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VALORAÇÃO DE EFLUENTES DE DIGESTÃO DE DEJETOS BOVINOS COM CULTIVOS MISTOS DE MICROALGAS

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Valoração de efluentes de digestão de dejetos bovinos com cultivos mistos de microalgas

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Engenharia Civil e Ambiental da Faculdade de Engenharia e Arquitetura da Universidade de Passo Fundo, como requisito para obtenção do título de Doutor em Engenharia.

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RESUMO

O rápido desenvolvimento da criação de bovinos de forma confinada, produziu uma grande quantidade de águas residuais, que podem resultar na eutrofização dos corpos d'água e contaminação das águas subterrâneas. Os cultivos de microalgas em efluentes podem contribuir para a remoção parcial de nitrogênio e fósforo, auxiliando no tratamento. Além das microalgas auxiliarem no tratamento do efluente, em diversos estudos, são consideradas biomassas promissoras e sustentáveis para a produção de biocombustíveis. A toxicidade dos efluentes pode afetar o crescimento algal, havendo a necessidade de estudo de concentrações de efluentes a serem utilizadas nos cultivos algais, bem como o uso de consórcios entre microalgas, que minimizem essa problemática. Dessa forma, objetivou-se estudar os cultivos das microalgas *Spirulina* e *Scenedesmus* isoladamente e em consórcio, em efluente de pós tratamento de dejetos de bovinos. Estudos de aumento de escala de cultivo foram realizados, avaliando-se o melhor modo de cultivo. Inicialmente, as diferentes microalgas foram cultivadas em fotobiorreatores individuais a fim de avaliar-se o melhor meio de cultivo para o crescimento de ambas microalgas (Zarrouk diluído a 20%, BG-11 e o meio BGZ modificado), sendo que a cinética de crescimento de ambas microalgas foi melhor com o meio Zarrouk 20%. Este meio também permitiu maior acúmulo de carboidratos intracelulares. Posteriormente foram realizados cultivos em escala laboratorial das microalgas *Spirulina platensis* e *Scenedesmus obliquus* em consórcios utilizando o efluente, a fim de viabilizar a produção de biomassa de microalgas para uso em biorrefinarias. As biomassas obtidas foram caracterizadas para avaliar o potencial para a produção de biocombustíveis e outros bioprodutos. O efluente foi utilizado em condições estéreis e não estéreis para melhor compreender a influência de outros microrganismos na remoção de N e P. A biomassa obtida com adição de 10% de efluente estéril em meio Zarrouk (20%) apresentou 44,12% e 34,62% de carboidratos, utilizando *Spirulina platensis* em monocultura ou os consórcios 50%/50% de *Spirulina* e *Scenedesmus*, respectivamente, esta biomassa apresentando potencial para ser utilizado na produção de bioetanol. As remoções de nitrogênio e fósforo foram maiores nas condições não estéreis e atingiram 92,7% e 49,66% de remoção de nitrogênio e fósforo, respectivamente, utilizando os consórcios e com adição de 30% de efluente no meio. Por fim, foi realizado o cultivo em escala piloto em raceways de 10 L, sendo que o melhor resultado foi escalonado em raceway de 100 L. Os ensaios foram realizados com a adição de efluente não esterilizado, com adição de 10% (v/v) de efluente nos tempos 1, 5 e 10 d, em modo batelada alimentada. As biomassas obtidas foram caracterizadas para avaliar o potencial para a produção de biocombustíveis e outros bioprodutos. Os cultivos que atingiram a maior massa seca foram 50% *Scenedesmus* + 50% *Spirulina* aos 15 dias de cultivo, sendo que a maior concentração de carboidratos foi alcançada no cultivo com 100% de *Spirulina*. Houve uma remoção de 16,75% de fósforo e 88,2% nitrogênio nos primeiros 5 dias de cultivo. O aumento de escala (raceway 100 L) apresentou resultados semelhantes em comparação ao cultivo realizado nos raceways de 10 L. O cultivo das microalgas em consórcio ou a *Spirulina* de forma isolada podem ser utilizados para auxiliar o tratamento do efluente concomitantemente à produção de biomassa para diferentes aplicações.

Palavras-Chaves: Biomassa, Biocombustíveis, Efluente, Bioetanol, Biogás, Biodiesel

ABSTRACT

The rapid development of cattle farming in a confined manner has produced a large amount of waste water, which can result in eutrophication of water bodies, contamination of groundwater. The cultivation of microalgae in effluents can contribute to the partial removal of nitrogen and phosphorus, helping in the treatment. In addition to the microalgae that assist in the treatment of the effluent, in several studies, they are considered promising and sustainable biomasses for the production of biofuels. Thus, the cultivation conditions in a consortium of microalgae *Spirulina* and *Scenedesmus* were studied. Initially, Zarrouk culture media diluted to 20%, BG-11 and modified BGZ medium cultivated with different microalgae in individual photobioreactors were studied. With the growth kinetics, it was observed that the microalgae cultivated with the Zarrouk medium 20% showed greater growth, as well as a greater accumulation of intracellular carbohydrates. Subsequently, laboratory-scale cultivation of the microalgae *Spirulina platensis* and *Scenedesmus obliquus* was carried out in consortia using effluents from the anaerobic treatment of bovine manure, in order to facilitate the production of microalgae biomass for use in biorefineries. The obtained biomasses were characterized to evaluate the potential for the production of biofuels and other bioproducts. The effluent was used in sterile and non-sterile conditions to better understand the influence of other microorganisms in the removal of N and P. The biomass obtained with the addition of 10% sterile effluent in Zarrouk medium (20%) presented 44.12% and 34.62% of carbohydrates, using *Spirulina platensis* in monoculture or the consortia 50% / 50% of *Spirulina* and *Scenedesmus*, respectively, this biomass presenting potential to be used in the production of bioethanol. The nitrogen and phosphorus removals were greater in non-sterile conditions and reached 92.7% and 49.66% nitrogen and phosphorus removal, respectively, using the consortia and with the addition of 30% of effluent in the medium. Finally, pilot scale cultivation was performed in 10 L raceways, the best result being replicated in a 100 L raceway. The tests were performed with the addition of non-sterile effluent, 10% (v / v) was added on days 1, 5 and 10, in the form of fed batch. The obtained biomasses were characterized to evaluate the potential for the production of biofuels and other bioproducts. The crops that reached the highest dry mass were 50% Sc + 50% Sp at 15 days of cultivation, and the highest concentration of carbohydrates was achieved at 100% Sp. There was a removal of 16.75% of phosphorus and 88.2% nitrogen in the first 5 days of cultivation. The scale increase (raceway 100L) showed similar results in comparison to the cultivation performed in the 10L raceways. The cultivation of microalgae in consortium or Spirulina in isolation can be used to assist the treatment of the effluent concomitantly with the production of biomass for different applications.

Key Words: Biomass, Biofuels, Effluent, Bioethanol, Biogas, Biodiesel

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1 INTRODUÇÃO GERAL

O rápido desenvolvimento da criação de bovinos de forma confinada, produziu uma grande quantidade de águas residuais, as quais contém altas concentrações de demanda química de oxigênio, nitrogênio e fósforo, sendo consideradas uma das águas residuais mais poluentes, que se não tratadas adequadamente, podem resultar na eutrofização dos corpos d'água, contaminação das águas subterrâneas e poluição do ar por volatilização de amônia (CAI; PARK; LI, 2013; LV et al., 2018). Os cultivos de microalgas em efluentes podem contribuir para a remoção parcial de nitrogênio e fósforo, auxiliando no tratamento (QUEIROZ et al., 2013). Dentre os efluentes agroindustriais, o efluente resultante de digestão anaeróbica de esterco bovino é rico em nutrientes e mantém uma proporção adequada de nitrogênio-fósforo para o crescimento de microalgas (MONFET; UNC, 2017).

No entanto, o efluente da digestão anaeróbica apresenta desafios únicos para o cultivo de microalgas que não são normalmente encontrados com meios quimicamente definidos, incluindo potencialmente alta turbidez e concentrações de compostos químicos, e a presença de microrganismos competitivos (LEVINE; COSTANZA-ROBINSON; SPATAFORA, 2011). A filtração e esterilização são aplicadas na maioria das pesquisas, porém são difíceis de serem utilizadas para o cultivo ao ar livre em grande escala. O método de pré-tratamento mais simples para evitar a toxicidade é a diluição da concentração do efluente, para que a concentração do efluente fique abaixo dos limites tóxicos, tais como os cultivos descontínuo alimentados e semicontínuos (JI et al., 2015; LUO et al., 2019; PECCIA et al., 2013).

Além das microalgas auxiliarem no tratamento do efluente, em diversos estudos, são consideradas biomassas promissoras e sustentáveis para a produção de biocombustíveis (BAICHA et al., 2016; NOVOVESKÁ et al., 2016). Isto se deve ao fato de que crescem rapidamente, não competirem com culturas alimentares por terras aráveis, e na produção é possível o uso de águas salgadas e residuais (RYAN GEORGIANNA; MAYFIELD, 2012). Entretanto, a produção em escala industrial, necessária para a implementação da produção de biocombustíveis algais, possui vários pontos críticos, limitando o desenvolvimento de plantas comerciais de cultivo. Um dos principais desafios no cultivo das microalgas é o custo, diretamente afetado pela disponibilidade de água e nutrientes necessários para o crescimento. Reduzir os custos de produção e melhorias no balanço energético da produção de biocombustíveis de microalgas é um desafio, que irá determinar a viabilidade comercial a longo prazo dos biocombustíveis microalgais (NOVOVESKÁ et al., 2016; VASSILEV; VASSILEVA, 2016).

Sendo assim, uma forma de reduzir os custos na produção seria pela reciclagem de nutrientes e reutilização da água dos cultivos, uma vez que as microalgas utilizam os nutrientes para as atividades metabólicas e síntese de biomassa. As células de microalgas armazenam nitrogênio, fósforo e carbono para a síntese de proteínas, lipídios, carboidratos e outras formas biomoleculares (JEBALI et al., 2018; ZENG et al., 2015).

Alguns estudos mostram que a redução dos custos no processo pode se dar pelo uso de águas residuais/efluentes como fonte eficiente de nutrientes e água (CANTER et al., 2015; PITTMAN; DEAN; OSUNDEKO, 2011). Além de utilizar o efluente como fonte de nutrientes e água, as microalgas são capazes de reduzir a carga orgânica, auxiliando no tratamento do efluente (SYDNEY et al., 2011).

Diversas espécies de microalgas já foram cultivadas em diferentes águas residuais, como efluentes urbanos, industriais e agrícolas, com objetivo de tratar o efluente ou utiliza-lo como fonte de nutrientes para a produção de biomassa microalgal. A biomassa obtida, por sua vez, pode ser utilizada como matéria prima para biocombustíveis, tais como biodiesel, bioetanol ou biometano (ARBIB; GARRIDO-PE, 2013; CAI; PARK; LI, 2013; CHOKSHI et al., 2016; MARKOU et al., 2018; REYIMU, 2017; WANG et al., 2010).

Além do aumento da produção de biomassa de baixo custo, a composição química da biomassa deve ser adequada ao biocombustível que se deseje obter. Portanto, as espécies conhecidas de microalgas que possuem taxas elevadas de crescimento podem ser manipuladas para produzir maiores concentrações, seja de lipídios, carboidratos ou proteínas (MARKOU; ANGELIDAKI; GEORGAKAKIS, 2012).

O controle do cultivo ou das condições ambientais é a forma comumente utilizada para a manipulação da composição da biomassa de microalgas, e maior acúmulo da substância desejada. Os cultivos e fatores ambientais mais frequentemente relatados, que afetam o teor de carboidratos, são o tipo e a concentração da fonte de nutrientes, intensidade de luz e temperatura. Além disso, o modo metabólico (autotróficos, heterotróficos, e mixotróficos) afeta a composição da biomassa (DRAGONE et al., 2011). Porém, estas manipulações geram estresse às células de microalgas, ocasionando normalmente baixas produtividades de biomassa, e, em geral, baixa produtividade de carboidratos. Portanto, o estabelecimento de estratégias que permitam alcançar a melhor combinação de conteúdo de carboidratos e taxa de produção de biomassa deve ser aplicado (HO et al., 2013).

Vários estudos têm demonstrado que o cultivo sob condições deficientes de nitrogênio eleva o teor de lipídios ou carboidratos, porque o nitrogênio em condição de esgotamento favorece que lipídios ou carboidratos sejam sintetizados preferencialmente ao

invés de proteínas (DRAGONE et al., 2011; HO et al., 2013). O fósforo é um elemento essencial e a restrição deste nutriente afeta a estratégia global de energia das microalgas, resultando na diminuição da síntese de proteínas e acúmulo de carboidratos e/ou lipídios (MARKOU; ANGELIDAKI; GEORGAKAKIS, 2012).

Portanto, a integração do tratamento de águas residuais e produção de algas, fornece benefícios cumulativos, eliminando a necessidade de água externa e nutrientes, proporcionando serviços de tratamento de efluentes eficientes, compensando assim uma parcela significativa no custo de produção dos biocombustíveis (STURM; LAMER, 2011).

Além disso, esses sistemas de tratamento de efluentes permitem a utilização de multiespécies, ao contrário de instalações comerciais, nas quais há o cultivo de apenas uma única espécie de microalga, com rendimentos elevados de lipídios ou carboidratos ou outras características desejáveis, porém a manutenção de monoculturas é difícil e possui custo elevado. O cultivo com policultura pode aumentar a produtividade das microalgas através de duas vias principais: eficiência na utilização dos recursos e estabilidade da comunidade (CARDINALE; NELSON, 2012).

Várias espécies ocupam diferentes nichos funcionais, usam recursos de forma mais eficiente por causa de seus diferentes espectros de absorção, necessidades de nutrientes e fisiologia geral. Este princípio aplica-se a fontes de nutrientes como nitrogênio, fósforo, carbono, e na quantidade e qualidade da luz presente (BEHL; DONVAL; STIBOR, 2011; GAMFELDT; HILLEBRAND, 2011).

Este projeto faz parte da linha de pesquisa em Desenvolvimento de processos aplicados ao tratamento de ar, água, efluentes e solos do Programa de Pós-Graduação em Engenharia Civil e Ambiental, e ao grupo de pesquisa em Saneamento Ambiental do CNPq, uma vez que concomitante à produção da biomassa microalgal ocorre a valoração e tratamento do efluente.

A tese está organizada em 4 capítulos. No capítulo I é apresentada uma revisão de literatura referente ao cultivo de microalgas em consórcio com adição de efluente, e apresenta os possíveis bioprodutos que podem ser gerados a partir da biomassa microalgal. No capítulo II, foi estudado qual o melhor meio de cultivo que pode ser utilizado para o cultivo do consórcio microalgas *Spirulina platensis* e *Scenedesmus oblíquos*. No capítulo III, é apresentado o cultivo em consórcio das microalgas, com adição de efluente da digestão anaeróbia de dejetos bovinos de forma descontinua aos meios de cultivo algais, avaliando-se os efeitos também na composição da biomassa microalgal. Já no capítulo IV, foi estudada a adição do efluente em modo descontinuo alimentado aos cultivos algais, com estudo de

aumento de escala. Além desses capítulos, uma introdução geral e objetivos estão apresentados, bem como a conclusão geral da tese.

1.1 Objetivos

1.1.1 Objetivo Geral

Realizar cultivos sustentáveis de microalgas utilizando efluente da biodigestão de esterco bovino para produção de biomassa microalgal.

1.1.2 Objetivos Específicos

Os objetivos específicos são:

a) Selecionar meio de cultivo padrão para o cultivo em consórcio das microalgas *Spirulina* e *Scenedesmus*;

b) Estudar se as concentrações iniciais de inóculo das microalgas crescendo em consórcio afetam a cinética de crescimento e as concentrações de carboidratos da biomassa em meio padrão;

c) Avaliar o efeito do efluente estéril e não estéril sobre o crescimento das microalgas puras e em consórcio em cultivos descontínuos;

d) Estudar os modos descontínuos alimentados para realizar o aumento de escala dos cultivos das microalgas.

e) Avaliar a composição química das biomassas para verificar a aplicação das mesmas para a produção de biocombustíveis.

O objetivo “a” foi cumprido no CAPÍTULO II - Definition of means for consortium and standardization of cell concentration determination. Os objetivos “b” e “c” estão apresentados no CAPÍTULO III - Microalgae consortia for post-treating effluent of anaerobic digestion of cattle waste and evaluation of biochemical composition of biomass. O objetivo “d” está apresentado no CAPÍTULO IV - Cultivation of microalgae in consortia adding effluent in fed batch mode and scale up to biomass production. Finalmente, o objetivo “e” está apresentado nos CAPÍTULOS III e IV.

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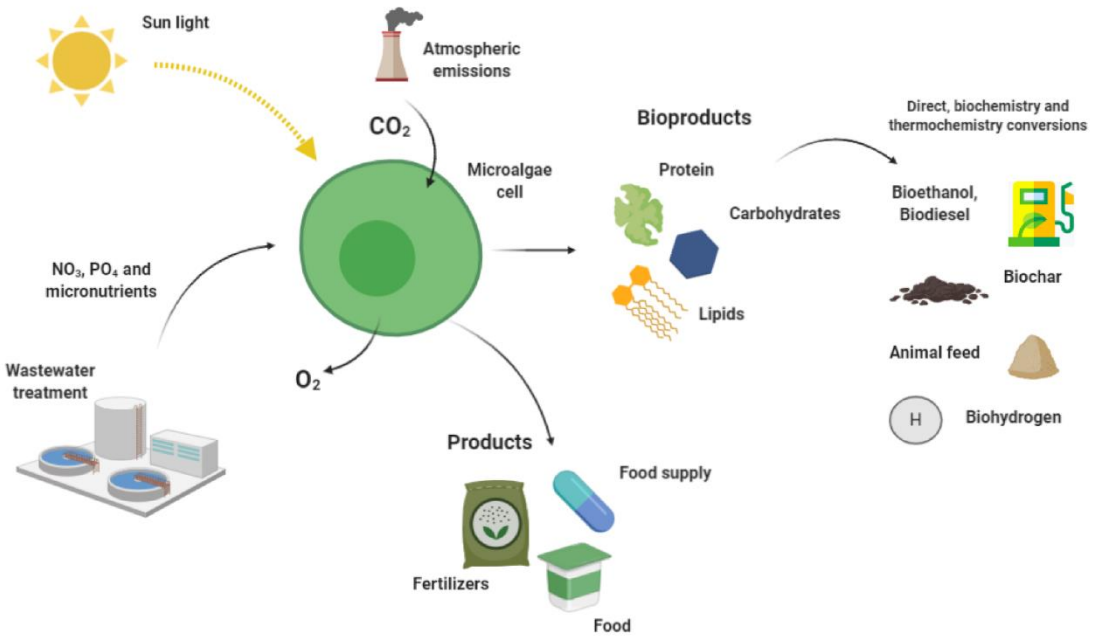
2 CAPÍTULO I - MICROALGAE CONSORTIA CULTIVATION USING EFFLUENTS FOR BIOPRODUCT MANUFACTURE

Graphical Abstract



Abstract

The aim of the present study was to analyze studies that cultivate microalgae in a consortium using effluent as a nutrient source, and later use the biomass to produce other biocompounds. The production of microalgal biomass, which is associated with the remediation of effluent, production of biofuels, and by-products of high added value (such as bioethanol, biomethane, biodiesel, biofertilizers, and biochar), has been highlighted among the types of sustainable refineries. Further, consortium cultures, with interactions between microalgae and bacteria, fungi, and protozoa at the cellular level, can generate mutual relationships in the conversion and use of nutrients. Large-scale microalgae cultivation enables the mitigation of the presence of inorganic carbon in the atmosphere through microalgal cellular respiration. To assess the economic and environmental sustainability of the promotion of technologies that use microalgae, the relationships between the bioeconomy and renewable energy must be established. The present study sought to demonstrate the relationship between the factors associated with the cultivation of microalgae using effluents and the use of microalgal biomass in the production of biocomposites. Furthermore, whether these factors could favor the reduction of costs related to the production processes and boost the biorefinery industry were also investigated.

Keywords: Microalgae, Biorefinery, Biofuels, Bioproducts, Sustainable Energy

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1 Introduction

The growing demand for energy as well as the concerns of natural resource depletion have led to unsettling thoughts regarding the future of the global energy matrix (AVAGYAN, 2018; PRÉAT et al., 2020). Alternative energy sources have thus been continuously developed to serve as potential solutions to these concerns. In particular, bio-based energy sources have been demonstrated to be promising; however, this favorable feature is only observed between the advances in alternative energy sources. According to the report by the International Energy Statistics (EIA, 2018), bio-based energy sources provide a relatively small contribution to global energy production. Among the efforts to create a cleaner and sustainable energy matrix, biorefineries have gained remarkable interest as a strategy to reduce production costs via the extraction of several products in a single step. This single step is comprised of several biomass conversion processes that are occurring simultaneously to obtain biofuels and other bioproducts, such as the food and feed sector, chemical and materials industry, and the pharmaceutical and personal care industries (AVAGYAN, 2018; BHATTACHARYA; GOSWAMI, 2020). Notably, biorefineries related to microalgae have been gaining attention as a renewable source of sustainable raw materials and a potential source of several bioproducts.

The discovery of microalgae as sources of bioproducts is not a recent revelation. In fact, ancient civilizations cultivated the microalgae of the *Spirulina* genus to achieve specific properties according to the content of nutrients, such as lipids, proteins, and carbohydrates (RICHMOND et al., 1993). From these applications, the use of *Spirulina* has extended to environmental applications, such as toxic metal adsorption processes, removal of effluent nutrients, mitigation of the effects of atmospheric CO_2, etc. (FERRANDO; MATAMOROS, 2020; SHAHID et al., 2020; TASIC et al., 2020). More recently, obtaining bioproducts, including biofuels using the concept of integrated biorefineries, has also been achieved (CHANDRA; SHUKLA; MALLICK, 2020; NGUYEN et al., 2019; UMMALYMA; SUKUMARAN, 2014). Microalgae possess other advantages, including high exponential growth rates compared to other raw materials, as well as the potential to be produced throughout the year. With a lack of fertile areas for crop cultivation, microalgae can act as a renewable raw material and play an important role in a future bioeconomy (AVAGYAN, 2018; BUSSA et al., 2020). Owing to the potential uses of microalgae, growth optimization

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techniques and genetic engineering have been proposed (AVAGYAN, 2018; KHAN et al., 2018).

Biofuels synthesized from microalgae (third-generation biofuels) are an interesting option in the context of a circular economy. This concept of a circular economy allows the evaluation of the economic and environmental sustainability of a given technology, assessments of the life cycle, and establishment of a set of approaches to determine the sustainability of the system (AVAGYAN; SINGH, 2019; RAJESH BANU et al., 2020); this is increasingly sought after by policymakers and corporations (PALADINO; NEVIANI, 2020). However, the technologies used to convert microalgae to biofuel based on biomass phototrophic growth using fertilizers aggressively increase GHG emissions instead of mitigating these emissions (AVAGYAN 2017; AVAGYAN, 2018).

The use of effluents as a source of nutrients for the growth of biomass has great potential in the production of microalgae biofuels. In fact, crops aim to produce microalgal biomass by using a concomitant effluent treatment that fits the premise of biorefineries. Nagarajan et al. (2020) reiterated that effluents, in general, are rich in organic and inorganic compounds of carbon, nitrogen, and phosphorus, which can be used as a source of nutrients by microalgae. However, due to the variation in chemical profiles for the different types of effluents, there are no reports on the level of microalgae tolerance for each constituent (CHOONG et al., 2020).

During microalgal culture, cell metabolism causes a reduction in the amount of gases in the atmosphere that cause the greenhouse effect (AVAGYAN, 2018). Microalgae use inorganic carbon from the atmosphere (CO_2) and produce oxygen via photosynthesis, ultimately converting CO_2 into energy and water (SHOW et al., 2020). Thus, in an energy balance, according to Adamczyk et al. (2016), 1 kg of the microalgae, *Nannochloropsis gaditana*, could fix 1.5 kg of CO_2 in ten days.

Owing to the ongoing search to identify alternative technologies that use raw material in a sustainable manner, we sought to update the theme for the use of microalgae in the context of biorefineries by assessing the forms of consortium cultivation, use of nutrients present in effluents, and generation of bioproducts from biomass.

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2 Consortium of microalgae in effluent

When considering microalgae cultivation in effluent, the use of a single strain, which is common in regular cultivations with regular media, can be inefficient or could result in cell death (BÉLANGER-LÉPINE et al., 2020). However, on a large scale, the monoculture, especially when effluent is employed as a medium, is impeded due to contamination by undesirable microorganisms (WANG et al., 2013). Promoting cooperative interactions between microorganisms can diminish the problems that occur in the monoculture of microalgae strains; this is because the microbial interactions that occur through the exchange of metabolites can result in a general increase in biomass productivity, and therefore, the efficiency in nutrient removal (AVAGYAN, 2018; LÓPEZ ROCHA et al., 2020). Studies have demonstrated the use of mixed crops for many purposes, such as food production (CAMACHO; MACEDO; MALCATA, 2019; SUI; VLAEMINCK, 2020), biofuels (CHENG et al., 2019; QU et al., 2020), effluent treatment (AVAGYAN, 2018; FENG et al., 2020; LI et al., 2020), and microalgal biomass harvesting (NAZARI et al., 2020).

The main interactions between microorganisms occur between algae-algae and algae-bacteria consortia. Further, these interactions occur on a large scale with media containing industrial and municipal effluents. Figure 1 shows the crops with effluents. In this process, microalgae and bacteria (for example) coexist in granules, forming a mutually-beneficial symbiosis (i.e., microalgae can produce oxygen for bacteria to oxidize organic matter in the effluent using carbon dioxide generated by the bacteria in the presence of sunlight) (AVAGYAN, 2018; LIU et al., 2019).

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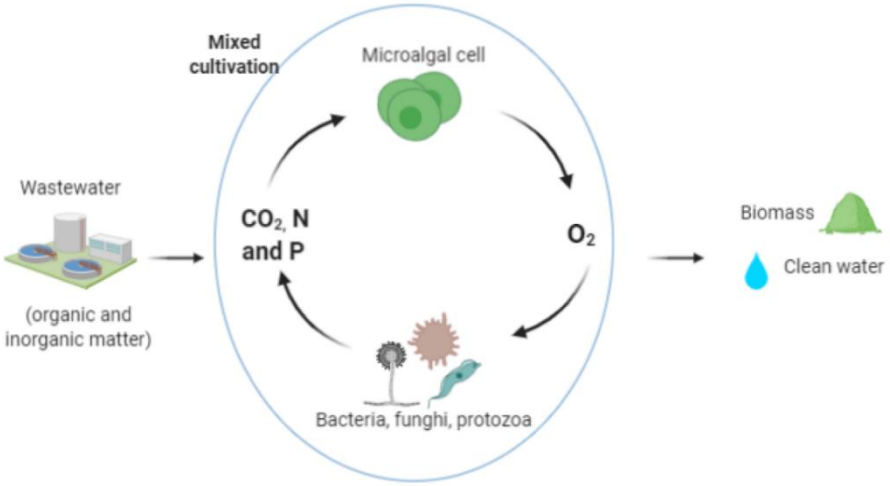
Figure 1. Scheme of mixed cultivation of microalgae in wastewaters.



The cultivation of microalgae in effluent is effective at removing nutrients from the effluent and assisting in its treatment. The use of microalgae consortia (several strains of microalga and including bacteria) using effluents could make the cultivation economically viable, reducing the effect of the effluent toxicity and making possible the obtaining of biomass for other applications (JORDAAN et al., 2018; LÓPEZ ROCHA et al., 2020)

2.1 Synthesis of nutrients

Nitrogen (N) and phosphorus (P) are the main nutrients required for the growth of biomass. Further, the N/P ratio in the culture medium must be adjusted for the efficient removal of both (XIN et al., 2010). For example, to produce 100 tons of biomass, up to 200 tons of CO_2, 10 tons of N, and 1 ton of P are consumed (ACIÉN FERNÁNDEZ; GÓMEZ-SERRANO; FERNÁNDEZ-SEVILLA, 2018).

Fortier and Sturm (2012) reported that microalgal biomass has a typical composition of $C_{106}H_{181}O_{45}N_{16}P$; therefore, the nutritional requirement can be estimated to be $106_C:16_N:1_P$ $\mu mol.L^{-1}$ or equivalent to 42C:7N:1P $mg.L^{-1}$, a proportion known as the Redfield Number (QIANG HU, 2004; ANDERSEN, 2005). Microalgae possess different mechanisms for capturing carbon, nitrogen, and phosphorus; absorbing carbons, such as HCO_3^- dissolving nitrogen from water, such as NH_4^+, NO_3^-, NO_2^-, or organic nitrogen; and using these elements to synthesize proteins, carbohydrates, and lipids (SNIFFEN; SALES; OLSON, 2018).

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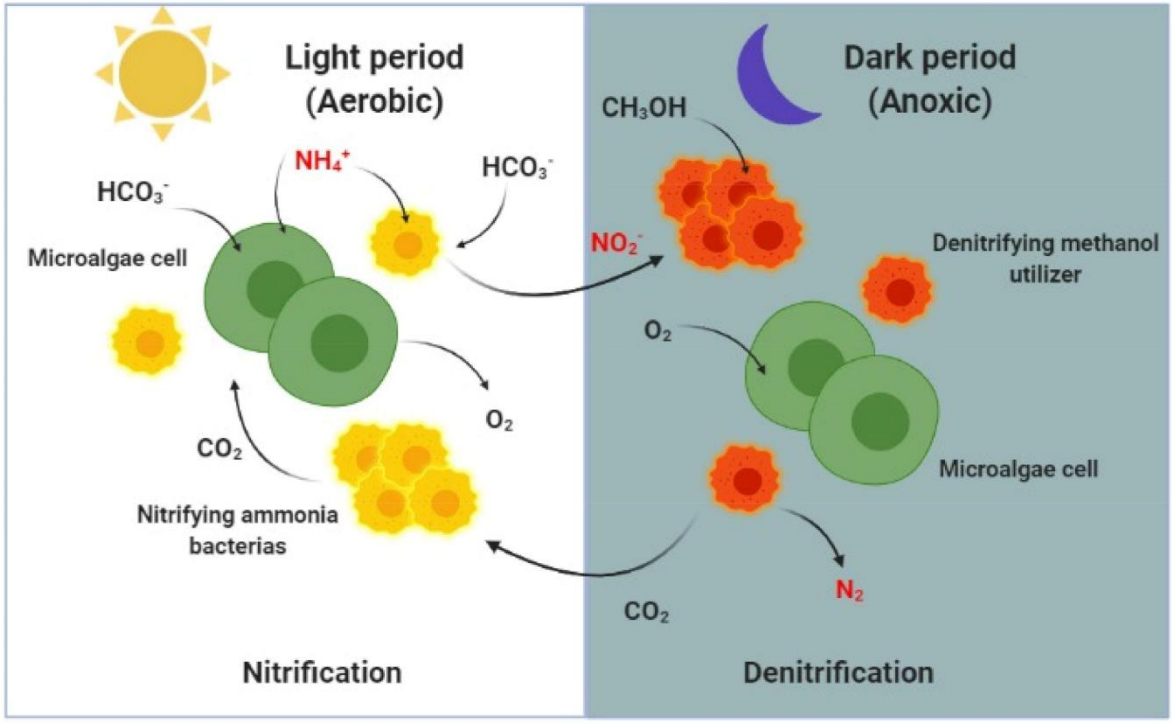
Microalgal consortia have received increased attention primarily due to bias in the concomitant effluent treatment process. Accordingly, more attention has been dedicated to research on such consortia. Nitrifying bacteria reduce the toxicity of NH_3 in the growth of microalgae and NH_4 by improving the removal of N ions in bacterial-microalgal consortium systems (RADA-ARIZA et al., 2017). Previously, Arun et al. (2019) analyzed alternating periods of light and darkness, in a row, to obtain complete biological nitrogen removal (BNR) without any external aeration and with the addition of methanol as the sole carbon source. During periods with lighting, the sum of ammonia and nitrite with a low concentration of dissolved oxygen was attributed to nitrification by oxidizing bacteria. However, in the absence of light, the nitrite produced was subsequently reduced by methanol using a denitrifier in the dark in the presence of methanol. As a result, a shortcut nitrogen removal was achieved by the consortium in the Photo Sequencing Bio-reactor. Figure 2 shows the interactions between algae and bacteria when grown in a consortium related to macronutrient nitrogen.

Figure 2. Scheme of N removal in mixed cultivation.



Adapted from Arun et al. (2019)

In the removal of nitrogen from effluents, the biological denitrification processes remove nitrate (NO_3^-), ultimately converting it into harmless gas (NO_2). Heterotrophic organisms thus require a carbon source that serves as an electron donor to grow quickly and

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form nitrate as an electron acceptor (REZVANI et al., 2019). According to Nagarajan et al. (2020), in addition to the organic matter present, animal manure in effluent increases the total and ammoniacal nitrogen content of the effluent.

Phosphorus is a key element in the metabolic route and synthesis of energy. As a result, the ability to efficiently capture and store P is an important advantage for microalgae (AVAGYAN, 2011; SOLOVCHENKO et al., 2019). Thus, the use of inorganic phosphorus present in effluents mainly contributes to the remediation and nutritional supplementation of cultivated species. With biological phosphorus removal via phosphorus-accumulating organisms, it has become possible to apply this technique in many large-scale effluent treatment processes (KHANZADA, 2020). Furthermore, under controlled and functional conditions for crops with high phosphorus content, microalgal biomass can be recovered and used as bio-solids (fertilizers) (OTA et al., 2016).

Notably, consortium cultivation contributes to the treatment of the effluent through the utilization of nutrients present in the effluent for microalgal growth; this is because each microalga/bacterial microorganism has a preference for nutrients with different chemical compositions (RADA-ARIZA et al., 2017; BÉLANGER-LÉPINE et al., 2020; LÓPEZ ROCHA et al., 2020).

2.2 Bioremediation of Effluent

Effluent and sewage treatment can be divided into three stages: physical, chemical, and biological treatments (RAWAT et al., 2011). The specifications of each treatment and their applicability are based on the type of effluent to be treated. Biological treatment is the most ecological and low-cost treatment as it uses microorganisms, such as microalgae and bacteria, to decompose organic matter and remove mineral nutrients from the effluent (TAN et al., 2018).

Among the sources of effluent (industrial and municipal), municipal effluent has a minor potential for growing microalgae (BHATNAGAR AND BHATNAGAR, 2010) as it does not possess a high concentration of nitrogen under normal circumstances, which would hinder their growth. However, the deprivation of nitrogen is a stress condition that could promote the production of microalgae biomass with more carbohydrates or lipids in its composition, being more usable to biofuel production. In this way, Sharma et al. (2020) evaluated the efficacy of two different microalgae consortia, which have the potential to

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concomitantly accumulate intracellular lipids, for the treatment of municipal sewage. High efficiencies were obtained for the treatment of the sewage effluent and a lipid content value of 31% was achieved on a dry basis. Ansari et al. (2020) investigated the bioremediation of sewage effluent and the accumulation of biochemical composites in cells of the species, *Scenedesmus obliquus*. The productivity of lipids reached 26.5 ± 1.5% of dry mass, which was higher than the control indexes, thereby corroborating the premise of biorefinery.

To perform the use of municipal or industrial effluents, many authors mentioned the use of microalgae consortia. Koreivienė et al. (2014) reported that the consortium of microalgae containing *Chlorella sp.* and *Scenedesmus sp.* proved to be more efficient at removing nitrogen and phosphorus from municipal effluent than the individual culture of *Chlorella sp.* and *Scenedesmus sp.* As a result, removals of 88.6 to 96.4% and 99.7 to 99.9% of nitrogen and phosphorus, respectively, were achieved after three weeks of cultivation. Thus, the use of consortium crops in municipal effluents is advantageous in relation to the cultivation of a single species.

Wang et al. (2010) investigated the growth of *Chlorella sp.* in four different types of effluent and proceeded to compare their ability to use and remove N, P, and chemical oxygen demand (COD). Based on their findings, they concluded that the algae growth profile and nutrient removal efficiency were proportional to the concentration of nutrients from the municipal effluent derived from different stages of the treatment process. In a similar study by Randrianarison and Ashraf (2018), which was carried out to assess the growth viability of *Chlorella sp.* on wastewater effluent, the algae removed ammonia, total nitrogen, total phosphorus, and COD at 93.9%, 89.1%, 80.9%, and 90.8%, respectively.

In industrial effluents, the microalgae strains that interact during remediation must withstand the most extreme conditions. The difficulty associated with the use of industrial effluents is caused by their composition. This is because of the high concentrations of nutrients in the industry, and in some cases, the presence of unknown compounds (ZHOU et al., 2014). For example, in a study by Acién Fernández et al. (2018), the levels of COD, N, and P in anaerobic digesters and agro-industrial effluent reached 3,000-16,000 $mg.L^{-1}$, 30-9,000 $mg.L^{-1}$, and 10-500 $mg.L^{-1}$, respectively, thereby reinforcing the idea of a high initial load for microalgal crops.

In a study that evaluated mixed microalgae crops using the species, *Tetraselmis sp.*, to treat final tannery effluents, Pena et al. (2019) obtained a maximum biomass concentration

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of 1.40 $g.L^{-1}$ in a crop with effluent; this resulted in reductions of 97.64%, 71.74%, and 50.37% for total phosphorus, total nitrogen, and COD, respectively.

Huy et al. (2018) used a consortium of microalgae that mainly contained *Chlorella sp.* and a smaller amount *of Scenedesmus sp.* to treat textile effluent, animal manure, and digested sludge. Cultivation in textile effluent resulted in removal efficiencies of 78.78% ± 0.86 for COD, 93.3% ± 2.98 for total nitrogen, and 100% for total phosphorus. However, the removal efficiencies in the digested sludge were 75.94% ± 2.58 for the COD, 72.31% ± 0.66 for total nitrogen, and 100% for phosphorus. Cultivation using animal manure showed the lowest removal efficiencies, with 19.95% ± 1.31% for COD, 16.72% ± 1.3% for total nitrogen, and 100% for phosphorus; the presence of heavy metals was identified as one of the factors influencing these yields.

Hultberg et al. (2017) demonstrated that the effluent resulting from anaerobic digestion during the biogas production process can be used to produce *Spirulina* biomass. Compared to the standard medium for *Spirulina*, which was optimized based on the composition of nutrients and the buffer capacity, a biomass production similar to the initial scheme was observed.

With the growing demand for quality water for industrial processes and effluent stricter emission standards by environmental policies, industries are progressively aiming to improve the effluent treatment system. The use of microalgae in the post treatment, or the use of natural growing microalgae to this proposal could be an alternative. In the case of the use of specific microalgae species, the studies presented previously showed that it is important to evaluate the combinations of species to each effluent, in order to knowing the toxic effects and the biomass compositions. In the case of using natural growing microalgae in the ponds of treatment plants the challenge will be, similarly, to envision applications for sludge or biomass originating in these treatment plants, creating economically and environmentally sustainable alternatives. Agreeing with Hussain et al. (2021), on an industrial scale in the bioremediation of effluents, aspects related to the harvest and biotechnological routes of conversion and production of bioproducts must be observed, mainly aiming at cost reduction. Another factor that must be considered is related to the behavior of different strains to the nutritional conditions in the effluent treatment plants.

Table 1 presents a compilation of studies that employed effluents to cultivate microalgae. Based on this compilation, the microalgal could be used to treat both municipal

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and industrial effluents, with studies achieving greater than 90% efficiency at removing nitrogen, phosphorus, and COD from the tested effluents.

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Table 1. Microalgae crops with different effluents.

Effluent	Reference	Microalgae/Bacteria species	Remotion Efficiency (%)			Biomass (g L^{-1})	Composition (%)		Cultivation Time (d)
			P	N	COD		Lipids	Carbohydrates	
BG-11 medium and synthetic effluent	(ARAVANTINOU; THEODORAKOPOULOS; MANARIOTIS, 2013)	*Scenedesmus rubescens*	11	-	-	4.25	4.97	-	30
		Neochloris vigensis	53			2.94	11.32		
		Chlorococcum spec	25			2.98	2.42		
Biogas process	(HULTBERG et al., 2017)	*Arthrospira platensis*	-	-	-	1.40	4.2	-	10
		Chlorella kessleri	95	96	-	2.70	7.4	44.6	11
		Chlorella vulgaris	98	99		2.91	11.3	36.2	
Dairy Effluent	(YADAVALLI et al., 2014)	*Chlorella pyrenoidosa*	99	97	86	-	7	28	10
		Euglena gracilis	97	95	80		11	32	7
Municipal effluent	(CHOI; LEE, 2015)	*Chlorella vulgaris*	20-80	78-97	-	0.40 – 2.97	-	-	15
Municipal effluent	(DI TERMINI et al., 2011)	*Scenedesmus*	80 – 99.9	90 – 99.9	-	-	-	-	7
Carpet mill	(CHINNASAMY et al., 2010)	*Consortium: Chlamydomonas globosa, Chlorella minutissima* and *Scenedesmus bijuga*	-	-	-	5.9 g $m^{-2}.d^{-1}$	5.3	15.7	8
Cattle slaughterhouse effluent	(MARONEZE et al., 2014)	*Phormidium sp.*	52	57	90	-	15.4	15.9	42
Municipal effluent	(NUNEZ et al. 2001)	*Scenedesmus sp.*	83.3	100	-	-	16	-	21
		Chlorella vulgaris	80.3	60.1			-		

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Dairy Effluent	(CHOKSHI et al., 2016)	*Acutodesmus dimorphus*	100	100	90	32.3 x 10^6 cells/ml	25	30	8
Municipal effluent	(FOLADORI; PETRINI; ANDREOTTOLA, 2018)	Indigenous species activated sludge	-	98±2	87±5	1.3 g TSS/L	-	-	-
Synthetic municipal effluent	(FERRO et al., 2019)	*Chlorella vulgaris* and *Rhizobium sp.*	100	59	79-83% to TOC	50 mg L^{-1} of biomass dry weight	-	-	5
Municipal effluent	(ANSARI et al., 2019)	*Scenedesmus obliquus*	94	NH_4^+ (81%), NO_3 (100%)	71	1.3 mg.L^{-1} in BG11 medium and 0.88 g.L^{-1} in effluent	20.3 ± 1.1 in BG-11 medium and 26.5 ± 1.5 in effluent	14.4 ± 0.9 in BG-11 medium and 21.4 ± 1.2 in effluent	16
Palm oil production effluent	(MOHD UDAIYAPPAN et al., 2020)	*Coelastrella sp. UKM4, Chlamydomonas sp. UKM6, Scenedesmus sp. UKM9, Actinobacteria, Bacteroidetes, Planctomycetes* and *Proteobacteria*	PO_4 >50%,	NH_4^+ >80%	DQO > 40% to *Scenedesmus*	10, 20 and 30% v/v (~400 mg.L^{-1})	-	-	30
Crude effluent processing whey	(MARAZZI et al., 2020)	*Scenedesmus acuminatus (SA)* and mists population (MP) of *Chlorella, Scenedesmus* and *Chlamydomonas spp*	69% (SA) and 73% (MP)	88% (SA) and 90% (MP)	93% (SA) and 94% (MP)	~2.3 mg.L^{-1} in mixed population and ~1.5 mg.L^{-1} *S. acuminatus*	-	-	69
Artificial effluents (AE) and dairy effluents (DE)	(MAKUT; DAS; GOSWAMI, 2019)	*Chlorella sorokiniana DBWC2 e* and *Chlorella sp. DBWC7;*		93.59 (AE) and 84.69 (DE)	82.27 (AE) and 90.49 (DE)	Experimental design 0.65 to 1.65 g.L^{-1}	-	-	7

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		Klebsiella pneumoniae ORWB1 and ~~e~~ *Acinetobacter calcoaceticus ORWB3*							
Activated sludge effluent treatment	(FAN et al., 2020)	*Chlorella sorokiniana* and ~~e~~ sludge bacteria	96	98 NH_4^+;	88	1:2 to 3:1 sludge ratio	-	-	30
Agricultural effluent treatment	(PIZZERA et al., 2019)	-	-	>70	-	0.5 $g.L^{-1}$	-	-	Hydraulic retention time of ~ 30 days
Effluent from anaerobic digestion	(PADDOCK; FERNÁNDEZ-BAYO; VANDERGHEYNST, 2020)	*Chlorella sorokiniana* ~~e~~ and indigenous bacteria of the genus *Pusillimonas*	-	34 - 67	60 – 14	~ 0.24 $g.L^{-1}$ for inoculation	-	-	~ 7
Winery effluent	(HIGGINS et al., 2018)	*Auxenochlorella protothecoides* ~~E~~ and *Proteobacteria, Bacteroidetes*	-	100	-38	0.11 and 0.13	-	-	4 and 5
Swine effluent	(WANG et al., 2020)	*Chlorella-Exiguobacterium* ~~e~~ and *Chlorella-Exiguobacterium/Bacillus licheniformis*	87.2	78.3 (NT) and 84,4 (NH_4^+)	86,3	7.7×10^6 $cells.mL^{-1}$ Chlorella and 15.4×10^6 CFU mL^{-1}	-	-	12

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3 Biofuels and bioproducts produced from algal biomass

Getachew et al. (2020) confirmed that the primary source of global energy (also called fossil fuels), such as carbon, oil, and natural gas, is not renewable. Biofuels have thus become an alternative and sustainable source from both economic and environmental points of view (BASTOS, 2018). Biofuels are classified into three generations. Although the first generation does not require technological advances, increasing production could lead to several issues, such as an increase in food prices, water scarcity, other problems associated with area recovery (SHAH et al., 2018), as well as increased GHG emissions instead of emission mitigation (AVAGYAN; SINGH, 2019).

According to Gaurav et al. (2017), the second generation of biofuels requires arable areas for growth, which can result in less food availability, and consequently, higher product prices. Microalgae fit within the third-generation biofuels generated by biotechnological processes. The remarkable advantages induced by the use of microalgae for biofuel production include the low content of lignin in the biomass, the rich biomass in composites of interest (carbohydrates and lipids), and rapid multiplication (SHAH et al., 2018). Figure 3 presents the procedures that are based on the conversion of biomass to bioproducts with high added value.

The metabolic route to achieve greater production based on nutritional factors can be directed for microalgae. By viewing a biocomposite of interest, Zaparoli et al. (2020) analyzed factors that could result in the accumulation of intracellular carbohydrates for bioethanol production. In addition, they examined the effects of stressors, such as UV light and micronutrients in the media. According to Zuccaro et al. (2020), the cultivation of microalgae requires specific environmental conditions, including temperature ranges, pH, light intensities, mixing conditions, and gas exchange. To increase the accumulation of the compounds of interest at the intracellular level, the conditions of cultivation can be altered by seeking a higher concentration of the biocomposite of interest, especially for biofuel production (ABINANDAN et al., 2018; AVAGYAN, 2018).

As will be presented, biofuels and microalgae bioproducts, produced in microalgae biorefineries have significant potential. By minimizing the impacts related to the fossil fuel network such as soil contamination, greenhouse gas emissions and geopolitical issues, microalgae biofuels, according to Ananthi et al. (2021), emerge as prosperous alternatives. Among some points that hinder the viability of this integration of the processes related to the microalgae biorefinery are high costs and complex biotechnological routes to be adapted to the industrial process, especially on a large scale. Emerging applications such as biofertilizers and

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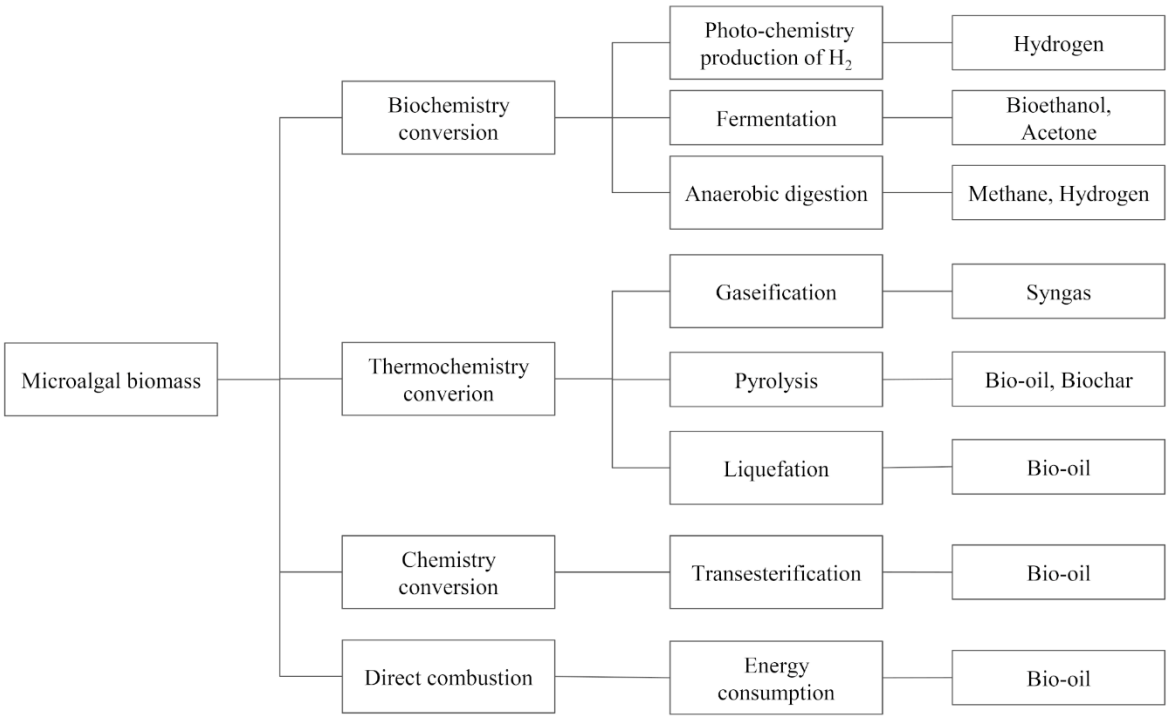
biochar, in turn, are even more recent, but no less important. The various applications of microalgae biomass make it possible to integrate routes and adapt processes, facilitating the economic and environmental feasibility of projects based on possible by-products.

The bioproduct that can be generated with a specific biomass is directly related to the biochemical composition of the microalgal cell. For each bioproduct, a biocomposite of interest is required; however, more than one bioproduct can be generated from the same biomass. With the production of microalgal biomass grown in effluent, the following bioproducts can be generated: bioethanol, biomethane, biodiesel, biofertilizers, and biochar.

Figure 3. Microalgal biomass conversion processes.



Adapted from Getachew et al. (2020).

3.1 Bioethanol

For bioethanol production, the biomass of the microalgae must be rich in sugars, which are the raw materials used for bioethanol production in fermentative processes. As a result, the productivity of carbohydrates in the cell must be high (MAGRO et al., 2016; AVAGYAN, 2018). Notably, microalgae are not composed of lignin. As a result, they can be converted into monosaccharides for bioethanol production (LAKATOS et al., 2019).

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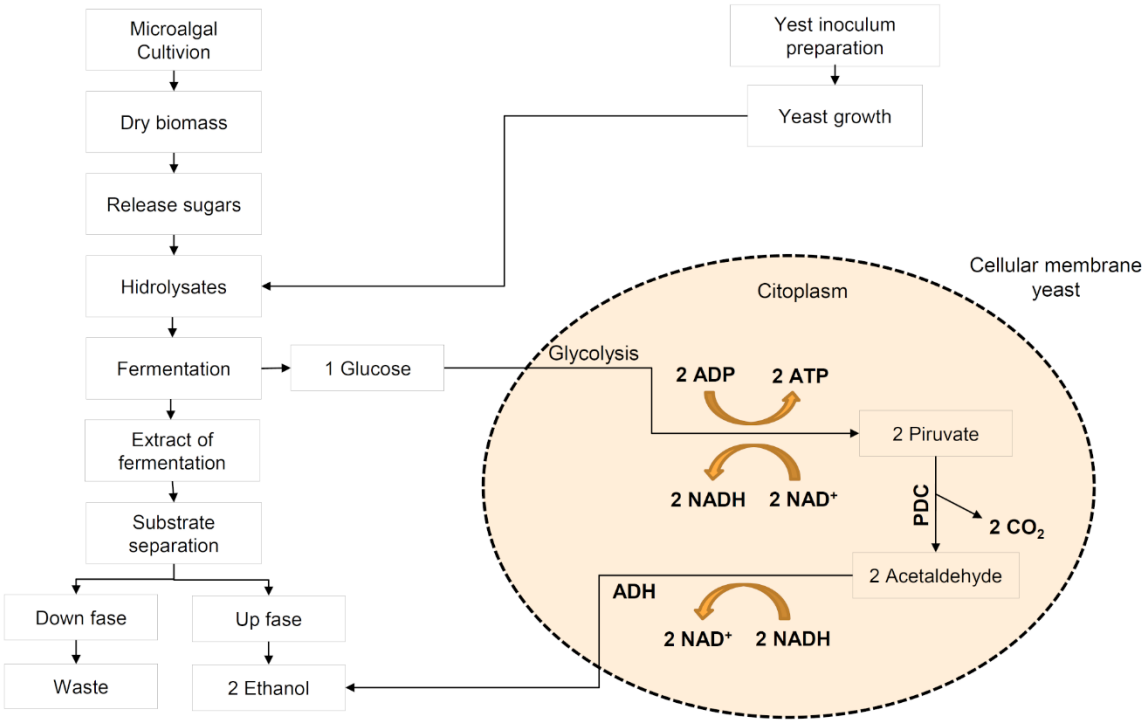
Bioethanol produced from microalgae carbohydrates can be directly used by the currently available internal combustion engine, without significant changes. In addition, the high octane rating and oxygen content of bioethanol fuel translate into higher engine performance and reduced emission rates (QUADER; AHMED, 2017).

The schematic in Figure 4 shows the process of bioethanol production from microalgae.

Figure 4. Scheme of bioethanol production from microalgae.



Adapted from Deb et al. (2019). (PDC: Pyruvate decarboxylase, ADH: Alcohol dehydrogenase).

The transformation process of microalgae carbohydrate is widely known. The major bottlenecks to achieve large-scale production of microalgal bioethanol include the accumulation of intracellular carbohydrate, which must be high; the concentration of biomass in the crops; and the need to decrease production costs. These bottlenecks can be overcome by cultivating microalgae in effluents, which ultimately reduce nutrient costs.

In the context of biorefinery, Tasic et al. (2020) recently cultivated *Chlamydomonas reinhardtii* CC-1093 in agroindustrial vinasse effluent for bioethanol production and the concomitant removal of nutrients. The maximum theoretical ethanol productivity obtained by the authors was found to reach 68.3% in one step of the process. Similarly, Qu et al. (2020) cultivated the newly isolated microalgal species, *Chlamydomonas sp.* QWY37, which reached a maximum carbohydrate production of 944 $mg.L^{-1}.d^{-1}$ and achieved high pollutant removal

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efficiencies (COD: 81%, total nitrogen: 96%, total phosphorous: approximately 100%) through semi-continuous operation. A maximum microalgal bioethanol yield of 61 $g.L^{-1}$ was also achieved. To the best of our knowledge, this is the first report to demonstrate the higher productivity of carbohydrates from microalgae using swine effluent with no pre-treatment associated with direct bioethanol production.

Based on the concept of circular economy and the reuse of by-products, Rempel et al. (2019) analyzed the production of bioethanol and the associated use of its waste for biomethane production. The energy potential for the direct conversion of *Spirulina* biomass into biomethane was 16,770 $kJ.kg^{-1}$, while that for the production of bioethanol from hydrolyzed biomass was 4,664 $kJ.kg^{-1}$.

The accumulation of the composites of interest intracellularly can be promoted via cellular stress. Zaparoli et al. (2020) studied the application of cell stresses in two-stage crops to accumulate intracellular carbohydrates for the later production of bioethanol. Among the best results, the limited nutrients in Zarrouk Medium diluted 20% and the greater light intensity (67.5 μmol photons m^{-2} s^{-1}) and photoperiod (18 h light/6 h dark) were identified as efficient strategies to achieve higher concentrations of intracellular carbohydrates (59.71%) and carbohydrate yields (55.85 $mg.L^{-1}.d^{-1}$) using the batch culture method. According to Bastos (2018), the positive effect of increasing light intensity on the accumulation of starch and lipids is feasible only up to a point; however, this is usually equal to the saturation of photosynthesis under the given conditions in a particular species.

In the context of industrial dairy production, Vieira Salla et al. (2016) obtained results of carbohydrate productivity in biomass of 60 $mg.L^{-1}.d^{-1}$ for crops with residues from the whey protein process. Based on their findings, the microalgae, *S. platensis*, is a promising raw material for the production of bioethanol. Aligning with the aforementioned study, Chokshi et al. (2016) demonstrated that the microalgae, *Acutodesmus dimorphus*, grown in dairy effluent reached a yield that allowed 1 kg of *A. dimorphus* biomass to produce approximately 195 g of biodiesel and 78 g of bioethanol. Essentially, this is 273 g of biofuels.

Ma et al. (2020) used residual microalgae from biodiesel production as a raw material to produce fermentable sugars through enzymatic hydrolysis. Further, Martın and Grossmann (2012) optimized the composition of the microalgae to simultaneously produce bioethanol and biodiesel.

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3.2 Biodiesel

The per unit area yield of oil from algae is estimated to be 20,000–80,000 L per acre per year (DEMIRBAS; FATIH DEMIRBAS, 2011). Further, the biodiesel yield from microalgae is 10–100-fold higher than that from vegetable oil (ORTIZ-MARTÍNEZ et al., 2019). Microalgae could produce biomass with lipids, and some microalgae have 50-80% lipid composition on a dry basis (AVAGYAN, 2018; UMMALYMA et al., 2020). The microalgal genera with high lipid content include *Botryococcus*, *Chlorella*, *Nannochloropsis*, *Scenedesmus*, *Neochloris*, *Phaeodactylum*~~,~~ and *Dunaliella* (GETACHEW et al., 2020).

To reduce the production costs of biodiesel, Choong et al. (2020) used synthetic agro-industrial effluent to promote the synthesis and production of intracellular lipids, and the removal of substances from the effluent. The best results for oil removal by microalgae were 41.56%, with 0.4 $L.min^{-1}$ of ozone flow, 3 $mL.L^{-1}$ of oil, 200 $mL.L^{-1}$ of microalgal biomass, and a pH value between 3 and 5.

To achieve a better adaptation of microalgal species to extreme environments, researchers have evaluated the acclimatization ability of cells. As a result, lipid-accumulating microalgae was found to acclimate to high doses of pollutants without the need for genetic adaptations (ARIF et al., 2020). This acclimatization ability is the ideal characteristic required to provide a structure for the development of greater removal efficiencies, use of nutrients, and effluent reuse with greater safety. Figure 5 shows the procedure used for microalgae biodiesel production.

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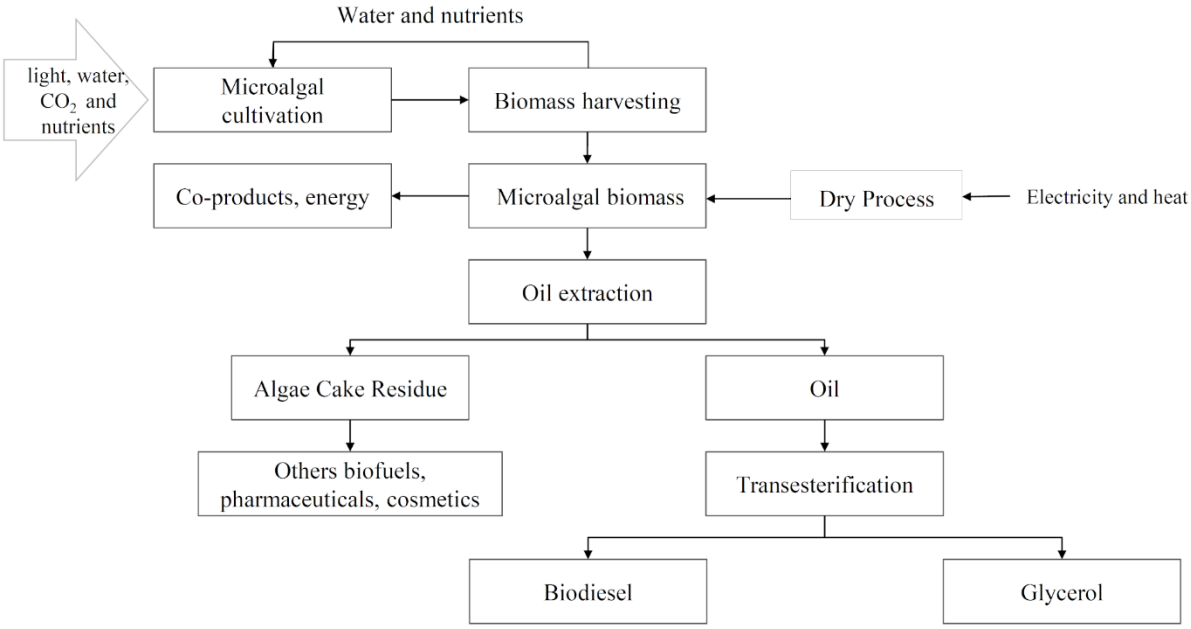
Figure 5. Biodiesel production scheme by microalgae.



Adapted from Sun et al. (2019).

In addition to using the effluent as a nutrient source in the context of biorefineries, it is important to use the entire biochemical composition of the cell. This is because only the lipids present in the cell are used to produce biodiesel and thus, the remaining carbohydrate can be used for bioethanol production. Moreover, the waste resulting from the process can be used to generate biomethane, biofertilizer, and biochar.

3.3 Biomethane

The fraction of any biomass could produce biomethane; however, the direct use of microalgal biomass to produce biomethane does not allow the potential transformation of the biomass into other biofuels, such as bioethanol and biodiesel. However, in the production of these biofuels, the waste generated can be used to produce biomethane, forming a transformation cycle based on the concept of biorefineries.

Biomethane (97% CH_4 after removing CO_2 from biogas) is a versatile renewable source of biofuel that can effectively replace natural gas in complex energy systems, transport, and agriculture (WALL et al., 2017; AVAGYAN, 2018). Further, Marín et al. (2018) proposed that the use of photosynthetic biogas from microalgae could overcome the economic and environmental disadvantages associated with the traditional modernization of biogas.

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Biomethane is produced via the process of anaerobic digestion. In this digestion process, the biomass of moist microalgae can be directly used to produce methane, thereby eliminating the energy required for dehydration and therefore, reducing the total energy consumption in biogas production. Besides anaerobic digestion, other conversion methods have been examined to extract energy from microalgal biomass, such as ethanol fermentation, lipid extraction, and anaerobic microbial cells (LAKANIEMI et al., 2013; PASSOS et al., 2014; AVAGYAN, 2018).

Owing to the presence of a wide range of carbohydrates (7%–69% of volatile solids [VS]), lipids (1-63% VS), and proteins (15%–84% VS), microalgae are attractive substrates for anaerobic digestion, directly or after the extraction of specific fractions (GANESH SARATALE et al., 2018).

Bose et al. (2020) reported the main benefits of gaseous biofuels produced by anaerobic digestion. These benefits include the potential use of gaseous biofuels in electricity, heat, and/or transportation; lower operating costs than other advanced bioenergy technologies, such as gasification and pyrolysis; and the ability to incorporate a wide variety of raw materials.

In the comparative analysis of biogas production by different microalgae species, *Chlorella kessleri* and *Scenedesmus obliquus* were found to display the lowest methane yields (218 and 178 $mL.g^{-1}$ VS, respectively), while other chlorophyll microalgae, such as *Chlamydomonas reinhardtii* (cell wall based on proteins without cellulose) and *Dunaliella salina* (without cell wall), had biomethane yields of 387 and 323 $mL.g^{-1}$ VS, respectively (MUSSGNUG et al., 2010).

Studies have addressed the production of biomethane associated with effluent treatment. For example, Brar et al. (2020) evaluated the efficiency of bioremediation and the productivity of biomethane in crops with municipal effluent after primary treatment. Based on their findings, biogas productivity in crops reached values ranging from 618-925 $mg.L^{-1}$, with a percent biomethane of 48-65%. Figure 6 displays a biomethane production scheme with cogeneration and biogas purification that covers the energy demand.

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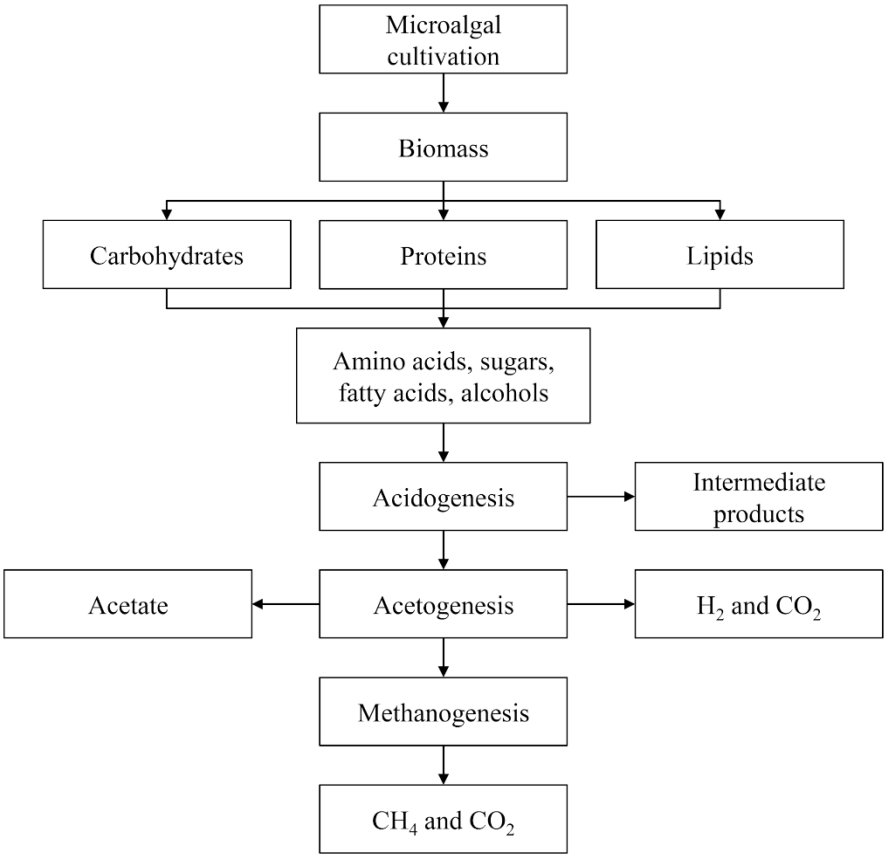
Figure 6. Scheme of microalgal culture in biomethane production.



Adapted from (Cavinato et al. 2017).

3.4 Biofertilizers

Current agricultural practices intensively exploit available arable land for cultivation, which has resulted in the loss of nutrients that are essential for optimal plant growth (BHALAMURUGAN; VALERIE; MARK, 2018). As a result, there is an indiscriminate use of fertilizers that induce a series of environmental liabilities, such as eutrophication, soil infertility, and loss of biodiversity (KÖHLER; TRIEBSKORN, 2013). Biostimulants and biofertilizers are considered ecological and low-cost alternatives to synthetic products, such as fertilizers, products for crop protection, and plant growth regulators (KAWALEKAR 2013; AVAGYAN, 2018). Furthermore, the microalgae, *C. vulgaris* and *S. platensis*, are considered essential biofertilizers and are mainly studied because of their commercial importance as sources of proteins, vitamins, amino acids, and fatty acids (DINESHKUMAR et al., 2020).

Biofertilizers can be produced from two processes, aerobic digestion and anaerobic digestion. The aerobic digestion process employed for biofertilizer production includes

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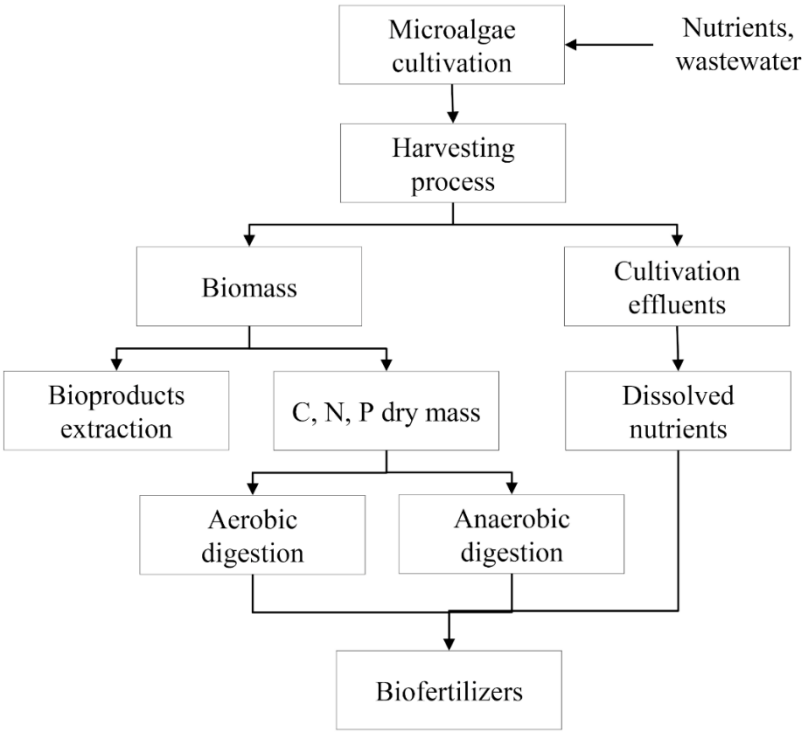
traditional solid compost and liquid compost. According to Demeke and Gabbiye (2020), in solid composting, biomass, CO_2 and water generate stabilized compounds that can be applied as biofertilizers in the soil when the environmental factors are favorable. For the biofertilizers produced through liquid composting, heterotrophic microorganisms are known to degrade organic matter.

Anaerobic digestion consists of complex associative interactions that transform organic matter into CH_4 and CO_2 (APPELS et al., 2008), based on the steps of hydrolysis, acidogenesis, acetogenesis, and methanogenes.

Organisms, such as algae, bacteria, and fungi transform organic and inorganic compounds containing macroelements into other compounds that are easily digestible by plants. Microalgae contribute to biorefineries by reducing gases in the atmosphere through nutrient accumulation and metabolism, which directs the accumulation of C, N, P, and S. According to Ferreira et al. (2019), most cyanobacteria can fix atmospheric nitrogen, which can be effectively used as biofertilizers.

As shown in Figure 7, during microalgal cultivation, biomass mineralizes nutrients incorporated into organic compounds. For example, organic P is available for absorption by microalgae or plants after it is hydrolyzed by extracellular phosphatases, which releases the inorganic phosphate, thereby causing the phosphodiester bonds to divide (DYHRMAN, 2016).

Figure 7. Scheme of biofertilizer production of microalgae cultivation.



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Although only few studies have investigated the use of microalgae biomass as a fertilizer in agricultural practices (AVAGYAN, 2018; AVAGYAN, 2021), some have revealed the positive effect of microalgae on plant growth and the high yield of several crops (EKINCI et al., 2019). Previous studies conducted under field and greenhouse conditions indicated that dried or wet application of microalgae (*Chlorella vulgaris*) to the soil caused a higher nutrient uptake, and shoot and root growth of maize (SHAABAN, 2001).

According to Wuang et al. (2016), the use of *S. platensis* to treat effluent from aquaculture and the subsequent application of algal biomass in fertilizer studies demonstrated their viability. In fact, because the concentrations of ammonia and nitrate in water could be removed, its ability to treat water despite being inadequate at removing nitrite was demonstrated. Supplementation of leafy vegetables with *S. platensis* resulted in greater plant growth in all tested vegetables than the controls. However, the *Spirulina*-based fertilizer had a comparable performance to the chemical fertilizer used to examine most plant growth parameters. Further, this fertilizer was identified to be favorable for the tested species, Arugula.

In addition to microalgal biomass as a biofertilizer, the effluent resulting from microalgal cultivation could be used as a fertilizer. In fact, if the efficiency of nutrient removal by microalgae is not high, nutrients that can be used by plants still remain, which mitigates the presence of residues from this process.

3.5 Biochar

Biochar is a carbon-rich carbon from any biomass produced by thermal decomposition of the organic raw material under a limited supply of oxygen (O_2) at a relatively low temperature (ALHASHIMI; AKTAS, 2017). Microalgal biomass can be converted into biochar through thermochemical conversion from temperatures ranging from 200 to 750 °C (SORIA-VERDUGO et al., 2017; AVAGYAN, 2018; ONG et al., 2019).This thermal transformation, which requires further studies and elevates the costs of a process, is a disadvantage during the comparison of biochar with biofertilizers, as the latter uses microalgae directly as a fertilizer, without the need for a transformation process.

According to Maguyon and Capareda (2013), biodiesel production corresponds to only 17% to 43% of the initial biomass for products derived from microalgae with properties similar to some liquid fuels. Further, the remaining biomass was observed to be transformed into syngas (8%–25%) and biochar (34%–63%). Accordingly, searches for alternatives to this specific by-

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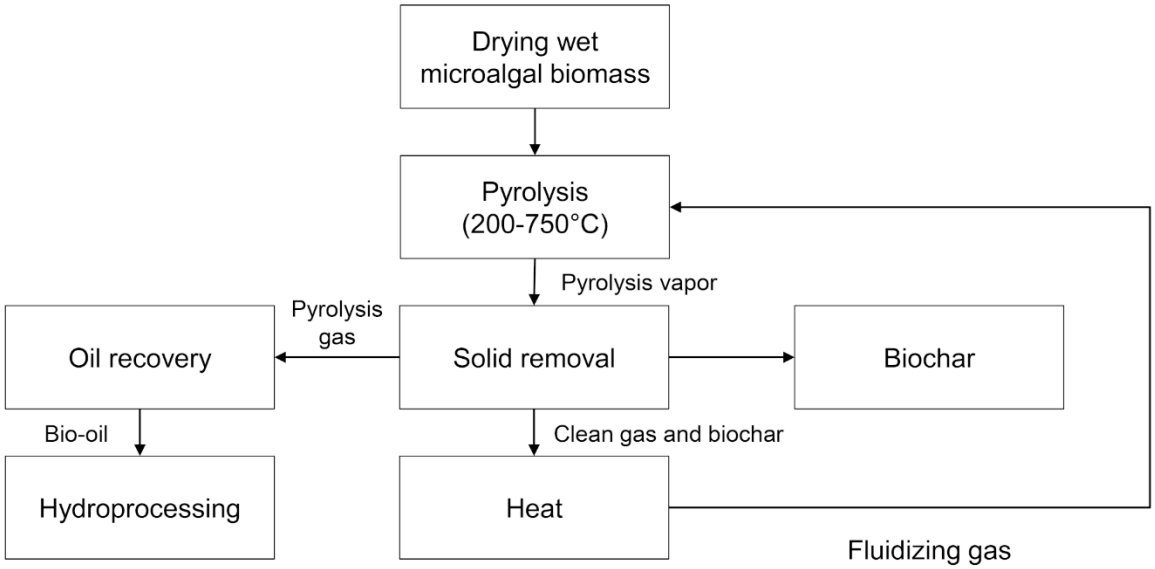
product are ongoing owing to its potential in areas, such as product making, agriculture (SANTOS; PIRES, 2018), and the environment (ROSS et al., 2015; SARKAR; SHIMIZU 2015). Biochar production can thus be considered as one of the potential value-added strategies for microalgae cultivation in effluent treatment based on its multiple utilities in the context of biorefinery (SARKAR et al., 2015).

Biochar based on microalgae consists of large aggregates ranging from 10 to 100 μm in size and an irregular porosity of 1 μm (YU et al., 2017). According to Choi et al. (2020), biochar acts as an ecological and economical adsorbent that can be used to remove antibiotics from water sources. As shown in Figure 8, biochar originates from products established via pyrolysis.

Figure 8. Biochar production based microalgal biomass.



Adapted from Yu et al. (2017)

Gan et al. (2020) studied wet roasting associated with microwaves for the co-production of biochar and sugar from microalgal biomass. These researchers used two different strains of *Chlorella vulgaris* as the microalgal, each of which exerted a characteristic of greater carbohydrate and protein accumulation. Based on their findings, biochar produced with organic acid is desirable as a solid fuel; however, using sulfuric acid is more suitable for producing sugar for bioethanol production.

Yu et al. (2018) showed that the cultivation of the microalga, *C. vulgaris*, and the production of its respective microalgal biochar through pyrolysis could be employed as a potential clean technology for carbon sequestration and microalgal biorefinery in a sustainable environment. Using a CO_2 concentration of 2.5%, *C. vulgaris* cultivation was found to have a

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maximum biomass productivity of 0.87 $g.L^{-1}.d^{-1}$. Further, the pyrolysis conversion process of the microalgal biomass generated 26.9% of the total biochar yield.

Roberts et al. (2013) revealed the viability of an integrated biomass production process for algae grown in municipal effluent as a nutrient source. The production was carried out on a pilot scale in raceways, with a mixed culture of algae. Notably, hydrothermal processing was found to result in the formation of 18.4 ± 4.6% ash-free dry weight aqueous co-products and 45.0 ± 5.9% dry weight solid biochar.

4 Future perspectives

The cultivation of microalgae in consortium has advantages for bioremediation of effluents and for obtaining biomass and bioproducts, however this technology presents challenges. How to promote cooperative interactions between microorganisms that result in an overall increase in biomass productivity and nutrient removal efficiency (LÓPEZ ROCHA et al., 2020) is an important challenge to be solved, as each environmental condition constitutes a new problem to be studied, related to its toxicity to the microorganisms involved. Another challenge lies in defining the best type of interaction to be used (microalgae/microalgae and microalgae/bacteria) in the treatment of effluents from different sources. Furthermore, it is necessary to define the mechanisms involved in the removal of nutrients by microalgae or microalgae/bacteria and how the interactions between the microorganisms that make up the consortia can improve the process of removing contaminants and nutrients (FOUILLAND, 2012; GONÇALVES et al., 2016). Still, it is important to understand the potential of microalgae cultivation for the production of bioproducts, which have some limitations, mainly related to the costs of achieving commercial production. Furthermore, the best cultivation conditions observed for nutrient removal or algal growth may not be the best conditions for bioproducts accumulation.

Obtaining sufficient biomass for the production of microalgae biofuels can be hampered by the need to perform pre-treatments on effluents (for example, sterilization, dilution) in order to reduce the inhibition of microalgae growth and increase yield of energy production, as reported by Lu et al. (2015) and Cheng et al. (2019). This need can make obtaining bioethanol from microalgae very expensive (SANDEFUR et al., 2016), making it necessary to develop systems where it is not necessary to perform pre-treatments on the effluent.

Hena et al. (2015) demonstrated the production of biodiesel from microalgae cultivated consortium in dairy farm wastewater. To improve the economic viability in general, high-value

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products such as arachidonic acid and eicosapentaenoic acid can be extracted from biomass, and the rest of the oil can be converted into biodiesel. And after lipid extraction, the energy stored in the residual biomass can be recovered through biomethane. This is an example of the use of all potential of the microalgal biomass in the context of biorefineries. The same can be done after the production of bioethanol by microalgae biomass, as shown by Dar et al. (2019), Garfí et al. (2019) and Rempel et al. (2019), who produced biomethane using the residues from the production of bioethanol, in order to reduce the impacts of these production processes on the environment and add value to these renewable raw materials.

Another future perspective to be better explored is the production of biofertilizers using microalgae biomass, as the quality of this products is largely a function of processing as well as the quality of the microalgal biomass used. This type of microalgae-based products is more common in agriculture every day and will continue to grow due to its demonstrated positive effects in increasing plant growth and production (ACIÉN FERNÁNDEZ et al., 2018).

Relative to biochar production, one challenge is the reduction of production costs, especially with drying and pre-treatments that are still expensive for industrialization. As the wet biomass needs to be dehydrated before the pyrolysis process, an increasing in the production cost is observed. Regarding this problem, microwave-assisted pyrolysis may be the solution to reduce the cost of pre-treatment, as this technology is capable of handling moderately wet samples to produce pyrolytic products (LEE et al., 2020).

Thus, it appears that the need to define the best conditions for cultivation case by case, for each type of effluent and related to each microalgal consortium to be proposed. The biomass obtained, in turn, will depend on the defined conditions, and it will be possible, from this, to determine the best options for bioproducts. Given the infinity of propositions that can be made, further studies on the characterization of effluents and consortia, as well as the quality of the biomass obtained, should be carried out in order to make safe propositions in the context of integrated biorefineries.

5 Conclusion

Microalgae have been emerging as the most promising organisms in assessments of the demand for sustainable energy production. The present review sought to analyze and present the most recent advances in the biotechnological processes of microalgae cultivation using effluents and the use of biomass for the production of several bioproducts. The main challenges in this process involve the adaptation of the microalgae to different types of effluents. As a

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result, assessing the most resistant species, as well as the consortia between microalgae that allow the achievement of high cell concentrations is critical, and will enable the use of biomass to obtain biofuels, such as bioethanol, biodiesel, biomethane, or even biofertilizers or biochar. These bioproducts are important alternatives to the algal biomass obtained after cultivation with effluents as the sources of nutrients. This is due to the limitations associated with the use of these biomasses for purposes of nutrition, which contribute to the mitigation of the damages induced by environmental pollution (potentially caused by effluents) and their recovery.

Some industrial and municipal wastewater treatment plants already use decanters with microalgae to carry out post-treatment or final polishing of the effluent. As a result, the quality parameters are achieved in these instances. In a practical sense, this would be the most efficient and profitable route for industries and cities to implement algal biomass collection systems, ultimately directing it to biotechnological conversions that generate high value-added bioproducts. The future use of microalgae for bioproduct manufacture will thus depend on advances in the cultivation of microalgae in effluents, which is observed as a low-cost cultivation-free medium. Further, the entire biochemical composition of the cell could be used to generate different types of bioproducts.

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3 CAPÍTULO II - DEFINITION OF MEANS FOR CONSORTIUM AND STANDARDIZATION OF CELL CONCENTRATION DETERMINATION

Abstract
There is a need to meet current demand for fossil fuels through alternative sources of renewable energy. Among the sources for the sustainable production of biofuels, microalgal biomass capable of accumulating carbohydrates, which is necessary for the subsequent synthesis of bioethanol, is a promising one. The use of different species of microalgae becomes promising when evaluated on a large scale, at which point the sterile condition ceases to exist. Therefore, the objective of this work was to evaluate the best conditions for cultivation in a consortium of *Spirulina* and *Scenedesmus* microalgae, for subsequent bioethanol. Initially, the culture media Zarrouk diluted to 20%, BG-11 and the modified BGZ medium cultured with the different microalgae in individual photobioreactors were studied. With the growth kinetics, it was observed that the microalgae grown with the Zarrouk medium 20% had higher growth, as well as greater accumulation of intracellular carbohydrates.

Keywords: Microalgae, Biorefinery, Biofuels, Bioproducts,

1 Introduction

Over the years, the industrialization and the population growth has been demanding for high energy consumption. The world economy is focused on the use of non-renewable sources of energy has been rethinking her performance. Around the globe, highlight investments in alternative energy sources.

In relation of fuels, however, according to Li; Liu; Liu, (2014), the most of energy parts still provides by fossil fuels, how petroleum, coal and natural gas. Researches, however, are seeking to develop biofuels obtained different types of biomass.

Photosynthetic microorganisms has a big potential as sources of transformation of solar energy in chemical energy, when have high rates of biomass production, much higher than vascular plants. The development of process and technologies that allow the extraction of these compounds from biofuels of 3rd generation production is, that way, a highly desirable solution, with the advantage that they can be produced in marginal lands, using salty waters, brackish or residual (BAICHA et al., 2016).

The importance of a consortium cultivation is that, when it presence of organic sources of carbon or compounds that has toxicity, in the case of effluents, the microalgae may present synergy, presenting better results in the biomass production. In this sense, the objective was

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determining the best conditions for growth of *Spirulina platensis* and *Scenedesmus oblíquos* in a consortium for the bioethanol acquisition

2 Material and Methods

At first, the standard culture medium with which microalgae were best developed was defined. For this, *S. platensis* (Sp) and *S. obliquus* (Sc) cultivated in the different culture media were used: Zarrouk diluted to 20% (Z) commonly used in S. platensis, BG 11 commonly used in S. obliquus and the BGZ medium (BG 11 medium with addition of 3,36 g. L^{-1} sodium bicarbonate) (Table 1).

Table 1. Composition of culture media Zarrrouk, BG11 (BG), and BG-11 added sodium bicarbonate (BGZ).

Nutrient	Zarrouk medium 20% (Z)	BG11 (BG)	BG-11 + $NaHCO_3$ (BGZ)
$NaHCO_3$ (g/L)	3.36	-	3.36
Na_2CO_3	-	0.02	0.02
K_2HPO_4 (g/L)	0.1	0.04	0.04
$NaNO_3$ (g/L)	0.5	1.5	1.5
K_2SO_4 (g/L)	0.2	-	-
NaCl (g/L)	0.2	-	-
$MgSO_4.7H_2O$ (g/L)	0.04	0.075	0.075
$CaCl_2$ (g/L)	0.008	-	-
$CaCl_2.2H_2O$	-	0.036	0.036
$FeSO_4.7H_2O$ (g/L)	0.002	-	-
EDTA (g/L)	0.016	0.001	0.001
Citric acid	-	0.006	0.006

Strains isolated from the microalgae *Spirulina platensis* and *Scenedesmus obliquus* were used in the Laboratory of Biochemistry and Bioprocesses of the University of Passo Fundo. In all cultures performed in this work the assays were conducted in duplicate in 1 L erlenmeyer type photobioreactors, with a useful volume of 900 mL. Subsequently, the cultures were conditioned in non-sterile thermostatic greenhouses with photoperiod 12 h light/dark with 2000 lux illumination (Table 2).

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Table 2. Experiments performed to define the culture medium to be used in microalgae consortia and results of kinetic parameters obtained.

Exp.	CT (d)	Xf ($g.L^{-1}$)	Δ_{log} (d)	P_{max} ($g.L^{-1}.d^{-1}$)	μ_{max} (d^{-1})	Td (d)
Z-Sp	29	1.780 ± 0.080^{a}	21	0.101 ± 0.002^{a}	0.050 ± 0.007^{c}	12.13 ± 0.08^{a}
BG-Sp	29	0.840 ± 0.007^{c}	24	0.063 ± 0.009^{a}	0.055 ± 0.002^{c}	12.55 ± 0.55^{a}
BGZ-Sp	29	1.670 ± 0.150^{ab}	24	0.111 ± 0.005^{a}	0.077 ± 0.001^{b}	8.93 ± 0.04^{bc}
Z-Sc	28	1.730 ± 0.020^{a}	21	0.113 ± 0.020^{a}	0.101 ± 0.002^{a}	6.83 ± 0.15^{c}
BG-Sc	28	1.310 ± 0.060^{b}	24	0.101 ± 0.030^{a}	0.084 ± 0.004^{ab}	8.18 ± 0.39^{bc}
BGZ-Sc	28	1.340 ± 0.160^{b}	22	0.074 ± 0.001^{a}	0.089 ± 0.001^{ab}	7.77 ± 0.07^{bc}

Cultivation time (CT), Final biomass concentration (Xf) ($g.L^{-1}$), Δ_{log}: duration of exponential growth phase (d), P_{max}: maximum cell productivity ($g.L^{-1}.d^{-1}$), μmax: maximum specific growth rate (d^{-1}), Td: Time of duplication (d). Sc: *Scenedesmus*; Sp: *Spirulina*.

Mean values of tests performed in duplicates ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level ($p > 0.05$).

2.1 Data processing and statistical analysis

Microorganism growth curves versus time were constructed. The final biomass concentration (X_f, $g.L^{-1}$ or number of cells per mL^{-1}), maximum biomass productivity ($P_{máx}$, $g.L^{-1}.d^{-1}$) (Equation 1), and maximum specific growth rate ($\mu_{máx}$, d^{-1}) (Equation 2) were evaluated (SCHMIDELL et al., 2001).

$$P_{máx}(g.L^{-1}.d^{-1}) = \frac{X - X_0}{t - t_0} \quad (1)$$

$$\mu_{max}(d^{-1}) = \frac{1}{\Delta t}.Ln\frac{X_2}{X_1} \quad (2)$$

Where P_{max} is the maximum biomass productivity ($g.L^{-1}.d^{-1}$), X is the biomass concentration ($g.L^{-1}$) in time t (d), and e X_0 is the biomass concentration ($g.L^{-1}$) in time t_0 (d). X_1 and X_2 are the biomass concentration ($g.L^{-1}$) in the begging and in the end of exponential phase, Δt is the duration time (h) of exponential phase, and μmax = maximum specific growth speed (d^{-1}).

Productivity of carbohydrates and proteins in cultivation ($g.L^{-1}.d^{-1}$) was obtained according to Equation (3 and 4) (MARGARITES et al., 2016).

$$\text{Carbohydrate Productivity } (g.L^{-1}.d^{-1}) = \frac{X_f \times CHO}{100 \times t_c} \quad (3)$$

$$\text{Protein Productivity } (g.L^{-1}.d^{-1}) = \frac{X_f \times PROT}{100 \times t_c} \quad (4)$$

Where X_f is the final biomass concentration ($g.L^{-1}$), CHO is the carbohydrate percent in biomass (%), PROT is the protein content in biomass (%), and e t_c is cultivation time (d).

Differences between the means of the evaluated parameters were analyzed using analysis of variance at the 95% confidence level followed by Tukey's post-hoc test. All tests were performed in duplicates. The results were expressed as the average ± standard deviation.

2.2 Characterization of microalgae biomass

The biomasses obtained in cultivation were characterized in relation to carbohydrate and protein contents. The samples for quantification of carbohydrate and protein content were prepared via sonication of 5 mg of dry biomass in 10 mL of distilled water and sonication for five 59 s cycles in a cell disruptor device (Unique Tip Model DES500). Carbohydrate content was determined using the phenol sulfuric method (DUBOIS et al., 1956). The protein content in algal biomass was determined according to the methodology proposed by Lowry (LOWRY, 1951). The contents of carbohydrates and proteins are presented on a dry basis.

3 Results and Discussion

Figure 7 shows the growth curves of microalgae grown individually in Zarrouk 20%, BG-11 and BGZ media. The microalgae *Spirulina* cultivated in the BG-11 (BG) medium showed the lowest growth. This microalgae is normally grown in Zarrouk medium, which has a high concentration of nutrients when compared to BG-11 medium. In addition, the BG-11 medium has a low concentration of carbon source (0.02 $g.L^{-1}$ of sodium carbonate) compared to the Zarrouk medium, in which 3.36 $g.L^{-1}$ of sodium bicarbonate are added. Therefore, the low concentration of carbon source in the BG-11 medium may have negatively affected the

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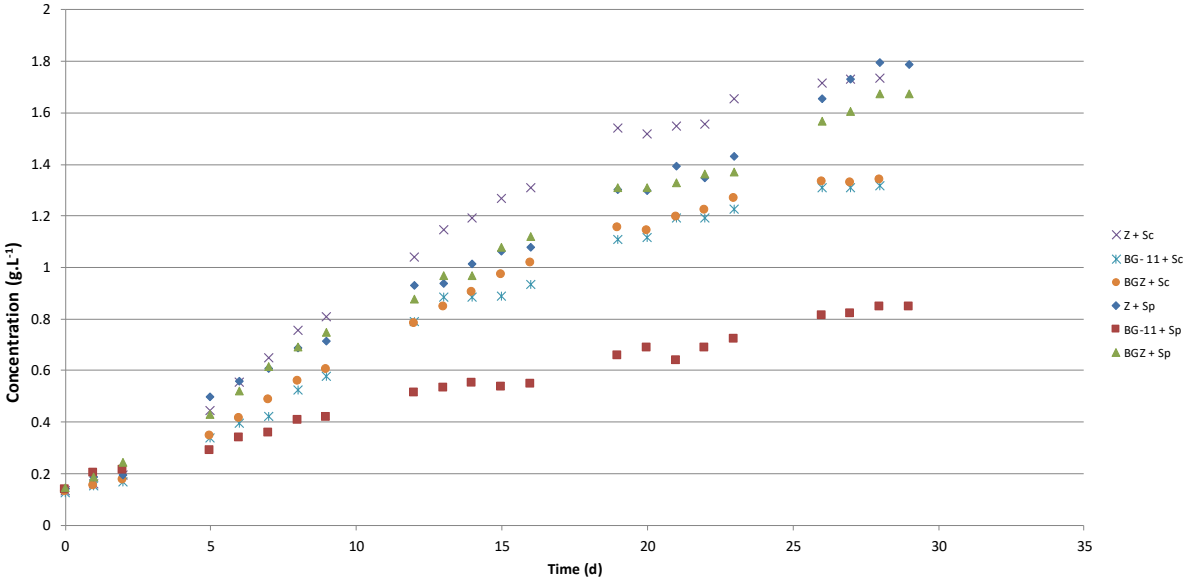
growth of the *Spirulina* microalgae. According to Cai; Park; Li, (2013), carbon is considered one of the most important nutrients, since it constitutes about 50% of microalgal biomass. Its high demand stems from the fact that this component is the main constituent of all organic substances synthesized by cells (proteins, carbohydrates, nucleic acids, vitamins, lipids, among others).

The most appropriate culture medium for the growth of the *Spirulina* and *Scenedesmus* microalgae was the Zarrouk (Z) medium, which in comparison to the BG-11 and BGZ media has high concentrations of nutrients. The *Scenedesmus* microalgae, which is usually grown in BG-11 medium, showed better growth in Zarrouk medium.

Figure 1. Curvas de crescimento dos cultivos realizados



The highest final biomass concentrations were reached in the Z + Sc, Z + Sp and BGZ + Sp tests (Table 3), with no significant difference between them ($p > 0.05$), demonstrating that the Zarrouk medium was the one that offered the best condition for the highest concentration of both microalgae.

Table 3 presents the results of final biomass concentration, maximum specific growth speed, generation time and maximum productivity. There was no significant difference ($p > 0.05$) in the maximum productivity results.

Table 3. Experiments performed to define the culture medium to be used in microalgae consortia and results of kinetic parameters obtained.

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Exp.	Tc (d)	Xf ($g.L^{-1}$)	Δlogl (d)	Pmax ($g.L^{-1}.d^{-1}$)	μmax (d^{-1})	tg (d)
Z + Sp	29	1,78±0,08[a]	21	0,1011±0,002[a]	0,050±0,0066[c]	12,13±0,08[a]
BG 11 + Sp	29	0,84±0,007[c]	24	0,0627±0,009[a]	0,055±0,0024[c]	12,55±0,55[a]
BGZ + Sp	29	1,67±0,15[ab]	24	0,1114±0,005[a]	0,077±0,0003[b]	8,93±0,04[bc]
Z + Sc	28	1,73±0,02[a]	21	0,1133±0,02[a]	0,101±0,0023[a]	6,83±01,5[c]
BG 11 + Sc	28	1,31±0,06[b]	24	0,1007±0,03[a]	0,084±0,0041[ab]	8,18±0,39[bc]
BGZ + Sc	28	1,34±0,16[b]	22	0,074±0,0008[a]	0,089±0,0008[ab]	7,77±0,07[bc]

Cultivation time (CT), Final biomass concentration (Xf) ($g.L^{-1}$), Δ_{log}: duration of exponential growth phase (d), P_{max}: maximum cell productivity ($g.L^{-1}.d^{-1}$), μmax: maximum specific growth rate (d^{-1}), Td: Time of duplication (d). Sc: *Scenedesmus*; Sp: *Spirulina*.

Mean values of tests performed in duplicates ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level ($p > 0.05$).

Figure 2 (a) shows the concentrations of carbohydrates and proteins, obtained in the cultivation of microalgae in different media.

The microalgae cultivated in the Zarrouk medium showed greater capacity to accumulate carbohydrates: the Z + Sp test presented the highest concentration of intracellular carbohydrates, being followed by the Z + Sc test. This fact is possibly due to the addition of lower concentrations of the nitrogen source 0.5 $g.L^{-1}$ ($NaNO_3$) in the Zarrouk medium, thus occurring a high C/N ratio, while in other media 1.5 g was added/L ($NaNO_3$). Due to the fact that nitrogen depletion directs the metabolism of microalgae previously turned to cell multiplication for the production of reserve components, such as saturated fatty acids, preparing the cell for a period of nutritional deprivation (ALONSO et al., 2000; XU et al., 2012). It was also found that the microalgae grown in both the BGZ and the BG-11 medium showed high concentrations of proteins, but small concentrations of carbohydrates.

Figure 02 (b) shows the carbohydrate productivities ($g.L^{-1}.d^{-1}$). It is possible to observe that the highest yields for both microalgae were obtained in cultivation with the Zarrouk medium. As the cultivation time (d) suffered only one day variation for the different microalgae, what determined the productivity was the final cell concentration and carbohydrate concentration.

Figure 2. Concentrations of intracellular carbohydrates and proteins (%) (a) and productivities ($g.L^{-1}.d^{-1}$) (b) for the assays performed to define the culture medium to the cultivation of microalgae in consortia

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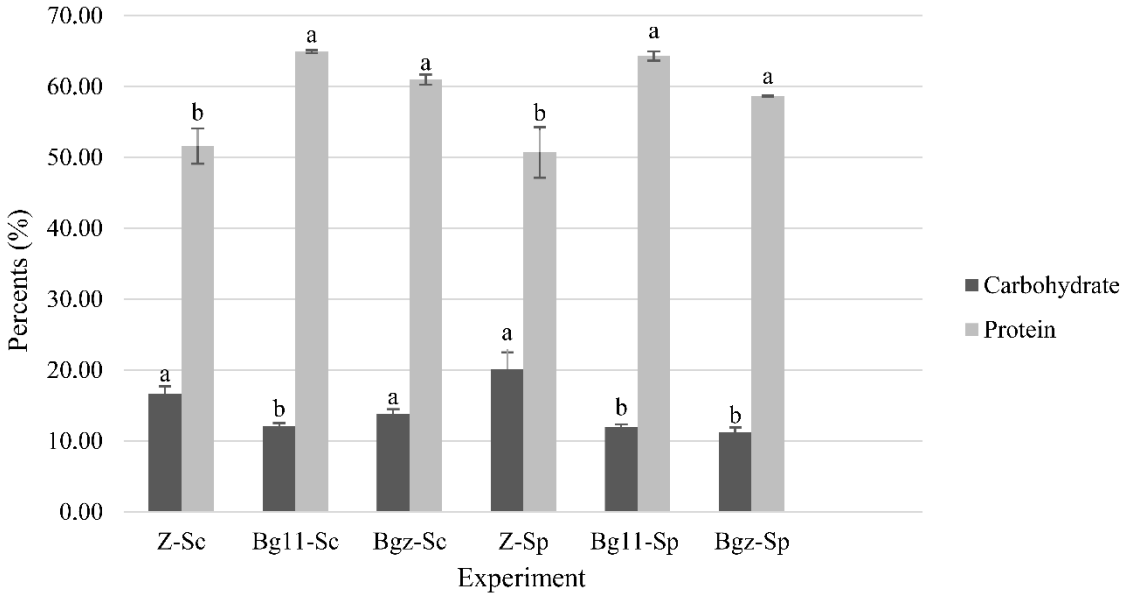
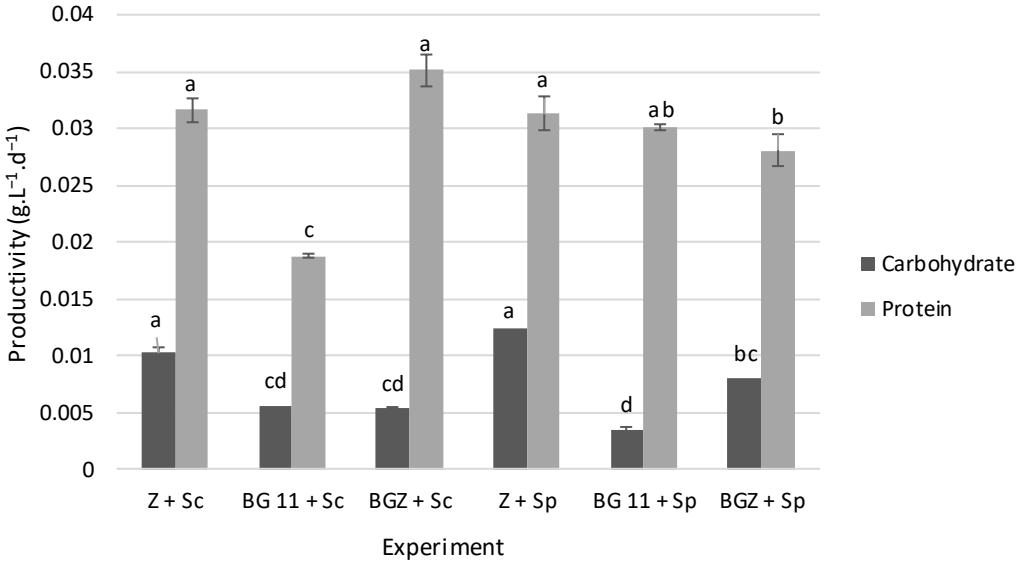


(a)

Mean values of tests performed in duplicates ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level (p> 0.05).



(b)

Mean values of tests performed in duplicates ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level (p> 0.05).

Nitrogen makes up on average about 7-10% of the dry weight of microalgal biomass and is essential for the constitution of structural and functional proteins in algal cells. (QIANG HU, 2004). When inorganic nitrogen is available in the crop, there is an increase in the concentrations of proteins, carotenoids and chlorophyll, however, as the nitrogen in the medium is limited, the quantities of these substances are reduced (LOURENÇO, 2006).

According Ho; Chen; Chang, (2012), the production of lipids and carbohydrates by *S. obliquus* CNW-N was significantly increased using an appropriate light intensity and nitrogen deprivation strategies, since nitrogen deprivation period is an important factor that influences the accumulation of lipids and carbohydrates.

Vasileva et al. (2015) investigated the influence of different nitrogen sources (ammonium nitrate, urea and ammonium nitrate + urea) on the growth, protein, carbohydrate and lipid content of *Scenedesmus* sp. cultivation, the use of medium with each of them separately provided a better biomass yield during the entire cultivation period. The best growth was observed in medium containing urea, where the concentration of biomass reached 9.0 $g.L^{-1}$. Carbohydrates, followed by proteins and lipids, dominated the biochemical composition of *Scenedesmus* sp.

4 Conclusion

The culture media influenced the growth of the two microalgae species, and it was possible to observe that the cultures reached the highest cellular concentration and carbohydrates were those cultivated in Zarrouk medium for both microalgae. While the cultures carried out with the BG-11 and BG-11 media added with sodium bicarbonate contain high concentrations of proteins.

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4 CAPÍTULO III - MICROALGAE CONSORTIA FOR POST-TREATING EFFLUENT OF ANAEROBIC DIGESTION OF CATTLE WASTE AND EVALUATION OF BIOCHEMICAL COMPOSITION OF BIOMASS

Abstract
The aim of this study was to cultivate *Spirulina platensis* and *Scenedesmus obliquus* microalgae in consortia using effluents of cattle waste anaerobic treatment, in order to give possibilities to the production of microalgae biomass to biorefineries uses. The biomasses obtained were characterized to evaluated the potential for the production of biofuels and other bioproducts. The effluent was used in sterile and non-sterile conditions to better understanding the influence of others microrganisms in N and P removal. The biomass obtained with addition of 10% of sterile effluent in Zarrouk media (20%) presented 44.12% and 34.62% of carbohydrates, using *Spirulina platensis* in monoculture or the 50%/50% consortia of *Spirulina* and *Scenedesmus*, respectively, this biomass presenting potential to be used to bioethanol production. Nitrogen and phosphorous removal were higher in non-sterile conditions and reached 92.7% and 49.66% of nitrogen and phosphorous removal, respectively, using the consortia and with the addition of 30% effluent in the media. The cultivation of microalgae in a consortium may be used to assist the treatment of water concurrently with the production of biomass to different applications.

Keywords: Bioeconomy; Bioethanol; Carbohydrates; Proteins; *Spirulina, Scenedesmus*.

1 Introduction

The need for sustainable use of natural resources, as well as the growing demand for renewable energy sources, in addition to involving environmental security related to changes, drive the current ones towards the transformation of a traditional linear economy to a circular economy. Among the challenges for such green environmental initiatives is the recovery and efficient reuse of solid waste and effluents generated by human activities, such as industrial, domestic and agricultural practices (VADIVELOO; NWOBA; MOHEIMANI, 2019).

Besides that, concerns about energy dependence, security and climate change have led to an increased interest in the production of biofuels, as bioethanol. Microalgae have potential to offer sustainability to the future of the planet as an alternative energy resource using bioremediation processes or mitigating the effects of global warming, and applications for obtaining bioproducts that may be used to improve human health and food safety (MIRANDA; PASSARINHO; GOUVEIA, 2012; SOUZA et al., 2019; VIEIRA SALLA et al., 2016a).

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Integrated biorefineries use a biofuels approach in combination with other value-added products from microalgae biomass (SARKAR; SHIMIZU, 2015). The production of bioproducts in biorefineries based on microalgae biomass includes the following steps: microalgae cultivation, biomass harvesting, cell disruption and biocompound extraction, fractionation and purification (MARKOU; NERANTZIS, 2013).

The use of biofuels adds less carbon to the environment compared to fossil fuels because the carbon released via this action already exists as part of the carbon cycle (POPP et al., 2014), which minimizes the environmental impact related to greenhouse gas emissions, the acidification and eutrophication of aquatic environments, and water use (COLLA et al., 2019). To obtain biofuels, microalgae have the advantages of fast and sustainable growth (MYEONG; HOON; BAE, 2017), and no competition with food for arable land, which was needed for the first-generation biofuels from sugary or starchy raw materials (MAN et al., 2018). The environmental impact of second-generation biofuels obtained from lignocellulolytic biomass may include the generation of effluents containing chemicals and the eutrophication and acidification of soils, and energy costs related to the processes involved in biomass disruption (DAYLAN; CILIZ, 2016).

Colla et al. (2019) demonstrated that microalgae cultures, despite having high CO_2 emissions in open lagoons, removed this gas from the atmosphere in photoautotrophic cultivation, and the high use of water was solved with the use of salty water or liquid effluents, which had a positive impact on the eutrophication and acidification that may be caused by this source. One of the environmental impacts cited in the production of bioethanol is the emission of CO_2 during fermentation. The concept of microalgae biorefinery suggests that these gases could return to the algal culture system.

The use of effluent to cultivate microalgae may reduce the costs of algal culture and contribute to make large-scale production economically viable. Several recent studies used microalgae for effluent treatment (JEBALI et al., 2018; MOHAMMADI et al., 2018), however, it is necessary to evaluate the influence of the media composition on the biochemical composition of biomass, since this can influence its final use. In addition to the benefits in terms of cost reduction, microalgae are proving to be a promising alternative for treating effluents with emerging pollutants such as acetylsalicylic acid, caffeine present fluoxetine, paracetamol, and diazepam (REMPEL et al., 2021).

Some microalgae accumulate considerable amounts of carbohydrates depending on the cultivation techniques, for example, *Spirulina platensis* grown under stress, can alter its

metabolic pathways, leading to the accumulation of carbohydrates (JOHN et al., 2011; ZAPAROLI et al., 2020), being the microalga biomass absent of lignin, which makes these organisms suitable for bioethanol production (HARUN; DANQUAH; FORDE, 2010; WANG et al., 2015). *Chlorella*, *Chlamydomona*, *Dunaliella*, *Scenedesmus*, *Tetraselmis* and *Spirulina* accumulate a large amount of carbohydrates (> 40% dry weight) (JOHN et al., 2011; MAGRO et al., 2017).

Many studies investigated microalgal consortia for effluent phycoremediation (ALMOMANI; ÖRMECI, 2016; DAVIS et al., 2015; MARAZZI et al., 2020; RUIZ-MARTINEZ et al., 2014; WANG et al., 2020). Microalgal consortia are more resistant to competition from other microorganisms, and exhibit a more robust cultivation compared to the loss of individual algae during cultivation, which is one of the main advantages of microalgae consortia in effluent treatment (PIRES; MARTINS; SIMÕES, 2012). Different degrees of resistance between species may be a competitive advantage for the use of these biomasses in the production of biofuels associated with effluent treatment from various studies (BATISTA et al., 2015; POSADAS et al., 2015).

Koreivienè et al. (2014) reported that the microalgae consortia of *Chlorella* sp. and *Scenedesmus* sp. was more efficient in removing nitrogen and phosphorus from municipal wastewater compared to the individual cultures of *Chlorella* sp. and *Scenedesmus* sp. after three weeks of cultivation, which exhibited 88.6 to 96.4% and 99.7 to 99.9% removal of nitrogen and phosphorus, respectively. However, few reports optimized microalgae consortia for bioethanol production (CASTRO et al., 2015). And, the literature still does not have reports on the level of tolerance of microalgae in relation to each constituent, due to the variation of chemical profiles in different types of wastewater (CHOONG et al., 2020).

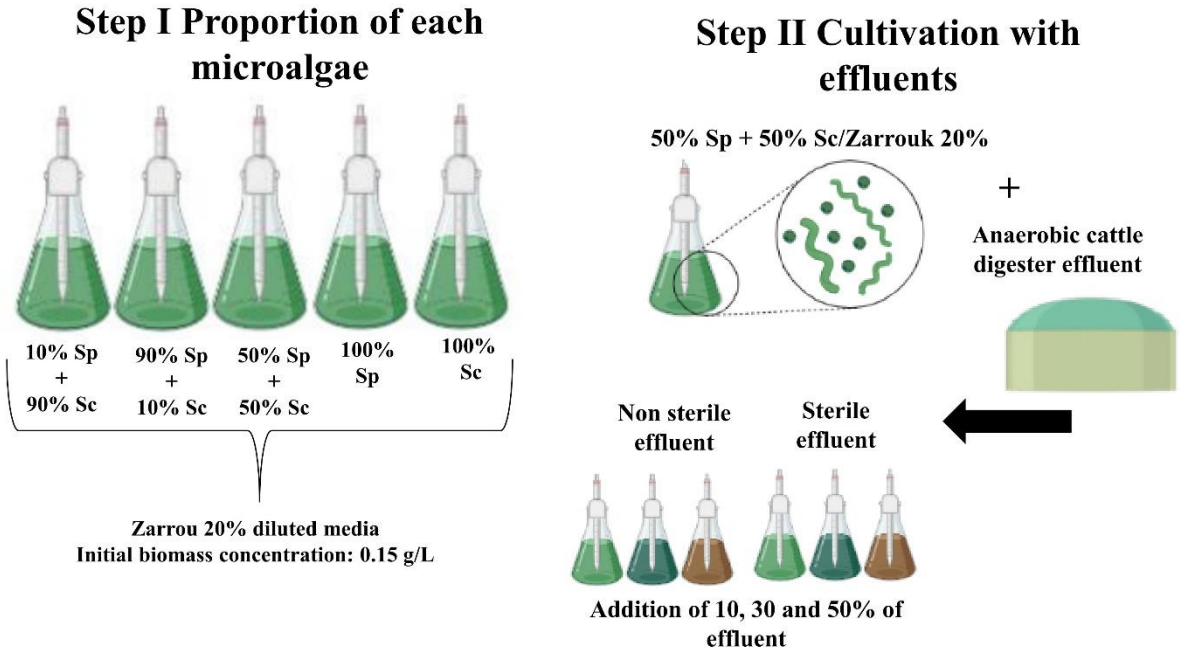
The aim of this study was to cultivate *Spirulina platensis* and *Scenedesmus obliquus* microalgae in consortia using effluent supplementation in synthetic media and to evaluate the biochemical potential of this biomass for several uses.

2 Material and Methods

The study was realized in two steps. First it was necessary the standardization of the methodology of cell counting was realized, once the microalgae *Spirulina platensis* and *Scenedesmus obliquus* obtained from the strain bank of the Laboratory of Biochemistry and Bioprocess of University of Passo Fundo (UPF), presents different characteristics in relation to

cell size and shape as well as cell mass. These step was realized using standard Zarrouk medium (ZARROUK, 1966). After, the study with the addition of effluent of cattle was accomplished. Figure 01 shows a flow diagram of the steps.

Figure 1. Flow diagram of the steps



2.1 Microorganisms, inoculum preparation and cultivation medium

The inoculum preparation and experiments using *S. platensis* LEB 52 and *S. obliquus* were performed in Zarrouk's medium diluted to 20% in sterile conditions. The composition of culture media Zarrrouk diluted to 20% is: $NaHCO_3$ (3.36 $g.L^{-1}$), K_2HPO_4 (0.1 $g.L^{-1}$), $NaNO_3$ (0.5 $g.L^{-1}$), K_2SO_4 (0.2 $g.L^{-1}$) NaCl (0.2 $g.L^{-1}$), $MgSO_4.7H_2O$ (0.04 $g.L^{-1}$), $CaCl_2$ (0.008 $g.L^{-1}$), $FeSO_4.7H_2O$ (0.002 $g.L^{-1}$), EDTA (0.016 $g.L^{-1}$).

2.2 Step I - Definition of initial cell concentrations of cultures in consortia

The cultivation of microalgae in consortia using the media Zarrouk at a 20% concentration was performed to verify the effect of the initial inoculum concentrations on growth kinetics and standardize the cell counting methods of mixed cultures because microalgae have differentiated morphologies. Different proportions of microalgae were

evaluated in the initial cultivation, and the initial total cell concentration of both microalgae was set at 0.15 $g.L^{-1}$ (VIEIRA SALLA et al., 2016b). The volume of culture medium was constant, according to the design presented in Table 1. For comparison purposes, control assays were performed in axenic conditions. Cultures were performed in 1 L Erlenmeyer flasks with a working volume of 900 mL at an initial cell concentration of 0.15 $g.L^{-1}$ with constant agitation and air injection using diaphragm pumps (Boyu model U-2800) with flow rate of 2 $L.min^{-1}$). The cultures were incubated in a temperature-controlled of 30 ºC greenhouse (TE model 4020 E-1, Tecnal, Piracicaba, São Paulo, Brazil) with a 12-h light/dark photoperiod and 2,000 lux of luminosity (DE MORAIS; COSTA, 2007). All tests were performed in duplicate. At the end of the cultures, the cells were collected by centrifugation at 3500 rpm for 10 min (centrifuge 5810, Eppendorf, Hamburg, Germany) and dried in an oven at a temperature of 50 °C to determine the carbohydrate and protein contents of the biomass.

2.3 Step II – Microalgae cultivation using effluents

2.3.1 Effluents

The effluents used were obtained after the anaerobic digestion of cattle waste, which generally contains high concentrations of chemical oxygen demand (COD), nitrogen and phosphorus, and is considered one of the most polluting effluents (LV et al., 2016). The effluent was filtered on cotton and Whatman nº 40 filter paper. Tests were performed using only filtered or filtered and sterilized effluent for 30 minutes at 121º C to verify the effects of the nutrients present in the effluent on cultivation and inhibiting the effects of commonly present bacteria.

Effluent of cattle waste was characterized according to the parameters of Total Nitrogen Kjeldahl (TKN) (Volumetric Method 4500-N_{org} B), COD (Colorimetry 5220 D), and Total Phosphorus (Potassium Persulfate Method 4500-PF), cited by American Public Health Association (APHA, 1995), and pH (potentiometric method 4500-H^+ B) according to (AOAC, 2000).

2.3.2 Cultivation

Cultures were performed in duplicate in 1-L Erlenmeyer photobioreactors with a working volume of 900 mL. Different proportions of microalgae inoculums were evaluated,

and the total initial cell concentration was set at 0.15 $g.L^{-1}$ (VIEIRA SALLA et al., 2016b). Sterile and non-sterile effluents were added at the initial cultivation time in different proportions (10 to 50% v/v). The volume of Zarrouk 20% medium added (365 mL) was constant in each photobioreactor and filled to the total volume (900 mL). Effluent and inoculum were added, and sterile distilled water was used to compensate for differences in the quantities of the inoculum used based on the volumes needed for each microalga, as outlined in Table 2. For comparison, assays were performed growing each microalga in monoculture. The cultures were incubated in a non-sterile temperature-controlled greenhouse (TE model 4020 E-1, Tecnal, Piracicaba, São Paulo, Brazil) with a 12-h light/dark photoperiod at 2,000 lux measured inside the greenhouse (DE MORAIS; COSTA, 2007), and cultivated for 15 days. All tests were performed in duplicate. At the end of the cultures, the cells were collected by centrifugation at 3500 rpm for 10 min (centrifuge 5810, Eppendorf, Hamburg, Germany) and dried in an oven at a temperature of 50 °C to determine the carbohydrate and protein contents of the biomass.

2.4 Analytical determinations during cultivation and biomass and data processing

The monitoring of microalgae growth in the consortium assays of Step I was performed by counting cells in a Neubauer chamber (GÓMEZ-SERRANO et al., 2015), and the results are expressed as cell $number.mL^{-1}$. In parallel, optical density (OD) measurements were taken at 670 nm (spectrophotometer model UV-1600, Pró-Tools, Porto Alegre, RS, Brazil) (COSTA; COLLA; FILHO, 2002) based on a pre-established relationship between biomass dry mass and absorbance. The results are expressed in $g.L^{-1}$.

The biomasses obtained in the axenic cultivation or in consortia were characterized in relation to carbohydrate and protein contents. The samples for quantification of carbohydrate and protein content were prepared via sonication of 5 mg of dry biomass in 10 mL of distilled water and sonication for five 59 s cycles in a cell disruptor device (Unique Tip Model DES500). Carbohydrate content was determined using the phenol sulfuric method (DUBOIS et al., 1956). The protein content in algal biomass was determined according to the methodology proposed by Lowry (LOWRY, 1951). The contents of carbohydrates and proteins are presented on a dry basis.

During the cultivation with effluent, nutrient consumption was monitored using Total Phosphorus and Total Nitrogen determinations (APHA, 1995), and the initial and final concentrations were monitored after biomass cultivation and separation.

2.5 Data processing and statistical analysis

Microorganism growth curves versus time were constructed. The final biomass concentration (X_f, $g.L^{-1}$ or number of cells per mL^{-1}), maximum biomass productivity ($P_{máx}$, $g.L^{-1}.d^{-1}$), and maximum specific growth rate ($\mu_{máx}$, d^{-1}) were evaluated (SCHMIDELL et al., 2001). Productivity of carbohydrates and proteins in cultivation ($g.L^{-1}.d^{-1}$) was obtained (MARGARITES et al., 2016). For all statistical analyses, Statistica 5.5 software was used. Differences between the means of the evaluated parameters were analyzed using analysis of variance at the 95% confidence level followed by Tukey's post-hoc test. All tests were performed in duplicates. The results were expressed as the average ± standard deviation.

3 Results and discussions

3.1 Step I - Definition of initial cell concentrations of cultures in consortia

Table 1 presents the results of growth of each microalga in Zarrouk media based on the number of cells.mL^{-1}. The microalgae added in the smallest proportion at the initial time also showed growth with no death or inhibition. The cultivations that reached the highest dry mass at the end of cultivation were 100% Sp (1.69 ± 0.01 $g.L^{-1}$) and 10% Sc + 90% Sp (1.72 ± 0.04 $g.L^{-1}$), which shows that the microalga *Spirulina* directly influences the highest biomass concentrations in cultivation. The lowest concentrations were obtained for 90% Sc + 10% Sp (0.79 ± 0.05 $g.L^{-1}$) and control 100% *Scenedesmus* (0.97 ± 0.05 $g.L^{-1}$). The 50% Sp + 50% Sc (1.18 ± 0.10 $g.L^{-1}$) consortia obtained an intermediate biomass concentration, being used in the experiments of Step II.

Table 1. Design for the study of different initial inoculum concentrations of *Spirulina* and *Scenedesmus* in cultivations in consortia and results of cultivation kinetic parameters

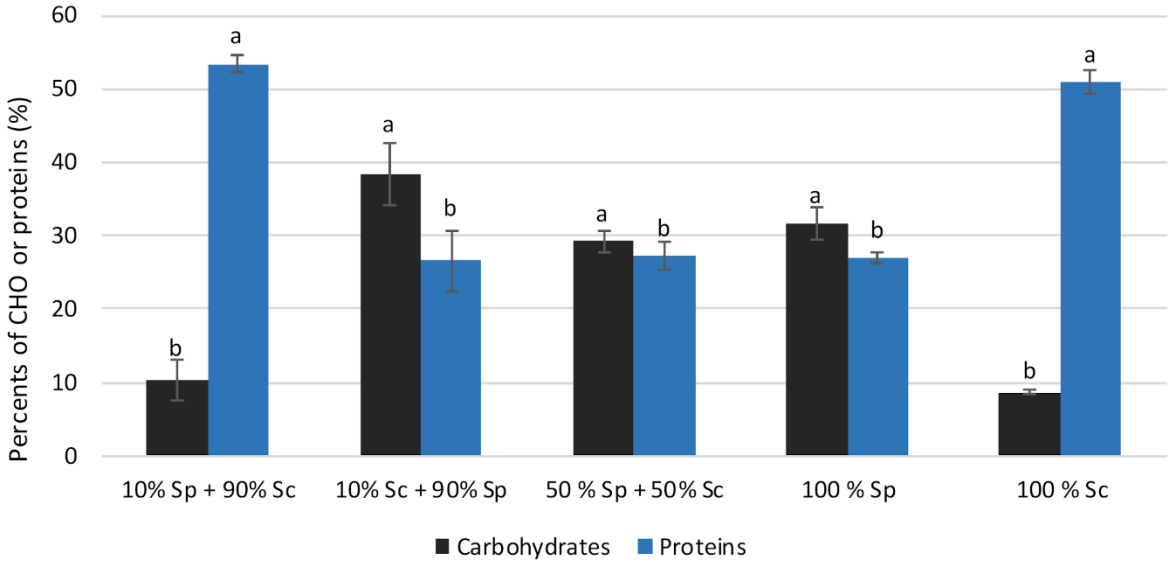
	Scenedesmus obliquus		***Spirulina platensis***	
Proportions of each microalga to obtain the initial concentration of 0.15 g_{cells}/L Experiments	**X_{max} (cells/mL)**	μ_{max} (d^{-1})	**X_{max} (cells/mL)**	μ_{max} (d^{-1})
10% Sp + 90% Sc	$9.50.10^6 \pm 1.75.10^5$	0.75±0.05	$3.53.10^3 \pm 9.69.10^2$	0.31±0.05
90% Sp + 10% Sc	$3.75.10^5 \pm 2.50.10^4$	0.77±0.11	$2.47.10^4 \pm 5.94.10^2$	0.19±0.02
50% Sp + 50% Sc	$3.34.10^6 \pm 3.44.10^5$	1.00±0.01	$1.37.10^4 \pm 2.00.10^3$	0.23±0.03
100% Sp	-	-	$2.43.10^4 \pm 1.25.10^3$	0.26±0.01
100% Sc	$1.40.10^7 \pm 9.38.10^4$	0.66±0.04	-	-

- Fields without values refer to pure cultivation, with no cells of these species.

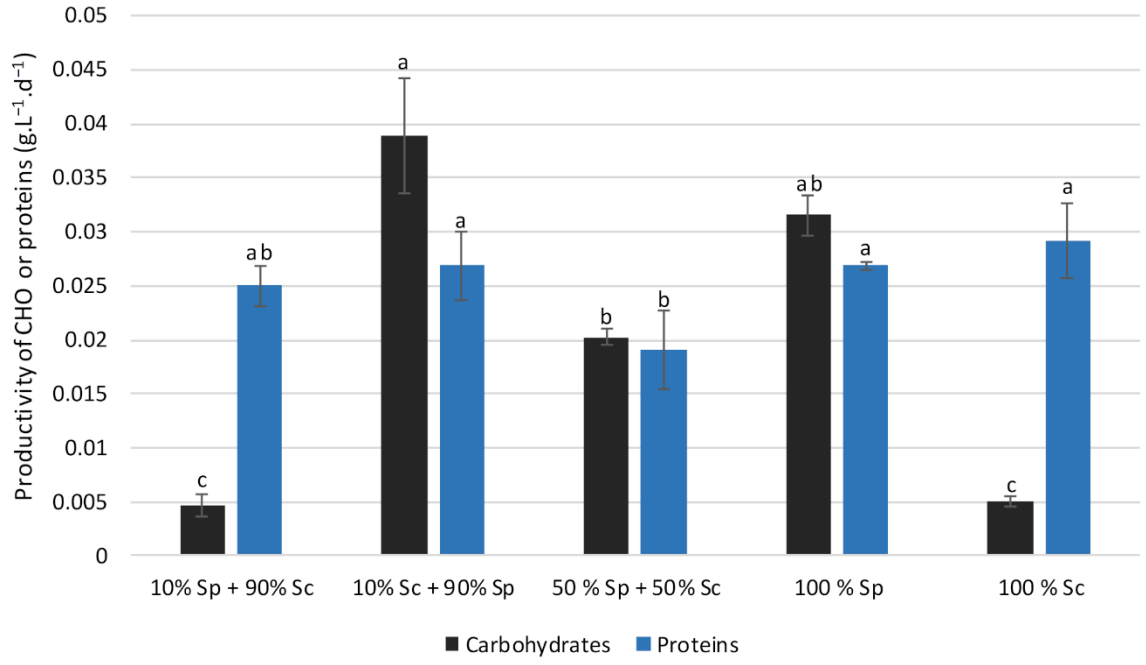
μ_{max}: maximum specific growth rate (d^{-1})
X_{max}: Final biomass concentration (Xf) (cells/mL),

The highest concentrations of carbohydrates in the assays were obtained with 100% Sp, 10% Sc + 90% Sp and 50% Sp + 50% Sc (p> 0.05). The cultures that obtained the highest carbohydrate concentrations were the cultures with the highest *Spirulina* concentrations. Therefore, the *Spirulina* microalgae cultivated under the conditions of the tests performed have a greater capacity to accumulate intracellular carbohydrates (Figure 2a). Many microalgae produce a substantial amount of protein, and this behavior primarily occurs when the growth medium is rich in nutrients, mainly nitrogen (DISMUKES et al., 2008). The highest carbohydrate productivities were obtained in the trials with 100% Sp and 10% Sc + 90% Sp, which were not significantly different (p>0.05) (Figure 2b). Productivity is directly related to carbohydrate concentration and final biomass concentration (MARGARITES et al., 2016). Therefore, the cultures with a higher proportion of *Spirulina* obtained the highest productivity because *Spirulina* had the highest final biomass concentrations and obtained the highest carbohydrate concentrations.

Figure 2. Intracellular Carbohydrate and Protein Concentration (a) and Carbohydrate and Protein Productivity ($g.L^{-1}.d^{-1}$) (b) obtained for the different tests performed



(a)



(b)

Mean values of tests performed in duplicates (n = 2) ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level (p> 0.05).

3.2 Step II - Cultivation of microalgae in monoculture or in consortium with effluent addition

3.2.1 Effluent characterization

The physical and chemical characteristics of the effluent used were:

- For sterile effluent the results of Total Nitrogen Kjeldahl (TKN), pH, COD and Total phosphorus were 66.2 ± 2.7 $mg.L^{-1}$, 6.5 ± 0.2, 1294.4 ± 40.8 $mg.L^{-1}$ and 10.12 ± 0.3 $mg.L^{-1}$, respectively;
- For non-sterile effluent the results of Total Nitrogen Kjeldahl (TKN), pH, COD and Total phosphorus were 72.6 ± 3.3 $mg.L^{-1}$, 6.7 ± 0.2, 1662.85 ± 30.2 $mg.L^{-1}$ and 19.8 ± 1.2 $mg.L^{-1}$, respectively.

Phosphorus is as important as nitrogen in microalgae cultivation, and it is primarily consumed in inorganic form and with the help of enzymes in its organic form. Therefore, the effluent used has potential for cultivation of microalgae because both nutrients are present in its composition. Autoclaving slightly reduced phosphorus and nitrogen concentrations of the effluent.

3.2.2 Effects on microalgal growth

Table 2 shows the effect of the addition of sterile and non-sterile effluent on the growth parameters of *Spirulina* and *Scenedesmus* microalgae growing in isolation or consortia. The sterilization of the effluent didn't influence the growth (cell/mL) of *Scenedesmus* in the addition of 10 or 30% of effluent, as can be seen by the comparisons of means of growth in sterile and non-sterile conditions (p>0.05, lower cases letters after the means in Table 2). For the addition of 50% of effluent, the microalgae *Scenedesmus* was able to growth only in the sterile condition. For the microalgae *Spirulina,* in the assays added of 10 % of effluent, a higher cell concentration was observed with the effluent not sterilized (p<0.05), however, for 30% or 50% of addition of effluent, the same pattern observed to *Scenedesmus* occurred. In general, it can be concluded that that the native microorganisms in the effluent competed with the microalgae and affected their growth.

Table 2. Study of the effect of addition of sterile and non-sterile effluent on growth parameters of *Spirulina* and *Scenedesmus* microalgae growing in isolation and in consortia.

Microalgae concentration in inoculum composition and percentage of effluent added at initial time	**Sterilization**	***Scenedesmus obliquus***		***Spirulina platensis***	
		X_{max} (cells.mL^{-1})	μ_{max} (d^{-1})	**X_{max} (cells.mL^{-1})**	μ_{max} (d^{-1})
100% *Scenedesmus* + 10 % effluent	yes	$8.01.10^{7}\pm1.15.10^{7\ aAB}$	0.24 ± 0.01^{aC}	-	-
100% *Scenedesmus* + 30 % effluent	yes	$9.93.10^{7}\pm5.21.10^{7\ aA}$	0.35 ± 0.02^{aB}	-	-
100% *Scenedesmus* + 50 % effluent	yes	$2.06.10^{7}\pm1.24.10^{6\ BC}$	0.24 ± 0.09^{C}	-	-
100% *Spirulina* + 10 % effluent	yes	-	-	$4.41.10^{5}\pm2.30.10^{4\ aA}$	0.23 ± 0.01^{aA}
100% *Spirulina* + 30 % effluent	yes	-	-	$2.48.10^{5}\pm9.19.10^{4\ aB}$	0.48 ± 0.10^{A}
100% *Spirulina* + 50 % effluent	yes	-	-	$3.38.10^{4}\pm4.42.10^{4\ C}$	-
50% *Scenedesmus*/50% *Spirulina* + 10 % effluent	yes	$6.70.10^{7}\pm7.07.10^{6\ aABC}$	0.35 ± 0.15^{aB}	$1.23.10^{5}\pm3.18.10^{4\ aBC}$	0.22 ± 0.06^{bA}
50% *Scenedesmus*/50% *Spirulina* + 30 % effluent	yes	$3.49.10^{7}\pm2.17.10^{7\ aABC}$	0.25 ± 0.04^{aC}	$8.13.10^{4}\pm4.42.10^{4\ aBC}$	0.18 ± 0.04^{aA}
50% *Scenedesmus*/50% *Spirulina* + 50 % effluent	yes	$2.12.10^{7}\pm1.60.10^{7\ BC}$	0.63 ± 0.11^{AB}	$4.13.10^{4}\pm5.30.10^{3\ C}$	0.16 ± 0.02^{A}
100% *Scenedesmus* + 10 % effluent	No	$4.56.10^{7}\pm4.07.10^{6\ aABC}$	0.94 ± 0.16^{bA}	-	-
100% *Scenedesmus* + 30 % effluent	No	$6.63.10^{6}\pm4.42.10^{6\ aC}$	0.60 ± 0.06^{bB}	-	-
100% *Scenedesmus* + 50 % effluent	No	*Without growth*			
100% *Spirulina* + 10 % effluent	No	-	-	$9.75.10^{4}\pm3.18.10^{4\ bBC}$	0.39 ± 0.22^{aA}
100% *Spirulina* + 30 % effluent	No	-	-	$5.25.10^{4}\pm3.54.10^{3\ aC}$	-
100% *Spirulina* + 50 % effluent	No	*Without growth*			
50% *Scenedesmus*/50% *Spirulina* + 10 % effluent	No	$2.18.10^{7}\pm4.02.10^{6\ bBC}$	0.21 ± 0.01^{aC}	$1.16.10^{5}\pm8.66.10^{4\ aBC}$	0.53 ± 0.02^{aA}
50% *Scenedesmus*/50% *Spirulina* + 30 % effluent	No	$5.50.10^{6}\pm1.06.10^{6\ aC}$	0.11 ± 0.02^{aC}	$4.63.10^{4}\pm2.30.10^{4\ aC}$	0.37 ± 0.11^{aA}
50% *Scenedesmus*/50% *Spirulina* + 50 % effluent	No	*Without growth*			

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Mean values of tests performed in duplicates (n = 2) ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level (p> 0.05). The lower case letters compare individually assays in the condition of sterile or non-sterile tests; the upper case letters compare all the tests in the column. To perform the mean comparisons of data of cell growth (cells/mL) the data was previously transformed to log (cells/mL).

The comparisons of means considering all experiments (upper case letters after the means in Table 2) showed that the highest cell concentrations were reached in the cultivation with the lowest effluent concentrations (10% and 30%). Microalgae in the non-sterile culture did not survive with the addition of 50% effluent, affecting negatively the cellular concentration of microalgae. In sterile conditions, the initial effluent concentration interfered with the cellular growth of microalgae cultured individually or in consortia.

The highest added effluent concentration (50%) resulted in the lowest optical densities (data not shown) probably due to the effluent toxicity to microalgae. Some studies have provided different limiting concentrations for ammonium in effluents to the growth of *Chlorella* species. Tuantet et al. (2014) reported algae inhibition at 140 $mg.L^{-1}$ at *Chlorella sorokiniana*. He et al. (2013) observed a decrease in algal growth when the concentrations of ammonium increased in the influent from a maximum observed growth rate on of 0.92 d^{-1} at 30 $mg.L^{-1}$ to 0.33 d^{-1} at 143 $mg.L^{-1}$ at *Chlorella vulgaris*. Few studies used *Spirulina* or *Scenedesmus* to treat effluents, what makes the study relevant, once this application associated with the subsequent application of biomass. In the work, was measured the total nitrogen concentration, observing that concentrations near to 35 $mg.L^{-1}$ of total nitrogen were present in the experiments added of 50% of effluent, showing the sensibility of the both microalgae used. According to Sniffen et al. (2018), algae can quickly and efficiently remove dissolved nitrogen, typically in either the form of ammonia or nitrate, from an aqueous system under a variety of conditions. In non-axenic systems based on algae that contain other microorganisms, it is important to consider nitrogen transformation pathways in addition to bio assimilation by microalgae, such as nitrification (the sequential oxidation of NH_3 to NO_2^- and then NO_3^-) or denitrification (sequential reduction of NO_3^- to N_2). Inorganic nitrogen in the form of ammonia and nitrate can be used as a source of nutrients for algae growth, while bacteria can often use these sources of nitrogen as well as nitrite and organic nitrogen for growth.

Besides of this, the addition of 30% of effluent in microalgae cultivation is a good result, being this proportion of effluent considered high comparing with other studies. For example, Hultberg et al. (2017) used the effluent from the biogas process to the growth of *Spirulina platensis* LB 2340. The concentration of 1.5% (v/v) of the effluent was added at the start as a nutrient source, a similar volume of effluent was added after 3 days of growth, and a final addition of 3% (v/v) of the initial volume was added after 6 days of growth. In comparison, Morales-Amaral et al. (2015) used about 30% of addition of effluent from anaerobic digestion

from a real urban wastewater in an outdoor production of *Scenedesmus* sp. in thin-layer and raceway reactors; above this value the culture's performance reduced, probably due to ammonium excess (above 122 $mg.L^{-1}$).

The best result obtained for the concentration of 50% of effluent was the sterile test performed in a consortium 50% *Spirulina*/50% *Scenedesmus* + 50% effluent, demonstrating that the consortia presented better results than monocultures in this condition. This result occurred because the species occupy different functional niches and use resources more efficiently because of their different absorption spectra, nutrient requirements and overall physiology (BEHL; DONVAL; STIBOR, 2011; GAMFELDT; HILLEBRAND, 2011).

Gonçalves et al. (2016) reported that microalgae consortia showed better resilience than microalgae monoculture when growing in food processing effluents and that the loss of one microorganism in a consortium may be compensated by the other microorganisms, which increases the resistance of the microalgal system to competition from other microorganisms. In this study, the microalgae in consortia presented the same resilience that in monoculture, considering the data presented in Table 2. The main effect observed in the tests was the increase of effluent concentration, that caused reduction of growth cell in the addition of 50% for both microalgae growing in monoculture or consortia.

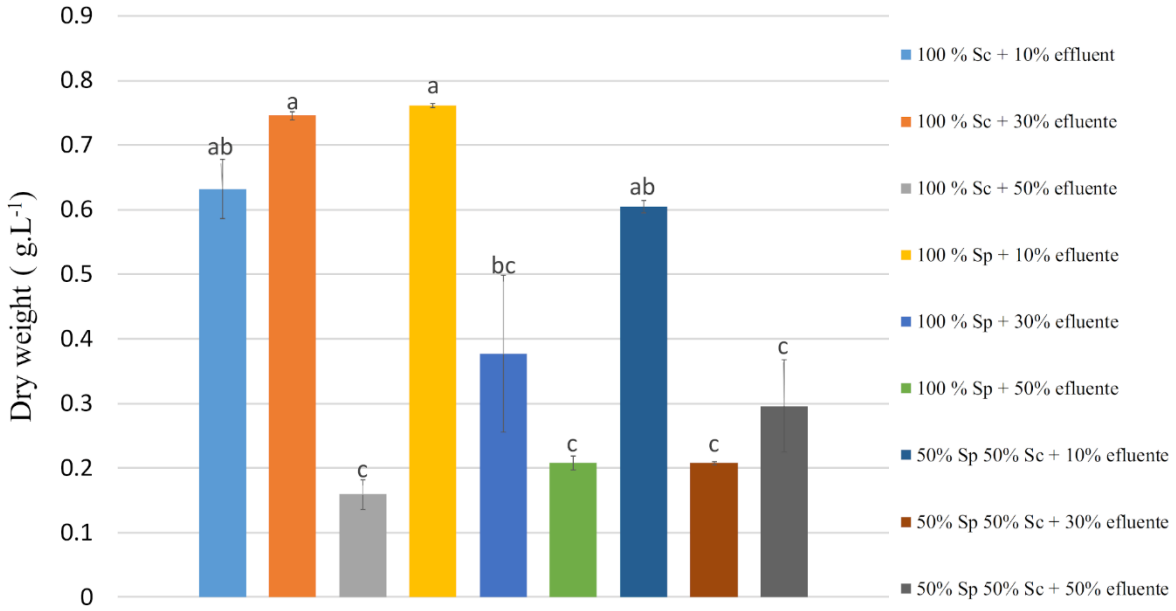
Analyzing the individual growth of each microalgae (number of $cells.mL^{-1}$) with the addition of sterile effluent (Supplementary material 1), it was observed that the *Scenedesmus* microalgae presented higher growth compared to *Spirulina* because *Spirulina* had a smaller number of cells for the same dry mass, because of the cell size. The microalga *Scenedesmus* showed an increased number of cells in all tests, even with the highest effluent concentration, but the microalgae *Spirulina* grown individually in 50% effluent showed a reduced number of cells, probably due to the toxicity of the effluent to *Spirulina*, as explained before.

The analysis of the time course of individual growth of each microalga cultivated in non-sterile effluent (Supplementary material 1) showed that both microalgae presented a decreased number of cells during the cultivation time when inoculated alone. *Scenedesmus* obtained the greatest cell growth when cultivated with 10% effluent, and *Spirulina* obtained the greatest growth when cultivated with 30% effluent. The growth with non-sterile effluent was lower compared to cultivation with sterile effluent.

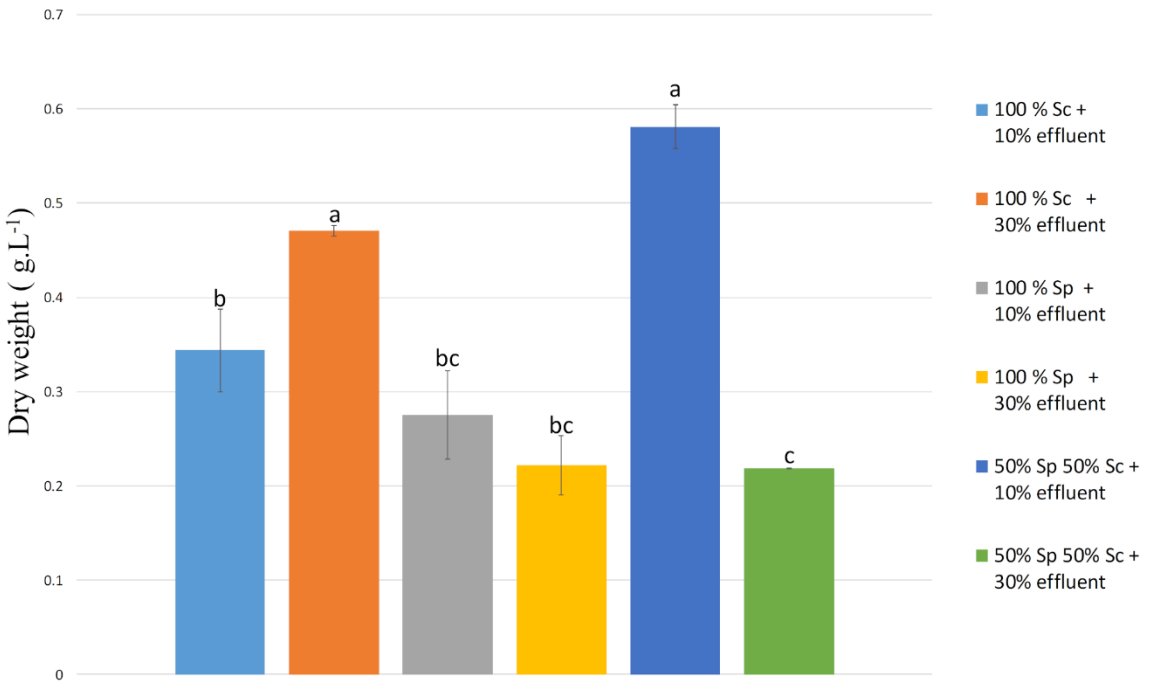
3.2.3 Effects on the dry weight and on the biochemical composition of biomass

To evaluate the possibility of use of biomass to bioethanol production, the dry weight obtained in the end of cultivation is important, in balance with the quantity of carbohydrates in the biomass. Figure 3a shows that the cultures with sterile effluent realized with the consortia of 50% *Spirulina*/50% *Scenedesmus* and 10% of effluent presented similar dry weigh (p>0.05) that the experiments accomplished in monocultures and with the addition of 10% of effluent.

Figure 3. Biomass the end of cultivation with addition of the sterile effluent (a) and nonsterile (b)



(a)



(b)

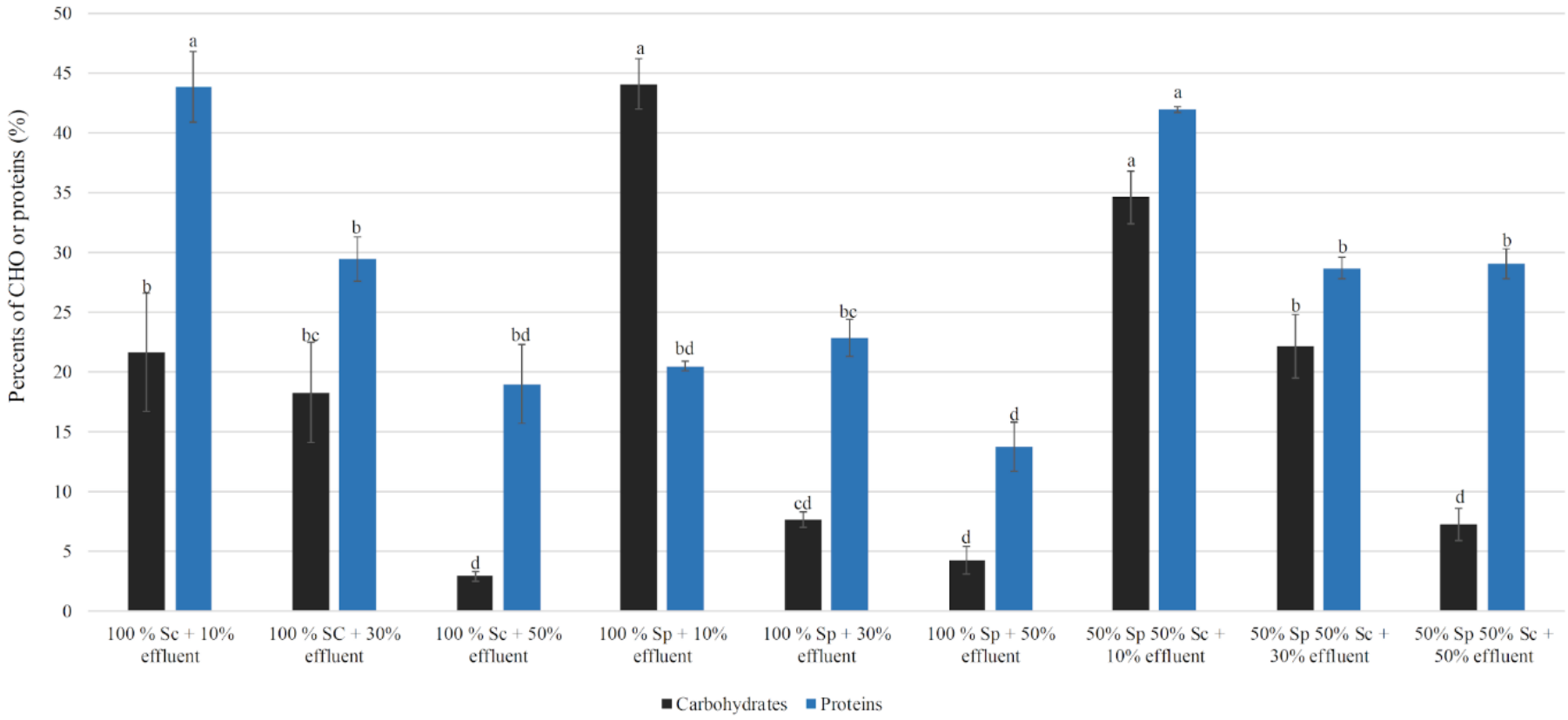
Mean values of tests performed in duplicates (n = 2) ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level (p>0.05)

The monoculture of *Scenedesmus* was able to growth with 30% of effluent, showing a good capacity of this microalgae to growth in this effluent; however, as can be seeing in the Figure 4a, the biomass obtained in the experiment with *Scenedesmus* and 30% of effluent presented near to 30% of proteins and less than 20% of carbohydrates, being not properly to production of bioethanol. On the other hand, the biomass obtained in the consortia of 50% of *Spirulina* and 50% of *Scenedesmus* with the addition of 10% of effluent, presented almost 35% of carbohydrates (Figure 4a). Hena et al. (2018) cultivated *Arthrospira platensis* in dairy farm wastewater for biodiesel production. The microalgae accumulated 31.89% carbohydrates, 30.45% lipid and 16.81% proteins. Mendonça et al. (2018) cultivated *Scenedesmus obliquus* microalgae in cattle wastewater in vertical alveolar flat panel photobioreactors, after previous digestion in a hybrid anaerobic reactor. In the batch mode, the levels of proteins obtained were 32% of proteins and 27% of carbohydrates.

The highest carbohydrate concentrations in sterile effluent cultivation (p<0.05) were achieved in the 100% Sp + 10% effluent and 50% Sp + 50% Sc + 10% effluent trials (p>0.05) comparing only the both trials). This is an indication that the biomasses with higher concentrations of carbohydrates were the cultures with the highest initial concentrations of

Spirulina, while the presence of *Scenedesmus* tends to bring biomass with more quantities of proteins, which could be more appropriated to the production of biofertilizers and biomethane. The highest concentrations of protein were achieved in the assay of 100% *Scenedesmus* + 10% effluent and 50% *Scenedesmus*/50% *Spirulina* + 10% effluent. Therefore, the largest accumulations of proteins were achieved in tests with the highest concentration of *Scenedesmus* microalgae.

Figure 4. Intracellular carbohydrate and protein concentration obtained in the microalgal pool with addition of sterile effluent (a), carbohydrate and protein productivity (b)



(a)

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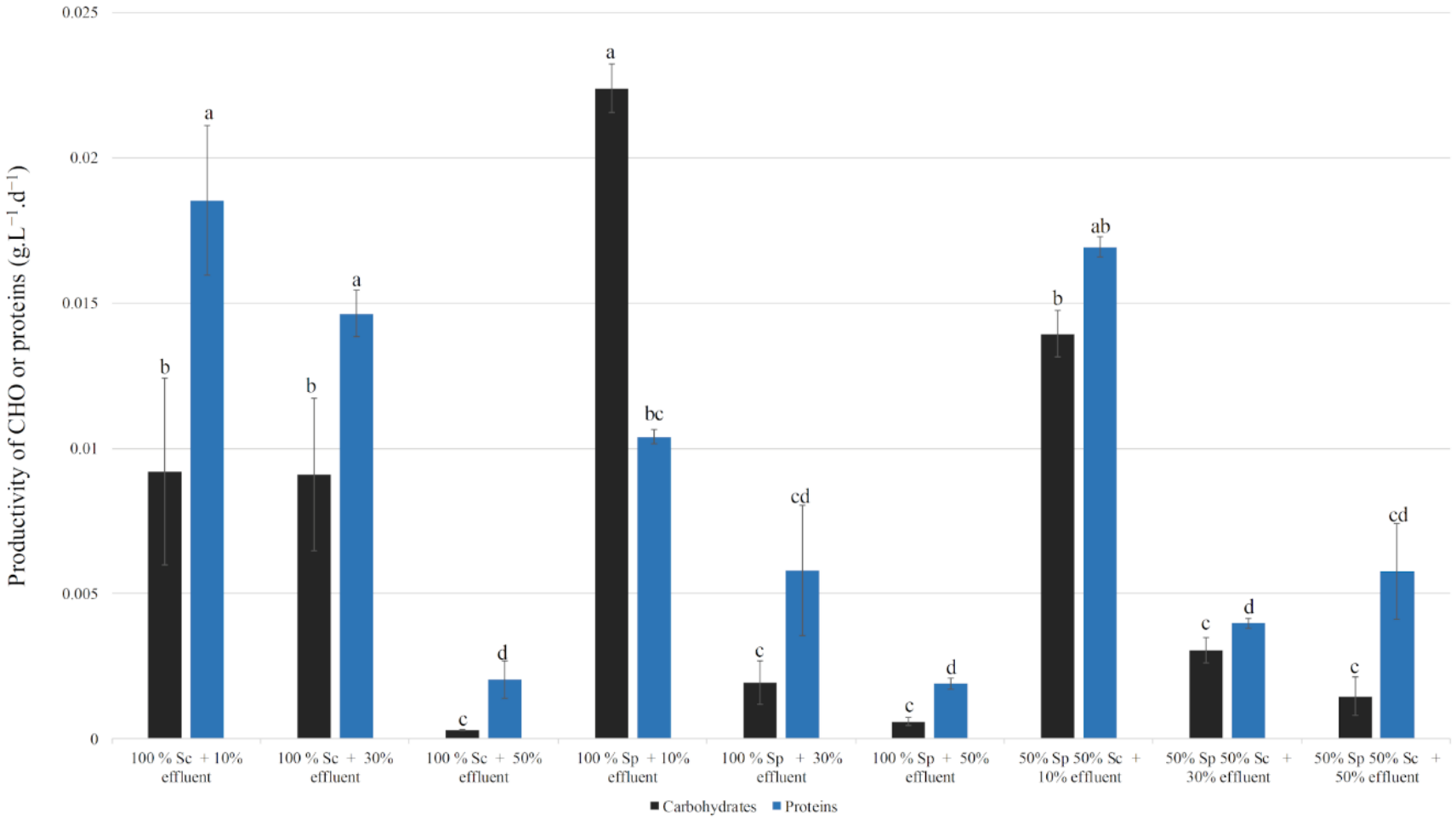


(b)

Mean values of tests performed in duplicates (n = 2) ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level (p>0.05). The comparisons were done considering columns with the same color.

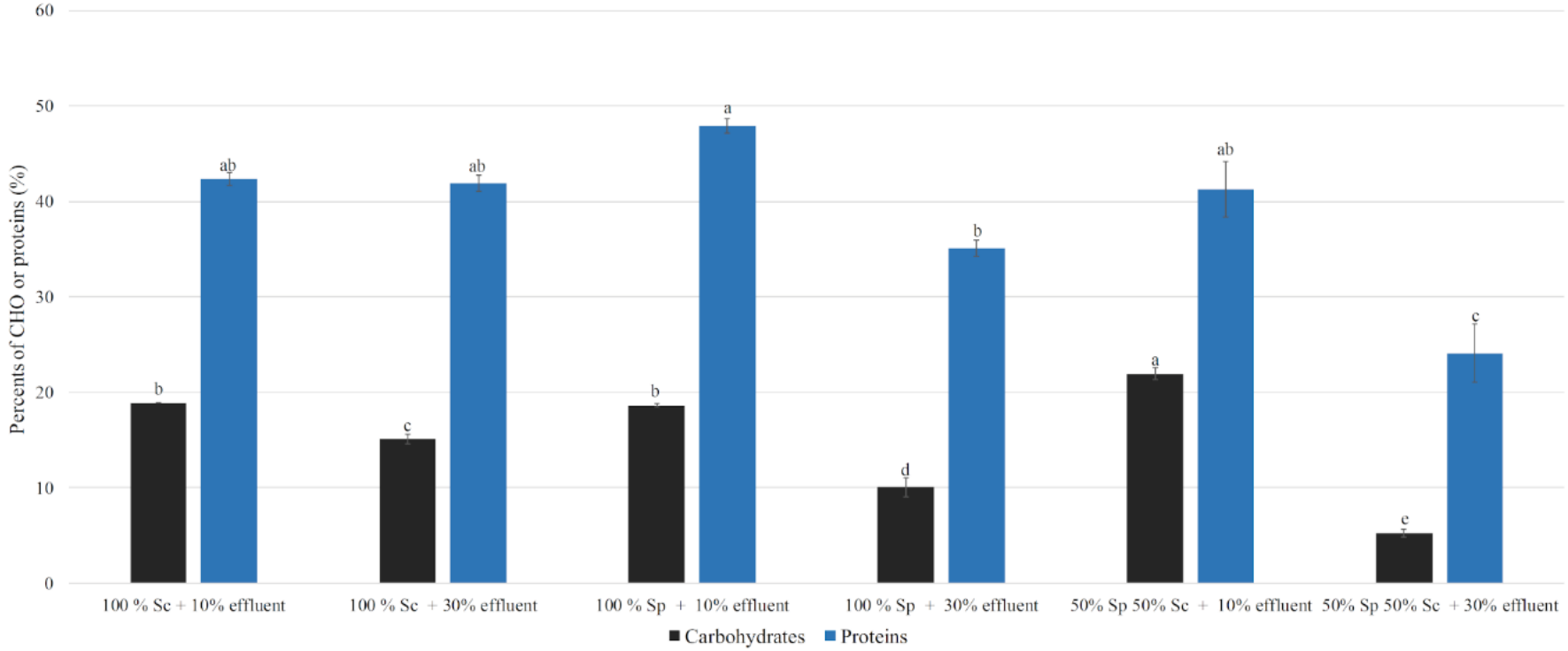
Publicado no Periódico *Bioenerg. Res.* (2021). https://doi.org/10.1007/s12155-021-10270-8
Francisco G. Magro, João F. Freitag, André Bergoli, Jorge Alberto Vieira Costa e Luciane M. Colla

The experiment that reached the highest dry mass at the end of cultivation with non-sterile effluent was 50% *Scenedesmus*/50% *Spirulina* + 30% effluent, followed by 100% *Scenedesmus* + 30%, with no significant difference between these groups (Figure 3b). However, Figure 5 shows that the composition of biomass obtained was composed more with proteins than carbohydrates compared with the experiments accomplished with sterile effluent, possible because of the influence of the presence of other microorganisms, as bacteria. According to Paddock et al. (2020), the relationships between microalgae and bacteria in wastewater are poorly understood due to the complexity of the interactions between biota. Complex forms of carbon com pounds produced by microalgae are utilized by bacteria and bacteria can convert macro and micronutrients into usable forms for as well as secrete vitamins and other cofactors that are required for growth by some microalgae. The bacteria may also produce growth hormones that enhance microalgae growth and the microalgae, because of the production of O_2, can favor the growth of bacteria for respiration which in turn produce CO_2 for use by microalgae.

The highest concentration of carbohydrates in the non-sterile effluent tests was obtained in the 50% *Spirulina* + 50% *Scenedesmus* + 10% effluent test, followed by the 100% *Scenedesmus* + 10% effluent and 100% *Spirulina* + 10% effluent tests, which was similar to the use of sterile effluent (Figures 5a and 4a).

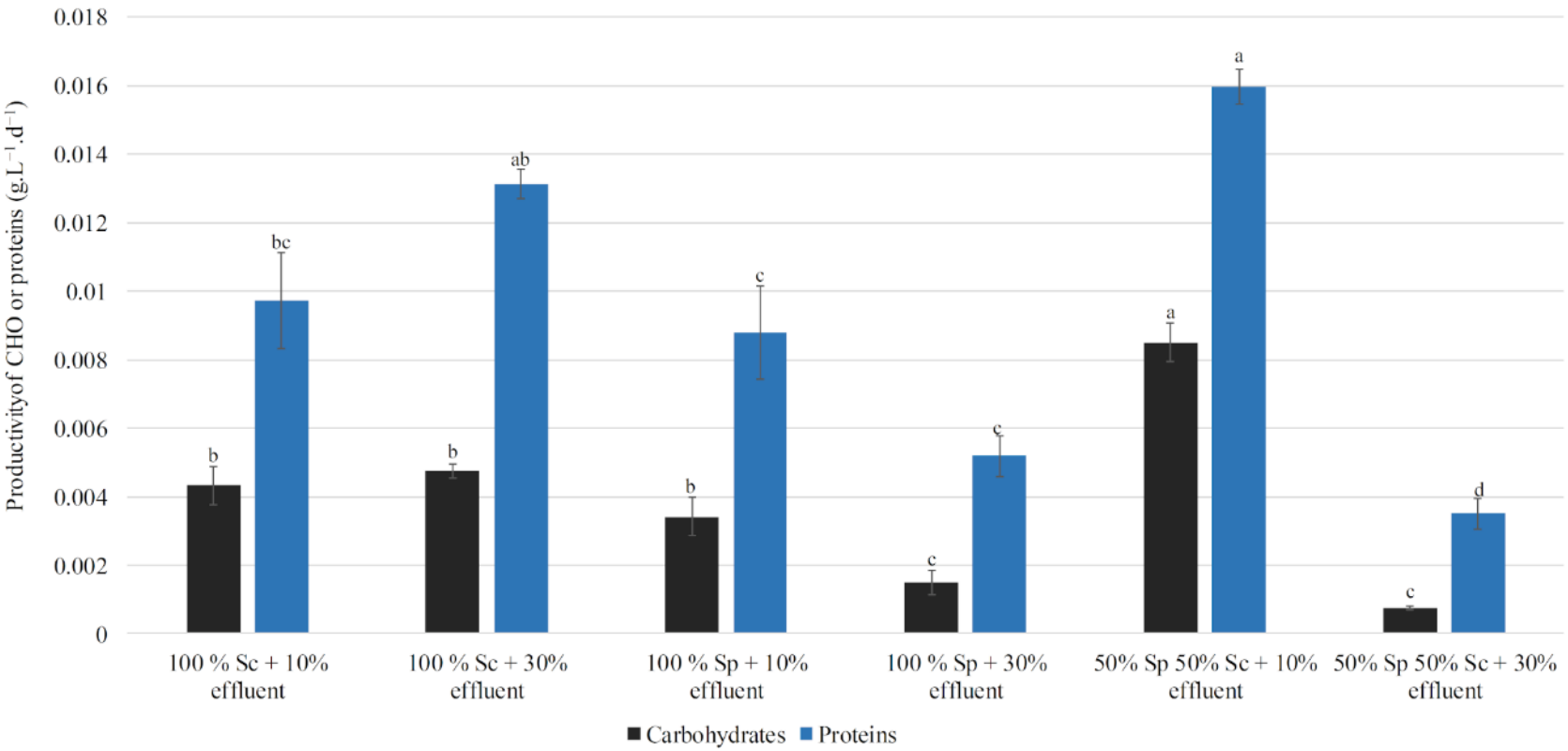
Was observed that with the increase in effluent concentration in the culture proportionately decreased intracellular carbohydrate concentrations, likely due to the high concentration of nitrogen in the effluent, which directs the cellular metabolism to protein accumulation and reserves other substances, such as carbohydrate (Figure 4). Biomass with a high concentration of carbohydrate could be used for the production of bioethanol and biomass with a higher concentration of protein could be used for the production of bio methane.

Figure 5. Intracellular carbohydrate and protein concentration obtained in the microalgal consortium with non-sterile effluent added



(a)

Publicado no Periódico *Bioenerg. Res.* (2021). https://doi.org/10.1007/s12155-021-10270-8
Francisco G. Magro, João F. Freitag, André Bergoli, Jorge Alberto Vieira Costa e Luciane M. Colla



(b)

Mean values of tests performed in duplicates (n = 2) ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level ($p > 0.05$). The comparisons were done considering columns with the same color.

3.2.4 Effects on nitrogen, COD and phosphorus removal

Nitrogen removal was high in all tests (Table 3), which demonstrates that the microalgae could remove nitrogen from the effluent in monocultures or in consortia. Nitrogen removal was higher in non-sterile effluent assays, which indicates that other microorganisms present in the effluent had a positive influence on nitrogen removal. Phosphorus removal was also higher for non-sterile effluent assays, likely because the other microorganisms present assisted in the removal. These results suggest a symbiotic relationship in microalgae-bacteria interactions. Microalgae release organic compounds during photosynthesis that may be used by bacteria as a source of carbon, energy and O_2. Bacteria release CO_2 that is needed for photosynthetic reactions (GONÇALVES; PIRES; SIMÕES, 2016). Rada-Ariza et al. (2017) studied the removal of ammonium from artificial wastewater by microalgae-bacterial pools and only microalgae. The microalgal-bacterial consortium removed ammonium at higher rates (100 ± 18 $mg.L^{-1}.d^{-1}$) than the microalgae consortium (44 ± 16 $mg.L^{-1}.d^{-1}$), when the system achieved a stable performance with a hydraulic retention time of 2 days.

Table 3. Efficiency of nitrogen, phosphorus and COD removal in sterile and non-sterile effluent assays.

Microalgae concentration in inoculum composition and percentage of effluent added at initial time	Sterilization	Initial Nitrogen concentration ($mg.L^{-1}$)	Final concentration Nitrogen ($mg.L^{-1}$)	Nitrogen removal efficiency (%)	Initial Phosphorus concentration ($mg.L^{-1}$)	Final Phosphorus concentration ($mg.L^{-1}$)	Phosphorus removal efficiency (%)	Initial COD concentration ($mg.L^{-1}$)	Final concentration CDO ($mg.L^{-1}$)	CDO removal efficiency (%)
100% Sc + 10 % effluent	yes	7.78±0.28	6.9±0.1	$11.28±1.24^{h}$	7.51±0.06	5.07±0.32	$32.46±5.37^{bc}$	348.82±4.57	310.69±14.14	$10.93±2.88^{h}$
100% Sc + 30 % effluent	yes	21.27±0.14	6.65±1.05	$68.71±7.18^{cdefg}$	4.85±0.03	4.45±0.12	$8.14±4.50^{e}$	595.70±21.42	406.13±0.64	$31.82±2.34^{cdefg}$
100% Sc + 50 % effluent	yes	34.38±0.16	13.3±2.1	$61.29±8.82^{efg}$	6.76±0.04	6.12±0.02	$9.41±1.27^{e}$	915.70±65.72	565.26±7.15	$38.25±3.66^{bcd}$
100% Sp + 10 % effluent	yes	7.83±0.16	3.85±0.35	$50.88±5.25^{g}$	7.3±0.30	6.30±0.02	$13.50±5.48^{de}$	317.09±26.15	256.98±23.16	$18.95±4.56^{defgh}$
100% Sp + 30 % effluent	yes	21.12±0.15	3.9±1.1	$81.50±7.50^{abcde}$	3.97±0.04	3.40±0.03	$14.34±2.54^{de}$	502.99±46.68	333.13±13.81	$33.76±3.41^{cde}$
100% Sp + 50 % effluent	yes	34.6±0.41	14.25±1.65	$58.77±7.23^{fg}$	5.98±0.09	5.25±0.04	$12.24±3.04^{e}$	831.49±30.41	477.62±18.34	$42.55±4.31^{abcd}$
50% Sc + 50% Sp + 10 % effluent	yes	7.7±0.25	2.6±0.2	$66.15±4.79^{defg}$	7.41±0.05	6.30±0.04	$14.97±0.09^{de}$	367.92±17.39	315.44±21.55	$14.27±9.92^{gh}$
50% Sc + 50% Sp + 30 % effluent	yes	21.23±0.07	6.25±0.75	$70.57±4.88^{bcdefg}$	4.33±0.13	3.82±0.13	$11.74±0.56^{e}$	578.11±102.45	375.95±35.85	$34.96±5.40^{cd}$
50% Sc + 50% Sp + 50 % effluent	yes	34.43±0.06	6.65±0.35	$80.68±1.47^{abcde}$	6.38±0.08	5.12±0.11	$19.70±4.04^{de}$	813.61±34.39	407.3±11.80	$49.93±0.66^{abc}$
100% Sc + 10 % effluent	No	7.41±0.12	1.75±0.37	$76.63±6.98^{abcdef}$	10.27±0.03	7.87±0.08	$26.57±0,79^{cd}$	396.72±9.99	337.26±16.46	$14.98±2.00^{fgh}$
100% Sc + 30 % effluent	No	19.38±0.29	1.70±0.3	$91.30±2.29^{ab}$	12.50±0.07	8.62±0.34	$31.03±3.52^{c}$	745.34±28.25	421.95±27.42	$43.38±5.83^{abcd}$
100% Sp + 10 % effluent	No	6.97±0.12	1.05±0.35	$84.68±7.46^{abcd}$	9.37±0.02	6.22±0.34	$33.53±5.31^{bc}$	374.09±20.51	282.10±10.20	$24.58±6.87^{efgh}$
100% Sp + 30 % effluent	No	18.94±0.23	1.8±0.3	$90.48±2.25^{abc}$	11.08±0.10	9.08±.0.36	$18.06±5.46^{d}$	783.24±37.81	352.42±5.48	$55.00±2.87^{ab}$
50% Sc + 50% Sp + 10 % effluent	No	7.23±0.08	0.5±0.2	$93.08±3.91^{a}$	10.12±0.21	5.66±0.22	$44.07±3.09^{ab}$	372.96±32.10	278.95±5.65	$25.20±4.94^{defgh}$
50% Sc + 50% Sp + 30 % effluent	No	19.20±0.2	1.4±0.7	$92.70±5.15^{a}$	11.92±0.12	6.00±0.5	$49.66±1.86^{a}$	745.59±56.13	315.61±8.28	$57.66±2.09^{a}$

Mean values of tests performed in duplicates (n = 2) ± standard deviation. Equal letters in the trials indicate that they showed no significant difference in the 95% confidence level (p> 0.05).

Considering the sterile assays, higher removal of nitrogen was obtained in the experiments realized with the addition of 30% of effluent (61.29% and 81.5% for *Scenedesmus* and *Spirulina* monocultures, respectively). The greatest removal of COD was in crops with the highest initial concentrations of COD (30% and 50% addition of effluent). In a similar study a mix of the species *Scenedesmus* sp. growing in an alternative medium consisted of diluted cattle manure effluent in water (30% v./v.), it was observed an decreasing in all physicochemical parameters, indicating efficient treatment of effluent through the cultivation of microalgae. There was a reduction of 92.5% of total nitrogen, 51.9% of phosphorus, 62.9% of potassium, 53.6% of COD and 22.4% of BOD (SCHERER et al., 2017). Hena et al. (2018) assessed the capacity of *Arthrospira platensis* cultivated in dairy farm wastewater for biodiesel production, concluding that the *A. platensis* was able to reduce 98.4 % COD, 98.8 % PO_{-4} and 99.6% $NO_{3.}$ Luo et al. (2019), studied the cultivation of the microalga *Scenedesmus sp.* with anaerobic digestion of cattle wastewater combined with municipal wastewater, which was diluted with modified BG-11 medium at 0-30%, and showed that *Scenedesmus* sp. could grow rapidly in the anaerobic digestion of 10% cattle wastewater with secondary effluent as a diluent without sterilization, achieving more than 90% COD, NO_3^- and NH_4^+ removal efficiency.

The consortia presented the best removal of nitrogen in the assay with 50% of addition of effluent (80.68%). In non-sterile conditions, all removals were statistically equal (p>0.05) and higher than in the sterile conditions, varying of 76 to 93% (Table 3). Regarding the removal of phosphorous, higher removals were obtained with the consortia in non-sterile condition. Various studies have reported similar improvement in growth and nutrient removal efficiency for co-cultivation of microalgae and bacteria as compared to microalgae alone (MAKUT; DAS; GOSWAMI, 2019).

A similar study revealed that a *Chlorella/Scenedesmus* consortium eliminated up to 99.7–99.9% of inorganic phosphorus and up to 88.6-96.4% of inorganic nitrogen from municipal wastewater within three weeks without the presence of other microorganisms (KOREIVIENE et al., 2014).

Choi et al. (2018) study the consortium of *Scenedesmus dimorphus* and nitrifiers bacteria, as result, the consortium system showed enhancement in both nitrogen (N) and phosphorous (P) removal compared to each single culture of microalgae. Especially, total N removal efficiency and P removal efficiency in consortium reactor were enhanced 3.4 and 6.5 times compared to nitrifiers bacteria only in reactor, respectively. These results demonstrate

that the effluent of cattle waste may be used as an alternative source of nutrients for the microalgae cultivation, and the microalgae studied reduce the concentration of up to 93% of nitrogen and 49% phosphorus in the effluent as a beneficial treatment.

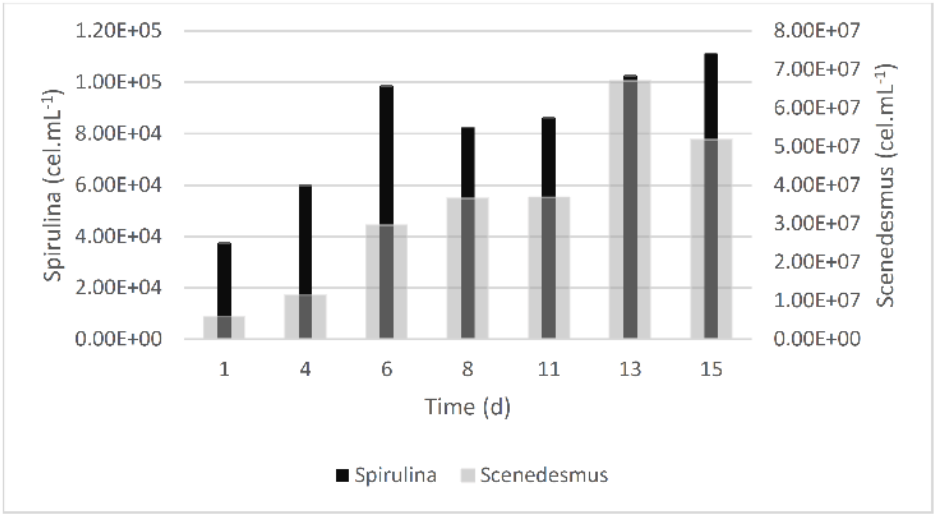
4 Conclusion

The present study demonstrated that it is possible to use post-treating effluent of anaerobic digestion of cattle waste sterile or non-sterile in the cultivation of microalgae. The consortia allow the post-treatment of the effluent concurrently with the production of microalgal biomass, which can be used for different applications. Sterile/non-sterile conditions cause different patterns of biomolecules accumulation in biomass and high removals of N and P were obtained using 50%/50% consortia in non-sterile conditions.

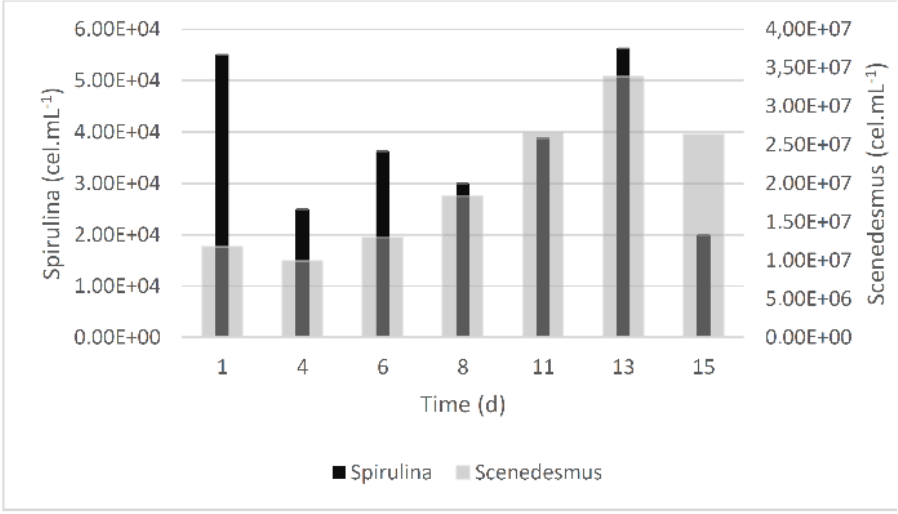
SUPPLEMENTARY MATERIAL 1

Cell growth of microalgae (cell. mL^{-1}) added in different proportions in the culture, with addition of STERILE effluent.

(a) 50% *Spirulina* e 50% *Scenedesmus* + 10% effluent, (b) 50% *Spirulina* e 50% *Scenedesmus* + 30% effluent, (c) 50% *Spirulina* e 50% *Scenedesmus* + 50% effluent, (d) 100% *Spirulina* + 10% effluent, (e) 100% *Spirulina* + 30% effluent, (f) 100% *Spirulina* + 50% effluent, (g) 100% *Scenedesmus* + 10% effluent, (h) 100% *Scenedesmus* + 30% effluent e (i) 100% *Scenedesmus* + 50% effluent

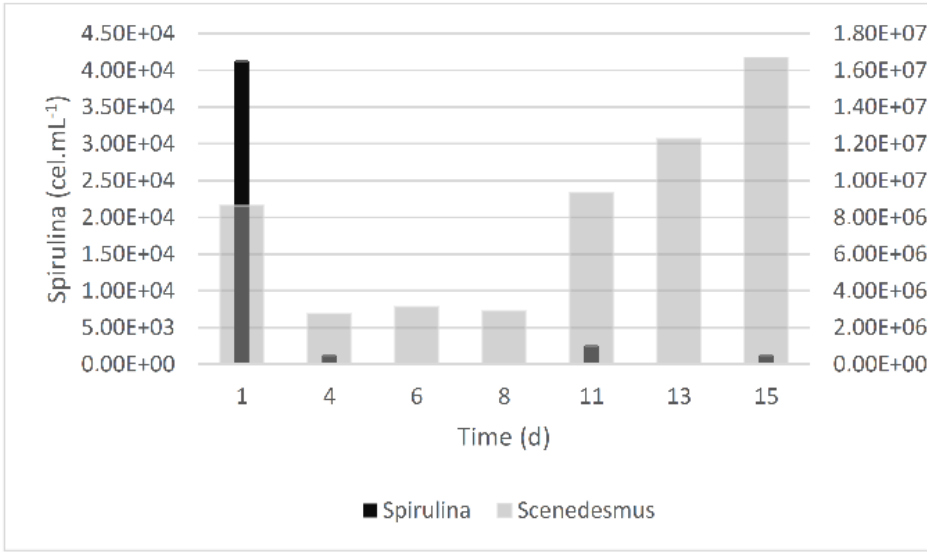


(a)



(b)

Publicado no Periódico *Bioenerg. Res.* (2021). https://doi.org/10.1007/s12155-021-10270-8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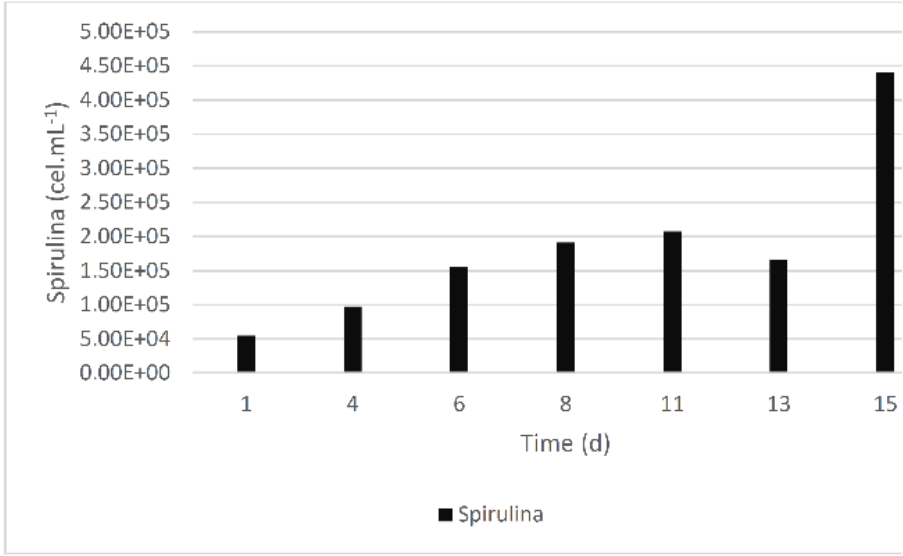
1.00E+04
5.00E+03
0.00E+00
Spirulina (cel.mL-1)
1.80E+07
1.60E+07
1.40E+07
1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
1
4
6
8
11
13
15
Time (d)
Spirulina
Scenedesmus

(c)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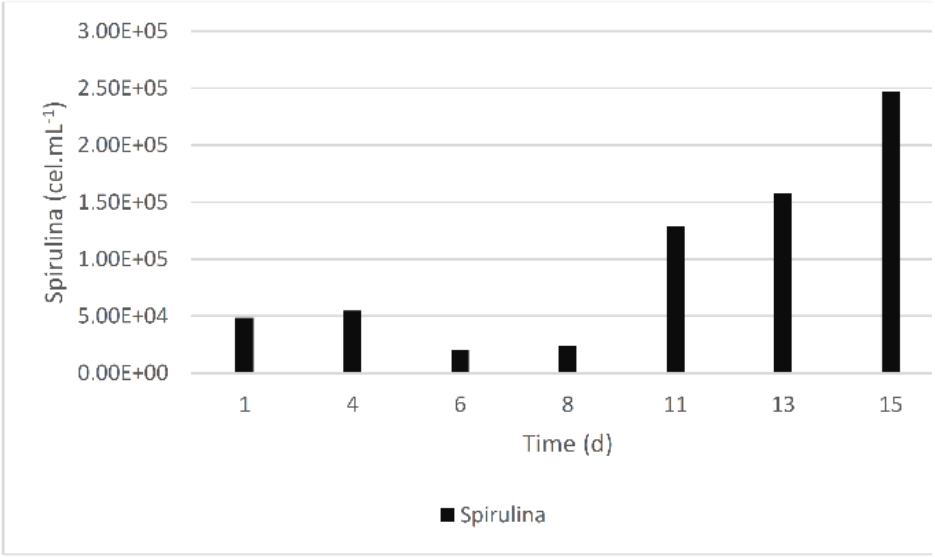
3.00E+05
2.50E+05
2.00E+05
1.50E+05
1.00E+05
5.00E+04
0.00E+00
Spirulina (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Spirulina

(d)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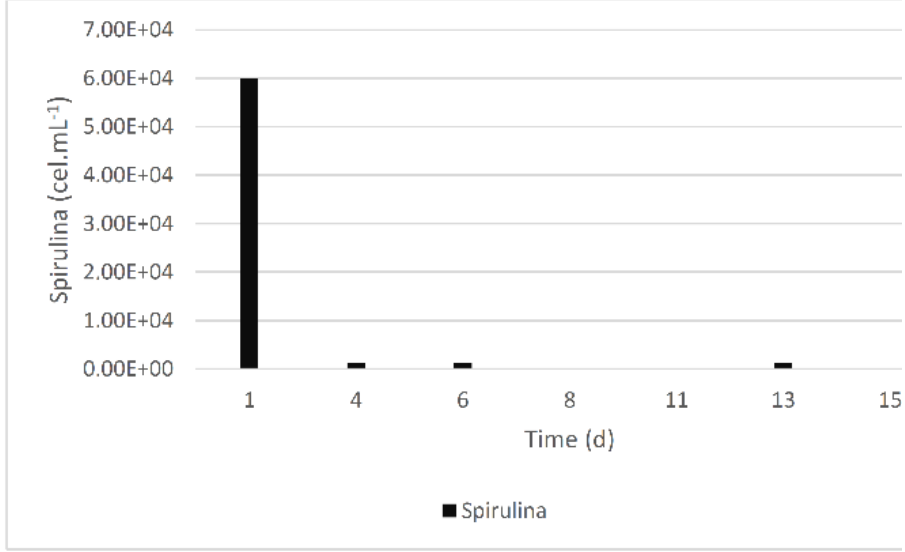
Spirulina (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Spirulina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4.00E+04
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2.00E+04
1.00E+04
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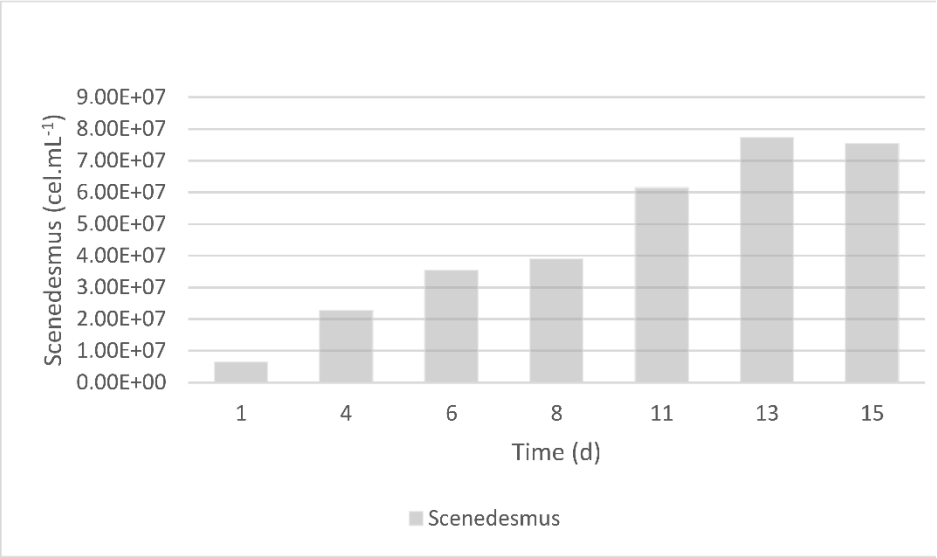
Spirulina (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Spirulina

(e)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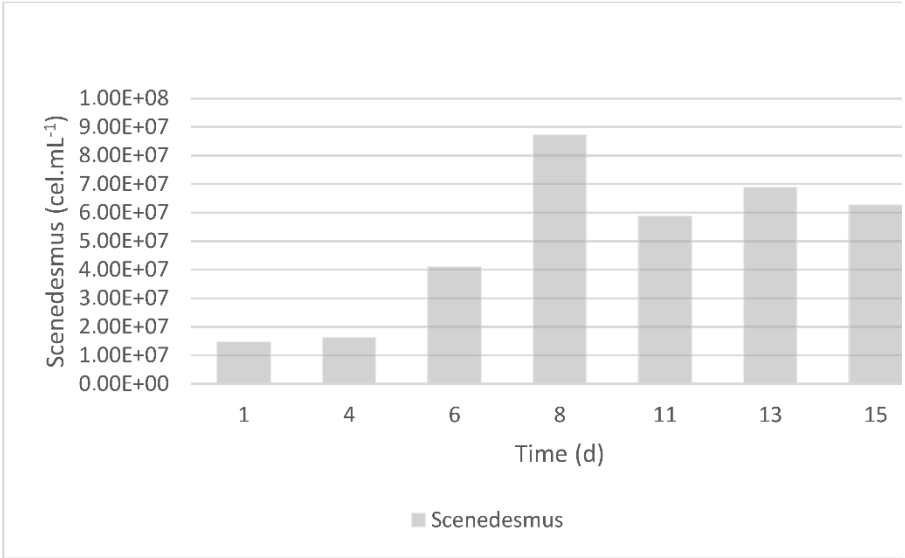
8.00E+07
7.00E+07
6.00E+07
5.00E+07
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
Scenedesmus (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Scenedesmus

(f)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9.00E+07
8.00E+07
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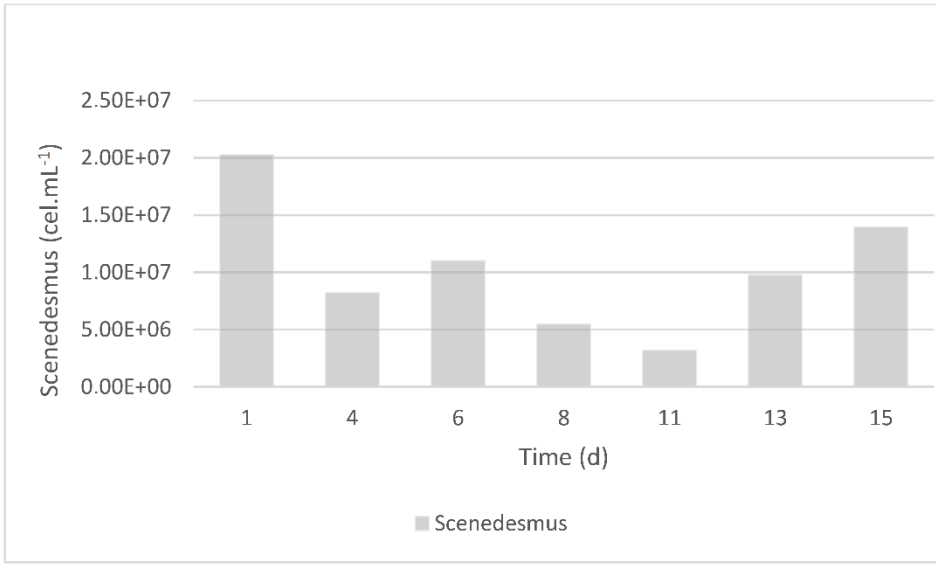
6.00E+07
5.00E+07
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
Scenedesmus (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Scenedesmus

(g)

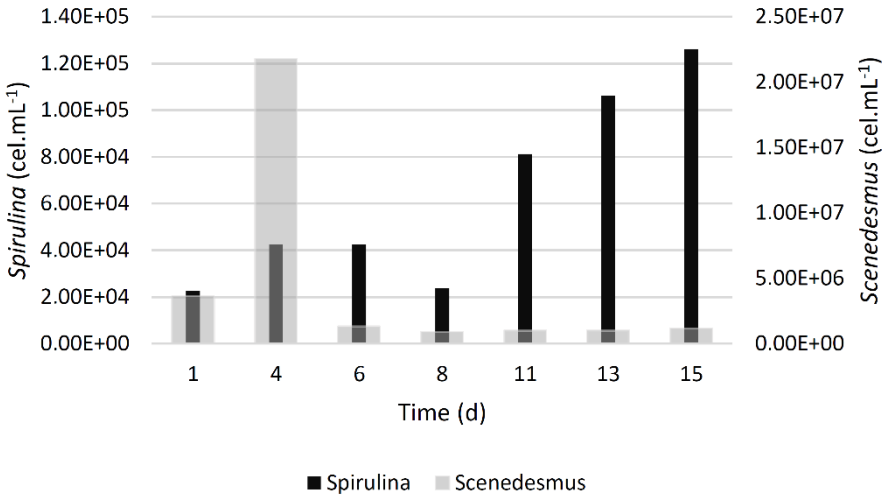
(h)



(i)

Cell growth of microalgae (cells.mL^{-1}) added in different proportions in the culture, with NON-STERILE effluent addition.

(a) 50% *Spirulina* e 50% *Scenedesmus* + 10% effluent, (b) 50% *Spirulina* e 50% *Scenedesmus* + 30% effluent, (c) 100% *Scenedesmus* + 10% effluent 100%, (d) 100% *Scenedesmus* + 30% effluent 100%, (e) *Spirulina* + 10% effluent, (f) *Spirulina* + 30% effluent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8.00E+04
6.00E+04
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2.00E+04
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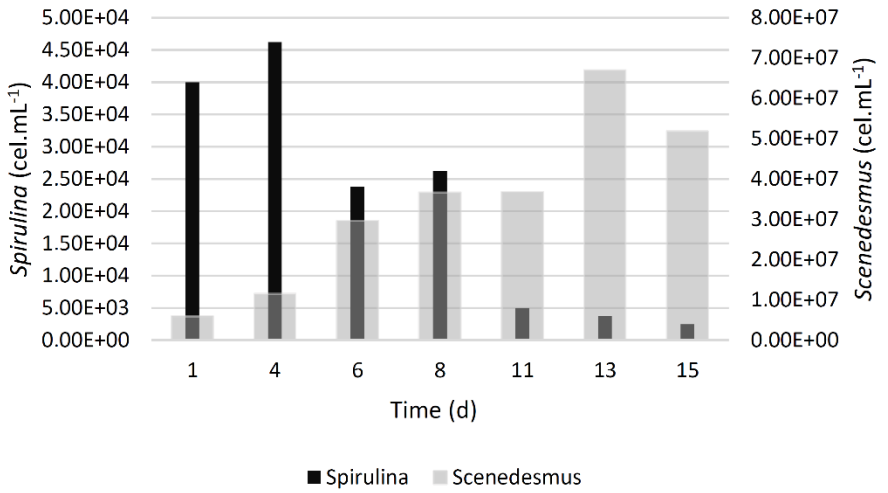
Spirulina (cel.mL-1)
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
Scenedesmus (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Spirulina
Scenedesmus

(a)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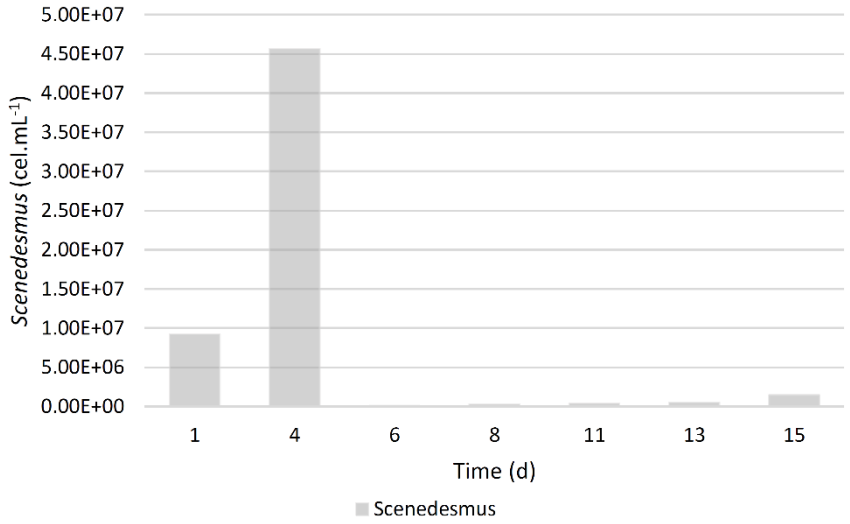
1.00E+04
5.00E+03
0.00E+00
Spirulina (cel.mL-1)
8.00E+07
7.00E+07
6.00E+07
5.00E+07
4.00E+07
3.00E+07
2.00E+07
1.00E+07
0.00E+00
Scenedesmus (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Spirulina
Scenedesmus

(b)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2.00E+07
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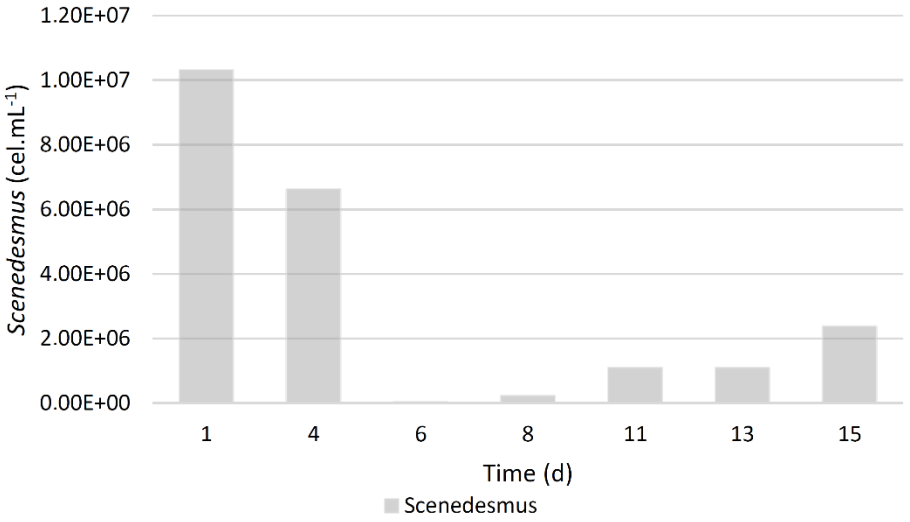
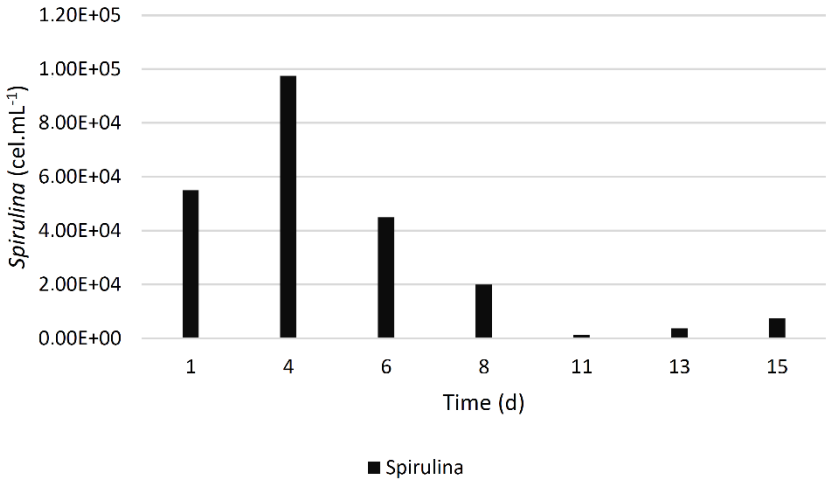
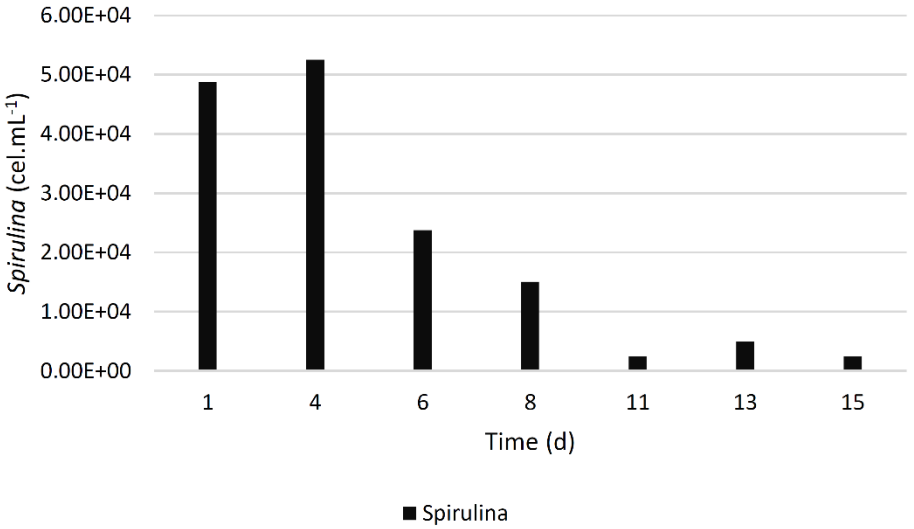
1.00E+07
5.00E+06
0.00E+00
Scenedesmus (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Scenedesmus


1.20E+07
1.00E+07
8.00E+06
6.00E+06
4.00E+06
2.00E+06
0.00E+00
Scenedesmus (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Scenedesmus

(c)


1.20E+05
1.00E+05
8.00E+04
6.00E+04
4.00E+04
2.00E+04
0.00E+00
Spirulina (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Spirulina

(d)


6.00E+04
5.00E+04
4.00E+04
3.00E+04
2.00E+04
1.00E+04
0.00E+00
Spirulina (cel.mL-1)
1
4
6
8
11
13
15
Time (d)
Spirulina

(e)

(f)

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5 CAPÍTULO IV - CULTIVATION OF MICROALGAE IN CONSORTIA ADDING EFFLUENT IN FED BATCH MODE AND SCALE UP TO BIOMASS PRODUCTION

Abstract
The search for a sustainable development has led several production processes to seek the implementation of biorefineries. The present work evaluated the cultivation of *Spirulina platensis* and *Scenedesmus obliquus* in consortium, with the addition of effluents of cattle waste in fed batch mode, to obtain biomass. In addition, a pilot scale was performed. Cultures were conducted in Zarrouk 20% medium with addition of sterile and non-sterile effluent in fed batch process. 10% (v/v) of effluent was added in the first day of cultivation and subsequently in 5 and 10 days. The obtained biomasses were characterized, evaluating the potential for biofuels and other bioproducts. The cultivation that reached the highest dry mass was the one with 50% Sc + 50% Sp with 15 days of cultivation, and the highest concentration of carbohydrates (43.82%) was achieved in the 100% Sp one. Phosphorus was removed during the cultivation, either in crops with only Zarrouk 20% as a medium and in crops with addition of effluent, demonstrating a significant phosphorus removal, nitrogen, on the other hand, had a significant decrease in cultivation with the addition of effluent, where it reduced the concentration by more than 50% when compared to day 1 and day 15. The scale-up (raceway with a 100L) showed similar results in comparison to the cultivations performed in 10 L raceways. The cultivation of microalgae in consortium and *Spirulina* can be used to assist water treatment with a simultaneously production of biomass for different applications.

Keywords: *Spirulina platensis*, *Scenedesmus obliquus*, pilot-scale, biorefineries.

Highlights:

- We added effluents of cattle waste in fed batch mode to produce *S. platensis* and *S. obliquus* in consortia
- We observed the growth of microalgae evaluating the number of cells and cellular mass behavior
- We evaluate the combined effects of effluents microorganisms and microalgae using non sterile effluent

1 Introduction

The search for a more sustainable development has encouraged several production processes to seek the implementation of biorefineries, which consists in using renewable raw materials in a production process that does not generate waste, or with minimal production of waste at the end of the process. The biorefineries process allows, in addition to a more

sustainable production through the reduction of residues and polluting gases, the obtainment of several products, among them: biofuels, biofertilizers and functional foods (CHERUBINI, 2010; FERNANDO et al., 2006). As a potential raw material for biorefineries, microalgae have emerged as an option, as they are photosynthetic microorganisms that, thanks to the possibility of directing their crops to obtain biomass with specific compositions, allow their use in the production of various products, which can dilute costs of microalgal crops (ASTOLFI et al., 2020; PRÉAT et al., 2020; ZHU, 2015). In this context, seeing how the culture medium is considerable share of the costs, several studies have sought alternative culture media, including the use of effluents as a source of nutrients (HULTBERG et al., 2017; MAHDY et al., 2015; ZENG et al., 2015).

The use of effluents from the most diverse sources is reported in several studies aiming to the cultivation of microalgae (ANSARI et al., 2019; HULTBERG et al., 2017; MARKOU, 2015; PADDOCK; FERNÁNDEZ-BAYO; VANDERGHEYNST, 2020), bringing strategic advantages for microalgal cultivation, as the possibility of accumulating reserve compounds with the addition of effluent, which may be caused due to the cellular stress, thus leading the microalgae to accumulate reserve compounds, such as carbohydrates, which are essential for the production of bioethanol biofuel (SALLA et al., 2016). Some microalgae accumulate considerable amounts of carbohydrates depending on the cultivation techniques, for example, *Spirulina platensis* grown under stress, can alter its metabolic pathways, leading to the accumulation of this compound (JOHN et al., 2011; ZAPAROLI et al., 2020). *Chlorella*, *Chlamydomona*, *Dunaliella*, *Scenedesmus*, *Tetraselmis* and *Spirulina* can accumulate a large amount of carbohydrates (> 40% dry weight) (JOHN et al., 2011; MAGRO et al., 2017).

In addition to the possibility of treating the effluent, due to removal of phosphorous and nitrogen present in the effluent as nutrients, it is possible to obtain a cooperation between microorganisms already present in the effluent and the microalgae, bringing benefits as the increase in biomass production and removal of the compounds mentioned above (MAHDY et al., 2015; WANG et al., 2020). Another cooperation, which also brings benefits, can be obtained by cultivation in consortium using more than one species of microalgae (HUY et al., 2018; KOREIVIENE et al., 2014). The consortium has been shown to increase nutrient removal efficiency in wastewater while generating microalgae biomass for use in co-product production (BÉLANGER-LÉPINE et al., 2020; BHATNAGAR et al., 2011).

The literature still does not have reports on the level of tolerance of microalgae in relation to each constituent, due to the variation of chemical profiles in different types of

effluents (CHOONG et al., 2020). Thus, a strategy that has been shown to be successful in preventing effluent toxicity is the fed batch culture mode, whereby the effluent is gradually added to the culture medium (MARKOU, 2015).

Thus, the use of microalgae grown with effluents and in the context of biorefineries, has the potential to reduce the costs of cultivation and the generation of more than one value-added product, thus, providing an environmentally sustainable and economically viable process (XIN et al., 2016; ZHU, 2015). The available literature on the combination of nutrient removal and cultivation of microalgae without sterilization, disinfection or chemical pre-treatment in external photobiotrators on a pilot scale is limited, however, this knowledge is necessary to implement economical commercialization of microalgae-based biofuels (LU et al, 2015).

The present work evaluated the cultivation of a consortium of microalgae, *Spirulina platensis* and *Scenedesmus obliquus*, with the addition of bovine culture effluent, in fed batch mode, aiming to obtain biomass for the possible generation of value-added bioproducts. In addition, a pilot scale was performed with the best results.

2 Material and Methods

2.1 Effluent

The effluents used were obtained after the anaerobic digestion of cattle waste, which generally contains high concentrations of chemical oxygen demand (COD), nitrogen and phosphorus, and is considered one of the most polluting effluents (LV et al., 2016). The effluent was filtered on cotton.

Effluent of cattle waste was characterized according to the parameters of Total Nitrogen Kjeldahl (TKN) (Volumetric Method 4500-N_{org} B), COD (Colorimetry 5220 D), and Total Phosphorus (Potassium Persulfate Method 4500-PF), cited by American Public Health Association (APHA, 1995), and pH (potentiometric method 4500-H^+ B) according to (AOAC, 2000).

The physical and chemical characteristics of the effluent used in 10 L raceways were:

- For effluent the results of Total Nitrogen Kjeldahl (TKN), pH, COD and Total phosphorus were 97.3 ± 0.98 $mg.L^{-1}$, 7.2 ± 0.2, 370.14 ± 21.5 $mg.L^{-1}$ and 18.71 ± 1.11 $mg.L^{-1}$, respectively;

The physical and chemical characteristics of the effluent used in 100 L raceways were:

- For effluent the results of Total Nitrogen Kjeldahl (TKN), pH, COD and Total phosphorus were 372.23 ± 4.65 mg.L^{-1}, 7.1 ± 0.2, 3048.14 ± 108.9 mg.L^{-1} and 55.24 ± 1.53 mg.L^{-1}, respectively;

2.2 Cultivation

The inoculum preparation and experiments using *S. platensis* LEB 52 (Sp) and *S. obliquus* (Sc) obtained from the strain bank at the Biochemistry and Bioprocess Laboratory of Passo Fundo University (UPF) were performed in a Zarrouk medium diluted to 20% in sterile conditions. The composition of the Zarrouk culture media diluted to 20% is: $NaHCO_3$ (3.36 g.L^{-1}), K_2HPO_4 (0.1 g.L^{-1}), $NaNO_3$ (0.5 g.L^{-1}), K_2SO_4 (0.2 g.L^{-1}) NaCl (0.2 g.L^{-1}), $MgSO_4.7H_2O$ (0.04 g.L^{-1}), $CaCl_2$ (0.008 g.L^{-1}), $FeSO_4.7H_2O$ (0.002 g.L^{-1}), EDTA (0.016 g.L^{-1}).

The cultivations were conducted with the microalgae *Spirulina* isolated, and using the consortia of *Spirulina* and *S. obliquus* in an initial cell concentration of 0.15 g.L^{-1}. The cultivation of *Scenedesmus* isolated was not carried out due to the lack of adaptation on the 10 L raceway (Table 01) (MAGRO et al., 2021). Cultures were performed in duplicate in *raceways* with a working volume of 10 L, in a greenhouse with temperature control between 20 ºC and 30 ºC. The agitation of the cultures was carried out by submerged pumps of 220 L.h^{-1} (HBO-300, China).

The experiments were realized with and without the addition of effluent. In the essays in which the effluent was added, 10% (v/v) of effluent was added in 1, 5 and 10 days of cultivation, in a fed batch regime. Cultures were conducted for 15 days, with closure due to the arrival in the stationary or decline phase.

2.2.1 Pilot-scale

A cultivation of *Spirulina* was conducted in 100 L raceway. The initial cell concentration was set at 0.20 g.L^{-1} in order to prevent the occurrence of long lag phase, and the eaffluent was added to the culture only after it reached the concentration of 0.5 g.L^{-1}. The addition of 10% (v/v) of effluent was realized on days 1 (when the culture reached 0.5 g.L^{-1}), 5 and 10, in the form of fed batch. Cultures were conducted for 15 days.

2.3 Analytical determinations during cultivation and biomass

The monitoring of microalgae growth was performed by counting cells in a Neubauer chamber (GÓMEZ-SERRANO et al., 2015), and the results are expressed as cell number.mL^{-1}. In parallel, optical density (OD) measurements were taken at 670 nm (spectrophotometer model UV-1600, Pró-Tools, Porto Alegre, Rio Grande do Sul, Brazil) (COSTA; COLLA; FILHO, 2002). And every 5 days, samples were collected to determine the dry mass by filtration in cellulose filters with 0.45 µm pores.

In 1, 5, 10 and 15 days, samples were collected, centrifuged at 3,500 rpm for 10 min (centrifuge 5810, Eppendorf, Hamburg, Germany) and dried in an oven at a temperature of 50 °C, while the supernatant was kept frozen until the performance of the Total Nitrogen Kjeldahl (TKN) (Volumetric Method 4500-N_{org} B), COD (Colorimetry 5220 D), and Total Phosphorus (Potassium Persulfate Method 4500-PF), cited by American Public Health Association (APHA, 1995).

The biomasses obtained in cultivation were characterized in relation to carbohydrate and protein contents. The samples for quantification of carbohydrate and protein content were prepared via sonication of 5 mg of dry biomass in 10 mL of distilled water and submitted sonication for five 59 s cycles in a cell disruptor device (Unique Tip Model DES500). Carbohydrate content was determined using the phenol sulfuric method (DUBOIS et al., 1956). The protein content in algal biomass was determined according to the methodology proposed by Lowry (LOWRY, 1951). The contents of carbohydrates and proteins are presented on a dry basis.

2.4 Data processing and statistical analysis

Microorganism growth curves versus time were constructed. The final biomass concentration (X_f, $g.L^{-1}$ or number of cells per mL^{-1}), maximum biomass productivity ($P_{máx}$, $g.L^{-1}.d^{-1}$), and maximum specific growth rate ($\mu_{máx}$, d^{-1}) were evaluated (SCHMIDELL et al., 2001). Productivity of carbohydrates and proteins in cultivation ($g.L^{-1}.d^{-1}$) was obtained (MARGARITES et al., 2016). For all statistical analyses, Statistica 5.5 software was used. Differences between the means of the evaluated parameters were analyzed using analysis of variance at the 95% confidence level followed by Tukey's post-hoc test. All tests were performed in duplicates. The results were expressed as the average ± standard deviation.

3 Results and Discussions

In the tests carried out in order to verify the growth of microalgal with and without the addition of effluent it was found that *Spirulina platensis* presented the highest number of cells when grown in consortium with *Scenedesmus obliquus* without the addition of effluent, and in isolation when cultivated with the addition of effluent (Table 1).

Table 1. Design for the study of *Spirulina* and *Scenedesmus* in cultivations in consortia and results of cultivation kinetic parameters

	Scenedesmus obliquus		*Spirulina platensis*	
Proportions of each microalga to obtain the initial concentration of 0.15 g_{cells}/L Experiments	**X_{max} (cells.mL^{-1})**	**μ_{max} (d^{-1})**	**X_{max} (cells.mL^{-1})**	**μ_{max} (d^{-1})**
50% Sp + 50% Sc	$1.15.10^{4}\pm7.51.10^{2a}$	0.11 ± 0.01^{b}	$1.02.10^{4}\pm2.21.10^{2a}$	0.40 ± 0.00^{a}
50% Sp + 50% Sc + Effluent	$2.11.10^{4}\pm3.89.10^{3a}$	0.19 ± 0.02^{a}	$1.56.10^{3}\pm1.77.10^{2c}$	0.25 ± 0.03^{b}
100% Sp	-	-	$5.05.10^{3}\pm2.19.10^{2b}$	0.10 ± 0.01^{c}
100% Sp + Effluent	-	-	$8.44.10^{3}\pm8.84.10^{2a}$	0.25 ± 0.02^{b}

- Fields without values refer to pure cultivation, with no cells of these species.

μ_{max}: maximum specific growth rate (d^{-1})
X_{max}: Final biomass concentration (Xf) (cells.mL^{-1})

The best result in cellular concentration was obtained in the consortium with 50% Sp + 50% Sc (Figure 1a) demonstrating that the microalgal consortium presented a greater adaptation phase when compared to the cultivation of Spirulina only, which had its exponential growth starting on the third day of cultivation. *Spirulina*, when grown isolated, resulted in the increase of OD values with the addition of the effluent, due to the addition of the effluent, contributing to the concentration of nutrients in the culture medium.

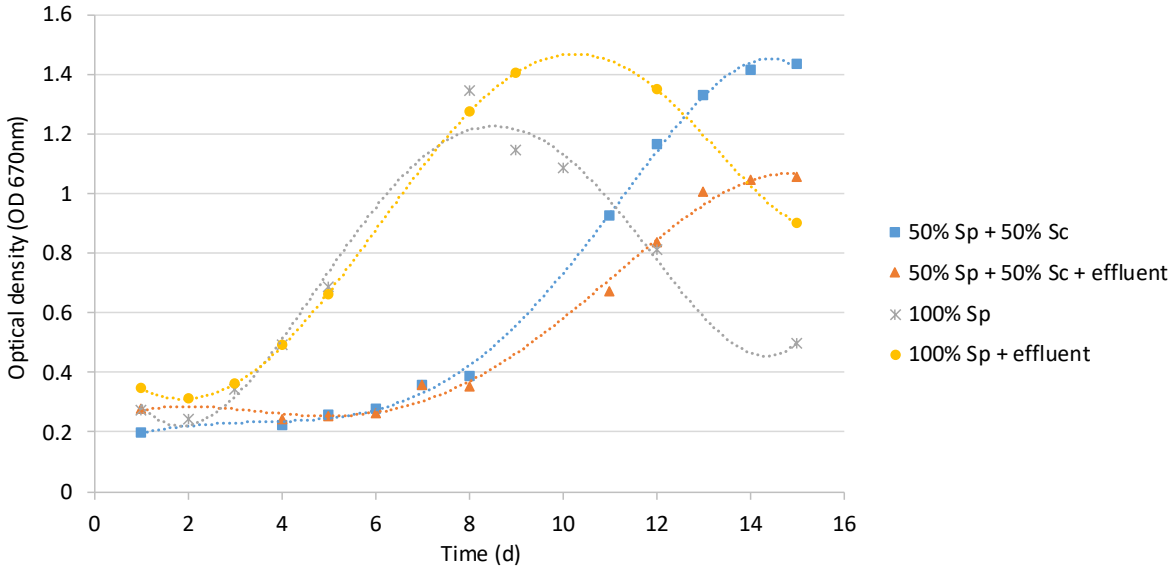
And it is also clear that *Spirulina* grown isolated reached its peak of growth between 8 and 9 days of growth, after this period it entered the decline phase, differently from the cultivation carried out in a consortium that entered the stationary phase between 13 and 14 cultivation days. In the interval of 1 to 4 days, the lowest optical densities were observed, possibly caused by cell death and adaptation of cells to the medium.

Analyzing the individual growth of each microalgae, through the number of cells.mL^{-1}, it was verified that *Scenedesmus* showed greater growth in relation to *Spirulina*, due to the fact that *Spirulina* presents a lower number of cells for the same dry mass (Figure 1b).

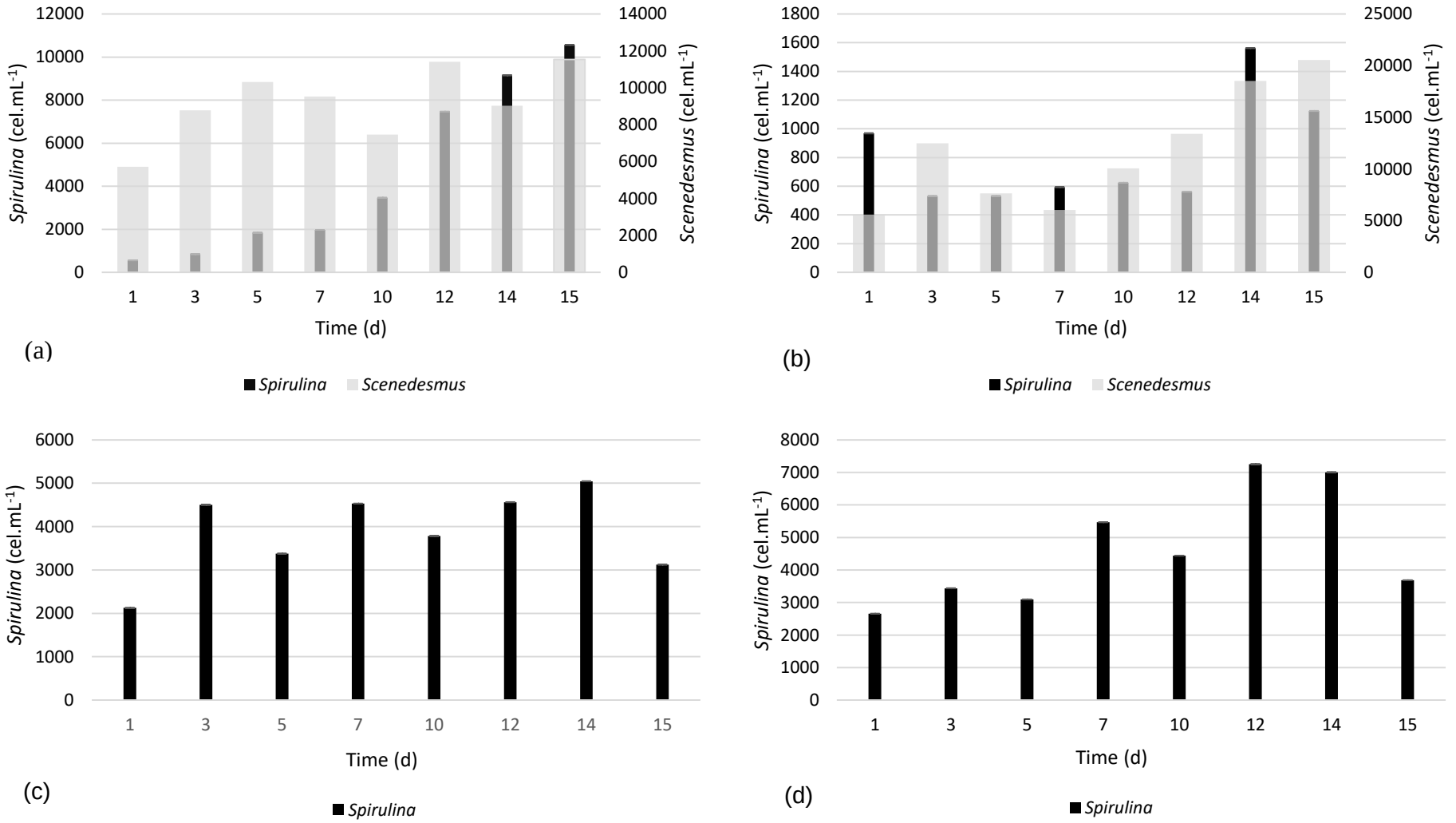
In consortium crops it was also observed that the *Scenedesmus* microalgae increased the number of cells when cultivated with the addition of effluent. *Spirulina*, on the other hand, decreased the number of cells, when in consortium there may be competition for both light and nutrients between the two microalgae, causing *Spirulina* to grow less in the consortium when compared in isolation. *Spirulina* when cultivated isolated showed the highest number of cells.mL^{-1}, after 12 days of cultivation with the addition of effluent.

Since this dispute for luminosity may have made *Scenedesmus* survive in the consortium, which did not occur in isolation, as the high luminosity of the open environment may have caused cell death. Ho et al. (2012) demonstrated, that further increases in light intensity to 540 µmol m^{-2}s^{-1} resulted in a marked drop in both CO_2 fixation rate and biomass productivity of *Scenedesmus*, suggesting that excessive illumination would inhibit the biomass production and CO_2 fixation ability, which is commonly recognized as the photo-inhibition effect.

Figure 1. Growth curve (OD_{670}) for tests carried out with and without effluent (a) and Cell growth of microalgae (cell. mL^{-1}), with and without addition of effluent (b).



(a)



(a) 50% *Spirulina* e 50% *Scenedesmus*, (b) 50% *Spirulina* e 50% *Scenedesmus* effluent, (c) 100% *Spirulina* (d) 100% *Spirulina* + effluent.

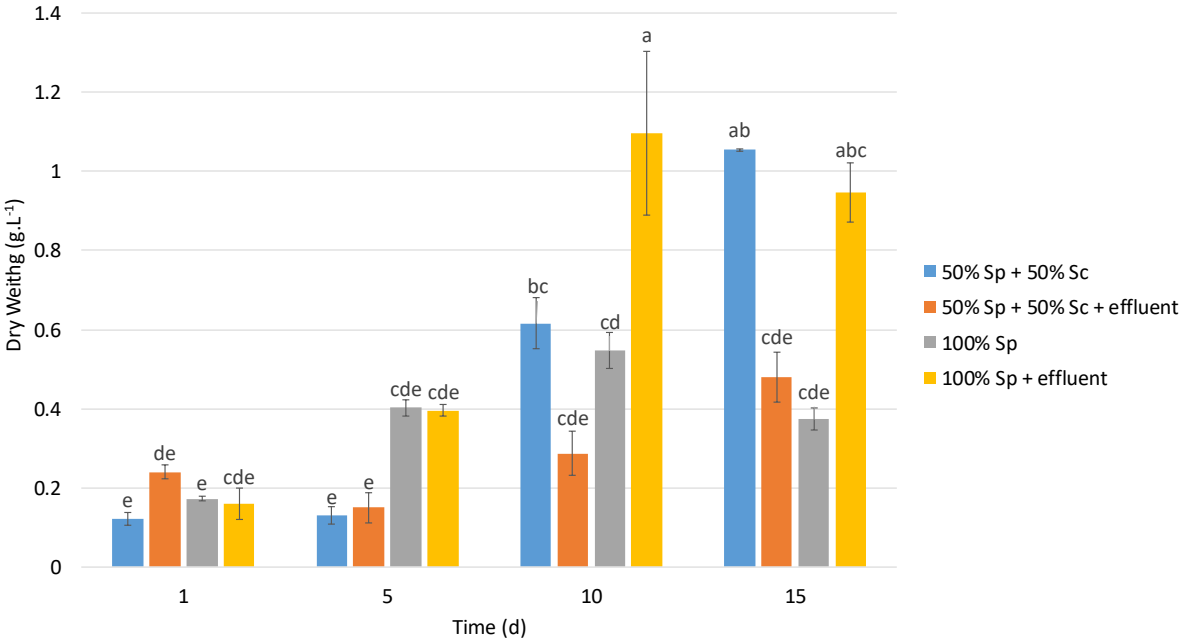
(b)

3.1 Effects on the dry weight and biomass composition

The cultivation that reached the highest dry mass were 50% Sc + 50% Sp at 15 days of cultivation, 100% Sp + effluent at 10 days of cultivation and 100% Sp + effluent at 15 days of cultivation, with no significant difference between them (Figure 2). It is possible to observe that the biomass concentration increases over the cultivation days, except in the 100% Sp cultivation, which reached its maximum concentration at 10 cultivation days.

In a similar study by Hultberg, (2017), who tested the effluent of a biogas processing plant as a source of nutrients for the cultivation of *Spirulina* and compared it with a conventional medium of *Spirulina*. The biomass production observed in the effluent-based medium was equal to that of the *Spirulina* medium during the first 6 days. After that, a decrease in biomass was observed in the effluent-based medium, while the amount of biomass in the *Spirulina* medium remained stable.

Figure 2. Biomass throughout cultivation with the addition of the effluent and without the addition of the effluent



Average values of tests performed in duplicates ± standard deviation. Same letters in the tests indicate that they showed no significant difference in the 95% confidence level (p> 0.05).

The highest concentration of carbohydrates was achieved in the 100% Sp test in the 15 days of cultivation (p> 0.05), followed by the 100% Sp tests in the 10 days of cultivation and 100% Sp + effluent and 50% Sp + 50% Sc both in the 15 days of cultivation. Therefore, it was found that the cultures that obtained the highest concentrations of carbohydrates are those that

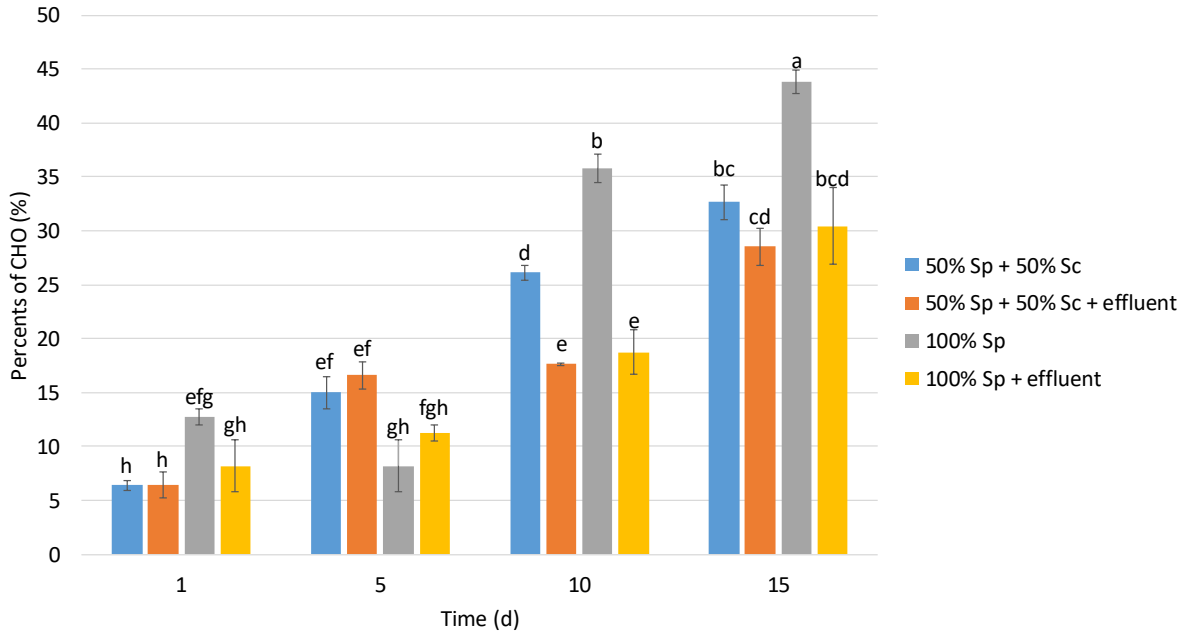
have the highest concentrations of *Spirulina*. In this sense, it is possible to affirm that the *Spirulina* microalgae grown under the conditions of the tests carried out have a greater capacity of accumulation of intracellular carbohydrates compared to the consortium with *Scenedesmus* with addition of effluent (Figure 3).

When comparing crops as to the addition of effluent, both trials (100% Sp + effluent and 50% Sp + 50% Sc + effluent) when compared with their peers without the addition of effluent obtained lower carbohydrate concentrations at 10 and 15 cultivation days, this possibly occurs due to the high nitrogen concentration in the effluent, which directs the cellular metabolism to the accumulation of proteins and not to the accumulation of reserve substances such as carbohydrate. Markou, (2015) in a study, cultivated in a fed batch regime *Spirulina platensis* and the microalgae *Chlorella vulgaris* in ammonia-rich wastewater derived from the anaerobic digestion of poultry litter, *A. platensis* presented different biochemical compositions in the four levels of fed batch addition. A general observation was that increasing the addition level of total ammonia, proteins, lipids, phycocyanin, chlorophyll, and total carotenoids increased, while carbohydrates decreased.

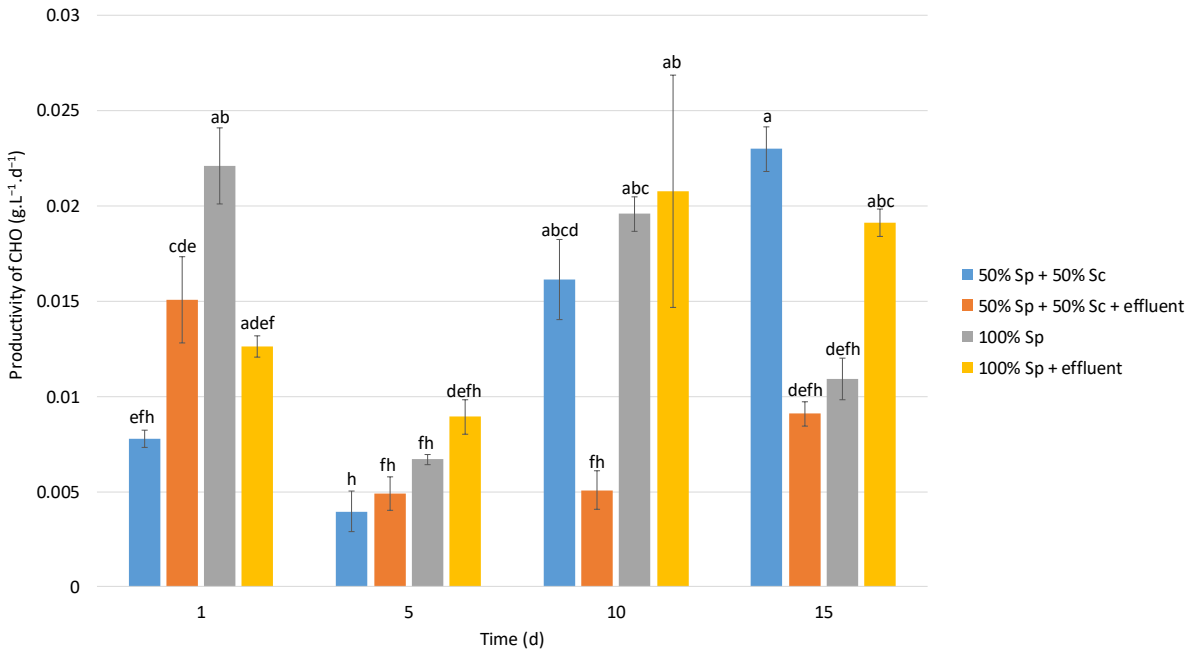
And it is possible to observe that throughout the cultivation period the concentration of carbohydrate increases, being higher after 15 days of cultivation in all tests This occurs because the nutrients of the medium are being depleted with the passing of time, causing the microalgae to direct the metabolism to reserve substances in the case of carbohydrates.

As for carbohydrate productivity, taking into account the concentration of carbohydrates, dry mass and cultivation time, the highest yields were found on days 10 and 15 of cultivation, due to the higher concentration of carbohydrates and dry mass on those days, with the exception of 100 % Sp on the first day of cultivation.

Figure 3. Intracellular carbohydrate concentration obtained in tests with and without the addition of effluent (a), carbohydrate productivity (b).



(a)



(b)

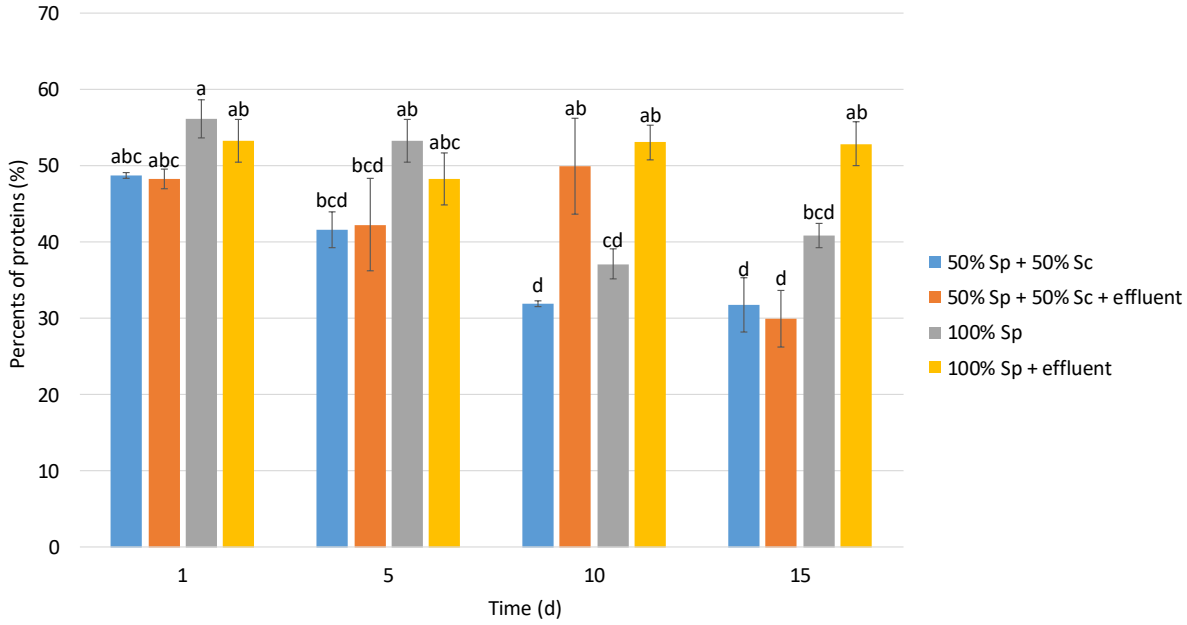
Average values of tests performed in duplicates ± standard deviation. Same letters in the tests indicate that they showed no significant difference in the 95% confidence level (p> 0.05).

The highest concentrations of protein as opposed to carbohydrate concentrations were higher in the first days of cultivation, due to the higher concentrations of nitrogen at the beginning of cultivations. And considering the 10th and 15th days it is possible to observe that the protein concentrations were higher in the crops with the addition of effluent, except in the

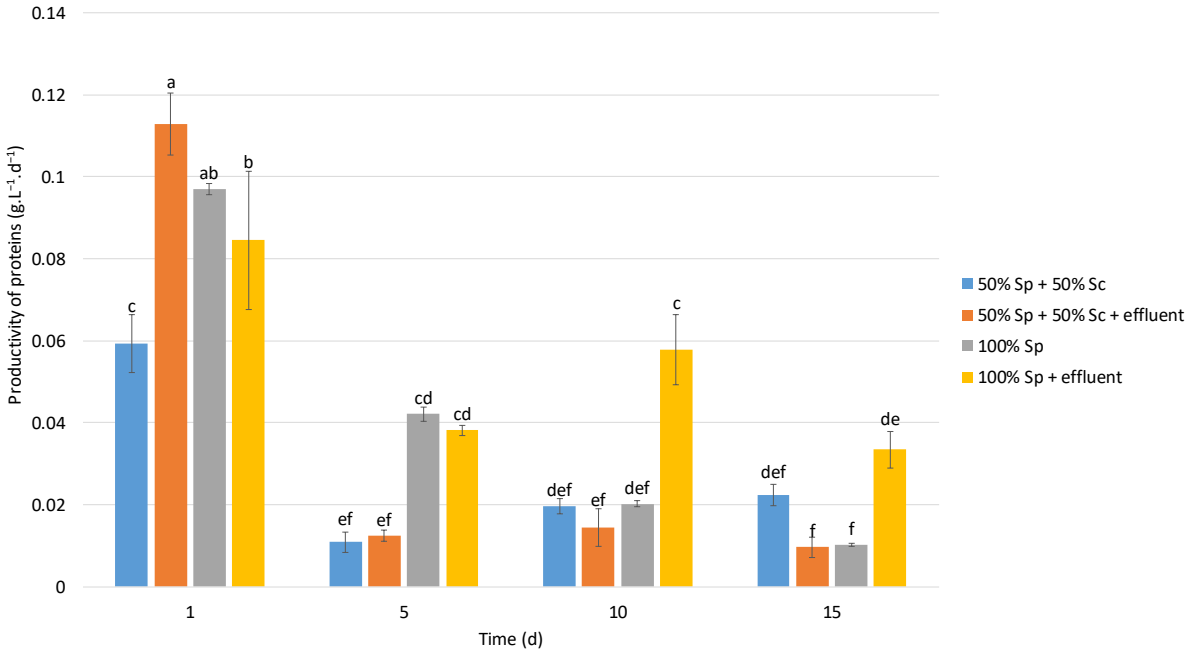
cultivation with 50% Sp + 50% Sc + effluent, due to the fact of the addition of nutrients present in the effluent. Protein yields were lower with the course of cultivation, the lowest for all crops after 15 days of cultivation (Figure 4).

Protein concentrations at 15 days of culture, when compared to tests with and without the addition of effluent (50% Sc + 50% Sp and 50% Sc + 50% Sp + effluent) and (100% Sp and 100% Sp + effluent) showed no significant difference ($p > 0.05$). Hultberg, (2017) in a similar study using biogas effluent from vegetable waste processing, obtained, in the biomass harvested after 6 days of growth, the total protein concentration, expressed in % of dry mass, of 60.5 ± 6.2 and 63.3 ± 2.7 for *Spirulina* medium and effluent-based medium, respectively, where no significant differences were observed between treatments.

Figure 4. Intracellular protein concentration obtained in tests with and without the addition of effluent (a), protein productivity (b).



(a)



(b)

Average values of tests performed in duplicates ± standard deviation. Same letters in the tests indicate that they showed no significant difference in the 95% confidence level (p> 0.05).

3.2 COD, nitrogen and phosphorus concentrations

Throughout the cultivation, in the tests without the addition of effluent, the COD remained in the same range, with no significant difference (p> 0.05) except for the assay with 50% Sc + 50% Sp, probably due to the fact that in the supernatant after the centrifugation there were still cell residues which increased the COD on this day. In cultures added with effluent, the COD concentrations are higher, due to the addition of effluent in the volume of 10% (v/v) with the concentration of 370.14 ± 21.5 mg.L^{-1} of COD, however after the addition on day 5, it had no significant difference (p> 0.05) (Figure 5a)

The highest concentrations of nitrogen were in crops with the addition of effluent, on day 01, after which there was a reduction in concentration, increasing again over the period of cultivation due to the addition of the effluent. For crops without adding effluent, the concentration remained the same (Figure 5b).

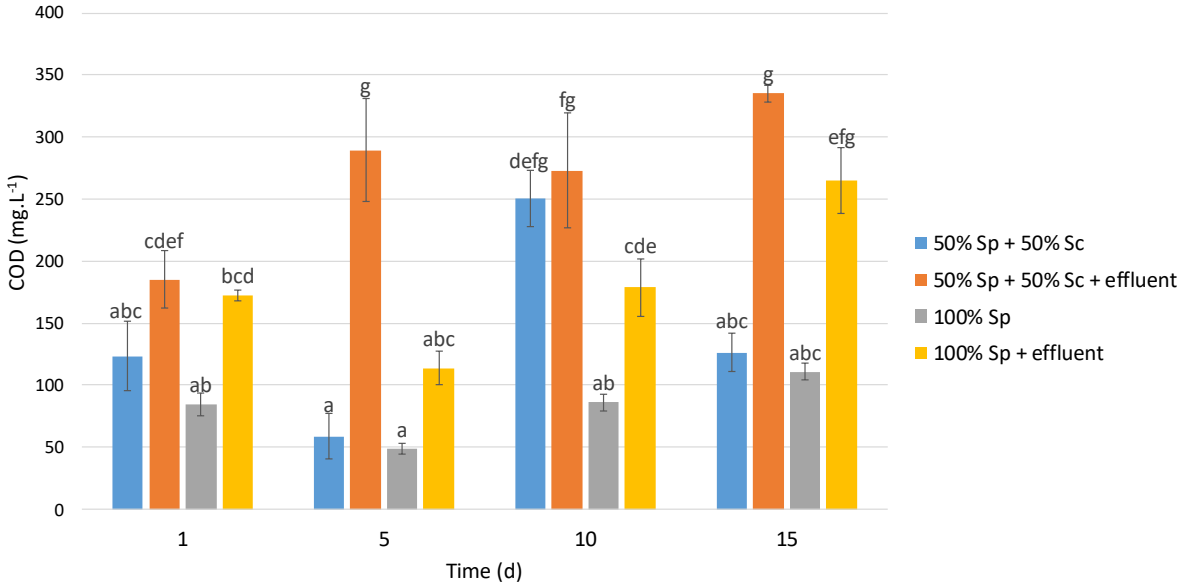
Figure 5c shows that phosphorus was removed during cultivation, both for crops in Zarrouk 20% medium and for crops with addition of effluent, demonstrating that phosphorus removal is significant (p> 0.05), since with each addition of effluent, approximately 18.71 ± 1.11 mg of phosphorus was added to the culture. Phosphorus is essential for algae growth as it

is involved in many cellular processes, although it constitutes less than 1% of the biomass (MORALES-AMARAL et al., 2015).

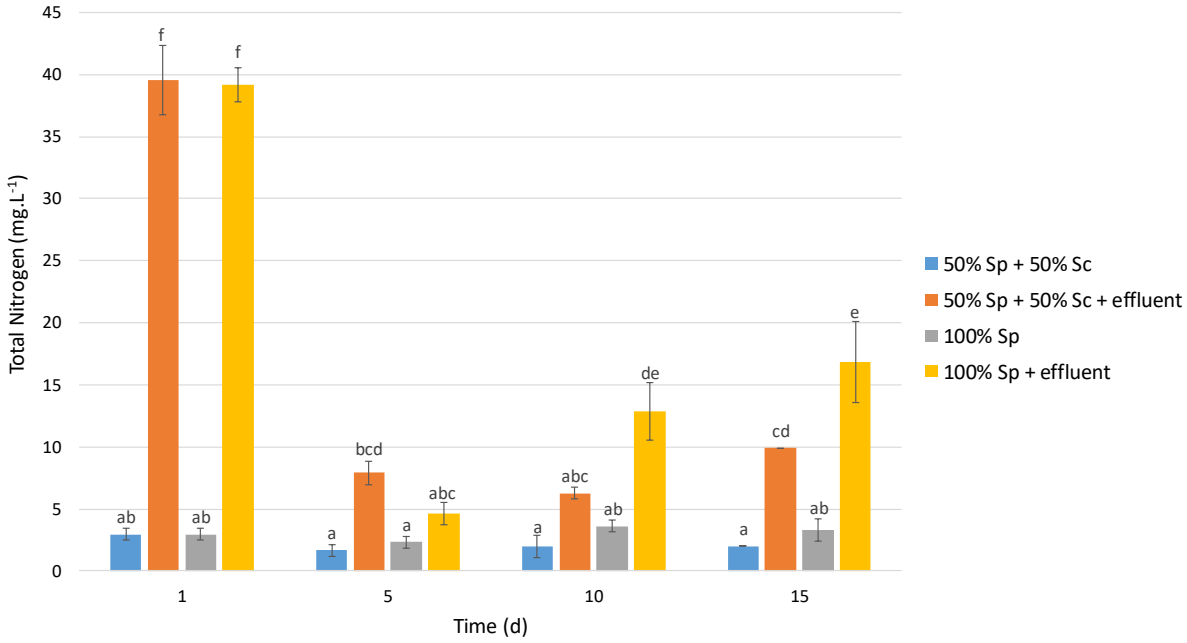
Koreiviene et al., (2014) reported that *Chlorella/Scenedesmus* consortium eliminated up to 99.7–99.9% of inorganic phosphorus and up to 88.6–96.4% of inorganic nitrogen from the wastewater within three weeks. Scherer et al., (2017) showed that a microalgae *Scenedesmus* sp biomass cultivation in cattle manure effluent caused a decrease in all physicochemical parameters, with a reduction of 92.5% of total nitrogen, 51.9% of phosphorus, and 53.6% of COD.

In a study carried out with *Arthrospira platensis* (*Spirulina*) grown in a dairy farm effluent, for the production of biodiesel, the concentrations of COD, phosphorus and nitrogen had a reduction of above 98%, in 4-5 days of culture (HENA et al., 2018).

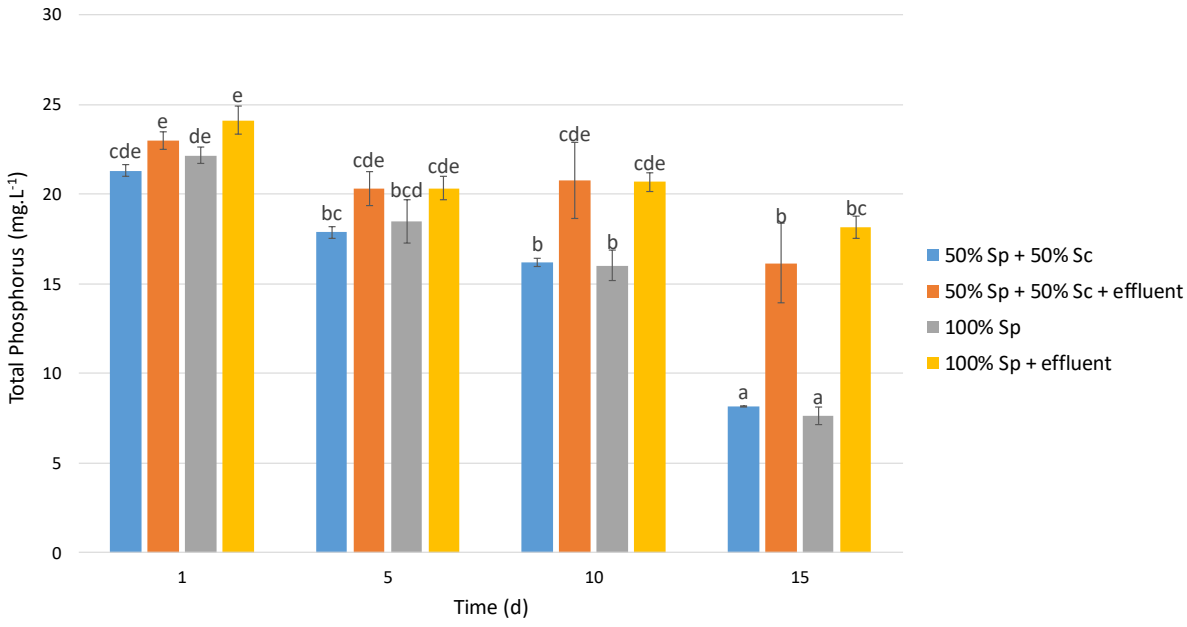
Figure 5. Concentration of COD (a), nitrogen (b) and phosphorus (c) in the culture medium



(a)



(b)



(c)

Average values of tests performed in duplicates ± standard deviation. Same letters in the tests indicate that they showed no significant difference in the 95% confidence level (p> 0.05).

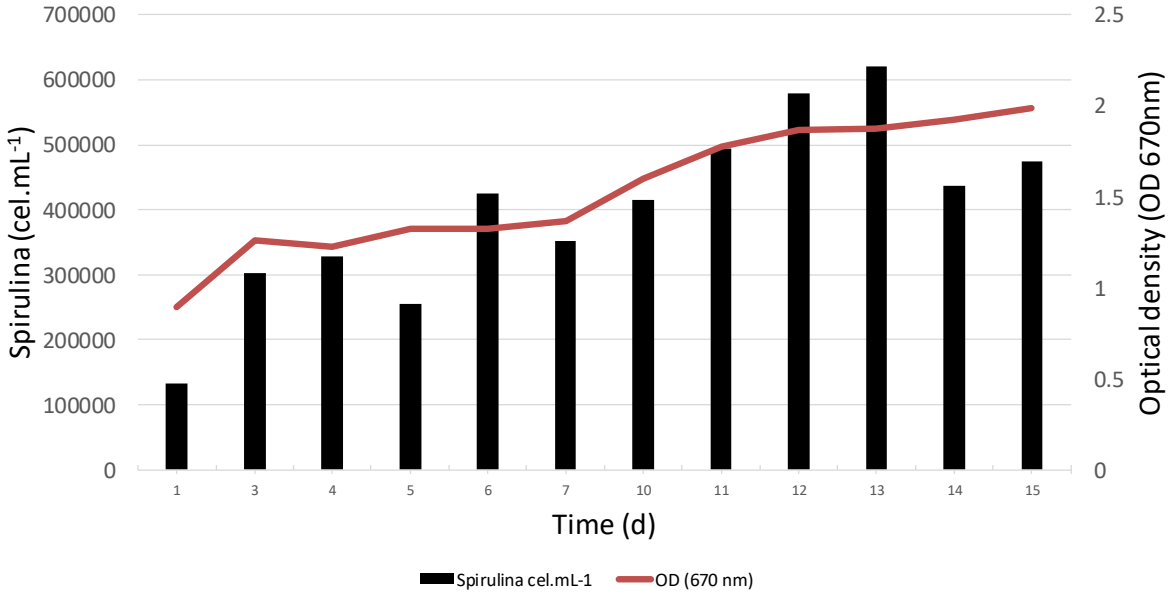
3.3 Pilot-scale

The scale-up test was performed with Spirulina in isolation, as it was the microalgae that reached the highest dry mass (0.94 $g.L^{-1}$) and highest productivity in CHO (0.019 $g.L^{-1}.d^{-1}$) with addition of the effluent.

Through the growth curves (OD_{670}) (Figure 6) it is possible to note that *Spirulina* achieved lesser exponential growth than when growing in 10 L raceways.

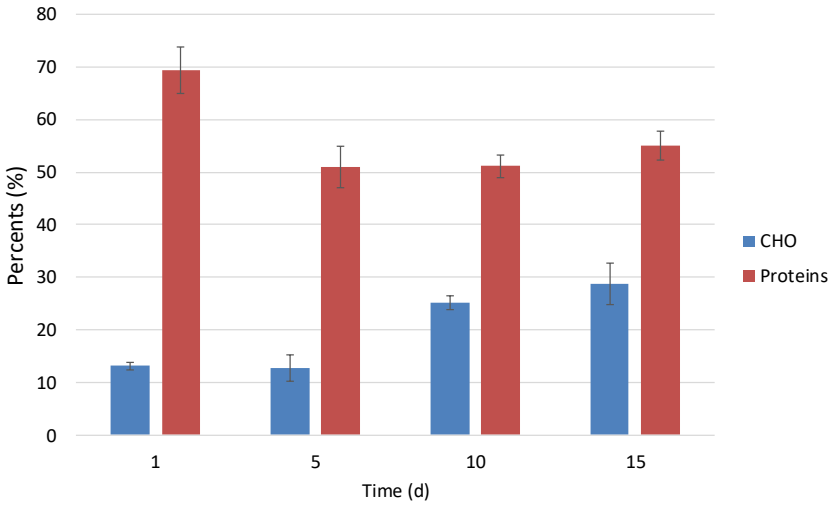
Analyzing the number of cells (cells.mL^{-1}) (Figure 6), it is noticed that the most significant growth occurs after the addition of the effluent on days 1, 5 and 10, and over the days the number of cells decreases , probably due to a shortage of nutrients in the medium. The cultivation that reached the highest dry mass were at 15 days of cultivation, it is possible to observe that the biomass concentration increases over the cultivation days. On day 1, 5, 10 and 15 the dry mass concentration was 0.63 ± 0.04 $g.L^{-1}$, 0.79 ± 0.02 $g.L^{-1}$, 1.12 ± 0.04 $g.L^{-1}$ and 1.65 ± 0.04 $g.L^{-1}$ respectively.

Figure 6. Growth curve (OD_{670}), Cell growth of microalgae (cell. mL^{-1}) and Biomass throughout cultivation (c)

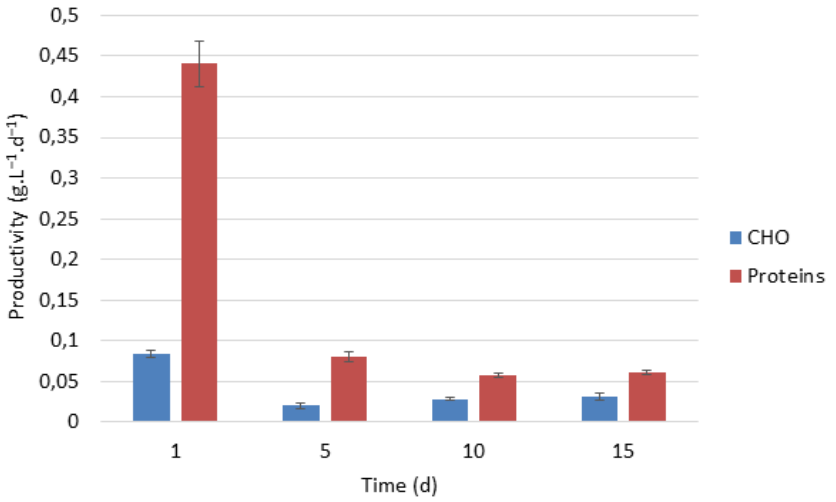


The highest concentration of carbohydrates was achieved in the in the 15 days of cultivation, reaching 28.8%. The cultivation carried out with *Spirulina* in the 10 L raceway with the addition of effluent reached 30.4%, so it is possible to affirm that the scale increase did not affect the CHO concentration in the cell. And the cultivation showed the same pattern in carbohydrate accumulation, increases with the cultivation period, being higher after 15 days of cultivation. Since the same characteristics were observed for the concentration of proteins (Figure 7).

Figure 7. Intracellular carbohydrate and protein concentration (a), carbohydrate and protein productivity (b).



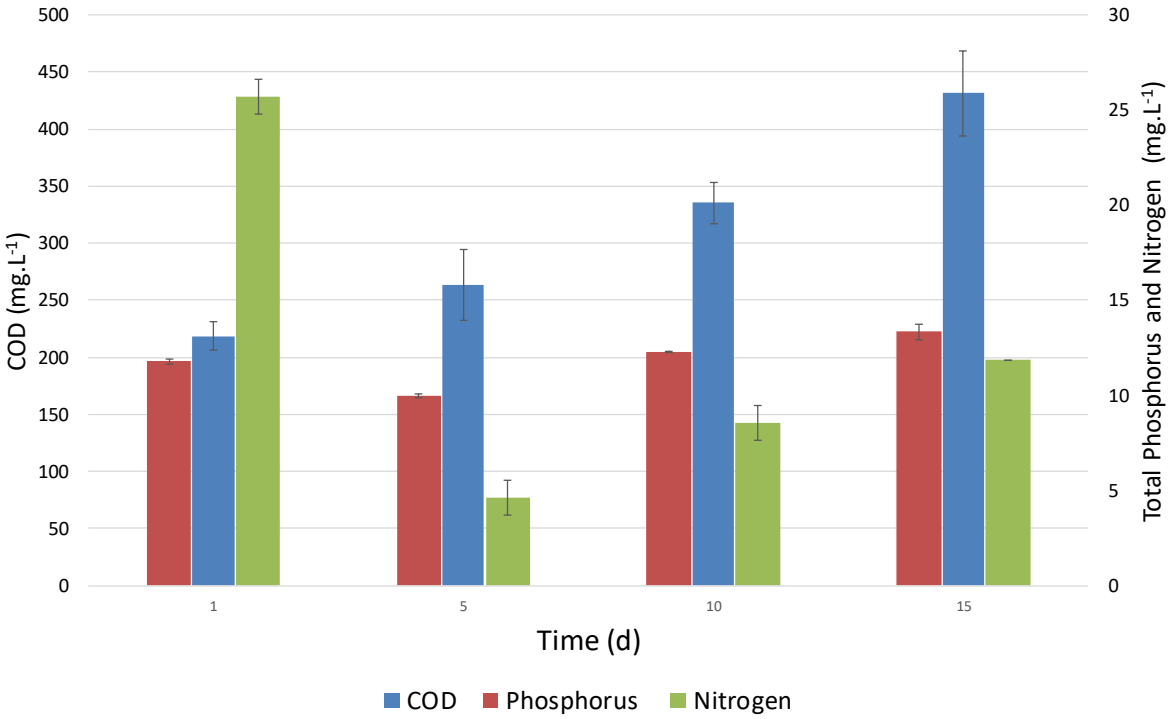
(a)



(b)

In the increase in scale, the COD increased over the days of cultivation, due to the addition of effluent in the volume of 10% (v/v) similar to what occurred in the 10 L raceways. Iit is possible to observe that the nitrogen concentration throughout the cultivation also presented characteristics similar to the cultures carried out in the 10 L raceways, with a higher concentration on day 01, lower on day 05, and increasing again on days 10 and 15 cultures, due to the addition of the effluent with Total Nitrogen Kjeldahl concentration of 372.23 ± 4.65 $mg.L^{-1}$. The concentration of phosphorus was lower on day 05 of cultivation, increasing on days 10 and 15 due to the addition of effluent and decreased consumption by microalgae (Figure 8).

Figure 8. Concentration of COD, nitrogen and phosphorus in the culture medium



4 Conclusion

The crops that reached the highest dry mass were 50% Sc + 50% Sp at 15 days of cultivation, and 100% Sp + effluent at 10 and 15 days of cultivation, thus, the cultivation that most generated biomass with the addition of effluent, demonstrating that *Spirulina* when grown alone with the addition of effluent in 10 L raceways reached the highest concentrations of biomass. The highest concentration of carbohydrates was achieved at 100% Sp without adding the effluent, since the addition of effluent increased the concentration of proteins and decreased the intracellular carbohydrates in the cultivations performed.

Phosphorus and nitrogen was removed during cultivation, both for cultivation in Zarrouk 20% medium, and for crops with added effluent, demonstrating that the removal of phosphorus and nitrogen is significant. The cultivation of microalgae in consortium and only *Spirulina* on can be used to assist the water treatment with the simultaneously production of biomass for different applications.

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6 CONSIDERAÇÕES FINAIS

Considerando a proposta da tese de doutorado, na revisão de literatura identificou-se que a lacuna do conhecimento referente ao cultivo de microalgas está relacionada aos processos envolvem a adaptação das microalgas a diferentes tipos de efluentes e a definição de espécies mais resistentes a cada tipo de efluente, bem como a necessidade de conhecer seu comportamento em cultivos em escala. Em função disto foram delineados os objetivos desta tese.

Em relação ao objetivo "a" (selecionar um meio de cultivo padrão para o cultivo em consórcio das microalgas), concluiu-se que os meios de cultura influenciaram o crescimento das duas espécies de microalgas, sendo possível observar que as culturas atingiram a maior concentração celular e concentração de carboidratos quando cultivados em meio Zarrouk 20% Desta forma foi o meio selecionado para o cultivo de ambas de forma isolada e em consórcio.

Referente aos objetivos "b" (estudo de concentrações iniciais de inoculo das microalgas, "c" (avaliar o efeito do efluente estéril e não estéril sobre o crescimento microalgal) e "e" (avaliar o efeito dos cultivos sobre a composição química das microalgas) concluiu-se que as microalgas adicionadas em menores concentrações inicias (10%), também apresentaram crescimento sem morte ou inibição. Os cultivos que atingiram a maior massa seca ao final do cultivo e maiores concentrações de carboidratos foram tinham as maiores concentrações inicias da microalga *Spirulina*. Nos cultivos descontínuos realizados em escala laboratorial conclui-se que é possível utilizar do efluente da digestão anaeróbia de dejetos bovinos estéreis ou não estéreis no cultivo de microalgas. E os consórcios das microalgas permitem o pós-tratamento do efluente concomitantemente à produção de biomassa microalgal, que pode ser utilizada para diversas aplicações. As condições estéreis/não estéreis causaram diferentes padrões de acúmulo de biomoléculas na biomassa e altas remoções de nitrogênio e fósforo foram obtidas usando consórcios 50% *Scenedesmus* + 50% *Spirulina* em condições não estéreis.

As conclusões parciais referentes aos objetivos "d" (estudar os modos descontínuos alimentados para realizar o aumento de escala dos cultivos das microalgas) e "e" (avaliar composição química das biomassas obtidas) foram que a microalga *Spirulina* cultivada isoladamente em modo descontínuo alimentado com adição de efluente atingiu maior produtividade em 10 e 15 dias de cultivo. A *Spirulina* quando cultivada isolada com a adição de efluente em r*aceways* de 10 L atingiu as maiores concentrações de biomassa, e a maior

concentração de carboidratos foi alcançada a 100% *Spirulina* sem adição do efluente. Houve uma remoção de 16,75% de fósforo e 88,2% nitrogênio nos primeiros 5 dias de cultivo.

Como conclusão geral, laboratorial em reatores fechados e modo descontínuo com adição de efluente estéril e não estéril conclui-se que o consórcio apresentou melhor resultado quanto a remoção dos nutrientes e concentração de biomassa com adição do efluente não estéril, sendo que as maiores concentrações de biomassa foram atingidas nos cultivos com as menores concentrações de efluente (0,58 $g.L^{-1}$) As maiores concentrações de carboidratos foram atingidas nos cultivos com maior concentração de *Spirulina* sem a adição do efluente. Em escala de 10 L em reatores abertos e modo descontínuo alimentado a microalga *Scenedesmus* não sobreviveu de forma isolada e o cultivo que atingiu a maior massa seca foi de *Spirulina* de forma isolada com a adição de efluente, porém em todos os ensaios quando comparados aos reatores fechadas a concentração da biomassa foi superior. Já a composição celular teve resultado semelhantes, ocorrendo a diminuição a concentração de carboidratos e aumento da concentração de proteína com a adição do efluente.

Para a condição em específico estudada, se buscar o maior acúmulo de carboidratos não se deve adicionar efluente, porém para cada tipo de microalga e características físico-químicas do efluente deve ser realizado um novo estudo.

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