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SACAROSE COMO PLATAFORMA PARA CULTIVOS HETEROTRÓFICOS MICROALGAIS

Santa Maria, RS
2017

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Dissertação apresentada ao Curso de Pós-Graduação em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Ciência e Tecnologia dos Alimentos**

Orientador: Prof. Dr°. Eduardo Jacob Lopes

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RESUMO

SACAROSE COMO PLATAFORMA PARA CULTIVOS HETEROTRÓFICOS MICROALGAIS

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As microalgas apresentam a capacidade de obtenção de energia a partir do consumo de substratos orgânicos na ausência de luminosidade. O cultivo heterotrófico suportado por uma fonte exógena de carbono é uma importante forma de produção de metabólitos de interesse comercial. A escolha de insumos de baixo custo para a formulação de meios de cultura é de grande importância para a economia global de processos biotecnológicos, uma vez que representam um percentual significativo no custo final do produto. Em face disto o trabalho teve por objetivos: (i) formular um meio de cultura sintético baseado em diferentes concentrações de sacarose para uso em cultivos heterotróficos, (ii) avaliar a cinética de consumo de sacarose e produção de biomassa, (iii) definir o perfil qualitativo da fração lipídica, (iv) caracterizar os bioprodutos de natureza intracelular, (v) determinar a análise do ciclo de vida do processo (balanço de energia, emissões de CO_2 e balanço de água. Os resultados obtidos demonstraram que a microalga *Phormidium autumnale* teve melhor desempenho no processo na relação C/N de 40, atingindo uma produtividade de óleo de 18,9 mg/L/h. Na melhor condição, os resultados mostraram que a produção de biodiesel de microalgas tem uma produção de energia positiva (50,59 MJ) associada a um baixo consumo de água (28,38 m^3 / kg) e baixas emissões de CO_2 (9,18 kg CO_2-eq / kg). A composição deste óleo foi predominantemente saturada (45,20%), monoinsaturados (34,70%) e poliinsaturados (19,90%), resultando em características de qualidade do biodiesel adequadas às normas nacionais (ANP 255) e internacionais (ASTM 6751 e EN 14214).

Palavras chave: microalga, heterotrófico, biodiesel, analise do ciclo de vida

ABSTRACT

SUCROSE AS A PLATFORM FOR MICROALGAE HETEROTROPHIC CULTIVATION

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Microalgae present the capacity to obtain energy from consuming organic substrates in the absence of luminosity. The heterotrophic cultivation supported by an exogenous source of Carbon is an important form of metabolites production of commercial interest and the choice of low-cost inputs to the formulation of culture mediums is of great importance to the global economic of biotechnological processes since it represents a significant percentual on the final cost of the product. In this sense, the aims of this work were: (I) formulate a culture medium synthetic based on different concentrations of sucrose for the use in heterotrophic cultivation, (II) evaluate the kinetic of sucrose consume and the biomass production, (III) determine the qualitative profile of the lipid fraction, (IV) characterize the bioproducts of intracellular nature, (V) determine the analysis of the water cycle of the process (energy balance, CO_2 emissions, and water balance). The results obtained demonstrated that the microalgae *Phormidium autumnale* had its best performance in the process in the relation C/N de 40, reaching an oil productivities of 18.9 mg/L/h. On the best condition, the results showed that the biodiesel production of microalgae has a positive energy production (50.59 MJ) associated with a low water consumption (28.38 m^3/ kg) e low CO_2 emissions (9.18 kg CO_2-eq). The composition of this oil was predominately saturated (45.20%), monounsaturated (34.70%) and polyunsaturated (19.90%), resulting in quality characteristics of biodiesel adequate on the national (ANP 255) and international norms (ASTM 6751 e EN 14214).

Keywords: microalgae, heterotrophic, biodiesel, Life cycle assessment

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SUMÁRIO

INTRODUÇÃO

O crescente aumento da concentração de CO_2 na atmosfera, associado ao esgotamento dos combustíveis fosseis não renováveis e a alta demanda energética, tem aumentado o interesse comercial em desenvolver tecnologias para a produção de combustíveis alternativos e sustentáveis. Diante desse cenário, busca-se aliar o desenvolvimento econômico com proteção ambiental, desenvolvendo-se novos produtos, novas alternativas de processos e técnicas eficientes no combate e remediação da poluição, tornando assim, a atividade industrial menos impactante ao meio ambiente (SAINGER et al., 2017; TRIVEDI et al., 2015).

As microalgas possuem um grande potencial para a produção de biocombustíveis, como o biodiesel de 3ª geração, devido a sua diversidade metabólica (autotrófico, heterotrófico e mixotrófico) e a possibilidade de acumularem lipídios que vem sendo utilizado como ferramenta alternativa para a produção de óleos e obtenção de biocombustíveis (QUEIROZ et al., 2011; RASHID et al., 2014). Esses recursos permitem que as microalgas apresentem diversas vantagens sobre culturas oleaginosas convencionais como o milho, soja e girassol (HLAVOVA et al., 2015).

Contudo, um fator determinante nos cultivos heterotróficos microalgais é a escolha da fonte de carbono orgânico utilizada, que representa um dos principais obstáculos para a aplicação industrial. O substrato orgânico é estimado em cerca de 80% do custo total do processo, podendo inviabilizar economicamente o processo. Uma alternativa aos elevados custos é a substituição de determinadas fontes de carbono orgânico por substratos de baixo custo (WANG 2016). A sacarose constitui até 60% da biomassa seca de algumas plantas como a beterraba e a cana de açúcar, e têm sido consideradas alternativas promissoras para a minimização do custo dos meios de cultura para o cultivo de microalgas (PEREZ-GARCIA et al., 2011; QUEIROZ et al., 2013).

Em contrapartida, o sucesso de qualquer sistema de produção de biocombustíveis dependerá dos custos ambientais. Assim, a fim de avaliar o impacto ambiental e viabilidade de tais empreendimentos dessa tecnologia, a metodologia de Avaliação do Ciclo de Vida (ACV) é uma valiosa ferramenta para avaliar o desempenho ambiental destes sistemas, procurando analisar o custo energético total de um processo de produção, considerando as entradas de energia e o impacto

ambiental de cada etapa. Quando combinado com outras análises, o ACV pode ajudar a identificar áreas de alto custo e determinar se uma estratégia de produção de biocombustíveis visando se o processo é sustentavelmente viável (GUO, et al., 2017; CHEW, et al., 2017; DUTTA, et al., 2016).

Neste sentido, a estratégia de suportar a geração desta biomassa em meio de cultivo sintético adicionado de uma fonte de carbono orgânico, economicamente viável, poderá contribuir para o avanço da viabilidade ambiental deste tipo de processo.

OBJETIVOS

Objetivo Geral

- Desenvolver biorrefinarias microalgais suportadas com sacarose de forma economicamente sustentável que sirva como alternativa para processos convencionais de produção.

Objetivos Específicos

- Formular um meio de cultura sintético fundamentado em diferentes concentrações de sacarose para uso em cultivos heterotróficos;
- Avaliar a cinética de consumo de sacarose e a produção de biomassa;
- Definir o perfil qualitativo da fração lipídica;
- Caracterizar bioprodutos de natureza intracelular
- Determinar a análise do ciclo de vida do processo (balanço de energia, emissões de CO_2 e balanço de água.

CAPÍTULO 1

REVISÃO BIBLIOGRAFICA

1 MICROALGAS

As microalgas são em grande parte um grupo diversificado de micro-organismos que compreendem dois grupos: as do reino protistas, sendo fotoautotróficos e eucarióticos e as cianobactérias procarióticas (SINGH e SAXENA, 2015). Microalgas são organismos microscópicos fotossintetizantes que crescem rapidamente em diferentes condições ambientais devido a sua estrutura celular simples, unicelular ou multicelular (MATA et al., 2010; SCOTT et al., 2010). A fotossíntese é a forma de obtenção de energia mais utilizada, e em casos que não há contato com a luz, a obtenção da energia ocorre por meio da respiração.

O interesse por esses micro-organismos reside no seu potencial de utilização de biomassa para alimentação, rações, produtos químicos finos, tratamento de resíduos e aplicações energéticas. Sob o ponto de vista de aplicação biotecnológica, são consideradas os organismos mais versáteis, uma vez que podem mediar até três metabolismos em paralelo para a obtenção de energia e manutenção de suas estruturas (QUEIROZ et al., 2013).

A principal vantagem do cultivo das microalgas é a obtenção de seus produtos metábolitos, que são utilizados na alimentação de organismos aquáticos e terrestres, ou como suplementos alimentares para os seres humanos, ou para seu uso em processos ambientais, como tratamento de águas residuais, fertilização dos solos, biocombustíveis e fitorremediação de resíduos tóxicos (PEREZ-GARCIA et al., 2011).

A composição das microalgas são fundamentalmente carboidratos, lipídeos, proteínas e ácidos nucleicos, sendo que as proporções variam amplamente entre as espécies e de acordo com as condições de cultivo. As proporções de lipídeos presente nesses microrganismos variam normalmente de 5% a 75% de biomassa seca, dependendo da microalga em questão, sendo que, as espécies estudadas em sua maioria apresentam em torno de 20% a 50% de lipídeos. Os triglicerídeos das microalgas conhecidas apresentam composição em ácidos graxos semelhante à dos óleos vegetais usados na produção de biodiesel (MATA et al.,2010).

Muitas microalgas têm demonstrado facilidade em crescer rapidamente em cultivos heterotróficos. Tais algas apresentam a capacidade de crescimento na ausência de luz, substituindo a fixação de CO_2 atmosférico das culturas autotróficas por consumo de substratos orgânicos dissolvidos no meio de cultura. *Phormidium autumnale* é uma microalga filamentosa de 3 a 4 μm de diâmetro. Sua espécie é

conhecida por viver em ambientes extremos, como fontes termais, solos de desertos e lugares contaminados. Por esta razão, apresenta grande potencial para o uso em processos biológicos (KOLLER et al., 2014).

2 PROCESSOS BASEADOS EM MICROALGAS

A crescente demanda por produtos e serviços tem elevado drasticamente a atividade industrial, gerando cada vez mais resíduos e elevando a utilização dos recursos naturais, os quais estão tornando-se escassos e muitas vezes encontram-se poluídos e degradados (ONCEL, 2011). Diante desse cenário, busca-se aliar desenvolvimento econômico com proteção ambiental, desenvolvendo-se novos produtos, novas alternativas de processos e técnicas eficientes no combate e remediação da poluição, tornando assim, a atividade industrial menos impactante ao meio ambiente.

Os processos biotecnológicos tornaram-se uma alternativa interessante no combate à poluição e na geração de novos produtos, uma vez que esses processos se utilizam do metabolismo microbiano para degradar e remover poluentes, bem como para transformar matérias primas gerando produtos menos nocivos ao meio ambiente (GADD, 2008). Nesses processos, existe uma gama de micro-organismos atuantes, como bactérias fungos, algas e microalgas.

A utilização de microalgas para tratamento de águas residuais é particularmente atraente devido as suas habilidades em assimilar nutrientes como matéria orgânica, NO^{3-}, PO_4^{3-}, NH_4^{+}, CO_2 e metais pesados (PEÑA-CASTRO et al., 2004). O bio-tratamento de efluentes líquidos se dá em biorreatores heterotróficos, onde a matéria orgânica e nutrientes inorgânicos são simultaneamente convertidos em biomassa na ausência de luminosidade. Esses processos são considerados uma alternativa barata para as formas convencionais de tratamento de efluentes secundários e terciários (QUEIROZ et al., 2013). Tem-se intensificado os estudos em processos biológicos para tratamento de efluentes, surgindo assim, a biossorção que consiste na absorção de metais tóxicos por microrganismos (QUINTELAS et al., 2008). Esse processo apresenta-se como uma tecnologia promissora e em atual expansão, apresentando vantagens como baixo custo e boa eficiência. Dentre os micro-organismos utilizados na biossorção, as microalgas possuem destaque em

função da sua capacidade de retenção e imobilização de metais (MÓDENES et al., 2009).

O óleo extraído de microalgas apresenta características semelhantes às dos óleos de plantas oleaginosas e se enquadra dentro das principais características exigidas pela ANP (Agencia Nacional de Petróleo) para qualidade do biodiesel, como o ponto de fulgor mínimo de 115°C e baixo índice de acidez (menor que 0,8 mg KOH/g) (TEIXEIRA, 2008). Entre as diversas matérias-primas para a produção de biodiesel, a biomassa de microalgas é aquela que apresenta a possibilidade de produção de biodiesel, que poderá servir como uma alternativa ao diesel (cerca de 40 bilhões de litros por ano) e de modo ambientalmente sustentável. As microalgas produzem compostos poliinsaturados, o que leva à diminuição da estabilidade do biodiesel produzido, entretanto, devido à presença de ácidos graxos poliinsaturados, uma vantagem apresentada pelo biodiesel de microalgas é o alto rendimento em temperaturas baixas, característica que não é apresentada pelo biodiesel de oleaginosas convencionais, as quais apresentam pouco rendimento em temperaturas relativamente baixas (KOLLER et al, 2014).

O acúmulo de lipídios em microalgas para a produção de biodiesel tem sido um foco atual de trabalho de inúmeros pesquisadores em todo o mundo. A utilização de lipídios de microalgas, apresenta vantagens em comparação com as fontes convencionais de lipídios, tais como a elevada produtividade em lipídios por área cultivada, em comparação com culturas oleaginosas agricultúraveis; capacidade de sintetizar e acumular grandes quantidades de lipídios neutros; elevadas taxas de crescimento; crescimento em ambientes inóspitos, os quais não são agricultúraveis; utilização de fontes de nutrientes, tais como nitrogênio e fósforo, de uma variedade de fontes de águas residuais, contribuindo para o tratamento destas águas residuárias; sequestro de CO_2; produção de co-produtos de elevado valor agregado (por exemplo, biopolímeros, proteínas, polissacarídeos, pigmentos, alimentação animal, fertilizantes e H_2); crescimento em fotobiorreatores durante todo o ano com uma produção anual de biomassa com produtividade, com base na superfície, superior ao dos ecossistemas terrestres, por cerca de dez vezes (HU et al., 2008; WIJFFELS e BARBOSA, 2010; SCOTT et al., 2010).

Finalmente, microalgas apresentam a capacidade de acumular substâncias poliméricas extracelulares (SPE) na superfície da célula, como uma forma de proteção

para as mesmas. SPE's são matrizes heterogêneas de polímeros de polissacarídeos, proteínas, ácidos nucléicos e fosfolipídos (MCSWAIN et al., 2005). Os exopolímeros microalgais têm múltiplas aplicações industriais. Neste sentido, podem ser aplicados na indústria de alimentos como espessantes e gelificantes. Na indústria farmacêutica, podem ser empregados como matriz hidrofílica de libertação controlada de medicamentos e no desenvolvimento de vacinas bacterianas e para aumentar a imunidade inespecífica. Além disso, algumas SPE's têm características de biosurfactantes e estão sendo utilizados na biorremediação de águas e solos (MISHRA et al., 2011).

3 METABOLISMO HETEROTRÓFICO

O crescimento e a composição das microalgas são definidos pelas condições de cultivo, dependendo de sua espécie, apresentam três tipos principais de metabolismo: autotrófico, com utilização da luz como fonte única de energia que é convertida em energia química por meio de reações fotossintéticas; heterotrófico: utilização apenas de compostos orgânicos dissolvidos como fonte de carbono e energia; e mixotrófico: conseguem simultaneamente realizar a fotossíntese e consumir carbono inorgânico e orgânico, o que permite aumentar a sua produtividade. (BRENNAN e OWENDE, 2010; MATA et al., 2010; PEREZ-GARCIA et al., 2011; BHATNAGAR et al., 2011).

A principal característica do metabolismo heterotrófico é a necessidade de uma fonte orgânica externa para obtenção de energia e carbono. Microalgas em cultivo heterotrófico não realizam fotossíntese, e por isto não necessitam de luz. Segundo BUMBAK et al. (2011), o metabolismo heterotrófico das microalgas é semelhante ao de outros microrganismos, como as leveduras utilizadas na produção da cerveja e de etanol.

Segundo Azma et al. (2011), a capacidade de crescer heterotroficamente está presente em vários gêneros de microalgas e tem relação principal com as seguintes características: (1) permeabilidade celular à fonte de carbono orgânica, (2) transporte ativo da fonte de carbono orgânico e (3) fatores enzimáticos presentes no interior da célula. Assim, a maior parte das características determinantes para o metabolismo heterotrófico refere-se à entrada da fonte de carbono orgânico na célula sendo as mais empregadas são o acetato e a glicose.

O metabolismo inicial da glicose nas microalgas ocorre pela via metabólicas das pentoses fosfatos e ocorre no citosol (PEREZGARCIA et al., 2011; YEH e CHANG, 2012). A via das Pentoses é a rota inicial mais utilizada em cultivos de microrganismos heterotróficos. Entre os produtos finais desta via destacam-se a frutose 6-P e o gliceraldeído 3-P, que são compostos intermediários da glicólise via Embden-Meyerhof. Percebe-se que a via das Pentoses pode ser considerada uma preparação para gerar ATP pela via Embden-Meyerhof/ Ciclo do Ácido Cítrico (PEREZ-GARCIA et al., 2011; YEH e CHANG, 2012).

Outro aspecto que se deve levar em consideração em sistemas heterotróficos é a relação carbono/nitrogênio (C/N), que podem influenciar o teor de lipídeos acumulados pela célula, controlando a síntese de lipídios (Gordillo, 1998). Altas taxas de C/N favorecem a formação de lipídios, decorrente do esgotamento de nitrogênio na cultura (RATLEDGE, 1989).

Estudos com microalgas indicaram a glicose como a melhor fonte de carbono em cultivos heterotróficos, atingindo altas concentrações de biomassa e lipídeos (HUANG et al., 2012). No entanto a glicose é uma fonte carbono de custo elevado, responsável por 60-75% do custo total do biodiesel (CANAKCI, 2008).

A sacarose um dos produtos de armazenamento no citoplasma das células vegetais constitui até 60% da biomassa seca de algumas plantas e pode ser obtida principalmente a partir da cana de açúcar e beterraba. Dentre as diversas finalidades da sacarose, ela pode ser utilizada na produção de etanol e em outros processos fermentativos. A produção de etanol a partir da cana de açúcar promove a formação de um resíduo que contém sacarose, podendo ser utilizado em cultivos fermentativos (WANG, et al., 2016).

4 BIOPRODUTOS DE ORIGEM MICROALGAL

A biodiversidade e consequente variabilidade na composição bioquímica das microalgas, aliada ao emprego de melhoramento genético e ao estabelecimento de tecnologia de cultivo em grande escala, vêm permitindo que as microalgas sejam utilizadas em diversas aplicações (BOROWITZKA, 2013). Recentemente as microalgas estão recebendo uma atenção considerável devido à sua capacidade de sintetizar compostos valiosos (por exemplo, pigmentos), e por acumularem compostos de alta energia (por exemplo, lipídios, carboidratos). Portanto, sendo consideradas

como uma matéria-prima de terceira geração para a produção de biocombustíveis. (TRIVEDI, et al., 2015)

A conversão de biomassa de microalgas para biocombustíveis e produtos de alto valor agregado estão globalmente ganhando um destaque significativo e tem sido o principal foco de pesquisadores que buscam vencer as barreiras econômicas e ambientais para o setor de energias renováveis (HERRERO e IBÁÑEZ, 2015). A combinação da produção de biocombustíveis de microalgas com as aplicações convencionais é excelente para prosperar a indústria de biorrefinaria microalgal de forma sustentável (MA, et al., 2015)

Finalmente, os sistemas globais usados na produção de biocombustíveis incluem primariamente a produção de biomassa. Esta produção pode ser mediada via biotecnológica, no qual cianobactérias e clorofíceas apresentam importante potencial para a produção de óleos, adequados à manufatura de biodiesel (CHISTI, 2007).

O histórico da produção brasileira de biodiesel mostra que o setor está em crescente dependência de uma única matéria-prima, a soja, que se destaca como a principal fonte de matéria-prima lipídica utilizada na produção de biodiesel no Brasil, correspondendo a aproximadamente 80% do biodiesel produzido e comercializado (ANP, 2016). Entretanto, alguns problemas vêm sendo relatados quanto à utilização de fontes oleaginosas para a síntese de biodiesel. Um dos gargalos identificados é o uso do solo, pois é preciso avaliar as extensões de terras agricultáveis disponíveis pois pode haver conflito com a produção de alimentos, uma vez que muitos países enfrentam sérios problemas de fome e escassez, bem como a insuficiência de água e adubo (BRANDINI, 2016). Diante desse contexto pode-se observar, que o setor energético exige uma grande demanda por recursos naturais, sendo de suma importância o uso de fontes renováveis para geração de energia. Assim, a busca pela matéria-prima ideal tem se tornado constante e evoluído muito nos últimos anos. O cultivo de microalgas para a produção de biocombustíveis tem se tornado uma alternativa bastante promissora. O uso das microalgas como fonte viável de biomassa tem causado grande expectativa neste setor, apresentando vantagens como a não competição com alimentos, rápidas taxas de crescimento, acumulo de 35 lipídeos, alta tolerância às condições extremas do meio ambiente, capacidade de serem cultivadas de maneira intensiva e grande potencial no uso em biorremediação e biofertilização. Além disso, tem a capacidade de produzir uma alta gama de bicombustíveis tais como:

biodiesel, biohidrogenio, biogás e bioetanol, bem como a produção de vários bioprodutos onde, objetivam a estruturação de plantas comerciais no conceito de biorrefinarias (CHISTI, 2007; GIRARD et al., 2014; ZHU, 2015).

5 BIORREFINARIAS MICROALGAIS

Diversos pesquisadores afirmam que para alcançar uma produção economicamente viável de microalgas para biocombustíveis é necessário explorar o completo potencial de produtos comerciais derivados da biomassa de microalgas por meio de uma infraestrutura de biorrefinarias (WIJFFELS e BARBOSA, 2010; RAWAT et al., 2013, YEN et al., 2013, ZHU et al., 2014).

Apesar das várias vantagens reivindicadas associadas com o desenvolvimento de biorrefinaria à base de microalgas (como melhorias potencialmente significativas na economia de biocombustíveis), existem ainda muitos desafios a serem superados (RIZWAN et al., 2015). Ferramentas e conceitos sobre sistemas de processo de engenharia podem ser aplicados para enfrentar esses desafios através do desenvolvimento de um quadro de modelagem sistemática para determinar as configurações ideais, em um custo eficaz, robusto e de forma ambientalmente sustentável (MATA et al., 2010).

A exploração comercial em larga escala das microalgas, segundo Spolaore et al. (2006) é motivada pelo elevado teor de proteínas da biomassa para utilização como recurso alimentar alternativo, tendo um alto valor de qualidade protéica comparado com fontes vegetais, como por exemplo, trigo, arroz e leguminosas, mas inferiores a fontes animais, como leite e carne (MATA et al., 2010). Em virtude das características da biomassa, processos baseados em cianobactérias têm sido considerados nos últimos anos potenciais tecnologias para converter resíduos industriais em insumos proteicos usados na formulação de rações animais (JACOB-LOPES et al., 2010).

Tendo como objetivo a utilização otimizada dos recursos disponíveis, as biorrefinarias combinam rotas de conversões químicas, bioquímicas e termoquímicas em um sistema de produção integrado. Por meio de processos termoquímicos e biológicos (liquefação, pirólise, gaseificação, extração e transesterificação, fermentação e digestão anaeróbia) microalgas podem ser convertidas em biodiesel, bio-etanol, bio-hidrogênio e bio-metano (DEMIRBAS, 2011). Como exemplo dessa versatilidade de usos da biomassa de microalgas, pesquisa sobre sua utilização para

a produção de eletricidade, utilizando células combustíveis microbianas, gerou butanol como um co-produto (LAKANIEMI e TUOVINENl, 2012). Células combustíveis microbianas são —dispositivos capazes de converter energia química em energia elétrica, por meio de reações químicas catalisadas por microrganismos (RACHINSKI et al., 2010). Diversas tecnologias são utilizadas para separar os principais constituintes da biomassa que podem ser posteriormente transformados em produtos de alto valor agregado por meio de outros processos com redução ou ausência de geração de resíduos.

Dentre todas as tecnologias para transformação da biomassa de microalgas em biocombustíveis, a que tem mais destaque, atualmente, é a que visa produção de biodiesel via transesterificação do óleo das microalgas (DEMIRBAS, 2011; GONZÁLEZ-FERNÁNDEZ et al., 2011). Todas elas, inclusive as de produção de outros biocombustíveis, no entanto, tem dois problemas cruciais: os custos do aproveitamento da biomassa e a eficiência energética de sua cadeia produtiva (HARWATI et al., 2012).

6 ANÁLISE DO CICLO DE VIDA

A Análise do Ciclo de Vida (LCA) é uma ferramenta muito importante para avaliação ambiental das cadeias de produção e é dividida em três partes: balanço energético, balanço de água e análise de gases de efeito estufa. Esta metodologia é amplamente utilizada e reconhecida por um número cada vez maior de cientistas e engenheiros em um número incontável de aplicações em todo mundo. Uma sistematização abrangente de seus requerimentos e etapas está contido nas normas ISO 14040/1997 até 14043/2000 (ISO 14040, 1997; ISO 14041, 1998; ISO 14042, 2000; ISO 14043, 2000).

As microalgas são consideradas uma das matérias-primas mais promissoras para a produção de biocombustíveis. Muitas espécies de algas apresentam alto potencial devido ao seu rápido crescimento celular e por possuírem alto teor de lipídeos que após esterificados são adequados para a produção de biodiesel, em comparação com biomassa terrestre (LOOR et al., 2016).

Esses recursos permitem que as microalgas apresentem diversas vantagens sobre culturas oleaginosas convencionais como o milho, soja e girassol como alta

produtividade lipídica e capacidade de duplicação da concentração celular em 24h (RASHID, et al., 2014). No entanto, a produção de biocombustível à base de algas ainda não é economicamente competitivo com as fontes de combustíveis fósseis tradicionais.

A substituição das fontes de matéria-prima para a produção de microalgas implica em alterações na geração de impactos ambientais nas várias fases associadas a esse processo. Estas fases podem ser resumidas em: produção da matéria prima, produção das microalgas e extração do biodiesel. De modo que os impactos ambientais devem ser avaliados sob esta perspectiva, incluindo todas as fases associadas ao seu simples uso. Neste sentido, a metodologia de Avaliação do Ciclo de Vida (ACV) é particularmente interessante, visto que permite determinar os impactos ambientais de todas as fases (ABU-GHOSH et al., 2015).

Esse método permite quantificar os fluxos de entrada e saída de matéria e energia ao longo de toda a cadeia produtiva, associando esses fluxos a categorias de impacto e apontando as etapas mais impactantes de cada processo (CHEHEBE, 1998). Também se podem indicar pontos da cadeia produtiva que poderiam ser alterados de forma a reduzir os impactos ambientais gerados.

Todas as operações envolvidas na produção de biocombustíveis a partir de microalgas demanda altas intensidades energéticas. Muitos LCA (Análise de Ciclo de Vida) com base em estudos de viabilidade, já foram testadas para a produção de combustíveis líquidos a partir de microalgas, porém a maioria dos estudos demostraram valores muito baixo ou valores negativos para o equilíbrio de energia (PRAGYA e PANDEV, 2016). Quanto a estudos referentes à Avaliação do Ciclo de Vida de microalgas para biodiesel no Brasil, foi identificado apenas o trabalho de Jorquera et al. (2010), que compara a produção de microalgas em lagoas abertas com a produção em fotobiorreatores. O estudo diz que ambos os processos podem ser considerados economicamente viáveis para o cultivo em massa da microalga *Nannochloropsis* sp., para fins de geração de biocombustíveis.

Avaliação do Ciclo de Vida (ACV) de biomassa de microalgas é de grande importância para permitir a inovação tecnológica viável, com intensidade energética reduzida e melhor desempenho ambiental global (GIOSTRI et al., 2016).

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CAPÍTULO 2

THIRD GENERATION BIODIESEL PRODUCTION FROM MICROALGAE *Phormidium autumnale*

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THIRD GENERATION BIODIESEL PRODUCTION FROM MICROALGAE *Phormidium autumnale*

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Abstract - The aim of this work was to evaluate third generation biodiesel production by the microalgae *Phormidium autumnale* using sucrose as exogenous carbon source. The study focused on optimization of the different C/N ratios and on the analysis of biofuel quality. The results indicate that a C/N ratio of 40 improved the performance of the system, reaching single-cell oil productivities of 18.9 mg/L in steady-state conditions. This oil has a composition predominantly saturated (45.2%) and monounsaturated (34.7%) suitable for biodiesel synthesis (ester content of 99.8%, cetane number of 58.5%, iodine value of 67.2 gI₂/100 g, unsaturation degree of 71.3% and a cold filter plugging point of 6.7 °C).
Keywords: Microalgae/cyanobacteria; Heterotrophic cultivation; Sucrose; Biodiesel 3G.

INTRODUCTION

The last decade has seen an emergence of biofuels due in part to social and political acknowledgement that fossil fuels are a finite resource. This is evidenced by a reduction in the discovery of new fossil fuel sources and the exploitation of more energy intense reserves such as shale gas and tar sands (Scaife *et al.*, 2015). As a result of this reality, biofuel research and development has progressed through several stages globally and within Brazil.

Currently, the biofuels are classified from first to fourth generation (Harun *et al.*, 2010; Martin and Grossmann, 2012). The third generation biofuels are obtained from microalgae biomass that possess high productivity of lipids, which after extraction are transesterified to obtain biodiesel, turning them into one of the most promising feedstocks for biofuel production (Wijffels and Barbosa, 2010).

There are two possible technological routes to microalgal biomass production: photosynthetic and heterotrophic cultivation. The photosynthetic culture of microalgae, based on CO_2 conversion, is limited by engineering related factors, since design and scale-up methodologies are poorly developed. Factors such as reactor configuration and material construction are considered the main difficulties, when closed photobioreactors are used. On the other hand, open pond technology is limited by biological factors such as organism survival, growth, CO_2 uptake, light utilization, seasonality, harvest and biosafety of transgenics. These questions are related mainly to photosynthetic oil production. An alternative process is based on heterotrophic metabolism of microalgae, in which

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the organic carbon source is converted in the absence of light. These processes can be conducted in conventional reactor configurations such as stirred tank and bubble column reactors, eliminating some disadvantages of the photosynthetic route (Queiroz *et al.*, 2011).

The microalgae heterotrophic culture, however, is severely limited by organic carbon availability. The organic substrate is estimated at about 80% of the total cost of the process and economically may make it unfeasible. An alternative to the high costs is the replacement of certain sources of organic carbon by low-cost substrates, such as sucrose, which can reduce costs by up to 40% (Xu *et al.*, 2006). According to Li *et al.* (2007), biodiesel 3G may in turn be produced by microalgae that use sucrose as the substrate, making it a more profitable alternative than ethanol from sugar cane. Additionally, Francisco *et al.* (2014), in a survey of potential carbon sources for microalgae production, identified sucrose as a suitable substrate to support heterotrophic microalgae cultivation. The heterotrophic microalgae possess structurally specific mechanisms for active transport of sucrose into the cell. This disaccharide is metabolized via the oxidative pentose-phosphate pathway after hydrolysis into monosaccharides (Knowles and Plaxton, 2003).

Thus, the aim of this work was to evaluate the third generation biodiesel production from heterotrophic cultivation of the microalgae *Phormidium autumnale* employing sucrose as exogenous carbon source. The study focused on optimization of the carbon/nitrogen ratio of the culture media, in the evaluation of different operational modes of the bioreactor and in the analysis of the biofuel quality.

MATERIAL AND METHODS

Microorganisms and Culture Media

Axenic cultures of *Phormidium autumnale* were originally isolated from the Cuatro Cienegas desert (26°59'N, 102°03'W-Mexico). Stock cultures were propagated and maintained in solidified agar-agar (20 g/L) containing synthetic BG11 medium (Ripka *et al.*, 1979). The incubation conditions used were 25 °C, a photon flux density of 15 μmol/m^2/s and a photoperiod of 12:12 h (light:dark). To obtain the inoculums in liquid form, 1 mL of sterile synthetic medium was transferred to slants, the colonies were scraped and then homogenized with the aid of a mixer tubes. The entire procedure was performed aseptically.

Bioreactor

Measurements were made in a bubble column bioreactor. The system was built of borosilicate glass and had an internal diameter of 15 cm and height of 20 cm, resulting in a height/diameter (h/D) ratio equal to 1.33 and a nominal working volume of 2.0 L. The dispersion system of the reactor consisted of a 2.5 cm diameter air diffuser located inside the bioreactor. The air flow was monitored by a flow meter (KI-Key Instruments®, Trevose-PA, USA) and the inlet of air and outlet of gases were filtered through filtering units made up of polypropylene membrane with a pore diameter of 0.22 μm and total diameter of 50 mm (Millex FG®, Billerica-MA, USA). The bioreactor including filtering units was previously sterilized by autoclaving at 121 °C for 40 min and then for 30 min containing the synthetic medium.

Obtaining Kinetic Data in an Experimental Bioreactor

Initial experiments were performed in a bioreactor operating under a batch regime, fed with 2.0 L of culture medium. The experimental conditions were as follows: initial concentration of inoculum of 100 mg/L, temperature of 30 °C, pH adjusted to 7.6, aeration of 1 VVM (volume of air per volume of culture per minute) and absence of light. The culture medium consisted of BG11 synthetic medium modified and supplemented with different concentrations of sucrose to obtain carbon/nitrogen ratios (C/N) of 20, 30, 40, 50, 60, 70 and 80. The concentration of sucrose was adjusted stoichiometrically (Francisco *et al.*, 2014).

In the continuous culture, after 120 h of batch culture, feed culture medium was added to the bioreactor at the dilution rate D=0.02 h^{-1}. At the same time, equal volumes of cell suspension were withdrawn from the bioreactor. The steady-state was considered to have been established after at least three volume changes, with a variation of cell dry weight less than 5%.

The experiments were performed twice, and in duplicate for each operational mode. Therefore, kinetic data refer to the mean value of four repetitions.

Kinetic Parameters

Biomass data were used to calculate the biomass productivity [$P_X = (X_t - X_{t-1})(t_t - t_{t-1})^{-1}$, mg/L/h] and the lipid productivity [$P_L = P_X L_C$, mg/L/h], in which X_t is the biomass concentration at the time t_t (mg/L) and X_{t-1} is the biomass concentration at the time t_{t-1} (mg/L), t is the residence time (h) and L_C is the lipid

content of the biomass (%). The concentrations of total organic carbon were used to calculate the substrate consumption rate (r_S=dS/dt, mg/L/h), and the biomass yield coefficient ($Y_{X/S}$=dX/dS, $mg_{cell}/mg_{substrate}$), where S_0 is the initial substrate concentration (mg/L), S is the substrate concentration (mg/L) and t is the time (h).

Sampling and Analytical Methods

Samples were collected aseptically in a laminar flow hood. The cell biomass, the pH dynamics and the consumption of organic carbon were monitored every 24 hours during the growth phase of microorganism.

The cell biomass was gravimetrically evaluated by filtering a known volume of culture medium through a 0.45 μm membrane filter (Millex FG®, Billerica-MA, USA), drying at 60 °C for 24 h.

The organic carbon concentration was expressed in terms of chemical oxygen demand (COD) and analyzed according to the closed reflux colorimetric method (APHA, 2005).

The total lipid concentration of the biomass was determined gravimetrically by the Bligh and Dyer (1959) method.

The saponification and esterification (methylation reaction) by the modified method of Hartman and Lago (1976) was used with the dried lipid extract to obtain the fatty acid methyl esters (biodiesel). An amount of 250 mg of oil was added to 5.0 mL of 0.50 mol/L NaOH in methanol. The mixture was then heated under reflux for 5 min. After adding 15.0 mL of the esterification reagent (prepared from a mixture of 2.0 g of ammonium chloride, 60.0 mL of methanol, and 3.0 mL of concentrated sulfuric acid for *ca.* 15min), the mixture was heated under reflux for another 3 min and subsequently transferred to a separation funnel containing 25.0 mL of petroleum ether and 50.0 mL of deionized water. After stirring the mixture and phase separation, the aqueous phase was discarded. Then 25.0 mL of deionized water was added to the organic phase. This mixture was stirred and, after phase separation, the aqueous phase was discarded. This procedure was repeated. The organic phase was collected, the solvent was evaporated in a rotary evaporator and the residue was removed under nitrogen flow. The methyl esters were solubilized in *n*-heptane before injection in the gas chromatograph. The fatty acid composition was determined using a VARIAN 3400CX gas chromatograph (Varian, Palo Alto-CA, USA). The fatty acid methyl esters were identified by comparison of the retention times with those of the standard (Supelco, St. Louis-MO, USA) and quantified by area normalization.

The fuel properties of biodiesel (ester content, EC; degree of unsaturation, DU; cetane number, CN; iodine value, IV and cold filter plugging point, CFPP) were determined according to the methodology proposed by Francisco *et al.* (2010).

The cetane number of the mixture was estimated by empirical equations. The cetane number, saponification value and iodine value were calculated in accordance with Eqs. (1)-(3)

$$CN = 46.3\frac{5458}{SV} - 0.225IV \quad (1)$$

$$SV = \frac{\sum(560N)}{M} \quad (2)$$

$$IV = \frac{\sum(254DN)}{M} \quad (3)$$

where CN is the cetane number, SV is the saponification value, IV is the iodine value, D is the number of double bonds, M is the molecular mass and N is the percentage of each fatty acid component.

The degree of unsaturation was calculated from empirical Eq. (4), taking into account the amount of monounsaturated and polyunsaturated methyl ester (wt%) present in the microalgae oil:

$$DU = (MUFA) + 2(PUFA) \quad (4)$$

where DU is the unsaturation degree (%), MUFA is the weight percentage of the monounsaturated fatty acids (wt%).

The long-chain saturated factor was obtained from empirical Eq. (5), taking into account the composition of fatty acids and assigning more weight to the composition of fatty acids with a long chain. This parameter was correlated with the cold filter plugging point, using Eq. (6):

$$LCSF = (0.1C16) + (0.3C18) + (1C20) + (1.5C22) + (2C24) \quad (5)$$

$$CFPP = 3.1417LCSF - 16.477 \quad (6)$$

where LCSF is the long-chain saturated factor; C16, C18, C20, C22, and C24 are the weight percentage of each of the fatty acids (wt%) and CFPP is the cold filter plugging point.

RESULTS AND DISCUSSION

The assessment of suitable concentration of the carbon sources for the production microalgal

biomass and bioproducts is a fundamental step in the consolidation of the process. The kinetic parameters using different C/N ratios for heterotrophic culture of *Phormidium autumnale* are shown in Table 1. The best results were evidenced in the range of C/N ratio between 30-50, with maximum kinetic performance at the C/N ratio of 40. In this condition, a maximum specific growth rate of 0.02 h^{-1}, generation times of 32.3 h, maximum cell density of 61.70 mg/L, average biomass productivity of 40.7 mg/L.h, average rate of sucrose consumption of 42.3 mg/L.h and a biomass yield coefficient of 0.44 $mg_{cell}/mg_{sucrose}$ were obtained. This biomass had a lipid content of 20.7%, resulting in an oil productivity of 8.46 mg/L.h.

According to Fay (1983), in general cyanobacterial culture requires a minimum C/N ratio of 20. Specifically for sucrose, the results obtained indicate a bell-shaped curve pattern in the range between 20 to 80. Comparatively, the results obtained are higher than those reported by Markou and Georgakakis (2011) that indicate maximum cell densities of 1180 mg/L for heterotrophic culture of *Phormidium* sp. using sucrose as exogenous carbon source.

Sucrose is metabolized by microalgae through the pentose-phosphate pathway (Smith, 1982). These microorganisms possess structurally specific mechanisms for the active transport of sucrose into the cell membrane. The heterotrophic microalgae have an inducible active carbohydrate symport system responsible for uptake of these molecules from the culture medium. The induction of this transport is achieved by some specific sugars. In general, in cultivations with suitable concentration and type of sugars, the symport system is induced to promote the alkalinization of the culture media by a net movement of protons accompanied by sugar uptake (Figure 1). The rate of the increase in pH fundamentally depends on the concentration and type of sugar used (Hong and Lee, 2007). Additionally, the disaccharides such as sucrose are only used in the pentose phosphate pathway, after a previous hydrolysis that transforms this sugar in monosaccharides, particularly fructose and glucose. Several enzymes, specifically invertase, are involved in these reactions and have been identified in microalgae cultures (Fuchs *et al.*, 1994; Gupta *et al.*, 2011).

Table 1: Kinetic parameters for different C/N ratios using sucrose as substrate in batch cultures.

C/N	μ_{max} (1/h)	t_g (h)	X_{max} (mg/L)	P_X (mg/L/h)	π (mg/L)	$Y_{X/S}$ ($mg_{cell}/mg_{sucrose}$)	L_C (%)	P_L (mg/L/h)
20	0.013±0.00	51.3±0.5	2775±56	14.7±0.4	30.9±0.8	0.47±0.00	8.9±0.2	1.32±0.03
30	0.020±0.00	34.1±0.3	5260±30	34.4±0.4	36.1±0.7	0.43±0.00	18.6±0.2	6.41±0.08
40	0.021±0.00	32.3±0.4	6170±28	40.7±0.4	42.3±0.4	0.44±0.00	20.7±0.3	8.46±0.15
50	0.024±0.00	28.8±0.1	5330±53	34.9±0.3	21.2±0.3	0.64±0.00	9.9±0.1	3.50±0.09
60	0.016±0.00	42.0±0.3	2925±18	18.2±0.2	25.0±0.2	0.72±0.00	8.1±0.1	1.46±0.05
70	0.014±0.00	48.8±0.3	1938±16	12.9±0.1	30.4±0.6	0.42±0.00	6.1±0.2	0.80±0.01
80	0.015±0.00	44.7±0.2	1683±22	10.3±0.1	58.1±0.7	0.18±0.00	5.1±0.1	0.53±0.01

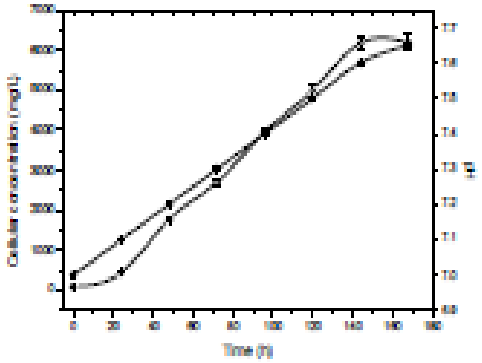

Figure 1: Variation of the pH (closed circle) and cellular concentration (open circle) *vs.* time at a C/N ratio of 40.

To further improve bulk oil production, continuous cultivations were performed (Table 2). Maintaining cells in the steady-state resulted in an oil productivity of 18.9 mg/L/h, an increase of 2.2-fold over batch cultivations. A faster product formation rate implies a higher productivity and corresponding reductions in plant operating time and operating cost, for an existing plant. On the other hand, for a new plant to be built, the increased rate implies, in addition to improved productivity, a smaller reactor and therefore a lower capital investment cost. Likewise, improved productivities imply a lower raw material cost and a lower capital investment for existing and new plants (Francisco *et al.*, 2015).

Scale-up projections for these values in the present scenario are limited by the large-scale application of heterotrophic microalgal bioreactors. However, in comparison with the productivity that can be achieved with soybeans, the Brazilian feedstock commonly used industrially for biodiesel, a scale-down projection analysis indicates that, for the Brazilian harvest of 2014, the average lipid productivity of soybeans was 0.46g_{oil}/m^2.day, considering a production cycle of 120 days (CONAB, 2015). Each hectare of arable Brazilian soil produces an average of 2,700 kg of soybean, containing up to 20% oil. The data obtained for *Phormidium autumnale* indicate that a continuous bioreactor with 1.0 L/m^3 of working volume, operating on a cycle of 120 days/year, would yield the same amount of lipids as that produced by soybean. This comparison indicates that the oil productivity of *Phormidium autumnale* can be increased by several fold by associating bioreactor optimal design with an operating cycle of 330 days/year.

Finally, in terms of oil composition and fuel properties of biodiesel (Table 3), the lipid fraction of biomass indicated eight different compounds, with oleic acid (26.2%) being the major. The single-cell oil showed a profile predominantly saturated (45.2%) and monounsaturated (34.7%), which determine the fuel properties of microalgal biodiesel (Francisco *et al.*, 2010). The biodiesel produced from microalgal oil had the following fuel properties: ester content of 99.8%, cetane number of 58.5, iodine value of 67.2 $gI_2/100$ g, degree of unsaturation of 71.3%, and cold filter plugging point of 6.7 °C. All these parameters comply with the limits established by the US, European, and Brazilian standards (ASTM, 2002, UNE-EN, 2003, ANP, 2003), besides being comparable with soybean biodiesel (Knothe, 2005). These results indicate the potential for the exploitation of this feedstock for biofuel production.

Table 2: Kinetic parameters of the steady-state process.

Parameter	Value
X_{max} (mg/L)	4800±698
P_X (mg/L/h)	92.1±1.5
r_S (mg/L/h)	56.3±1.2
L_C (%)	20.5±0.3
P_L (mg/L/h)	18.9±0.4

Table 3: Fatty acid profile and fuel properties of biodiesel 3G.

Fatty acid profile								
Methyl esters (g/100g)	Lauric (12:0)	Myristic (C14:0)	Palmitic (C16:0)	Palmitoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1n9c)	Linoleic (C18:2n6c)	γ-Linolenic (C18:3n6)
	4.9±0.1	7.3±0.1	22.5±0.5	8.5±0.1	10.5±0.2	26.2±0.8	17.8±0.5	2.10±0.08
Fuel properties								
Properties	EC (wt %)	CN	DU (wt %)	IV (gI/100g)	LCFS (wt %)	CFPP (°C)		
	99.8±0.1	58.5±0.9	71.3±1.8	67.2±0.9	7.4±0.2	6.70±0.06		

CONCLUSION

The results obtained indicate that sucrose is an exogenous carbon source with the potential to produce bulk oil and biodiesel by *Phormidium autumnale*, enabling oil productivities of 18.9 mg/Lh. This oil had a composition of predominantly saturated (545.2%) and monounsaturated (34.7%) fatty acids, suitable for biodiesel synthesis (ester content of 99.8%, cetane number of 58.5, iodine value of 67.2 $gI_2/100g$, degree of unsaturation of 71.3%, and cold filter plugging point of 6.7 °C.

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NOMENCLATURE

Acronyms

C/N	Carbon/nitrogen ratio
CFPP	Cold filter plugging point (°C)
CN	Cetane number
COD	Chemical oxygen demand (mg/L)
D	Number of double bonds
DU	Degree of unsaturation (%)
EC	Ester content (%)
IV	Iodine value ($gI_2/100g$)
Lc	Lipid content of the biomass (%)
LCSF	The long-chain saturated factor
M	Molar mass (g/mol)
MUFA	Weight percentage of the monounsaturated fatty acids (%)
N	Percentage of each fatty acid component (%)
PUFA	Weight percentage of the polyunsaturated fatty acids (%)
SV	Saponification value (%)
VVM	Volume of air per volume of culture per minute

Symbols

μ_{max}	Maximum specific growth rate (1/h)
P_L	Lipid productivity (mg/L/h)
P_X	Average cellular productivity (mg/L)
r_S	Substrate consumption rate (mg/L/h)
S	Substrate concentration (mg/L)
S_0	Initial substrate concentration (mg/L)
T	Residence time (mg/L)
tg	Generation time (h)
X_{max}	maximum cell biomass (mg/L)
$Y_{X/S}$	Biomass yield coefficient (mg/mg)

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CAPÍTULO 3

LIFE CYCLE ASSESSMENT OF THE THIRD-GENERATION BIODIESEL PRODUCED HETEROTROPHICALLY BY *Phormidium autumnale*

O artigo será submetido para a revista New Biotechnology

Life cycle assessment of the third-generation biodiesel produced heterotrophically by *Phormidium autumnale*

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ABSTRACT: The aim of this work was to perform a prospective life cycle assessment of the third-generation biodiesel (3G) produced from the heterotrophic cultivation of *Phormidium autumnale,* using sucrose as the carbon source. The study focused on evaluating the balance energy, water balance, CO_2 emissions and the analysis of the biofuel quality in diverse microalgae-based processes scenarios. In the best scenario, the results showed that the production of microalgal biodiesel has a positive energy production (50.59 MJ) associated with a low consumption of water (28.38 m^3/kg) and low CO_2 emissions (9.18 kg CO_2-eq/kg). The composition of this oil was predominantly saturated (45.20%), monounsaturated (34.70%) and polyunsaturated (19.90%) resulting in a biodiesel that complies with U.S, European and Brazilian standards.

Keywords: microalgae, heterotrophic, biodiesel, Life cycle assessment

1. Introduction

According to the International Energy Agency (IEA), more than 80% of the world primary energy supply derives from fossil fuels [1]. The expansion of the energy demand is associated to the growth of developing economies, which represents a considerable increase in the consumption of transport fuels, driving a shift towards biofuels. As a result of this reality, the biofuel research and development have

progressed through several stages globally and within Brazil [2]. The microalgal oil industry, though presently in its infancy, has a potential to provide future liquid transportation fuels that can improve world energy security.

In addition, microalgae-based fuels are third-generation biofuels and it may be a promising biofuel option. Their high growth rate and lipid content, after extraction, are transesterified to obtain biodiesel, thus turning them into one of the most promising feedstock [3,4]. In the heterotrophic culture of microalgae, glucose is the most commonly used organic carbon source [5]. However, the high cost of glucose, sometimes reaching up to about 80% of the total cost of the medium, makes the heterotrophic cultivation of microalgae economically infeasible [6]. An alternative to the high costs of production is the replacement of certain sources of organic carbon to low-cost substrates, such as sucrose, which can reduce costs by up to 40% [7]. Therefore, is important to know the life cycle performance of microalgae biodiesel production systems in order to establish the environmental benefits over conventional products.

Currently, every activity involved in the production of biofuels from microalgae is energy-intensive and it also produces greenhouse gases, besides having a high consumption of water; thus, an evaluation of the energy balance, CO_2 emissions and the water balance of microalgal fuels are, of course, essential [8]. Many Life cycle assessment (LCA) based viability studies, that use the net energy balance or net energy ratio as the viability indicators, have already been attempted for the production of liquid fuels from microalgae, but most studies have shown very low values or negative values for the energy balance [9, 10, 11].

Life cycle assessment is divided into three sections: energy balance, water balance and analysis of greenhouse gases. Worldwide, life cycle assessment (LCA) is recognized as a standardized and structured method for evaluating the environmental

impacts arising throughout the entire life cycle of a product, process or activity [12]. Finally, the biggest challenge faced in crafting an LCA is that it must address impact categories during early stage processes while ultimately accounting for the whole scope of product development. Additionally, the use of LCA is extremely helpful in determining the energy products and co-products which will demonstrate higher economic viability and environmental performances [13].

In this sense, the aim of this work was to perform a prospective life cycle assessment of the third-generation biodiesel produced from the heterotrophic cultivation of *Phormidium autumnale*, using sucrose as carbon source. The study focused on evaluating the balance energy, the balance of water, CO_2 emissions and in the analysis of the biofuel quality.

2. Material and methods

2.1. Goal and scope definition

The technical framework for the LCA methodology, according to the International Organization for Standardization (ISO) 14000 series [14], consists of four phases: goal and scope definition; inventory analysis; impact assessment and interpretation. The experimental data was obtained from laboratory experiments, where it was selected the process requirements and the inventory. Subsequently, the data was normalized for a functional unit of 1kg the biodiesel. The methodology was used to estimate the energy balance, the CO_2 emissions and the water balance.

Since microalgae biofuel industry is still a recent process, data on large scale microalgae production is lacking. In the present study, laboratory observations combined with published data of known industrial processes have been used and extrapolated [15, 9, 16]. Furthermore, the calculation experimental is based on

promising technologies which might be commercialized in the near future to determine the development potential.

As shown in (Fig. 1), the proposed process of the microalgae biodiesel production can be divided into nine sections.

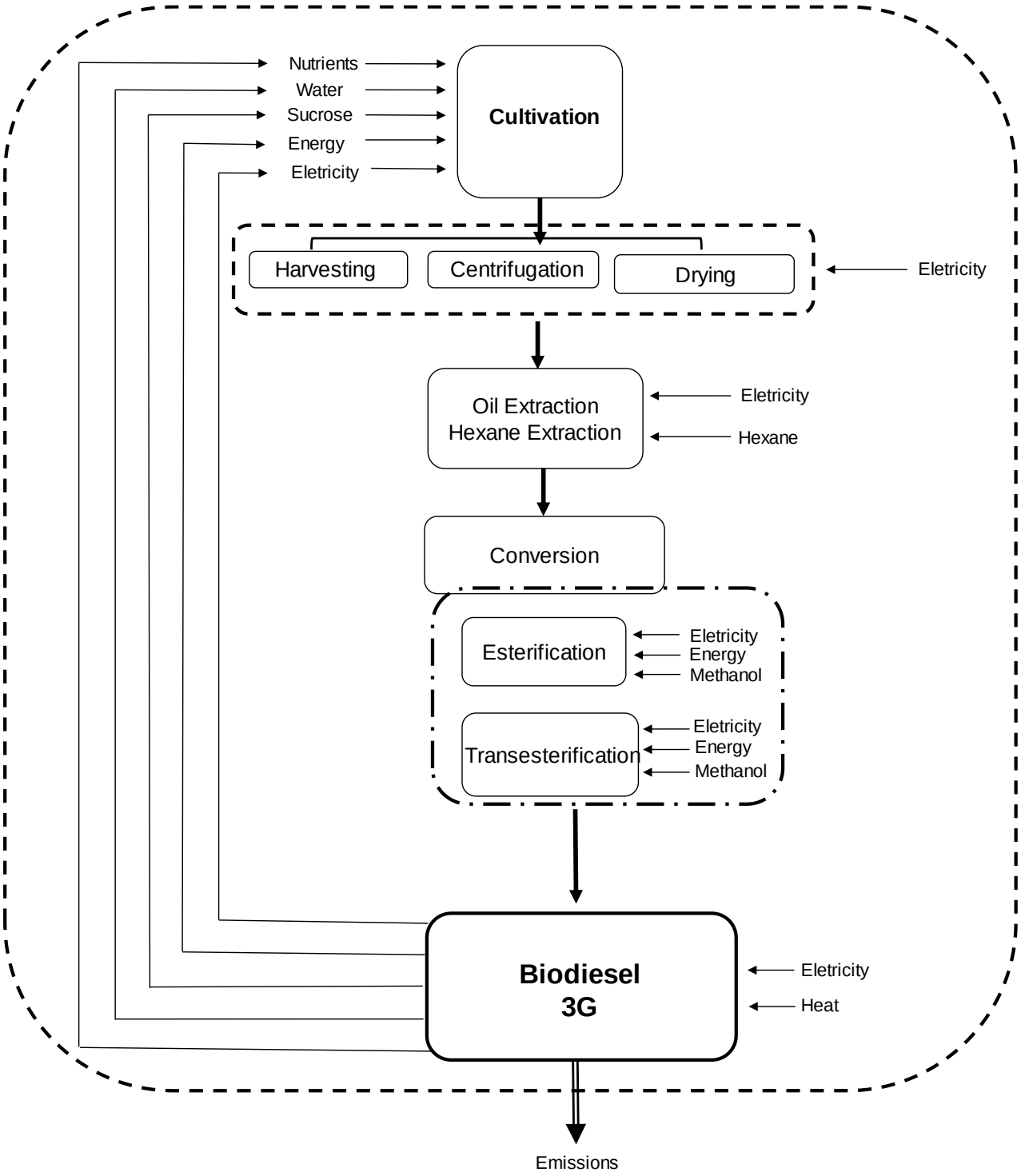

Figure 1. Flow diagram of the process microalgae biodiesel.

2.2 Microalgae biomass cultivation and harvesting

Axenic cultures of *Phormidium autumnale* were originally isolated from the Cuatro Cienegas desert (26º59'N, 102º03'W-Mexico). Stock cultures were propagated and maintained in solidified agar-agar (20 g/L) containing synthetic BG11 medium [17]. The incubation conditions used were temperature of 25ºC, a photon flux density of 15 µmol/m^2/s and a photoperiod of 12:12 h (light: dark). To obtain the inoculums in a liquid form, 1 mL of sterile synthetic medium was transferred to slants, the colonies were scraped and then homogenized with the aid of a mixer tubes. The entire procedure was performed aseptically.

2.3. Process description

Single-cell oil production was made in a bubble column bioreactor. The reactor specifications were followed according to Francisco [18].

The experiments were performed in a bioreactor operating under a batch regime, fed on 2.0 L of culture medium. The experimental conditions were as follows: initial concentration of inoculum of 100 mg/L, temperature of 30ºC, pH adjusted to 7.6, aeration of 1 VVM (volume of air per volume of culture per minute) and absence of light. The culture medium consisted of BG11 synthetic medium modified and supplemented with different concentrations of sucrose to obtain carbon/nitrogen ratios of 20 (C20), 30 (C30), 35 (C35), 40 (C40), 50 (50), 60 (C60), 70 (C70) and 80 (C80). The concentration of sucrose was adjusted stoichiometrically according to methodology proposed by Francisco [19].

The experiments were performed twice and in duplicate. Therefore, kinetic data refer to the mean value of four repetitions.

2.4. Microalgae oil extraction

The lipid fraction was extracted from the biomass by the modified Bligh and Dyer method [20], obtaining an immiscible system consisting of the sample water content and a mixture of chloroform and water. The total lipid concentration was determined gravimetrically from the chloroform extract by evaporating the chloroform in an atmosphere of nitrogen and subsequently drying to constant weight in a vacuum oven.

2.5 Biodiesel production

The method of Hartman and Lago [21] was used to saponify and esterify the dried lipid extract to obtain the fatty acid methyl esters (biodiesel). Fatty acid composition was determined using a gas chromatograph. Fatty acid methyl esters were identified by comparison of retention times with the authentic standards and quantified through area normalization by the Chromatography Station T2100p (Plus Edition) v 9.04 software.

The fuel properties of biodiesel (ester content, EC; cetane number, CN; iodine value, II; degree of unsaturation, DU; saponification value, SV; long-chain saturated factor, LCSF; cold filter plugging point, CFPP; cloud point, CP; allylic position equivalents, APE; bisallylic position equivalents, BAPE; oxidation stability, OS; higher heating value, HVV; kinematic viscosity, m and kinematic density, ρ) were determined for the best condition optimized of the study, and the properties of biodiesel were calculated by the BiodieselAnalyzer© 1.1 software, which estimates properties based on the fatty acid profile of the parent oil, through a system of empirical equations [22].

2.6 Life cycle inventory (LCI)

A summary of data sources and information obtained for process parameters and energy requirements are presented in Table 1. These data were analytically

evaluated in order to quantify and compile all the input and output flows for each stage within the process chain.

Table 1

Inputs/outputs inventory the process to produce 1 kg of biodiesel.

Stages	In/out	Utilities/materials	Base case	Amount (by scenarios)							
				C20	C30	C35	C40	C50	C60	C70	C80
Cultivation	Input	Water (m^3)	1								
		Electricity (kWh)	0.15								
	Output	Microalgal broth (kg)	-	2,7	5.26	5.6	6.17	5.33	2.9	1.9	1.68
Harvest	Input	Electricity (kWh)	0.13								
		Centrifugal pump (kWh)	0.75								
		Microalgae broth (L)	0.86								
		Drum dryer (kWh)	4								
	Output	Dry microalgae (kg)	-	2,7	5.26	5.6	6.17	5.33	2.9	1.9	1.68
Oil extraction	Input	Storage tank hexane (kWh)	0.38								
		Storage tank oil (kWh)	0.38								
		Centrifugal pump (kWh)	0.75								
		Evaporator/Stripper (kWh)	0.12								
		Centrifuge (kWh)	0.75								
		Desolventizer-Toaster-Dryer-Cooler (DTDC) (kWh)	0.35								
		Electricity (kWh)	0.13								

		Heat (MJ)	41								
	Output	Microlgal oil (kg)	-	0.54	1.05	1.12	1.23	1.06	0.58	0.38	0.33
Biodiesel production	Input	Microalgal oil (kg)	-	0.54	1.05	1.12	1.23	1.06	0.58	0.38	0.33
		Storage tank Methanol (kWh)	0.38								
		Storage tank hydrochloric acid (kWh)	0.38								
		Storage tank chloroform (kWh)	0.38								
		Centrifugal pump (kWh)	0.75								
		Evaporator/Stripper (kWh)	0.90								
		Desolventizer-Toaster-Dryer-Cooler (DTDC) (kWh)	0.35								
		Electricity (kWh)	0.13								
		Heat (MJ)	41								
	Output	Biodiesel (MJ)	-	22.14	45.11	44.31	50.59	43.70	23.78	15.58	13.77

2.6.1. Energy balance

The net energy ratio (NER) of a system is defined as the ratio of the total energy produced (energy content of the residual biomass) over the energy required for all plant operations [10]. Energy is reported in terms of mega joules(MJ) calculated according to Eq. (1):

$$NER = \frac{\sum E_{out}}{\sum E_{in}} \quad (1)$$

Where E_{out} is the renewable energy output and E_{in} is the fossil fuel energy input.

For the energy balance (EB) were considered all forms of energy of the system, and were calculated according to Eq. (2):

$$EB = \Sigma inputs - \Sigma outputs \quad (2)$$

The output energy was quantified by multiplying the energy potential of microalgal biodiesel, express in MJ [23].

2.6.2. Balance water

The water balance (WF) is determined by the sum of the water used in the system during all the stages of the process of microalgae biodiesel, express in m^3/kg the biodiesel.

2.6.2.1. Water balance blue

The WF blue refers to the amount of water incorporated in the product. It was determined by the evaporation rate according to Eq. (3):

$$WF_{blue} = Blue\ water\ evaporation + incorporation + return\ flow \quad (3)$$

2.6.2.2. Water balance green

The green WF refers to the volume of water consumed in a production process, plus the water incorporated into the harvested crop according to Eq. (4):

$$WF_{green} = Green\ water\ \ evaporation + incorporation + return\ flow \quad (4)$$

2.6.2.3 Evaporation rate

The evaporation rate of the process was calculated accordingly to Eq. (5) where the loss of mass (m) is obtained per unit time at a given temperature (°C).

$$ER = \frac{\Delta m}{\Delta t} \quad (5)$$

In this study, the water balance associated with the culture process is caused by evaporation and considering that microalgae have the pollution load removal capacity of water that will be thrown in the watershed, thus, were considered as the green and blue water balance.

2.6.3. Emissions greenhouse gases

The emission gas is calculated according to Eq. (6)

$$E = \sum_{i} Mi.\ Pi \quad (6)$$

Where Mi is the mass of substance i which contributes to impact E, and Pi are the characterization factors of substances i, express in kg CO_2-eq.

2.7. Statistical analyses

Analysis of variance (one-way ANOVA) and Tukey's test ($p<0.05$) were used to test differences between scenarios. The analyses were performed with the software Statistica 7.0 (StatSoft, Tulsa-OK, USA).

3. Results and discussion

3.1. Energy balance

To assess the sustainability of biofuel production system, the energy input analysis of the life cycle is needed. In this sense, biomass production, oil production

and the energy balance of the biodiesel 3G production from heterotrophic cultivation by *Phormidium autumnale* were examined. The results are summarized in Table 2.

Table 2

Production of biomass and single-cell oil, and analysis of the net energy ratio (NER) for production of 1kg of biodiesel.

			Balance energy			
Scenario	Biomass (kg/m^3)	Oil (kg/m^3)	Fossil energy input* (MJ)	Produced energy output (MJ)	Energy ratio	Energy balance (MJ)
C20	2.7^{a}±0.03	0.54^{a}±0.01	27.21^{a}	22.14^{a}±0.01	0.81^{a}±0.01	5.07^{a}±0.01
C30	5.26^{b}±0.03	1.05^{b}±0.01	27.21^{a}	45.11^{b}±0.01	1.65^{b}±0.01	-17.90^{b}±0.01
C35	5.6^{c}±0.03	1.12^{c}±0.01	27.21^{a}	44.31^{c}±0.02	1.62^{c}±0.01	-17.10^{c}±0.01
C40	6.17^{d}±0.03	1.23^{d}±0.01	27.21^{a}	50.59^{d}±0.01	1.85^{d}±0.01	-32.38^{d}±0.01
C50	5.33^{b}±0.03	1.06^{b}±0.02	27.21^{a}	43.70^{e}±0.01	1.60^{c}±0.01	-16.49^{e}±0.01
C60	2.9^{e}±0.03	0.58^{f}±0.01	27.21^{a}	23.78^{f}±0.01	0.87^{e}±0.01	3.43^{f}±0.01
C70	1.9^{f}±0.03	0.38^{g}±0.02	27.21^{a}	15.58^{g}±0.02	0.57^{f}±0.01	11.63^{g}±0.01
C80	1.68^{g}±0.03	0.33^{h}±0.01	27.21^{a}	13.77^{h}±0.02	0.50^{g}±0.01	13.44^{h}±0.01

Within the same column, means with different superscripts are significantly different ($p<0.05$) by Tukey's test.
*The energy input was considered the equal for all experiments.

The best performance of the process was evidenced in between scenarios 30-50, with maximum biomass production and oil at the scenario C40. In this condition, average biomass production was 6.17 kg/m^3 resulting in an oil production of 1.23 kg/m^3. These results show that in the heterotrophic metabolism, the production of intracellular oils by *Phormidium autumnale* is critically affected by the carbon/nitrogen ratio in the culture medium.

As shown in Table 2, the energy balance is a function of the oil production. The condition that presented the best performance was the scenario C40, with higher values fossil energy input (27.21 MJ), energy output (50.50 MJ), energy ratio (1,85

MJ) and energy balance (-32.38 MJ), followed by the scenarios C30, C35 and C50. These results show that primary energy contained in the biomass is greater than the primary energy input in all the system (NER>1), demonstrating that these processes have a great potential for energetic exploitation.

The other scenarios (C20, C60, C70 and C80) the balance energy was unfavorable. There is a linear behavior between biomass production and oil production and energy ratio. This is associated with the metabolism of microalgae; these microorganisms possess structurally specific mechanisms for the active transport of sucrose into the cell membrane. The heterotrophic microalgae have an inducible active carbohydrate symport system responsible for uptake of these molecules from the culture medium. The induction of this transport is achieved by some specific sugars. Where the concentration and type of sugar are in adequate amounts in the culture the symport system is induced to promote greater cell growth and consequently it will be possible to obtain higher yields of biomass and oil production [24].

Comparatively, the soybean oil is one of the most used oil crop for the production of biodiesel. According to Fore [25], to the production of biodiesel of soybean, are necessary the energy input of 4588 MJ, generating a fossil energy output of 21.401 MJ. In addition, with biodiesel 3G was possible to get on the best scenarios an energy input of 27.21 MJ, generating a fossil energy output of 50.50 MJ. This value of the energy produced by microalgae is twice as large when compared to the biodiesel produced by soybean.

On the other hand, the biodiesel 3G may be produced by microalgae that use sucrose as the substrate, making it a more profitable alternative than bioethanol from sugar cane. In sugarcane biorefinery, the bioethanol production requires a fossil

energy input of 1.92 MJ per kilogram of sugar cane, which generates 0.0083 MJ of output energy per kilogram of bioethanol produced. These values reached an unfavorable energy of 0.0043 MJ [26]. Moreover, it was considered that the total energy yield (bioethanol and bagasse) is 2.185 MJ per kg of cane, if consider only 11% of the total energy of bioethanol production using the microalgae, it is possible to achieve 3.52 MJ per kg of the sugar-cane. This value is larger compared to the total of energy (bioethanol) produced by sugarcane [27]. The microalgae biodiesel demonstrated a high potential of energetic exploration since the final yield of the process exceeded the demand for energy in the system input, promoting the sustainability of this technological route.

3.2 Life cycle CO_2 emissions

The environmental impact of converting sucrose to oil is analyzed based on the emissions during the complete life cycle. It is important to consider the CO_2 emission per unit of energy output, from products since the principal objective of the biodiesel system is to produce an energy carrier. Table 3 shows the CO_2 emissions of the eight scenarios calculated per unit of mass and energy output (MJ/kg).

Table 3

Comparison of CO_2 emissions from the scenarios C20, C30, C35, C40, C50, C60, C70, and C80 calculated per unit of energy from the biodiesel.

Scenario	Emissions by CO_2 (kg CO_2-eq/kg biodiesel)
C20	6.58^{a}±0.01
C30	8.23^{b}±0.01
C35	27.12^{c}±0.01
C40	18.09^{d}±0.01
C50	7.71^{e}±0.01
C60	10.51^{f}±0.01
C70	10.62^{g}±0.01
C80	16.47^{h}±0.01

Within the same column, means with different superscripts are significantly different ($p<0.05$) by Tukey's test.

As shown in Table 3, CO_2 emissions per kg of biodiesel were the lowest in scenario C20, followed by C50. The higher emissions of CO_2 were achieved by scenarios C35 followed by C60 and C80. However, the scenario C20 was the one which obtained the least emission of CO_2, but in energetic questions, it was the one which less produced energy. These results suggest that the less sucrose is added to the culture the less energy is required for the conversion of carbon from microalgae. Thus, during respiration, there will be less CO_2 liberation, since the use of carbohydrates in heterotrophic cultivation serves as the sole source of energy [28]. Furthermore, Posada [29], report that the energy demand required to supply the input of a lower emission of CO_2 achieves a higher energy expenditure than to emit a higher amount of CO_2. Under these conditions, the C40 scenario would still be the best result in energy balance and CO_2 emissions. These results are in accordance with the study

of Khoo [30], where it was observed that higher emissions of CO_2 will have a higher energy production, because it will lead to a greater final yield of microalgal biomass.

Comparatively, the emission of greenhouse gases from soybeans biodiesel varies between 51-101 kg CO_2-eq/kg, considering CO_2 emissions during the extraction of soybean oil and in the transesterification to obtain biodiesel [31]. Considering a higher CO_2 emission, which is observed in scenario C35, (27.12 kg CO_2-eq/kg) this value is still much lower than the achieved by soybean in the production of biodiesel.

On the other hand, the bioethanol utilization in Brazil must include the whole agro-industrial system. Essentially, a portion of fossil fuel is used for yielding bioethanol as biofuels for external use in addition to a proportion of the bagasse for sugar production [27]. According to Khatiwada [2] the CO_2 emissions of are estimated at 19.1 kg CO_2-eq/kg of bioethanol. These data were also supported by Souza [32] whom conducted an LCA of an integrated Brazilian sugarcane biorefinery with a similar framework. The results of CO_2 emissions presented were between 20 kg CO_2-eq/kg and 37 kg CO_2-eq/kg. These CO_2 emissions values are the highest when compared to all the scenarios under study.

3.3. Water balance

In additional, the water balance includes the inventories of the process water consumed, the water consumption associated with process energetic, of material inputs for each stage of the fuel cycle, and the water credits associated with the coproducts. In this sense, were calculated the green water balance, the blue water

balance and evaporation rate of the whole production process of biodiesel from microalgae and the results are summarized in Table 4.

Table 4

Life cycle of the water balance (WF), and the total evaporation (TE) of the biodiesel production from microalgae.

Scenarios	TE	Green WF	Blue WF	Total WF
	m^3 per kg of biodiesel	m^3 per kg of biodiesel	m^3 per kg of biodiesel	m^3 per kg of biodiesel
C20	0.1442^{a} ±0.01	14.19^{a} ±0.01	$3.95x10^{-5\ a}$±0.01	14.19^{a}±0.01
C30	0.2163^{b} ±0.01	21.28^{b}±0.01	$7.68x10^{-5\ b}$±0.01	21.28^{b}±0.01
C35	0.2523^{c} ±0.01	24.82^{c}±0.02	$8.19x10^{-5\ b}$±0.01	24.82^{c}±0.01
C40	0.2885^{d} ±0.01	28.38^{d}±0.01	$9x10^{-5\ c}$±0.01	28.38^{d}±0.01
C50	0.3607^{e} ±0.01	35.48^{e}±0.01	$7.75x10^{-5\ b}$±0.01	35.48^{e} ±0.01
C60	0.4328^{f} ±0.01	42.57^{f}±0.01	$4.24x10^{-5\ a}$±0.01	42.57^{f} ±0.01
C70	0.5050^{g} ±0.01	49.68^{g} ±0.01	$2.7x10^{-5\ d}$ ±0.01	49.68^{g} ±0.01
C80	0.5721^{h} ±0.01	56.28^{h} ±0.01	$2.41x10^{-5\ d}$ ±0.01	56.28^{h}±0.01

Within the same column, means with different superscripts are significantly different ($p<0.05$) by Tukey's test.

The blue WF represents the local water requirements for the microalgae-to-biofuels process. The blue WF varies between the scenarios; the lower consumption of blue water is represented by C80, C70 and C20, with $2.41x10^{-5}$ m^3/kg, $2.7x10^{-5}$ m^3/kg and $3.95x10^{-5}$ m^3/kg, respectively. In addition, for the balance of green water, the scenarios that demonstrated lower water consumption are C20 (14.19 m^3/kg), C30 (21.28 m^3/kg), C35 (24.82 m^3/kg) followed by C40 (28.38 m^3/kg).

Moreover, the total WF is the sum of blue and green WFs, representing the balance between them. In all the scenarios, total water balance increases with increase in sucrose concentration used during the process, as expected. The scenario that presented the lowest water consumption was the C20 ($3.95x10^{-5}$ m^3/kg), followed by C30, C35 and C40. However, the scenario C40 was the best scenario in

energy balance and CO_2 emission, considering a long-term process, this scenario becomes highly sustainable by having a low water consumption and positive energy balance associated with low CO_2 emissions.

The water balance of microalgae biodiesel was compared with the water balance (WF) for the production of bioethanol. According to [33], for the bioethanol, the total water balance produced from sugarcane is 7700 m^3/kg, or 4600 m^3/kg of green water, 2500 m^3/kg blue water, considering irrigation needs, and 500 m^3/kg of gray water. The microalgal biodiesel has a small water balance compared to bioethanol, because the microalgae have no gray WF, due the heterotrophic metabolism of these microorganisms have the capacity to the simultaneous conversion of the pollutants present in wastewater.

On the other hand, the water balance of biodiesel from soybean is much larger than the microalgae, because it requires relatively large amounts of irrigation in combination with smaller biodiesel yields per unit of crop [34]. Water balance for biodiesel from soybean is about $42x10^{-5}$ m^3 per kg of biodiesel producing this value is much higher when compared at the best scenario C40 (28.38 m^3/kg) of biodiesel microalgae. Thus, the microalgae biodiesel becomes a competitive alternative source, since it uses less water to 3G biodiesel compared to other conventional feedstock.

3.4. Oil composition and biodiesel properties

Finally, besides the environmental issues established by the life cycle analysis, the quality of biodiesel will determine the applicability of microalgal biodiesel. Thus, the properties of biodiesel from microalgae were evaluated. However, to ensure a final product of high quality, biodiesel must meet the EN 14214, ASTM 6751 or ANP

255 specifications in Europe, USA and in Brazil, respectively. The fatty acid profile and the combustion properties of the biodiesel in the best performance C40 are represented in Tables 5 and 6.

Table 5

Fatty acid composition of biodiesel microalgal in the C40 scenario.

Fatty acid profile	Methyl esters (%)
Lauric (12:0)	4.9±0.1
Myristic (C14:0)	7.3±0.1
Palmitic (C16:0)	22.5±0.5
Palmitoleic (C16:1)	8.5±0.1
Stearic (C18:0)	10.5±0.2
Oleic (C18:1n9c)	26.2±0.8
Linoleic (C18:2n6c)	17.8±0.5
γ-Linolenic (C18:3n6)	2.10±0.03
∑Saturated	45.2
∑Monounsaturated	34.7
∑Polyunsaturated	19.9

Table 6

Properties of microalgal biodiesel in the scenario C40 and its comparison with soybean and the standards used in the US (ASTM 6751), Europe (EN 14214) and Brazil (ANP 255).

Properties	Microalgae	Soybean[a]	ANP 255	ASTM 6751	EN 14214
EC (%)	99.8	96.9			min 96.5
CN	56.31	49.0	min 45	min 47	min 51
IV ($gl_2\ 100g^{-1}$)	70.04	128	-	-	max 120
DU (%)	74.5	143.8	-	-	-
SV	211.8	-	-	-	-
LCSF (%)	7.5	1.6	-	-	-
CFPP (°C)	7.09	-5.0	max 19	-	-
CP (°C)	6.87	-	-	-	-
APE	66.0	-	-	-	-
BAPE	22.0	-	-	-	-
OS (h)	8.52	1.3	-	min 3	min 6
HVV	39.5	-	-	-	-
μ ($mm^2\ s^{-1}$)	4.68	4.2	-	1.9-6.0	3.5-5.0
ρ ($g\ cm^{-3}$)	0.87	-	-	-	-

EC: ester content; CN: cetane number; IV: iodine value; DU: degree of unsaturation; SV: saponification value; LCSF: long-chain saturated factor; CFPP: cold filter plugging point; CP: cloud point; APE: allylic position equivalents; BAPE: bis-allylic position equivalents; OS: oxidation stability; HVV: higher heating value; m: kinematic viscosity; r: kinematic density. [a]Knothe (2017).

The composition of this oil (Table 5) indicated eight different compounds, with oleic acid (26.2%) being the main one. Microalgal oil showed a predominantly saturated (45.20%), followed by monounsaturated (34.70%) and polyunsaturated (19.90%) profile. This profile demonstrates the application potential of this type of biomass as an input for biodiesel production since oils with predominantly saturated and monounsaturated composition are the most suitable for biodiesel synthesis [35].

The biodiesel produced from microalgal oils has the following fuel properties (Table 6): ester content of 99.8%, cetane number of 46.31, iodine value of 70.04 $gl_2\ 100g^{-1}$, degree of unsaturation of 74.5%, saponification value of 211.8, long-chain saturated factor of 7.5%, cold filter plugging point at 7.09°C, cloud point at 6.87°C, allylic position equivalents of 66.0, bis-allylic position equivalents of 22.0, oxidation stability of 8.52 h, higher heating value of 39.5, kinematic viscosity of 1.28 $mm^2\ s^{-1}$, and kinematic density of 0.87 $g\ cm^{-3}$. All these parameters, comply with the limits

established by U.S., European, and Brazilian standards [36, 37, 38], and are comparable to soybean biodiesel [35]. These results indicate the potential of the use of microalgal biomass as a suitable lipid input for biodiesel manufacture.

4. Conclusions

Under the various scenarios tested, the best scenario for microalgae biodiesel production was ratio of C/N 40 which values for biomass production and oil production were 6.17 kg/m^3 and 1.12 kg/m^3, respectively. Life cycle assessment of the third-generation biodiesel produced from the heterotrophic cultivation of *Phormidium autumnale* in the best scenario showed a positive energy production (50.59 MJ) associated with a low consumption of water (28.38 m^3/kg), and a low CO_2 emission (18.09 CO_2-eq/kg). The high lipid production capacity potential obtained is interesting for the generation of quality biodiesel that meets or surpasses the most stringent US, European and Brazilian fuel standard requirements.

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CONCLUSÃO GERAL

O estudo empregando diferentes quantidades absolutas de nutrientes demostrou melhor eficiência do processo na relação C/N de 40, alcançando uma produtividade de óleo de 18,9 mg/L/h.

Na razão C/N otimizada, a microalga *Phormidium autumnale* apresentou a capacidade de assimilar sacarose como fonte de carbono orgânico demostrando ser um potencial para a produção de biodiesel de 3º geração.

Considerando a análise do ciclo de vida, o cultivo heterotrófico de *Phormidium autumnale* demostrou um balanço energético positivo na razão C/N de 40, associado a baixas emissões de CO_2 e um baixa demanda água.

Em termos de aplicabilidade do biodiesel, a composição do óleo microalgal cumpriu com as características de qualidade do biodiesel adequadas às normas nacionais (ANP 255) e internacionais (ASTM 6751 e EN 14214).

Ao comparar os resultados com o processo de produção do biodiesel de soja e com o etanol da cana de açúcar, a produção de biodiesel 3G de *Phormidium autumnale* confirma a viabilidade do uso deste biodiesel como uma alternativa para a mitigação das emissões de gases de efeito estufa, balanço energético e na demanda de água, contribuindo assim para o avanço da viabilidade sustentável deste tipo de processo.