increase dissolving of air-CO_2 thus favoring the photosynthesis. However, carbon consumption in culture B was higher probably because: a) presence of contaminations or; b) of the higher carbon decomposition rate caused by higher temperature), as depicted in Figure 2. The same trend was observed in heterotrophic cultures (A and F).

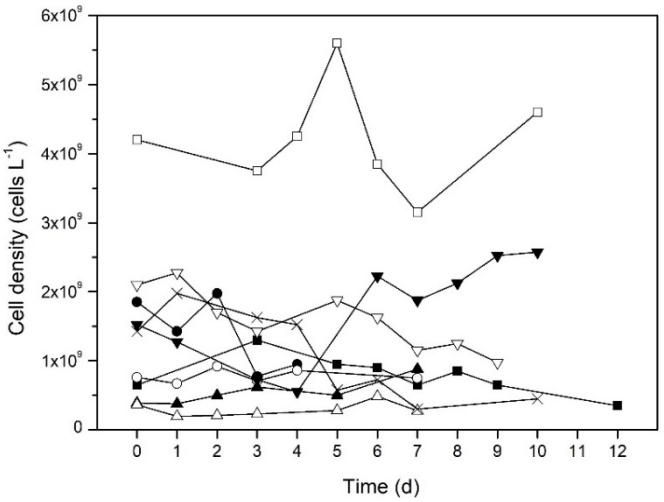

Figure 1: Cell density of Desmodesmus sp. *growth in vinasse, monitored using an optical microscope, for cultivations: A (■), B (□), C (●), D (○), E (▲), F (Δ), G (▼), H (∇), and I (×).*

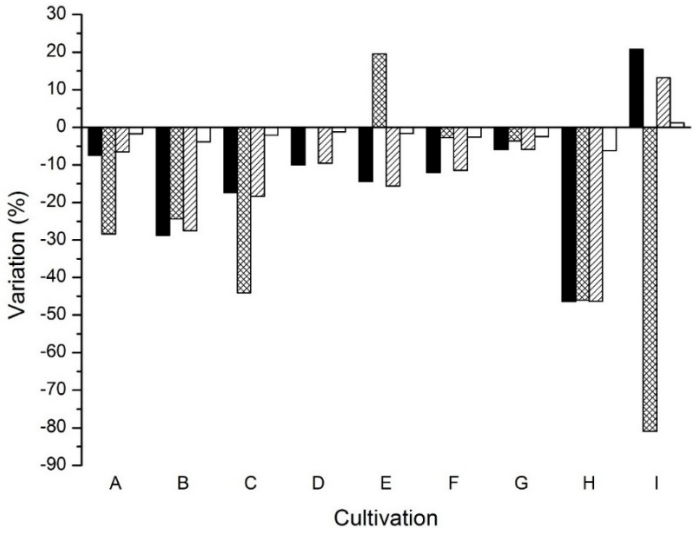

Figure 2: Percent reduction of total organic carbon (■), inorganic carbon (▩), total carbon (▨), and total nitrogen (□) in vinasse medium.

Due to the low nutrient consumption, cultures with raw vinasse recycled once (C and H), twice (D) and three times (E) were used for cultivation of *Desmodesmus* sp. Due to the depletion of nutrients, the recycling was limited at twice (as can be concluded from the results of cultivation E) and recycling gave better results with add-in nutrients, such as of K_2HPO_4 (culture H). Heterotrophic conditions (C and D cultivations) favored higher cell density using raw recycled vinasse, but the highest cell density value was obtained for mixotrophic (H) culture because of phosphate supplementation (Figure 1). According to Prokop, Bajpa, and Zappi (2014), phosphate is an essential nutrient for cell growth, and consequently, carbon consumption. Although higher cell density was obtained under heterotrophic conditions, carbon reduction was more drastic in the mixotrophic cultures (Figure 2). Which is due to initially higher cell density (culture C) and presence of phosphate supplementation (culture H).

In cultivation with biodigested vinasse (I), there was a decrease in the final cell density (three times less than in the initial) and an increase in the content of carbon. The decrease in cell density was the consequence of cell death and lysis due to the presence of the harmful components in the biodigested vinasse, mainly relative to the concentration of phenolic compounds (Candido and Lombardi, 2017) and presence of contaminats which resulted in nutrient shortages. On the other hand, cell death and lysis lead in an increase in carbon content (Kan and Pan, 2010).

Further studies aimed to test the potential of *Desmodesmus* sp, cultivated on various types of vinasse, as an alternative source of oil (lipids). Also, biomass's results were explained concerning algal biomass productivity rather than cell density or biomass concentration, since it is of commercial importance. As lipid and biomass data are interconnected when it comes to economic viability, it is plausible to analyze it concomitantly (Figure 3).

As observed in Figure 3, for raw vinasse cultures the highest lipid content (approximately 24%, cultures A, and F) and highest biomass productivity (culture F) were obtained using heterotrophic conditions. Among nutrients, nitrogen is of most importance for biomass production (Prokop, Bajpa and Zappi, 2014) and lipid content (Rios et al., 2015). Obtained highest lipid content was due to lowest TN reduction. As observed in Figure 2, there was a low consumption of nitrogen in all raw vinasse cultures, leading the accumulation of lipids.

In the analysis of recycled raw vinasse, the highest lipid content and biomass production were also obtained for heterotrophic conditions in once recycled vinasse (culture C). Although high biomass production and lipid content could also be noticed in mixotrophic cultivation (culture H), these increases were due to the addition of phosphate and increased CO_2 flux. Also, recycled cultivation results indicate better total carbon and nitrogen contents reduction (up to 46%).

Despite the decrease in cell density, biodigested vinasse showed to be preferable for biomass production rather than lipid content. However, the most significant difference comparing to other cultivations was the increase of both TC and TN content. In this case, TN increasing had no relation with lipid accumulation, but preferably with cell death and lysis.

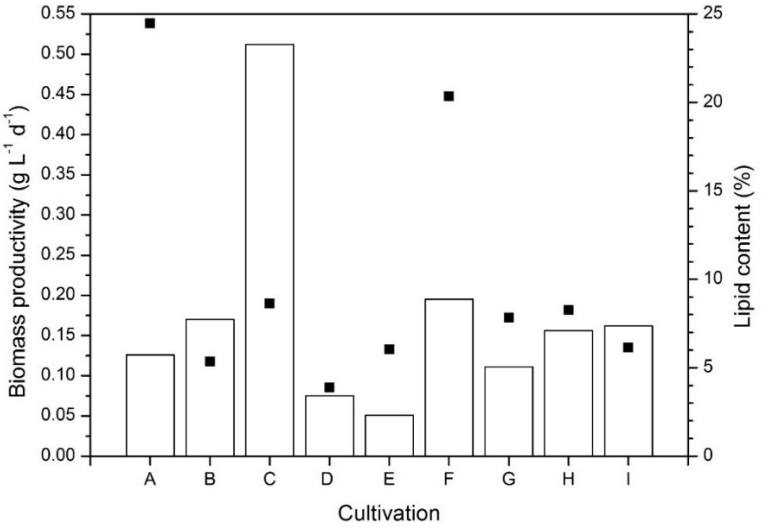

Figure 3: Biomass productivity (▭) and lipid content (■) for Desmodesmus sp*. cultivated in different types of vinasse.*

The lack of studies involving *Desmodesmus* sp. cultivation integrated to effluent treatment makes it difficult to compare with other's results. Santana et al. (2017) obtained lower biomass productivities than in this work, however, using different microalgae species, cultivated in 100% clarified and 50% diluted vinasse. Also, lower TOC reduction (around 8%) in comparison to the 14% obtained on average. Mattos and Bastos (2016) achieved higher nitrogen consumption in a 30-h batch cultivation of *Desmodesmus* sp in vinasse., but an initial biomass concentration of 1 g L^{-1}. Moreover, bacteria growth was also observed, with an approximate exponential behavior. Therefore, microalgae cultivation in vinasse is dependent of various experimental conditions and can be strongly influenced by contamination.

4. Conclusions and final remarks

Microalgae growth in a sustainable medium still faces challenges for scale-up and, mainly, to avoid contamination with other microorganisms. The presented study evaluated the possibility of growing microalga *Desmodesmus* sp. in a closed system, using vinasse as carbon and nutrient source. It was investigated the use of raw, recycled and biodigested vinasses. Results showed that under heterotrophic conditions, recycled raw vinasse and raw vinasse were the most suitable media for biomass production and lipid content, respectively. However, for effluent treatment, mixotrophic culture in once recycled raw vinasse with nutrient supplementation (phosphate) was more suitable.

Due to contamination with other microorganisms (fungi and bacteria), it was not possible to conduct all the experiments during the same period. Despite that, promising results aiming microalgae biomass production were obtained, and bioremediation analyzed through carbon and nitrogen reductions. Recycling raw vinasse was proved to be a potential step, and biodigested vinasse presents a harsh environment for this microalga growth. However, further study is necessary to make microalgae growth in vinasse feasible to be applied in large scale processes.

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References

Bligh E.G., Dyer W.J., 1959, A rapid method of total lipid extraction and purification, Canadian Journal of Biochemistry and Physiology, 37, 8, 911-917, DOI: 10.1139/o59-099.

Candido C., Lombardi, A. T., 2017, Growth of *Chlorella vulgaris* in treated conventional and biodigested vinasses, Journal of Applied Phycology, 29, 45-53, DOI: 10.1007/s10811-016-0940-2.

Cortes-Rodríguez E.F., Fukushima N.A., Palacios-Bereche R., Ensinas A.V., Nebra S.A., 2018, Vinasse concentration and juice evaporation system integrated to the conventional ethanol production process from sugarcane – Heat integration and impacts in cogeneration system, Renewable Energy, 115, 474-488, DOI: 10.1016/j.renene.2017.08.036.

Da Silva M.A., Barbosa G.H., Codato C.B., De Mattos L.F., Bastos R.G., Kieckbusch T.G., 2017. Heterotrophic growth of green microalgae *Desmodesmus subspicatus* in ethanol distillation wastewater (vinasse) and lipid extraction with supercritical CO_2, Journal of Chemical Technology & Biotechnology 92 (3), 573–79, DOI: 10.1002/jctb.5035.

Dos Santos R. R., Araújo O.Q.F., De Medeiros J.L., Chaloub, R.M., 2016, Cultivation of *Spirulina maxima* in medium supplemented with sugarcane vinasse, Bioresource Technology, 204, 38–48, DOI: 10.1016/j.biortech.2015.12.077.

Fuess L.T., Rodrigues I.J., Garcia M.L., 2017, Fertirrigation with sugarcane vinasse: Foreseeing potential impacts on soil and water resources through vinasse characterization, Journal of Environmental Science and Health. Part A, Toxic/hazardous Substances and Environmental Engineering, 52(11), 1063-1072, DOI: 10.1080/10934529.2017.1338892.

Georgianna, D.R., Mayfield, S.P., 2012, Exploiting diversity and synthetic biology for the production of algal biofuels, Nature, 488, 329-335, DOI: 10.1038/nature11479.

Kan Y., Pan J., 2010, A one-shot solution to bacterial and fungal contamination in the green alga *Chlamudomonas reinhardtii* culture by using an antibiotic cocktail, Journal of Phycology, 46, 1356-1358, DOI: 10.1111/j.1529-8817.2010.00904.x.

Katiyar R., Gurjar B.R., Biswas S., Pruthi V., Kumar N., Kumar P., 2017, Microalgae: An emerging source of energy based bio-products and a solution for environmental issues, Renewable and Sustainable Energy Reviews, 72, 1083-1093, DOI: 10.1016/j.rser.2016.10.028.

Mattos L.F.A., Bastos R.G., 2016, COD and nitrogen removal from sugarcane vinasse by heterotrophic green algae *Desmodesmus* sp., Desalination and Water Treatment, 57, 9465-9473, DOI: 0.1080/19443994.2015.1028454.

Moheimani N.R., McHenry, M.P., De Boer K., Bahri P.A. (eds), 2015, Biomass and Biofuels from Microalgae, Advances in Engineering and Biology, Biofuel and Biorefinery Technologies, 2, Springer, Switzerland.

Prokop A., Bajpai R.K., Zappi M.E. (eds), 2014, Algal Biorefineries, Volume 2: Products and Refinery Design, Springer, Switzerland.

Olguín E.J., Dorantes E., Castillo O.S., Hernández-Landa V.J., 2015. “Anaerobic digestates from vinasse promote growth and lipid enrichment in *Neochloris oleoabundans* cultures, Journal of Applied Phycology, 27 (5), 1813–22, DOI: 10.1007/s10811-015-0540-6.

Ramirez N.N.V., Farenzena M., Trierweiler J.O., Growth of Microalgae *Scenedesmus* sp in Ethanol Vinasse.” Brazilian Archives of Biology and Technology, 57 (5), 630–35, DOI: 10.1590/S1516-8913201401791.

Rios L.F., Klein B.C., Luz Jr. L.F., Maciel Filho R., Wolf Maciel M.R., 2015, Nitrogen Starvation for Lipid Accumulation in the Microalga Species *Desmodesmus* sp., Applied Biochemistry and Biotechnology, 175, 469-476, DOI: 10.1007/s12010-014-1283-6.

Rippka R., Deruelles J., Waterbury J.B., Herdman M., Stanier R.Y., 1979, Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria, Journal of General Microbiology, 111, 1-61, DOI: 10.1099/00221287-111-1-1.

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, 791-801, DOI: 10.1016/j.watres.2012.11.006.

Santana H., Cereijo C.R., Teles V.C., Nascimento R.C., Fernandes M.S., Brunale P., Campanha, R.C., Soares I.P., Silva F.C.P., Sabaini P.S., Siqueira F.G., Brasil B.S.A.F., 2017, Microalgae cultivation in sugarcane vinasse: Selection, growth and biochemical characterization, Bioresource Technology, 228, 133-140, DOI: 10.1016/j.biortech.2016.12.075.

Xin L., Hong-ying H., Ke G., Ying-xue S., 2010, Effects of different nitrogen and phosphorus concentrations on the growth nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp., Bioresource Technology, 101, 5494-5500, DOI: 10.1016/j.biortech.2010.02.016.