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Rosangela Rodrigues Dias

INFLUÊNCIA GEOESPACIAL NO DESEMPENHO DE FOTOBIORREATORES

Santa Maria, RS
2020

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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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Rosangela Rodrigues Dias

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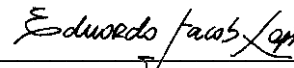

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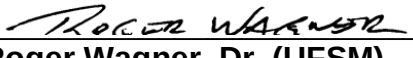

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A Alexandre dos Santos e Maicon Liberalesso.

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(Autor Desconhecido)

RESUMO

INFLUÊNCIA GEOESPACIAL NO DESEMPENHO DE FOTOBIORREATORES

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ORIENTADOR: Eduardo Jacob Lopes

Microalgas são microrganismos fotossintéticos dos quais uma variedade de compostos com potencial de aplicação em diferentes setores da indústria pode ser extraída. Como a produtividade de microalgas em sistemas de cultivo ao ar livre depende fortemente da luz solar, temperatura e fotoperíodo, a variabilidade climática em função da posição geográfica representa um desafio significativo para a padronização dos processos de produção em escala comercial. Em face disto, o trabalho teve por objetivos: (i) mapear a performance da microalga *Scenedesmus obliquus* CPCC05 em fotobiorreatores, (ii) avaliar o efeito da variabilidade climática em função da posição geográfica na produção de biomassa da microalga *Scenedesmus obliquus* CPCC05, (iii) avaliar a composição de lipídeos da biomassa microalgal, (iv) avaliar a composição de ácidos graxos da biomassa microalgal. Os resultados mostraram variações significativas na produtividade de microalgas e na proporção de ácidos graxos. Sob a intensidade de luz de 2100,9 $\mu mol.m^{-2}.s^{-1}$, temperatura de 28,75 °C e duração do dia de 12,52 h obteve-se a maior produtividade de biomassa (0,347 g/L.d) e lipídeos (0,048 g/L.d). A produtividade de biomassa variou de 0,009 a 0,347 g/L.d e a produtividade de lipídeos de 0,0008 a 0,048 g/L.d para as diferentes posições geográficas. Em paralelo, a proporção de ácidos graxos saturados variou de 31,39 a 65,68%, monoinsaturados de 8,28 a 28,72% e poli-insaturados de 21,37 a 57,78%. Os resultados deste trabalho fornecem informações importantes sobre os efeitos da variação espacial e temporal do clima no metabolismo fotossintético da microalga *Scenedesmus obliquus* CPCC05.

Palavras-chave: Microalga, *Scenedesmus obliquus*, localização espacial, clima.

ABSTRACT

GEOSPATIAL INFLUENCE ON THE PERFORMANCE OF PHOTOBIOREACTORS

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Microalgae are photosynthetic microorganisms from which a variety of compounds with potential for application in different industry sectors can be extracted. As microalgae productivity in outdoor cultivation systems depends strongly on sunlight, temperature, and photoperiod, climate variability as a function of geographic position represents a significant challenge for the standardization of commercial-scale production processes. Therefore, the objective of this work was: (i) to map the performance of the microalgae *Scenedesmus obliquus* CPCC05 in photobioreactors, (ii) assess the effect of the climate variability according to geographical position in the biomass production of the microalgae *Scenedesmus obliquus* CPCC05, (iii) assess the lipid composition of the microalgal biomass, (iv) assess the fatty acid composition of the microalgal biomass. The results showed significant variations in the microalgae productivity and in the proportion of fatty acids. Under the light intensity of 2100.9 $\mu mol.m^{-2}.s^{-1}$, temperature of 28.75 °C and day length of 12.52 h, it was obtained the highest productivity of biomass (0.347 g/L.d) and lipids (0.048 g/L.d). The biomass productivity ranged from 0.009 to 0.347 g/L.d and lipid productivity from 0.0008 to 0.048 g/L.d for the different geographic positions. In parallel, the proportion of saturated fatty acids ranged from 31.39 to 65.68%, monounsaturated from 8.28 to 28.72% and polyunsaturated from 21.37 to 57.78%. The results of this work provide important information about the effects of spatial and temporal variation of climate on the photosynthetic metabolism of the microalgae *Scenedesmus obliquus* CPCC05.

Keywords: Microalgae, *Scenedesmus obliquus*, spatial location, climate.

SUMÁRIO

INTRODUÇÃO

Considerando os desafios atuais associados ao aumento da demanda de energia e alimentos, há uma necessidade iminente de encontrar recursos sustentáveis adequados para atender a essas necessidades. O uso de fontes alternativas de proteínas, lipídeos, carboidratos e pigmentos desenvolvidos com foco na viabilidade econômica e sustentabilidade ambiental têm tornando-se uma tendência mundial (DE JESUS et al., 2018). Nesse contexto, as microalgas mostraram ter um grande potencial para a produção de numerosos bioprodutos (KOYANDE et al., 2019; MATOS, 2019).

A composição química da biomassa de microalgas a torna adequada para aplicação na alimentação humana e animal e para obtenção de moléculas altamente valiosas, como ácidos graxos poli-insaturados, carotenoides, ficobiliproteínas e polissacarídeos, que podem ser aplicados em produtos farmacêuticos e nutracêuticos (JACOB-LOPES et al., 2018, MOBIN et al., 2019). Dada a grande produtividade lipídica de algumas microalgas, esta tecnologia também pode ser explorada para a produção de biocombustíveis, como biodiesel (DIAS et al., 2019; MOFIJUR et al., 2019; SHOW et al. 2019).

No entanto, ao mesmo tempo que diversas vantagens são apresentadas pelas microalgas, ainda existem desafios específicos que precisam ser abordados para tornar os produtos à base de microalgas competitivos economicamente (BARSANTI e GUALTIERI, 2018; SUDHAKAR et al., 2019). Um dos gargalos do processo é a fase de cultivo, cuja operação é complicada tanto pela complexidade dos mecanismos biológicos envolvidos quanto pelas condições climáticas altamente flutuantes que afetam o sistema (DE-LUCA et al., 2018; GALÈS et al., 2019). Na prática, as condições de cultura externa diferem drasticamente das condições ótimas identificadas em laboratório. A variabilidade climática em função da posição geográfica influencia a eficiência fotossintética e, consequentemente, a produtividade das microalgas (KLEIN et al., 2018; GONZALEZ-CAMEJO et al., 2019).

Segundo Banerjee e Ramaswamy (2017) e Boruff et al. (2015) para determinar o potencial atual e futuro das microalgas é importante avaliar sua produtividade de acordo com a posição geográfica e condições climáticas prevalecentes. No entanto, um número limitado de estudos reproduziu o cultivo de microalgas considerando o clima local (PANKRATZ et al., 2019; LOZANO-GARCIA et al., 2019). Portanto, faz-se

necessário uma avaliação mais profunda acerca do efeito da variabilidade climática na performance de fotobiorreatorres, a fim de fornecer diretrizes para o cultivo de microalgas em escala comercial.

OBJETIVOS

OBJETIVO GERAL

Avaliar a influência geoespacial na performance de fotobiorreatores.

OBJETIVOS ESPESCÍFICOS

Simular os elementos atmosféricos (intensidade solar, temperatura e fotoperíodo) de diferentes posições geográficas da zona climática equatorial e tropical.

Avaliar a produção de biomassa da microalga *Scenedesmus obliquus* CPCC05 em função dos elementos atmosféricos e da tipologia climática.

Avaliar a composição de lipídeos da biomassa microalgal em função dos elementos atmosféricos e da tipologia climática.

Avaliar a composição de ácidos graxos da biomassa microalgal em função dos elementos atmosféricos e da tipologia climática.

CAPÍTULO 1

REVISÃO BIBLIOGRÁFICA

1 MICROALGAS

As microalgas abrangem um grupo heterogêneo de organismos unicelulares, na sua maioria fotossintéticos, incluindo eucariontes e procariontes (CADORET et al., 2012; MARONEZE et al., 2016). Até o momento, cerca de 30.000 espécies de microalgas foram identificadas, mas as estimativas sugerem que o número de espécies não descritas varia de centenas de milhares a milhões de espécies espalhadas pela biosfera (MOBIN e ALAM, 2017).

A classificação das microalgas é dada em uma variedade de classes, principalmente distinguidas em função da sua pigmentação fotossintética, ciclo de vida e estrutura celular básica. Entre essas classes, as mais importantes em termos de abundância são *Bacillariophyceae*, *Chlorophyceae* e *Chrysophyceae*. Em relação à exploração e uso biotecnológico as *Chlorophyceae* e *Cyanophyceae* são as mais relevantes (DEMIRBAS, 2010).

As *Cyanophyceae*, são exemplos de microalgas procarióticas, que não possuem organelas delimitadas por membranas. As *Chlorophyceae*, *Chrysophyceae* e *Bacillariophyceae*, por outro lado, são exemplos de microalgas eucarióticas, que possuem organelas delimitadas por membrana dedicadas a funções específicas. Estas, são providas de plastídios, os corpos com clorofila que realizam a fotossíntese. Porém, os pigmentos fotossintéticos associados a estrutura plastidial variam entre os grupos de microalgas e as várias cepas têm diferentes combinações de moléculas de clorofila, alguns têm apenas clorofila a, alguns a e b, enquanto outras cepas, a, c e d (PATEL et al., 2017; SUGANYA et al., 2016).

As microalgas podem ser cultivadas nos modos fotoautotrófico, heterotrófico, mixotrófico e fotoheterotrófico, sendo que a diferença entre estas modalidades de cultivo está na fonte de energia e de carbono (FRANCO et al., 2013). No cultivo fotoautotrófico as células requerem compostos inorgânicos e luz para o crescimento. Por outro lado, no cultivo heterotrófico requerem apenas compostos orgânicos (FRANCISCO et al., 2014). No cultivo mixotrófico, por sua vez, usam a luz, compostos orgânicos e inorgânicos como fonte de energia e, compostos orgânicos e inorgânicos como fonte de carbono e, no cultivo fotoheterotrófico, usam luz enquanto empregam compostos orgânicos como fonte de carbono. A principal diferença entre mixotrófico e fotoheterotrófico é que este último não pode absorver e metabolizar o CO_2 (CHEW et al., 2018).

Digno de nota, algumas espécies de microalgas crescem em pH extremo como, por exemplo, *Dunaliella acidófila* em pH 1, enquanto a microalga *Coccomyxa onubensis* possui uma tolerância de pH extremamente ampla, variando de 2,5 a 9. Microalgas de alta temperatura também são conhecidas, tais como a cianobactéria *Synechococcus* que cresce a temperaturas superiores a 70 ºC. Em contrapartida, espécies como *Chloromonas nivalis* pode crescer em gelo ou neve (BOROWITZKA, 2018). Dentre as microalgas, *Scenedesmus obliquus* surge com um grande potencial de exploração, uma vez que se destaca pela sua elevada produtividade de biomassa e, significativo teor lipídico (CHEAH et al., 2015).

2 METABOLISMO FOTOSSINTÉTICO

A fotossíntese é um processo que compreende duas etapas, um conjunto de reações luminosas, que ocorrem apenas quando as células são iluminadas e reações de fixação de carbono, também conhecidas como reações escuras, que ocorrem tanto na presença quanto na ausência de luz. No primeiro estágio, as células transformam a energia da luz em energia química, que é armazenada em compostos de alta energia para uso posterior nas reações de fixação de carbono (JACOB-LOPES et al., 2009; MARONEZE et al., 2016).

A fotossíntese ocorre nos cloroplastos que são envolvidos por uma membrana. Esta membrana contém um fluido aquoso chamado estroma, o qual contém o aparato bioquímico necessário para a fixação de CO_2. No estroma encontram-se os granum, pilhas em forma de disco delimitados por uma membrana chamada tilacóide (RAZZAK et al., 2013). Imerso nas membranas tilacóides estão os fotossistemas I e II onde ficam inseridos os pigmentos fotossintetizantes, representados principalmente por clorofilas, carotenoides e ficobilinas. Nesses fotossistemas, é possível perceber duas porções: o complexo antena e o centro de reação. O complexo antena apresenta moléculas de pigmento que captam a energia luminosa e levam-nas para o centro de reação, um local rico em proteínas e clorofila (RICHMOND e HU, 2013).

Inicialmente fótons de luz atinge moléculas de clorofila no fotossistema II, que são energizadas e essa energia é carreada até o centro de reação do fotossistema II. A molécula de clorofila do centro de reação é excitada, e seus elétrons são transferidos da clorofila para a plastoquinona. Para além dos elétrons, a platoquinona também recebe prótons do estroma. A cada elétron perdido pelo fotossistema II,

ocorre a substituição por um elétron proveniente da fotólise da água. A fotólise da água acorre pela ação da luz, gerando íons hidrogênio, elétrons e oxigênio (WILLIAMS e LAURENS, 2010).

A seguir, a plastoquinona transfere os elétrons para o citocromo b6f e os prótons recebidos anteriormente são liberados para o lúmen. Estas transferências são acompanhadas pelo bombear de íons hidrogênio para o espaço luminal pelo citocromo b6f. Posteriormente, os elétrons são transferindo do citocromo b6f para o fotossistema I por meio da plastocianina. No fotossistema I os elétrons são reenergizados e transferidos para ferredoxina, que transfere os elétrons para ferredoxina NADP redutase gerando NADPH pela adição dos elétrons e íons hidrogênio a NADP+ (KRISHNAN, 2016). Enquanto os elétrons passam pela cadeia transportadora de elétrons, a energia desses elétrons é utilizada para bombear íons de hidrogênio do estroma para os tilacóides gerando um gradiente de concentração que ativa a ATP sintase gerando ATP a partir da fotofosforilação do ADP + Pi. ATP, NADPH e O_2 são os produtos finais das reações luminosas oriundas do fluxo de elétrons acíclico (RICHMOND e HU, 2013).

Nas reações de fixação do carbono, o NADPH e o ATP produzidos anteriormente são usados para assimilação do dióxido de carbono. Nessa etapa ocorre uma série de reações denominadas de ciclo das pentoses ou ciclo de Calvin-Benson (RAZZAK et al., 2013). O ciclo inicia com a união de uma molécula de CO_2 a um açúcar de cinco carbonos conhecido como ribulose 1,5-bifosfato (RuBP), resultando em um intermediário de seis carbonos altamente instável, catalisada pela enzima ribulose-1,5-bisfosfato carboxilase/oxigenase (RuBisCO). O produto da reação é quebrado em duas moléculas de três carbonos chamada 3-fosfoglicerato. Na presença de ATP e NADPH, o 3-fosfoglicerato é reduzido a gliceraldeído 3-fosfato (G3P). Em soma, de cada seis moléculas de G3P formado uma é usada para síntese de açúcares e o restante é utilizado para regenerar RuBP de modo que o ciclo possa ser fechado (JACOB-LOPES et al., 2015; RAZZAK et al., 2013). Os principais produtos deste ciclo são carboidratos, ácidos graxos, aminoácidos e ácidos orgânicos também são sintetizados na fixação fotossintética de CO_2. Vários produtos finais podem ser formados sob diferentes condições de intensidade luminosa, concentração de CO_2 e nutrição (RICHMOND e HU, 2013).

3 FOTOBIORREATORES: PARÂMETROS DE PROCESSO E CONFIGURAÇÕES

Fotobiorreatores podem ser definidos como sistemas utilizados para o desenvolvimento de reações fotossintéticas, concebidos para a produção de biomassa (JACOB-LOPES et al., 2008). Estes equipamentos são projetados com base nos requisitos fisiológicos das microalgas, como fornecimento de luz, suprimento de nutrientes, condições de cultura e mistura (ACIÉN et al., 2018).

A disponibilidade de luz é um fator que regula a produtividade de microrganismos fotossintéticos (MARONEZE et al., 2016). O crescimento celular destes microrganismos é afetado pelo nível de intensidade luminosa, que pode cair em uma das três categorias: limitação de luz, saturação de luz ou inibição de luz. Quando a intensidade luminosa é baixa, o aumento deste parâmetro ocasiona a melhoria da eficiência fotossintética das células, até determinados níveis, em que o aparato fotossintético torna-se saturado. Com um aumento adicional da intensidade, ocorre a fotoinibição, causando danos irreversíveis ao aparato fotossintético (CHANG et al., 2017). Para maximizar a produtividade de biomassa, a intensidade da luz de saturação precisa ser distribuída por todo o fotobiorreator. Para este fim, otimizar o sistema de cultivo através do design adequado de sua geometria e orientação e melhorar a mistura das células são estratégias comumente adotadas para aprimorar a distribuição da luz e reduzir efeitos de sombreamento e fotoinibição (ACIÉN et al., 2018). Além da intensidade luminosa, o fotoperíodo, ou seja, a relação entre a duração dos ciclos de luz e escuro, é um fator a ser considerado no projeto de fotobiorreatores, uma vez que as oscilações de intensidade luminosa sofridas ao longo do dia ocasionam severas alterações na eficiência fotossintética dos sistemas (JACOB-LOPES et al., 2009).

No que se refere ao suprimento de nutrientes, as microalgas necessitam de quantidades específicas de macronutrientes e micronutrientes para serem capazes de produzir biomassa. No grupo dos macronutrientes, carbono, nitrogênio e fósforo, são essenciais. Considerando a razão Redfield C: N: P de 106: 16: 1 do fitoplâncton, todos os elementos essenciais devem estar presentes no meio de cultura em proporções e quantidades adequadas, de modo que o crescimento de microalgas não seja limitado. Além destes, o meio de cultivo deve conter vários outros nutrientes. Os micronutrientes mais relevantes são Mg, S, Ca, Na, Cl, Fe, Zn, Cu, Mo, Mn, B e Co (MARKOU et al., 2014). Águas residuais são uma boa fonte para a maioria desses

nutrientes (CHRISTENSON e SIMS, 2011; QUEIROZ et al., 2007). Na literatura, as composições dos meios de cultivo são frequentemente consideradas fixas. No entanto, a experiência de cultivar microalgas em vários tipos de efluentes com diversas composições nutricionais é a evidência de que a estequiometria do fitoplâncton diverge da relação Redfield sob condições específicas, sugerindo que o meio de cultivo pode ser flexível e pode ser adaptado ás necessidades metabólicas das diferentes espécies de microalgas (JACOB-LOPES et al., 2006; MARKOU et al., 2014).

Com relação as condições de cultura, as principais variáveis que afetam o crescimento de microalgas são pH e temperatura, consequentemente, fotobiorreatores devem ser projetados permitindo o controle dessas variáveis. O pH ótimo da maioria das espécies de microalgas está na faixa de 7 a 9. Algumas espécies, no entanto, têm pH ótimo em faixas mais ácidas ou básicas. Desvios do pH ótimo podem inibir a fotossíntese devido à sensibilidade do aparelho fotossintético, portanto, manter a cultura na faixa de pH ideal é crucial (WANG et al., 2012). A temperatura é um dos parâmetros mais críticos a serem controlados, por influenciar os processos celulares afetando a floração, capacidade fotossintética, crescimento e respiração das microalgas. A eficiência de sistemas que utilizam microalgas normalmente decresce em temperaturas excessivas ou reduzidas. A temperatura ótima para o desenvolvimento de microalgas situa-se na faixa de 18 a 24 ºC (ENZING et al., 2014). Para microalgas cultivadas com base na luz solar, as variações de temperatura dependem do ciclo de luz dia/noite e das mudanças sazonais. O controle de temperatura nos valores definidos como ideal para um determinado sistema pode ser obtido através do uso de camisas de aquecimento, serpentinas e trocadores de calor externo (ACIÉN et al., 2018).

A mistura é uma operação necessária em qualquer fotobiorreator. Quando outros fatores não limitam o crescimento de microalgas, a mistura eficiente é a fator mais importante para altos rendimentos de biomassa. A mistura garante a distribuição uniforme de nutrientes para as células e utilização eficiente de luz, facilita a transferência de calor, promove trocas gasosas, e mantém as células em suspensão (CHANG et al., 2017). Dependendo da escala e da escolha do sistema de cultivo, a mistura pode ser realizada por meio de aeração, bombeamento, agitação mecânica ou uma combinação desses meios. Deve-se notar que nem todas as espécies de

microalgas toleram elevadas taxas de agitação. A mistura vigorosa pode levar ao estresse hidrodinâmico, resultando em restrição ao crescimento de microalgas e atividade metabólica. Por isso, os sistemas de agitação e mistura devem ser dimensionados adequadamente (WANG et al., 2012).

Sem levar em consideração qualquer aspecto econômico, a configuração dos diferentes sistemas de cultivo, bem como suas variações, é desenvolvida e adaptada para atender as peculiaridades dos diferentes bioprocessos envolvendo microalgas. Em termos de configuração, fotobiorreatores são classificados em sistemas abertos, no qual os sistemas de cultivo são expostos em contato direto com a atmosfera e sistemas fechados em que o contato entre a cultura e a atmosfera é significativamente reduzido ou inexistente (CHANG et al., 2017).

Os fotobiorreatores abertos têm sido extensivamente utilizados para o cultivo em massa de microalgas. As principais configurações destes sistemas referem-se a lagoas circulares e tanques do tipo raceway (DEPRÁ et al., 2018). A limitação destes sistemas está fundamentada nas elevadas taxas de evaporação de água, controle limitado das condições operacionais e requerimento de amplas áreas para construção. Além disso, apresentam baixa produtividade devido à má utilização da luz e maior perspectiva de contaminação. Deste modo, esses sistemas são limitados a poucas espécies (VASUMATHI et al., 2012).

Uma alternativa aos fotobiorreatores abertos, são sistemas fechados, que possibilitam uma grande variedade de configurações e incrementam significativamente o desempenho dos cultivos. As principais configurações destes sistemas referem-se a fotobiorreatores tubulares, de placas planas e colunas verticais (WANG et al., 2012). Estes sistemas são caracterizados por apresentar elevada eficiência fotossintética associada à maior precisão e controle das variáveis operacionais, menor risco de contaminação e minimização das perdas de água por evaporação (YEN et al., 2014).

4 INFLUÊNCIA DO CLIMA NO CULTIVO DE MICROALGAS

Atualmente, mais de 90% da produção comercial de microalgas fotoautotróficas é realizada em sistemas de cultivo ao ar livre sob luz solar (CAÑEDO e LIZÁRRAGA, 2016). A luz solar é a fonte de energia para a formação de ATP e NADPH, e a qualidade, intensidade e período de luz podem ter efeitos significativos na

produtividade e composição química da biomassa (ZHUANG et al., 2018; JANKOWSKA, SAHU e OLESKOWICZ-POPIEL, 2017). Neste contexto, para o estabelecimento de uma unidade industrial de microalgas são necessárias considerações explícitas da tendência e variabilidade climática local (COLEMAN et al., 2014; PEROSA et al., 2015). Dentre os fatores relacionados à geografia que influenciam o cultivo de microalgas estão além da irradiação solar e duração do dia a temperatura (KLEIN et al., 2017).

Especificamente, o clima de um local é definido pela ação conjunta de elementos atmosféricos e fatores geográficos, e devido à sua extensão geográfica, o globo terrestre apresenta uma considerável tipologia climática. Existem hoje mais de 200 esquemas de classificação climática, a maior parte considerada empírica ou analítica e uma minoria, como genética ou dinâmica. Atualmente, o modelo analítico de classificação climática de Köppen-Geiger é o mais utilizado. O modelo considera cinco grupos climáticos: tropical (A), seco (B), subtropical (C), frio (D) e polar (E), que representam aproximadamente 19%, 30,2%, 13,4%, 24,6% e 12,8% da superfície terrestre, respectivamente. Estes grupos são subdividido em outros, que apresentam características especificas de cada localidade (MENDONÇA e DANNI-OLIVEIRA, 2007; PEEL et al., 2007). É importante salientar que o clima de qualquer região é composto pelos mesmos elementos (temperaturas, chuvas, pressão atmosférica e regime dos ventos); estes, no entanto, estão sujeitos a vários fatores, como latitude, altitude, massas de ar, continentalidade e maritimidade (ANTUNES, 1937).

Baseado em padrões climáticos o ano é subdividido em quatro estações (primavera, verão, outono e inverno), que ocorrem devido ao movimento translacional associado a inclinação do eixo de rotação da terra. Notoriamente, a zona climática equatorial e tropical, localizadas entre os trópicos de câncer e capricórnio, representam a parte do planeta que recebe a maior quantidade de luz solar, e geralmente apresentam por consequência elevadas temperaturas (HASTENRATH, 2012). Isso porque os raios atingem as regiões próximas ao paralelo do equador de forma perpendicular, isto é, de maneira mais intensa. À medida que se afasta, os raios atingem a superfície de forma mais inclinada e, consequentemente, com menor intensidade. Nesse sentido, as zonas climáticas localizada acima do trópico de câncer e abaixo do trópico de capricórnio são caracterizadas por apresentar temperaturas muito amenas, com uma grande amplitude térmica anual (REIS et al., 2018).

Neste contexto, baseado nos padrões climáticos do globo terrestre, a zona climática equatorial e tropical são as que, teoricamente, apresentam melhores condições de luz e temperatura para o cultivo de microalgas. Além disso, as microalgas podem ser colhidas continuamente, sem período de entressafra, uma vez que apresentam menor amplitude térmica anual comparada as demais zonas climáticas da terra.

5 COMPONENTES E APLICAÇÕES DE MICROALGAS

A biomassa microalgal é composta principalmente por proteínas, lipídeos e carboidratos, que pode servir de matéria prima para diferentes mercados. Os lipídios podem ser usados como elementos básicos na indústria química e óleos comestíveis para o mercado de alimentos e saúde. Os carboidratos podem ser usados para a produção de produtos químicos, como os usados em produtos farmacêuticos, e as proteínas purificadas podem ser usadas nos mercados de alimentos, rações e produtos químicos a granel (DEPRÁ et al., 2018).

As microalgas podem acumular uma alta porcentagem de lipídios, que correspondem por aproximadamente 30 a 50% do seu peso total (CHEW et al., 2017). Os dois principais grupos de lipídios nas microalgas consistem em lipídios polares e não polares. Os lipídios polares são constituídos principalmente por glicolipídeos e fosfolipídios, que constituem 41% a 92% do lipídeo total; enquanto os lipídeos não polares representam 5% a 51% dos lipídeos totais. Lipídeos não polares, como esteróis e ácidos graxos livres, também estão presentes em níveis mais baixos. O acúmulo de lipídios depende das espécies de microalgas, crescimento e condições ambientais (MIMOUNI et al., 2018). Em particular, as microalgas têm despertado interesse como fonte de ácidos graxos, especialmente os ácidos graxos poli-insaturados de cadeia longa, como o ácido γ-linolênico, o ácido araquidônico, o ácido eicosapentaenóico e o ácido docosahexaenóico, que chegam aos humanos através da cadeia alimentar ou são usados como suplementos alimentares. Além disso, dada a elevada produtividade de biomassa e teor lipídico de algumas microalgas, essa tecnologia tem sido explorada para a produção de biocombustíveis como biodiesel e biohidrogênio (DEPRÁ et al., 2018).

As microalgas normalmente têm um alto teor de carboidratos que é potencialmente superior a 50% do seu peso seco, pois tem uma eficiência de

conversão relativamente alta e pode armazenar carboidratos facilmente (YEN et al., 2013). Os carboidratos de microalgas podem ser encontrados na forma de amido, glicose, açúcares e outros polissacarídeos (SPOLAORE et al., 2006). Os polissacarídeos de microalgas podem modular o sistema imunitário e as reações inflamatórias, tornando-os altamente favoráveis como fontes de moléculas biologicamente ativas, tais como ingredientes alimentares funcionais e agentes terapêuticos naturais (CHEW et al., 2017).

O alto teor de proteína de várias espécies de microalgas é uma das razões para considerá-las como uma fonte não convencional de proteína. O conteúdo proteico das microalgas varia de 50 a 70% da sua composição. A proteína de microalgas pode ser usada para nutrição humana e animal (CHEW et al., 2017). A qualidade e o padrão de aminoácidos da maioria das microalgas se compara à de outras fontes convencionais (DRAAISMA et al., 2013). Algumas destas proteínas estão associadas à estimulação da produção do hormônio colecistocinina, que regula a supressão do apetite e, portanto, têm sido consideradas na formulação de alimentos funcionais contra a obesidade (PATIAS et al., 2018).

Devido à sua composição global, as microalgas são geralmente usadas no campo da nutrição humana e animal. No entanto, moléculas puras também podem ser extraídas quando suas concentrações são suficientemente altas. Isso leva a produtos valiosos como ácidos graxos poli-insaturados (PUFAs) e pigmentos (SPOLAORE et al., 2006). Os PUFAs mais comuns em microalgas são o ácido araquidônico, o ácido linolênico, ácido docosaexaenoico e o ácido eicosapentaenoico, que são farmacologicamente importantes para a dieta. Atualmente, microalgas são cultivadas principalmente para produzir ácido docosaexaenoico e eicosapentaenoico (JACOB-LOPES et al., 2018). Os ácidos graxos nas microalgas correspondem à maior fração lipídica e, em certas espécies, os PUFAs compõem entre 25 e 60% dos lipídios totais (DA SILVA VAZ et al., 2016; MATA et al., 2010).

Os pigmentos, por sua vez, são importantes para o metabolismo das microalgas fotossintéticas e apresentam diversas atividades biológicas benéficas, como atividade antioxidante e anti-inflamatória. As principais classes de pigmentos em microalgas são carotenoides e ficobiliproteínas (DA SILVA VAZ et al., 2016; RODRIGUES et al., 2015). Os carotenoides são usados principalmente em suplementos alimentares, corantes alimentícios, alimentos para animais e produtos

farmacêuticos, e as principais aplicações das ficobiliproteínas incluem corantes alimentícios e produtos farmacêuticos (CHEW et al., 2017).

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CAPÍTULO 2

MAPPING THE PERFORMANCE OF PHOTOBIOREACTORS: EQUATORIAL AND TROPICAL CLIMATE ZONE

O artigo será submetido para a revista *Bioresource Technology*[1].

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Mapping the Performance of Photobioreactors: Equatorial and Tropical Climate Zone

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Abstract

The aim of the present study was to map the performance of photobioreactors in the equatorial and tropical climatic zones. The climate of thirty-three geographical positions was simulated. The atmospheric elements considered were solar irradiance, temperature and photoperiod. The results showed significant variations in the microalgae productivity and in the proportion of fatty acids. Under the light intensity of 2100.9 $\mu mol.m^{-2}.s^{-1}$, the temperature of 28.75 °C and day length of 12.52 h, it was obtained the highest biomass productivity (0.347 g/L.d) and lipids (0.048 g/L.d). The biomass productivity ranged from 0.009 to 0.347 g/L.d and lipid productivity from 0.0008 to 0.048 g/L.d for the different geographic positions. In parallel, the proportion of saturated fatty acids ranged from 31.39 to 65.68%, monounsaturated from 8.28 to 28.72% and polyunsaturated from 21.37 to 57.78%. The results of this work provide important information about the effects of spatial and temporal variation of climate on the photosynthetic metabolism of the microalgae *Scenedesmus obliquus* CPCC05.

Keywords: microalgae, *Scenedesmus obliquus*, spatial location, climate.

1 Introduction

The natural resources are being used 1.75 times faster than the ecosystem can regenerate. Infinite growth in a finite world of resources will begin to impose limits on economic growth. In this context, the urgency of actions to reduce the negative impacts of global ecological spending is driving the exploitation of previously neglected unconventional sources (Global Footprint Network, 2019).

Alternative sources of food and energy developed with a focus on economic viability and environmental sustainability are on the ascension (Matos, 2019). In this perspective, microalgae have shown to be a promising resource. These microorganisms can valorize wasted resources, mitigate CO_2 and produce a variety of consumer products (Javed et al., 2019; Severo et al., 2019). Currently, the main microalgae-based products are single-cell protein, β-carotene, astaxanthin, eicosapentaenoic acid, and docosahexaenoic acid. These products are marketed as a food supplement, food coloring, and food additive (Jacob-Lopes et al., 2018).

In the current conjuncture, high costs, and low volumes of biomass production are obstacles to be overcome to make microalgae-based products a commodity (Zhang et al., 2016). As most microalgae products are intracellular, their economic viability depends strongly on biomass productivity, which in practice differs drastically of the identified in laboratory under ideal culture conditions (Maroneze et al., 2016). Under external conditions, the cultivation is complicated by both the complexity of the biological mechanisms involved as the highly fluctuating climate that affects the system (de Vree et al., 2016; De-Luca et al., 2018).

Today, about 90% of commercial microalgae production is outdoors under sunlight and with little or no control over the atmospheric elements (Cañedo and Lizárraga, 2016). The microalgae productivity is mainly influenced by solar radiation, temperature and photoperiod and the diversity of the spatial manifestation of these elements must to the action of geographic factors such as latitude and altitude. In this context, each zone, region, and position of the terrestrial surface has singular climate characteristics that are controlled by the conjugation of both. Considering this, it becomes crucial to assess the potential of microalgae as a source of biochemicals and bioenergy according to the geographic position and prevailing climatic conditions (de Jesus et al., 2018).

Namely, based on the climatic patterns of the terrestrial globe, the equatorial and tropical climatic zone are those that theoretically present the best light and temperature conditions for microalgae cultivation. As solar radiation in the troposphere differs latitudinally, reaching perpendicularly only the central zones, these receive more solar energy and their thermal values tend to be higher. Moreover, due to their more stable seasonal temperatures throughout the year, the microalgae can be harvested continuously without an off-season period (Chiu et al., 2016).

Associated with these aspects, the present study aimed to map the performance of photobioreactors in the equatorial and tropical climatic zones in order to provide guidelines for mass microalgae production. The climatic of diverse geographical positions were simulated. Here, we consider the vast climate typology of these two zones and the synergistic effect of different atmospheric elements. The study has focused on the biomass and lipids production and fatty acid profile of the microalgae *Scenedesmus obliquus* CPCC05.

2 Material and Methods

2.1 Microorganisms and culture media

Axenic cultures of *Scenedesmus obliquus* (CPCC05) were obtained from the Canadian Phycological Culture Centre. Stock cultures were propagated and maintained in synthetic BG-11 medium (Rippka et al., 1979). The incubation conditions used were 30 ºC, photon flux density of 15 µmol.m^{-2}. s^{-1} and a photoperiod of 12 h.

2.2 Photobioreactor design

Measurements were made in a bubble column photobioreactor, consisting of a reaction vessel, a photoperiod chamber, and a controller (Tecnal, Piracicaba-SP, Brazil). The system was built in 4 mm thick glass with an internal diameter of 9.0 cm, a height of 40 cm, and a nominal working volume of 2.0 L. The dispersion system for the reactor consisted of a 1.5 cm diameter air diffuser located in the centre of the column. The reactor was illuminated with

sixty Light-Emitting Diode (LED) lamps, located in the photoperiod chamber. The photoperiod controller was used to modulate the day length and the light intensity, which is linearly increased from the dark to the maximum light intensity and, after 3 hours, is likewise reduced to the dark period. The CO_2/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the airstream through three rotameters that measured the flow rates of carbon dioxide, air, and the mixture of gases, respectively.

2.3 Experimental conditions

The experiments were carried out in photobioreactors operating on batch mode, fed with 2.0 L synthetic BG-11 medium. The experimental conditions were as follows: initial cell concentration of 100 mg/L, continuous aeration of 1 VVM with an injection of air enriched with 15% carbon dioxide. The temperature and light conditions are defined in section 2.4.

The cell concentration was monitored every 24 h during the growth phase of the microorganism. All experiments were conducted until the stationary phase. In this phase, the biomass was separated from the culture medium by centrifugation, the supernatant was discarded and the remaining biomass was refrigerated until the lipid and fatty acids analysis. The tests were carried out in duplicate, and the kinetic data referred to the mean of four repetitions.

2.4 Simulation of atmospheric elements

It was simulated the atmospheric elements (day length/photoperiod, solar irradiance, and temperature) of thirty-three geographical positions of the equatorial (0° to 10° N and S) and tropical (10° to 25° N and S) climatic zone: Caracas-VEN, Jos-NGA, Bandundu-COD, Bimbo-CAF, Mopti-MLI, Darwin-AUS, San Salvador-SLV, Ubon Ratchathani-THA, Cuiabá-BRA, Nieuw Nickerie-SUR, Makassar-IDN, Manaus-BRA, Kundiawa-PNG, Kota Bharu-MYS, Aracaju-BRA, Toliara-MDG, Taif-SAU, Al-Fashir-SDN, Lobito-AGO, Nouadhibou-MRT, Roebourne-AUS, Ali Sabih-DJI, Tacna-PER, León-MEX, Kasane-BWA, Jamaame-

SOM, Potosí-BOL, São José dos Campos-BRA, Kisoro-UGA, Jabalpur-IND, Ibarra-ECU, Mackay-AUS, Lichinga-MOZ. The geographic positions were chosen to cover the climate typology of the equatorial and tropical zones based on the availability simultaneous of normal climatological data of temperature and solar irradiation of the websites.

The data of the annual average of solar irradiance, day length, and temperature of geographical positions were retrieved from the website's Solar Electricity Handbook (http://www.solarelectricityhandbook.com) and Weatherbase (https://www.weatherbase.com). The Köppen-Geiger climate classification was used to name the climatic type of each geographic position. The geographical positions of the study are shown in Figure 1. The climatic type and experimental conditions used for each geographical position are shown in Table 1.

Figure 1. World map of Köppen-Geiger climate classification with the geographic positions of the study.

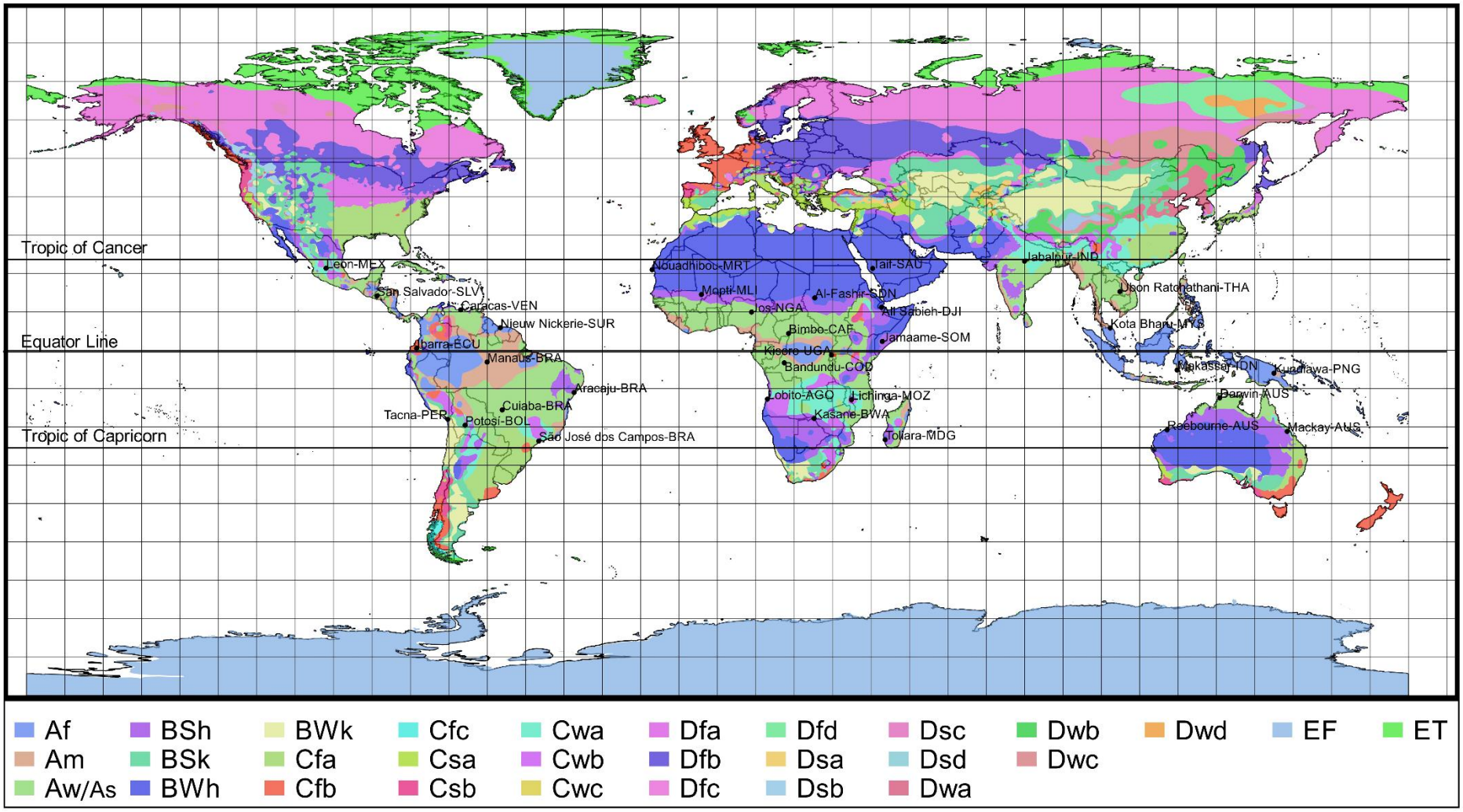

Table 1. Köppen-Geiger climate classification and experimental conditions for each geographic position.

Geographical Positions	Classification	Köppen-Geiger	Elevation (m)	Latitude	Longitude	Light intensity (μmol.m^{-2}.s^{-1})	Day length (h)	Temperature (°C)
Caracas-VEN	(A) Tropical	Aw	834	10° 30′ N	66° 53′ W	2015.4^{q}	12,48^{bcd}	22,75^{ijk}
Jos-NGA	(w) dry winter		1284	09° 52′ N	08° 54′ E	1982,4^{s}	12,49^{abcd}	21,92^{kl}
Bandundu-COD			322	03° 19′ S	17° 23′ E	1818,2^{A}	12,48^{bcd}	27,25^{bcd}
Bimbo-CAF			373	04° 15′ N	18° 25′ E	1926,0^{u}	12,47^{cd}	25,38^{h}
Mopti-MLI			271	14° 31′ N	04° 06′ W	2100,9^{m}	12,52^{abc}	28,75^{a}
Darwin-AUS			31	12° 26′ S	130° 53′ E	2219,1^{e}	12,48^{bcd}	27,25^{bcd}
San Salvador-SLV			620	13° 42′ N	89° 07′ W	2150,3^{i}	12,50^{abcd}	23,75^{i}
Ubon Ratchathani-THA			121	15° 14′ N	104° 52′ E	1871,4^{y}	12,50^{abcd}	26,42^{defg}
Cuiabá-BRA			189	15° 38′ S	56° 06′ W	1826,8^{z}	12,48^{bcd}	25,92^{fgh}
Nieuw Nickerie-SUR	(A) Tropical	Am	2	05° 57′ N	57° 02′ W	2186,0^{f}	12,48^{bcd}	26,50^{cdefg}
Makassar-IDN	(m) monsoonal		2	05° 08′ S	119° 25′ W	2129,0^{k}	12,46^{d}	27,50^{bc}
Manaus-BRA			84	03° 09′ S	59° 59′ W	1669,1^{E}	12,47^{cd}	27,17^{bcde}
Kundiawa-PNG	(A) Tropical	Af	1552	06° 01′ S	144° 58′ E	1764,6^{C}	12,50^{abcd}	20,05^{m}
Kota Bharu-MYS	(f) fully humid		4	06° 10′ N	102° 17′ E	1910,5^{v}	12,48^{bcd}	26,17^{efgh}
Aracaju-BRA	(A) Tropical (s) summer dry	As	9	10° 59′ S	37° 04′ W	1889,9^{x}	12,48^{bcd}	25,92^{fgh}
Toliara-MDG	(B) Dry	BWh	7	23° 23′ S	43° 44′ E	2285,4^{b}	12,51^{abcd}	23,50^{i}
Taif-SAU	(W) desert		1448	21° 29′ N	40° 33′ E	2127,7^{l}	12,53^{ab}	22,42^{jkl}
Al-Fashir-SDN	(h) hot arid		730	13° 37′ N	25° 20′ E	2238,7^{d}	12,50^{abcd}	25,25^{h}
Lobito-AGO			3	12° 22′ S	13° 32′ E	2034,1^{p}	12,48^{bcd}	23,42^{ij}
Nouadhibou-MRT			3	20° 56′ N	17° 02′ W	2157,3^{g}	12,53^{ab}	21,67^{l}
Roebourne-AUS			12	20° 46′ S	117° 08′ E	2337,3^{a}	12,51^{abcd}	26,75^{bcdef}
Ali Sabih-DJI			710	11° 09′ N	42° 43′ E	2242,9^{c}	12,46^{d}	25,64^{gh}
Tacna-PER	(B) Dry (W) desert (k) cold arid	BWk	696	18° 00′ S	70° 15′ W	2062,4^{o}	12,49^{abcd}	18,21^{o}
León-MEX	(B) Dry	BSh	1818	21° 08′ N	101° 41′ W	2074,9^{n}	12,53^{ab}	19,59^{mn}
Kasane-BWA	(S) steppe		973	17° 49′ S	25° 09′ E	2145,3^{j}	12,48^{bcd}	23,30^{ij}
Jamaame-SOM	(h) hot arid		11	00° 04′ N	42° 45′ E	2002,6^{r}	12,50^{abcd}	27,66^{b}

Potosí-BOL	(B) Dry (S) steppe (k) cold arid	BSk	3970	19° 35′ S	65° 45′ W	2156,6^{h}	12,53^{ab}	8,48^{r}
São José dos Campos-BRA	(C) Humid subtropical (f) fully humid (a) hot summer	Cfa	644	23° 13′ S	45° 51′ W	1579,1^{F}	12,50^{abcd}	19,29^{mn}
Kisoro-UGA	(C) Humid subtropical (f) fully humid (b) warm summer	Cfb	1927	01° 17′ S	29° 41′ E	1760,4^{D}	12,48^{bcd}	16,19^{p}
Jabalpur-IND	(C) Humid subtropical (s) summer dry (a) hot summer	Csa	392	23° 12′ N	79° 57′ E	1810,3^{B}	12,54^{a}	25,23^{h}
Ibarra-ECU	(C) Humid subtropical (s) summer dry (b) warm summer	Csb	2228	00° 24′ N	78° 06′ W	1429,0^{G}	12,50^{abcd}	15,00^{q}
Mackay-AUS	(C) Humid subtropical (w) winter dry (a) hot summer	Cwa	29	21° 07′ S	149° 13′ E	1899,2^{w}	12,51^{abcd}	22,08^{kl}
Lichinga-MOZ	(C) Humid subtropical (w) winter dry (b) warm summer	Cwb	1377	13° 16′ S	35° 14′ E	1952,2^{t}	12,49^{abcd}	18,83^{no}

Different letters within the same column express significant differences between the means ($p < 0.05$).

2.5 Kinetic parameters

Biomass data were used to calculate the biomass productivity [$P_X = (X_i-X_{i-1}).(t_i-t_{i-1})^{-1}$, g/L.d], the maximum specific growth rate [$\ln(X_i/X_0) = \mu_{max}.t$, 1/d], the generation time [$tg = 0.693/\mu_{max}$, d], and lipid productivity [$P_L = P_X.L_C$, g/L.d], where X_i is biomass concentration at time t_i (g/L) and X_{i-1} is biomass concentration at time t_{i-1} (g/L), t is the residence time (d) and L_C is the lipid content of the biomass (%). The residence time was defined as the elapsed time to reach the maximum biomass concentration.

2.6 Analytical methods

Cell concentration was evaluated gravimetrically by filtering a known volume of culture medium through a 0.45 μm filter (Millex FG, Billerica-MA, USA) and drying it at 60 °C for 24 h. The temperature was controlled by using thermostats. The flow rates of carbon dioxide, air, and CO_2 enriched air were determined with rotameters (AFSG 100 Key Instruments, Trevose-PA, USA).

The total lipid concentration of the biomass was determined gravimetrically by the modified Bligh and Dyer method (1959), described in Vendruscolo et al. (2018). The method of Hartman and Lago (1973) was used to saponify and esterify the dried lipid extract in order to obtain the fatty acid methyl esters (FAs). The FA composition was determined by using a VARIAN 3400CX gas chromatograph (Varian, Palo Alto-CA, USA). FAs were identified by comparison of the retention times with the authentic standards from FAME Mix-37 (P/N 47885-U, Sigma-Aldrich, St. Louis, USA) and quantified through area normalization by software T2100p Chromatography Station (Plus Edition) v9.04.

2.7 Statistical analysis

The analysis of variance (one-way ANOVA) and Tukey's test ($p<0.05$) was used to test differences between the geographical positions. To elucidate the relationship between the production parameters of biomass and lipid and the different climatic conditions simulated was

performed an exploratory factor analysis. The analyses were performed with the software Statistica 10.0 (StatSoft, Tulsa-OK, USA).

3 Results and Discussion

3.1 Effects of the geographic position on kinetic parameters

High productivity is the main reason why microalgae can be used as an alternative feedstock for the production of bioenergy and food. In photoautotrophic cultures, the productivity depends on biotic, abiotic and process-related factors. Process-related factors include biomass concentration, mixing intensity and harvest frequency. On the other hand, biotic factors include the presence of pathogens and abiotic include light, temperature, pH, salinity and nutrient composition (Okoro et al., 2019). Among these, light and temperature appear as the most important for microalgae productivity (Gonçalves et al., 2016). Besides, the light intensity and daily and seasonal variations of the sunlight and temperature that affect photosynthetic efficiency and, consequently, biomass production are also fundamental. In this context, Table 2 shows the dynamics of the kinetic parameters displayed by *Scenedesmus obliquus* CPCC05 in different geographical positions.

Table 2. Kinetic parameters for *Scenedesmus obliquus* CPCC05 in different geographic locations.

Köppen-Geiger	Geographical Positions	X_{max} (g/L)	P_X (g/L. d)	RT (d)	μ_{max} (d^{-1})	tg (d)	L (%)	P_L (g/L.d)
Aw	Caracas-VEN	1.96^{hij}±0.09	0.232^{g}±0.01	8^{abcd}±0.00	0.39^{hij}±0.02	1.77^{gh}±0.08	7.19^{v}±0.29	0.016^{kl}±0.00
	Jos-NGA	2.10^{fgh}±0.10	0.199^{i}±0.00	10^{ab}±0.00	0.34^{jkl}±0.01	2.03^{e}±0.10	11.79^{k}±0.47	0.023^{ghij}±0.01
	Bandundu-COD	2.06^{fghi}±0.08	0.279^{cd}±0.01	7^{abcde}±0.00	0.53^{cde}±0.02	1.30^{m}±0.06	11.37^{l}±0.45	0.031^{cdef}±0.00
	Bimbo-CAF	2.08^{fghi}±0.10	0.219^{h}±0.01	9^{abc}±0.00	0.58^{c}±0.03	1.19^{o}±0.05	14.57^{d}±0.36	0.032^{cde}±0.00
	Mopti-MLI	2.88^{a}±0.11	0.347^{a}±0.02	8^{abcd}±0.00	0.58^{c}±0.02	1.19^{o}±0.06	13.87^{f}±0.21	0.048^{a}±0.01
	Darwin-AUS	2.60^{b}±0.10	0.277^{cd}±0.01	9^{abc}±0.00	0.84^{a}±0.04	0.82^{q}±0.04	12.56^{i}±0.25	0.034^{bcd}±0.00
	San Salvador-SLV	1.80^{jk}±0.09	0.188^{ij}±0.00	9^{abc}±0.00	0.42^{ghi}±0.02	1.65^{i}±0.08	14.27^{e}±0.38	0.026^{fgh}±0.00
	Ubon Ratchathani-THA	2.18^{efgh}±0.10	0.259^{e}±0.01	8^{abcd}±0.00	0.55^{c}±0.02	1.26^{mn}±0.06	15.04^{b}±0.60	0.036^{bc}±0.00
	Cuiabá-BRA	1.70^{kl}±0.08	0.159^{l}±0.00	10^{ab}±0.00	0.54^{cd}±0.02	1.28^{m}±0.05	13.60^{g}±0.19	0.021^{hijk}±0.00
Am	Nieuw Nickerie-SUR	2.56^{bc}±0.12	0.307^{b}±0.02	8^{abcd}±0.00	0.82^{a}±0.03	0.84^{q}±0.03	6.76^{w}±0.17	0.020^{ijk}±0.00
	Makassar-IDN	2.10^{fgh}±0.10	0.285^{c}±0.01	7^{abcde}±0.00	0.48^{ef}±0.01	1.44^{kl}±0.05	8.69^{q}±0.22	0.024^{ghi}±0.00
	Manaus-BRA	2.28^{def}±0.11	0.242^{fg}±0.01	9^{abc}±0.00	0.30^{lm}±0.01	2.31^{c}±0.09	14.75^{c}±0.39	0.035^{bcd}±0.02
Af	Kundiawa-PNG	1.56^{l}±0.07	0.145^{m}±0.00	10^{ab}±0.00	0.32^{kl}±0.01	2.16^{d}±0.08	10.18^{p}±0.09	0.014^{l}±0.01
	Kota Bharu-MYS	2.44^{bcd}±0.12	0.234^{g}±0.01	10^{ab}±0.00	0.57^{c}±0.02	1.21^{no}±0.04	13.18^{h}±0.14	0.030^{def}±0.00
As	Aracaju-BRA	2.04^{ghi}±0.10	0.277^{cd}±0.01	7^{abcde}±0.00	0.47^{fg}±0.01	1.47^{k}±0.05	11.46^{l}±0.42	0.031^{cdef}±0.00
BWh	Toliara-MDG	2.18^{efgh}±0.11	0.259^{e}±0.01	8^{abcd}±0.00	0.49^{def}±0.01	1.41^{l}±0.05	7.82^{s}±0.21	0.020^{ijk}±0.00
	Taif-SAU	2.26^{defg}±0.	0.196^{i}±0.00	11^{a}±0.00	0.44^{fgh}±0.02	1.57^{j}±0.06	7.35^{u}±0.11	0.014^{l}±0.00
	Al-Fashir-SDN	2.54^{bc}±0.02	0.271^{d}±0.01	9^{abc}±0.00	0.66^{b}±0.03	1.05^{p}±0.04	12.06^{j}±0.14	0.032^{cde}±0.02
	Lobito-AGO	2.24^{defg}±0.09	0.237^{g}±0.01	9^{abc}±0.00	0.44^{fgh}±0.02	1.57^{j}±0.06	13.32^{h}±0.26	0.031^{cdef}±0.00
	Nouadhibou-MRT	2.10^{fgh}±0.10	0.249^{ef}±0.01	8^{abcd}±0.00	0.49^{def}±0.02	1.41^{l}±0.06	7.51^{t}±0.41	0.018^{jkl}±0.00
	Roebourne-AUS	2.22^{defg}±0.08	0.233^{g}±0.00	9^{abc}±0.00	0.68^{b}±0.03	1.01^{p}±0.04	13.54^{g}±0.23	0.031^{cdef}±0.00
	Ali Sabih-DJI	2.66^{ab}±0.13	0.284^{c}±0.01	9^{abc}±0.00	0.57^{c}±0.02	1.21^{no}±0.04	13.66^{g}±0.51	0.038^{b}±0.01
BWk	Tacna-PER	0.46^{m}±0.02	0.051^{no}±0.00	7^{abcde}±0.00	0.23^{n}±0.00	3.01^{a}±0.10	14.00^{f}±0.38	0.007^{m}±0.00
BSh	León-MEX	1.76^{jkl}±0.08	0.184^{jk}±0.00	9^{abc}±0.00	0.39^{hij}±0.01	1.77^{gh}±0.07	15.31^{a}±0.05	0.028^{efg}±0.00
	Kasane-BWA	1.86^{ijk}±0.09	0.175^{k}±0.00	10^{ab}±0.00	0.34^{jkl}±0.01	2.03^{e}±0.08	11.12^{m}±0.29	0.019^{ijkl}±0.00
	Jamaame-SOM	2.36^{cde}±0.11	0.272^{d}±0.01	9^{abc}±0.00	0.69^{b}±0.03	1.00^{p}±0.03	13.60^{g}±0.18	0.036^{bc}±0.00
BSk	Potosí-BOL	0.12^{n}±0.00	0.009^{q}±0.00	2^{d}±0.00	-	-	8.83^{q}±0.14	0.0008^{n}±0.00

Cfa	São José dos Campos-BRA	$0.46^{m}\pm0.02$	$0.060^{n}\pm0.00$	$6^{abcde}\pm0.00$	$0.25^{mn}\pm0.01$	$2.77^{b}\pm0.09$	$10.42^{o}\pm0.07$	$0.006^{mn}\pm0.00$
Cfb	Kisoro-UGA	$0.26^{mn}\pm0.01$	$0.039^{p}\pm0.00$	$4^{cde}\pm0.00$	$0.23^{n}\pm0.00$	$3.01^{a}\pm0.11$	$10.50^{no}\pm0.32$	$0.004^{mn}\pm0.00$
Csa	Jabalpur-IND	$1.86^{ijk}\pm0.09$	$0.219^{h}\pm0.01$	$8^{abcd}\pm0.00$	$0.40^{hi}\pm0.02$	$1.73^{h}\pm0.07$	$7.84^{s}\pm0.20$	$0.017^{kl}\pm0.00$
Csb	Ibarra-ECU	$0.24^{mn}\pm0.02$	$0.046^{op}\pm0.00$	$3^{de}\pm0.00$	$0.32^{kl}\pm0.01$	$2.16^{d}\pm0.08$	$8.50^{r}\pm0.25$	$0.003^{mn}\pm0.00$
Cwa	Mackay-AUS	$1.72^{kl}\pm0.08$	$0.162^{l}\pm0.00$	$10^{ab}\pm0.00$	$0.37^{ijk}\pm0.01$	$1.87^{f}\pm0.07$	$10.60^{n}\pm0.40$	$0.017^{kl}\pm0.00$
Cwb	Lichinga-MOZ	$0.36^{m}\pm0.002$	$0.053^{no}\pm0.00$	$5^{bcde}\pm0.00$	$0.38^{ij}\pm0.01$	$1.82^{fg}\pm0.09$	$13.18^{h}\pm0.09$	$0.006^{mn}\pm0.00$

Different letters within the same column express significant differences between the means ($p < 0.05$).
X_{max}: maximum biomass densities (g/L); μ_{max}: maximum specific growth rate (d^{-1}); L: lipid content (%); P_L: lipid productivity (g/L.d); RT: cellular residence time (d); tg: generation time (d); P_X: biomass productivity (g/L.d).

As can be seen, the spatial and temporal variation of light intensity, day length and temperature resulted in biomass and lipid productivities statistically different. These results emphasize the influence of the local climate on systems productivity. Among the simulations, Mopti-MLI presented the best condition for biomass (0.347 g/L.d) and lipid (0.48 g/L.d) productivity, with maximum biomass density of 2.88 g/L, maximum specific growth rate of 0.58 d^{-1}, generation time of 1.19 d and residence time of 8 d. The biomass productivity achieved under the climatic conditions of Nieuw Nickerie-SUR (0.307 g/L.d), Makassar-IDN (0.285 g/L.d), Ali Sabih-DJI (0.284 g/L.d), Bandundu-COD (0.279 g/L.d), Darwin-AUS (0.277 g/L.d) and Aracaju-BRA (0.277 g/L.d) and the lipid productivity of Ali Sabih-DJI (0.038 g/L.d), Ubon Ratchathani-THA (0.036 g/L.d), Jamaame-SOM (0.036 g/L.d), Manaus-BRA (0.035 g/L.d) and Darwin-AUS (0.034 g/L.d) were also high. In contrast, Potosí-BOL, Kisoro-UGA, Ibarra-ECU, Tacna-PER, Lichinga-MOZ and São José dos Campos-BRA presented the worst performances.

In the exploratory analysis (data not are shown), the temperature presented the highest factor loading (0.89). The results from table 2 clearly show that biomass production and, consequently, the productivity of *Scenedesmus obliquus* CPCC05 was positively affected when the temperature used ranged from 25.64 to 28.75 °C. Controversially, the climatic conditions from geographical positions with temperatures below 20 °C resulted in unsatisfactory performance. By way of comparison, to the low temperatures of the geographical positions evaluated by de Jesus et al. (2018) resulted in the lowest growth rates of *Spirulina* sp. LEB-18. Thus, based on the results, it is possible to suggest that the optimum growth temperature of the *Scenedesmus obliquus* CPCC05 is in the range of 25 to 29 °C. In parallel, El-Sheekh et al. (2017) when assessing the effect of temperature on growth of *Scenedesmus acutus* report a similar ideal temperature range, ranging from 25 to 30 °C.

According to Chang et al. (2017), the effect of temperature on microalgae growth presents a typical bell-shaped curve, in which growth rates increase with increasing temperature until the optimum temperature is reached and, posteriorly, an accented decline occurs. Therefore, at an optimal temperature, the culture expresses its maximum metabolic potential and is expected that reduced or excessive temperatures affect cellular metabolism, resulting in biomass losses and changes in metabolite production (Suparmaniam et al., 2019). Notoriously, although light intensity does not have present a significant factor load (0.53), it is unlikely that not impact on microalgae productivity, since light is the energy source to realize photosynthesis. The most reasonable cause for this gap would be that the range of light intensity used was within the ideal for cultivation, not causing deficits in the microalgae growth.

The *Scenedesmus obliquus* CPCC05 microalgae proved to be tolerant of a wide temperature range (8.48 to 28.75 °C) and light intensity (1429.0 to 2337.3 $\mu mol.m^{-2}.s^{-1}$), which makes it suitable for commercial exploitation under a variety of climate conditions. The selection of sites for the establishment of a microalgae industrial unit should take into account its trend and climate variability. The temporal and spatial variation of climate is mainly influenced by latitude and altitude. Normally, the farther the geographical positions of the equator line (0 ° latitude), lower the temperature, this because the incidence of sunlight is minor. However, in the same form, there is a relationship between temperature and altitude. Theoretically, how much higher the altitude, minor the temperature and vice versa. In this context, note that Jamaame-SOM and Ibarra-ECU, for example, have the same latitude, but temperatures of 27.66 °C and 15.00 °C, respectively. It this is because the altitude of Jamaame-SOM is 11 m, while that of Ibarra-ECU is 2228 m. In this sense, considering the influence of the geographic factors about the atmospheric elements essential for microalgae

cultivation is crucial for the implantation of microalgae-based production systems and processes.

With regard to the climatic classification of the evaluated geographical positions, it was possible to determine by the climate those with the best performances. Namely, the climates Af, As, Am, AW, BSh, BWh, Csa and Cwa presented satisfactory productivities while BSK, BWK, Cfa, Cfb, Csb, Cwb presented unsatisfactory productivities. It this can be explained since the determination of the climate types considers the seasonality and annual and monthly average values of air temperature. Lastly, it is possible to allege that the choice of a determined geographical position can be made in function of the climatic type and the temporal variation of the atmospheric elements essential for cultivation, i.e., light intensity, day length and temperature.

3.2 Effects of the geographic position on fatty acids profile

In our study, under the simulated climatic conditions, *Scenedesmus obliquus* CPCC05 accumulated from 6.76 to 15.31% of lipids in the dry weight of its cells (Table 2). The lipids consisted mainly of palmitic, oleic, linoleic and alpha-linolenic acid (Table 3). It this profile is typical of most microalgae and similar to that of higher plants. Its proportion in the different profiles can be attributed to the culture conditions. Beyond the fatty acids described in Table 3, were identified the arachidonic, docosahexaenoic, and eicosapentaenoic acids, but in small concentrations, which were considered in the sum of the minority fatty acids. These compounds, considered essential, are a valuable food complement (Shanab et al., 2018).

Table 3. Fatty acid profile of *Scenedesmus obliquus* CPCC05 under different climatic conditions.

		Fatty acids															
Köppen-Geiger	Geographical Positions	C11:0 (%)	C12:0 (%)	C13:0 (%)	C16:0 (%)	C16:1 (%)	C17:1 (%)	C18:0 (%)	C18:1n9c (%)	C18:2n6c (%)	C18:3n3 (%)	C20:2 (%)	C21:0 (%)	Minorities (%)	SFA (%)	MUFA (%)	PUFA (%)
Aw	Caracas-VEN	ND	ND	ND	37.87±0.49	3.89±0.06	ND	3.03±0.20	19.33±1.03	8.31±0.04	19.11±0.38	5.99±0.11	ND	2.47±0.11	42.31^{j}	23.36^{i}	34.33^{v}
	Jos-NGA	ND	ND	ND	29.99±0.78	3.82±0.23	ND	1.08±0.02	15.97±0.22	11.20±0.52	30.66±0.39	5.93±0.25	ND	1.35±0.06	31.84^{z}	19.87^{n}	48.29^{h}
	Bandundu-COD	ND	0.60±0.04	2.29±0.14	30.66±1.47	3.68±0.31	ND	1.70±0.09	14.24±0.27	10.30±0.36	31.30±0.85	ND	3.45±0.24	1.78±0.09	39.73^{mn}	18.08^{r}	42.19^{p}
	Bimbo-CAF	ND	0.40±0.02	1.46±0.01	29.40±0.52	3.11±0.09	ND	3.59±0.10	15.74±0.39	11.31±0.96	29.11±1.56	ND	3.24±0.08	2.63±0.13	39.35^{o}	19.28^{o}	41.37^{q}
	Mopti-MLI	1.70±0.00	ND	ND	26.45±0.71	0.34±0.01	4.89±0.04	10.40±0.43	11.65±0.10	8.45±0.05	33.78±0.15	ND	ND	2.35±0.03	39.56^{n}	17.06^{s}	43.39^{n}
	Darwin-AUS	1.24±0.03	ND	ND	26.01±0.04	0.35±0.01	4.70±0.13	5.40±0.01	14.88±0.76	10.40±0.04	34.82±0.59	ND	ND	2.19±0.04	33.60^{w}	20.16^{m}	46.25^{k}
	San Salvador-SLV	0.82±0.04	ND	ND	27.67±0.70	0.54±0.03	2.80±0.14	4.70±0.44	11.01±1.13	9.79±0.06	40.26±2.19	ND	ND	2.42±0.01	34.24^{u}	14.49^{v}	51.26^{e}
	Ubon Ratchathani-THA	2.46±0.17	ND	ND	32.47±0.08	0.49±0.04	2.03±0.06	8.46±0.43	17.89±0.15	10.66±0.09	20.69±0.69	ND	ND	4.86±0.13	44.77^{f}	20.78^{l}	34.45^{u}
	Cuiabá-BRA	0.78±0.03	ND	ND	28.96±0.06	0.47±0.02	3.31±0.10	3.28±0.25	14.75±0.86	10.96±0.20	34.60±0.89	ND	ND	2.88±0.05	34.11^{u}	18.85^{p}	47.04^{j}
Am	Nieuw Nickerie-SUR	ND	ND	ND	39.18±0.47	4.05±0.22	ND	2.99±0.13	23.27±0.05	7.01±0.15	17.41±0.25	4.06±0.31	ND	2.03±0.07	43.44^{i}	27.39^{c}	29.17^{x}
	Makassar-IDN	ND	ND	ND	42.43±0.98	2.10±0.09	ND	2.61±0.19	20.71±0.24	6.92±0.52	16.12±0.28	3.49±0.05	ND	5.61±0.03	47.05^{c}	26.01^{e}	26.94^{A}
	Manaus-BRA	0.56±0.02	ND	ND	26.74±0.21	0.31±0.01	4.53±0.21	6.74±0.05	12.98±0.06	10.49±0.14	35.34±0.30	ND	ND	2.32±0.06	34.92^{t}	18.03^{r}	47.06^{j}
Af	Kundiawa-PNG	ND	ND	ND	39.36±0.26	1.08±0.07	ND	2.57±0.03	21.34±0.43	7.88±0.38	18.75±0.43	5.25±0.45	ND	3.77±0.03	44.54^{g}	22.91^{j}	32.55^{w}
	Kota Bharu-MYS	0.94±0.04	ND	ND	28.91±0.34	0.31±0.01	3.21±0.11	8.78±0.83	17.33±0.86	9.47±0.34	28.90±0.20	ND	ND	2.77±0.13	39.73^{mn}	21.16^{k}	39.84^{r}
As	Aracaju-BRA	ND	0.55±0.04	2.13±0.14	31.32±1.48	3.35±0.32	ND	1.60±0.10	14.57±0.28	10.14±0.37	31.42±0.86	ND	3.18±0.24	1.73±0.09	39.78^{m}	18.08^{r}	42.14^{p}
BWh	Toliara-MDG	ND	ND	ND	45.48±1.41	0.33±0.02	ND	3.21±0.25	28.24±0.14	7.26±0.54	13.06±0.64	0.23±0.01	0.06±0.00	2.19±0.12	49.91^{b}	28.72^{a}	21.37^{C}
	Taif-SAU	ND	ND	ND	39.56±0.73	3.17±0.11	ND	3.46±0.13	24.43±0.38	7.80±0.43	15.12±0.39	4.62±0.04	ND	1.84±0.08	44.12^{h}	27.72^{b}	28.16^{z}
	Al-Fashir-SDN	ND	ND	ND	40.30±2.67	2.06±0.20	ND	2.77±0.26	22.95±0.62	9.13±0.89	19.24±1.20	0.03±0.00	0.04±0.00	3.50±0.12	46.01^{e}	25.10^{f}	28.89^{y}
	Lobito-AGO	ND	0.44±0.03	1.70±0.14	31.07±0.40	2.99±0.15	ND	2.56±0.22	15.39±1.14	11.79±0.25	29.04±1.14	ND	3.11±0.11	1.92±0.05	40.01^{l}	18.57^{q}	41.42^{q}
	Nouadhibou-MRT	ND	0.91±0.07	3.43±0.24	56.11±2.79	0.42±0.03	ND	1.78±0.12	6.11±0.59	5.58±0.52	19.41±1.67	ND	1.85±0.08	4.38±0.12	65.68^{a}	8.84^{B}	25.48^{B}
	Roebourne-AUS	1.05±0.08	ND	ND	26.80±0.37	0.32±0.00	3.59±0.01	4.50±0.11	19.55±0.63	10.53±0.53	31.19±0.32	ND	ND	2.46±0.00	33.38^{x}	23.76^{h}	42.86^{o}
	Ali Sabih-DJI	1.24±0.03	ND	ND	30.38±0.25	0.31±0.00	2.0±0.05	4.84±0.23	24.51±0.20	10.43±0.03	23.18±0.18	ND	ND	3.11±0.06	36.60^{r}	27.26^{d}	35.14^{t}
BWk	Tacna-PER	ND	2.27±0.22	1.36±0.12	27.65±0.16	0.60±0.04	ND	1.34±0.06	7.08±0.38	6.07±0.24	51.39±0.85	ND	ND	2.23±0.10	33.92^{v}	8.28^{C}	57.78^{a}
BSh	León-MEX	ND	2.36±0.07	1.40±0.03	28.85±0.26	0.33±0.02	ND	2.10±0.04	15.59±0.54	12.54±0.10	35.09±0.24	ND	ND	1.60±0.23	35.54^{s}	16.55^{u}	47.91^{i}
	Kasane-BWA	ND	2.37±0.06	1.44±0.07	29.52±0.74	0.41±0.02	ND	2.08±0.18	13.25±1.22	15.16±1.28	33.04±1.91	ND	ND	2.73±0.10	37.22^{q}	14.29^{w}	48.49^{g}
	Jamaame-SOM	1.03±0.07	ND	ND	23.36±1.18	0.43±0.02	5.01±0.11	7.12±0.51	11.14±0.59	9.60±0.62	39.74±2.41	ND	ND	3.12±0.61	32.93^{y}	16.82^{t}	51.13^{f}
BSk	Potosí-BOL	ND	1.37±0.09	0.74±0.05	23.28±0.27	0.57±0.04	0.08±0.01	2.10±0.19	8.89±0.88	10.28±0.05	44.62±0.37	ND	4.97±0.14	3.10±0.16	34.17^{u}	10.74^{A}	55.09^{c}
Cfa	São José dos Campos-BRA	ND	1.14±0.07	0.53±0.03	27.06±1.18	0.33±0.03	0.13±0.01	1.61±0.16	17.66±0.76	9.44±0.29	33.89±1.66	ND	5.55±0.37	2.64±0.15	37.10^{q}	19.20^{o}	43.70^{m}

Cfb	Kisoro-UGA	ND	1.84±0.00	1.04±0.01	34.93±0.23	1.76±0.03	ND	1.88±0.17	11.67±0.24	6.20±0.03	37.94±0.12	ND	ND	2.75±0.06	41.27^{k}	13.87^{x}	44.86^{l}
Csa	Jabalpur-IND	ND	ND	ND	41.07±0.52	1.71±0.04	ND	2.66±0.09	22.31±0.32	7.62±0.10	16.64±0.36	3.90±0.03	ND	4.08±0.07	46.46^{d}	24.56^{g}	28.98^{y}
Csb	Ibarra-ECU	ND	1.58±0.12	0.96±0.03	26.63±0.09	0.62±0.04	0.10±0.00	1.45±0.08	9.66±0.17	3.67±0.18	48.83±0.92	ND	3.70±0.17	2.80±0.19	35.65^{s}	11.67^{z}	52.68^{d}
Cwa	Mackay-AUS	ND	0.19±0.01	ND	29.56±0.68	2.88±0.19	ND	0.78±0.04	10.09±0.49	10.17±0.64	38.31±1.29	6.62±0.49	ND	1.40±0.02	31.39^{A}	13.07^{y}	55.54^{b}
Cwb	Lichinga-MOZ	2.51±0.01	ND	ND	25.95±1.14	3.83±0.44	1.26±0.09	7.62±0.08	17.54±0.23	6.14±0.05	29.63±1.07	ND	ND	5.52±0.02	38.55^{p}	23.39^{i}	38.07^{s}

Values with the same letter in the same column indicate that there is no statistically significant difference ($p \leq 0.05$). Values are the mean ± SD of three replicates.
ND: not detected; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

As can be seen, the proportion of SFAs, MUFAs and PUFAs varied from 31.39 to 65.68%, from 8.28 to 28.72% and from 21.37 to 57.78%, respectively. Twelve of the thirty-three profiles showed a higher content of SFAs and twenty-one of PUFAs, both comprising more than a third of the total fatty acids. These findings indicate that the local climate may induce lipid synthesis for a specific commercial application. Namely, profiles with a higher content of SFAs favor the biodiesel production, since contribute positively to important properties of this product such as oxidative stability. On the other hand, profiles with higher PUFAs content are suitable for application in food supplements (Vendruscolo et al., 2018; Dias et al., 2019).

The geographical positions that stood out by the content of SFAs were Nouadhibou-MRT (65.68%), followed by Toliara-MDG (49.91%), Makassar-IDN (47.05%) and Jabalpur-IND (46.46 %). On the other hand, those with the highest content of MUFAs were Toliara-MDG (28.72%), Taif-SAU (27.72%), Nieuw Nickerie-SUR (27.39%) and Ali Sabih-DJI (27.26%) and with the highest content of PUFAs were Tacna-PER (57.78%), Mackay-AUS (55.54%), Potosí-BOL (55.09%) and Ibarra-ECU (52.68%). Concomitantly, the positions that stood out for the high production of alpha-linolenic acid were Tacna-PER (51.39%), Ibarra-ECU (48.83%) and Potosí-BOL (44.62%).

According to previous studies, low-temperatures reduce the fluidity of the cell membranes and, to maintain structural integrity, the cells tend to synthesize unsaturated fatty acids. Thus, it is postulated that under low-temperature conditions the proportion of PUFAs is increased while the proportion of SFAs and MUFAs increases with increasing temperature (Aussant et al., 2018). It this trend was observed in the present study, in which the biomass of *Scenedesmus obliquus* CPCC05 cultivated under relatively low-temperatures (8-15 °C) showed an increased content of PUFAs, especially alpha-linolenic acid.

However, an effective standard has not been constated. It was observed that some geographical positions with somewhat high temperatures presented higher concentrations of PUFAs than SFAs and MUFAs. It this can be seen in the simulation of the climatic conditions of Jamaame-SOM. The temperature used to simulate the Jamaame-SOM climate was 27.66 °C. Under this temperature, was expected an increase in the content of SFAs and MUFAs, however, it presented one of the best conditions for the production of alpha-linolenic acid (39.74%) and PUFAs (51.13%). Considering this, it is noteworthy that, when simulating real climatic conditions, we evaluate the conjugation of three crucial variables for prospecting microalgae. As most studies are based on the modification of a single variable, temperature or light intensity, the combined effect and the spatial variability of both on the production of metabolites are neglected (Behera and Balasubramanian, 2018).

In addition, although the exploratory analysis shows only for temperature a significant factor load (0.73), it is known that in photoautotrophic cultures the intensity and duration of the light can undoubtedly affect the production of intracellular metabolites. Therefore, it is possible to conclude that the results were reflex not only of temperature but of the correlated effect between light intensity, day length and temperature.

Finally, with respect to climatic typology, it was not possible to distinguish, based on the climatic group, the one with the potential for greater production of saturated, monounsaturated or polyunsaturated fatty acids. However, it was observed a propensity based on the temperature criteria of each climate. Climates characterized by milder temperatures such as BWk and BSk, were among those with the best PUFAs values and the worst SFAs and MUFAs values. On the other hand, climates such as Am and BWh presented the opposite behavior.

4 Conclusions

The results evidence the effects of the spatial and temporal variation of the climate on the photosynthetic metabolism of the microalgae *Scenedesmus obliquus* CPCC05. The mapping of photobioreactor performance provides a data base for selecting places with potential for mass microalgae production. Under the premises of this study, the type of climate and the assessment of the conjugation between atmospheric elements and geographic factors of a spatial location may provide guidelines on their potential for large-scale cultivation of phototrophic microorganisms.

Declaration of Competing Interest

We declare that we have no conflict of interest.

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CONCLUSÃO GERAL

A performance de *Scenedesmus obliquus* CPCC05 em fotobiorreator foi influenciada pela variação espacial e temporal do clima. O efeito da latitude e altitude sobre parâmetros-chave para produção de biomassa e metabólitos intracelulares foi demonstrado.

Sob a intensidade de luz de 2100.9 $\mu mol.m^{-2}.s^{-1}$, temperatura de 28.75 °C e duração do dia de 12.52 h obteve-se a maior produtividade de biomassa (0.347 g/L.d) e lipídeos (0.048 g/L.d). A produtividade de biomassa variou de 0.009 a 0.347 g/L.d e a produtividade de lipídeos de 0.0008 a 0.048 g/L.d para as diferentes posições geográficas.

Variações na proporção de ácidos graxos saturados (31.39 a 65.68%), monoinsaturados (8.28 a 28.72%) e poli-insaturados (21.37 a 57.78%) sugerem que a posição geográfica pode influenciar na síntese de lipídeos para produção de biodiesel ou suplementos alimentares. Levando isso em consideração, seria possível canalizar o processo para uma aplicação comercial específica.

Sob as premissas deste estudo, o tipo climático e a avaliação da conjugação entre elementos atmosféricos e fatores geográficos de uma localização espacial podem fornece diretrizes sobre o seu potencial para o cultivo em larga escala de microalgas.

CAPÍTULO 3

APÊNDICE A - INTRODUCTORY CHAPTER: BIOTECHNOLOGY AND BIOENGINEERING

Capítulo de livro publicado em IntechOpen, 2019[1].

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[1]O manuscrito foi formatado conforme as normas exigidas pela Editora.

Chapter

Introductory Chapter: Biotechnology and Bioengineering

Rosangela Rodrigues Dias, Leila Queiroz Zepka and Eduardo Jacob-Lopes

1. A general overview on biotechnology and bioengineering

Biotechnology and bioengineering can be defined as "the integration of natural sciences and engineering sciences in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services" [1]. Although these areas overlap, depending upon the use of techniques and their applications, both have peculiar characteristics. While the focus of bioengineering is the implementation of engineering principles and design concepts in biology, biotechnology is more focused on the natural sciences [2].

These fields, today considered priority, are the fruit of strategies from various areas that aim to benefit humanity and its environment. Biotechnology and bioengineering, however, despite growing attention in recent decades, are not a new science. Humans have been developing them since the earliest beginnings, mainly in food production. Some ancestral examples include the preparation of fermented beverages from cereals in Babylon and Egypt (8000 to 6000 years BC); the production of bread, using ferment in Egypt (4000 years BC); and wine production in Greece (2000 years BC). Historically, the use of these traditional techniques in this period of history is called discoveries, and not development, once the underlying scientific principles were not understood [3, 4].

Indeed today, the biotechnology and bioengineering based on scientific progress find applications in several areas, including agriculture, livestock, human health, preservation of the environment, and manufacturing industry [5]. This wide applicability was only possible due to the combination of several fields of knowledge that include biochemistry, physiology, genetics, microbiology, virology, botany, zoology, ecology, computer science, and chemical engineering.

Therefore, this is a field of work typically multidisciplinary, which makes the effective collaboration and integration of professionals from different areas of knowledge absolutely indispensable so that all potential of biotechnology and bioengineering can be exploited. The interface between these fields is now understood not only as a "science" to learn about nature but also as a "technology" of susceptible alteration. The intersection between biotechnology and bioengineering and its kindred disciplines proved their economic importance, being capable to expand and promote the manufacture of products and services, besides modifying processes in favor of human benefit [6–8].

The current scenario points for biotechnology and bioengineering as being the main technology of the twenty-first century should be absorbed by the general public. Undoubtedly, knowledge of the principles of vital processes already achieved will proportionate changes significant in the society. Therefore, it is important to

ensure a broad awareness of what these two fields of knowledge involve and what the consequences of accepting or rejecting the innovations [9].

Thus, the chapters presented in this book are intended to help provide a deeper understanding about the recent progresses on biotechnology and bioengineering contributing substantially to the consolidation of bio-based processes and products.

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APÊNDICE B - BIODIESEL FROM MICROALGAE

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Chapter 6

BIODIESEL FROM MICROALGAE

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ABSTRACT

Biodiesel is an alternative to petroleum-derived fuels and its production from microalgae has attracted a lot of attention in recent years due to its environmental compatibility and biodegradability. Microalgae are promising sources of oil and their potential for biodiesel production is devoid the main disadvantages associated with first and second-generation fuels. The high achievable yields for both lipids and biomass, combined with their valuable co-products, if purposely exploited, can increase the economic viability of microalgae as a source of biodiesel. Thus, the aim of this chapter is to provide an overview of various aspects associated with biodiesel production from microalgae.

Keywords: algae, single-cell oil, biofuel

1. INTRODUCTION

Microalgae are photosynthetic microorganisms with simple growth requirements that convert sunlight, carbon dioxide and water into biomass in short periods of time. They can provide several different types of renewable biofuels. These include the biodiesel derived from microalgae oil (Ramanna, Rawat and Bux 2017). The idea of using

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microalgae as a source of fuel is not recent, but it is now being taken seriously because of the escalating price of petroleum and, more significantly, through the emerging concern about global warming that is associated to the use of fossil fuel (Tan and Lee 2016).

Research on microalgae for biofuels started in the 1920s in Germany where *Chlorella* strains were found to be capable of accumulating a large amount of lipids, mainly by nutritional stress (Gouveia et al. 2017). In the following decades, successive oil crises gave researchers the opportunity to reopen the field with highly ambitious projects. Thus, biodiesel microalgae-based so far has been the most studied biofuel.

Biodiesel ordinarily is produced by transesterification of either oil from crops, such as palm, soybean, rape, and sunflower, or from animal fat and used oils (Gonzalez-Fernandez and Muñoz 2017). However, the use of microalgae may be a more promising alternative, for being a good producer of oil and a more versatile biomass source (Shuba and Kifle 2018). The most productive oil crops, such as palm oil, do not approach microalgae to be able to sustainably supply the necessary amounts of biodiesel (Konur 2018).

In this context, the use of microalgal biomass as a third-generation biofuel feedstock is an alternative to first and second generations since this could allow to produce high-value compounds (Gonzalez-Fernandez and Muñoz 2017). Therefore, based on current projections of knowledge and technology, the biodiesel derived from microalgae is a technically auspicious energy resource.

Microalgae can contribute to a sustainable and economic way to produce biofuels if the right conditions are in operation. However, it is evident that considerable investment in technological development and technical expertise is still needed before algae biodiesel is economically viable and can become a reality. In this sense, the purpose of the chapter was evaluating: (1) the main microalgae species producing single cell oil, (2) the oil production and extraction processes, (3) the biodiesel synthesis, (4) the quality characteristics, (5) the environmental aspects and (6) the economic analysis of microalgae biodiesel.

2. Main Microalgae Species to Producing Single-Cell Oil

As superior plants, microalgae use sunlight to produce structural lipids and storage lipids, as well as a wide variety of metabolites (Tan and Lee 2016). The average lipid content in microalgae varies between 1 and 70% of dry weight. However, most common microalgae (*Chlorella*, *Crypthecodinium*, *Dunaliella*, *Isochrysis*, *Nannochloris*, *Nannochloropsis*, *Phaeodactylum* and *Tetraselmis*) have oil levels between 20 and 50% (Mata, Martins and Caetano 2010) (Table 1).

Table 1. Oil content of some microalgae

Microalgae	Oil content (% by dry weight)	Microalgae	Oil content (% by dry weight)
Ankistrodesmus sp.	24-31	*Dunaliella* sp.	17.5-67
Botryococcus braunii	25-75	*Dunaliella tertiolecta*	16.7-71
Chlorella emersonii	25-63	*Isochrysis* sp.	7.1-33
Chlorella minutissima	57	*Nannochloris* sp.	20-56
Chlorella protothecoides	14.6-57.8	*Nannochloropsis* sp.	12-53
Chlorella sorokiniana	19-22	*Neochloris oleoabundans*	29-65
Chlorella sp.	10-48	*Pavlova lutheri*	35.5
Chlorella vulgaris	5-58	*Phaeodactylum tricornutum*	18-57
Crypthecodinium cohnii	20 -51.1	*Scenedesmus obliquus*	11-55
Dunaliella primolecta	23.1	*Spirulina maxima*	4-9
Dunaliella salina	6-25	*Tetraselmis suecica*	8.5-23

Source: Adapted from Mata, Martins and Caetano (2010).

Chlorella is considered a good option for biodiesel production. However, other species are as efficient and productive as this one. That is why, the selection of species needs to take into account other factors, as for exemple, the ability of microalgae to develop using specific environmental conditions (Mata, Martins and Caetano 2010).

In addition, for biodiesel production, lipid productivity is a desirable key characteristic at microalgae. Lipid productivity (mg L^{-1} h^{-1}) is the product of lipid content (%) and biomass productivity (mg L^{-1}), hence, dependent on both.

The lipid content (%) may be enhanced under certain conditions, as for exemple, nutrient stress. Microalgae grown under nutrient starvation accumulate high content of lipids (%), however have low biomass productivity (mg L^{-1}), while microalgae grown under optimal conditions have large biomass productivity (mg L^{-1}), but with low lipid content (%). Producing lipids while maintaining high growth rates is vital for biodiesel production because high biomass productivity increases yield per harvest volume while high lipid content decreases the cost of extraction (Tan and Lee 2016).

Thus, it can be pointed out that application of nutrient stress might be useful for enhancing lipid content on dry weight basis, but fail to increases significantly lipid productivity, because the increase in lipid content occurs at the cost of biomass (Bhowmick, Koduru and Sen 2015). In this context, a two-stage culture with initial optimization of the biomass and final optimization of the lipid content is indicated for the high lipid productivity.

By eventuality, environmental and culturing condition variations, besides alter lipid production, also alter chemical composition of microalgae (Zhu, Li and Hiltunen 2016). For example, Fatty acid profile microalgal species *Chlamydomonas reinhardtii* varies under salt stress conditions. It is remarkable that palmitic acid (C16:0) and linolenic acid (C18:3n3) increase significantly at higher salinities concentrations (Hounslow et al.

2016). For biodiesel production, the suitability of lipids in terms of degree of saturation and proportion of total lipids constituted by triglycerides is relatively important, as well as their lipid productivity.

Insufficiently rigorous data on the impact of physiological stress on biomass productivity limit the comparison of lipid productivity under nutrient scarcity in many microalgae species. However, according Griffiths and Harrison (2009), under conditions of nutrient sufficient, three species stand out as having very high lipid productivities, above: *Amphora*, *Ettlia oleoabundans* (formerly *Neochloris oleoabundans*) and *Ankistrodesmus falcatus*. Productivity of *Amphora* is the product of a high lipid content and a modest growth rate. The lipid productivities of *E. oleoabundans* and *A. falcatus* are a function of their high growth rates and lipid contents.

Currently, the biotechnological application is focused on four main microalgae: a) Spirulina (Arthrospira platensis), b) Chlorella vulgaris, c) Dunaliella salina and d) Haematococcus pluvialis. The green algae (Chlorophyceae) Chlorella vulgaris, Dunaliella salina and Haematococcus pluvialis and the Cyanobacterium (also known as blue-green algae) Spirulina (Arthrospira platensis) are the most biotechnologically relevant microalgae that are widely commercialized. These microorganisms are commercialized mainly in the food industry as supplements, products pharmaceutical, cosmetics, animal nutrition and in the therapeutic field as nutraceutical compounds (Mobin and Alam 2017).

The *Chlorophyceae Haematococcus* and *Dunaliella* shall be highlighted as powerful pigment factories, and *Spirulina platensis* with outstanding capacity for protein accumulation. Some of the reported algae are used to produce edible oil, normally commercialized as single cell oil containing long chain fatty acids, as *Chlorella vulgaris* (α-linolenic acid) and *Spirulina* (γ-linolenic acid) (Matos 2017). These microalgae have peculiar characteristics and can survive well in extreme environments such as pH high (*Spirulina*) and high salinity (*Dunaleilla*), or they can grow very rapidly (*Chlorella*). The biochemical composition of *Spirulina* and *Dunaliella* is fundamentally composed of proteins and carbohydrates and for *Chorella* and *Haematococcus pluvialis* (green phase) is protein and lipids. In particular, the microalgae *Chorella* has the highest lipid content (2-46% of its dry weight) (Table 2).

Table 2. Biochemical composition of the main commercial microalgae

Microalgae	Carbohydrates (%)	Lipids (%)	Proteins (%)
Chlorella	12-28	2-46	11-58
Dunaliella	2-32	6-8	49-57
Haematococcus pluvialis (green stage)	15-17	20-25	29-45
Spirulina	8-16	4-9	46-71

Source: Adapted from Mobin and Alam (2017).

However, among various micro and macroalgae species, *Botryococcus braunii* is identified as the most promising for biodiesel production (content up to 75%, see Table 1) (Tasić et al. 2016). In particular, the species *Botryococcus braunii* has attracted broad interest due to its ability to accumulate large amounts of lipids, according to some special characteristics, which differentiate it from other organisms. Most of the hydrocarbons produced by *B. braunii* are accumulated in an extracellular matrix. Your extracellular location is one an advantage in comparison to other microalgae that accumulate triacylglycerol in the intracellular lipid body (Jin et al. 2016).

Still, B. braunii contain a high content of fatty acids monosaturates, as well as triglycerides, which makes it suitable for biodiesel production. The percentage of monounsaturated fatty acids in dry biomass is around 74% (Mondal et al. 2017). However, the main problem associated with this microalga is it is your relatively slow growth (doubling time of 72 hours). Other microalgae, unlike *B. braunii*, can fold the biomass within 24 hours or within 3.5 hours during the period of exponential growth. However, research that is more recent has shown that the doubling time in *B. braunii* can be reduced to 48 hours (Tasić et al. 2016).

Although lipid contents and biomass productivities are key characteristics for biodiesel production, they are not the only characteristics to be considered, since each species requires specific conditions for oil production and, consequently, the production of biodiesel.

3. Oil Production and Extraction Processes

3.1. Oil Production

For the formation of fatty acids and, therefore, lipids, microalgae can use a source of carbon which can be both inorganic or organic. The lipid classes basically are divided into neutral lipids or non-polar (e.g., triglycerides, sterols and waxes) and polar lipid (e.g., glycolipids) (Schüler et al. 2017). The triglycerides as neutral lipids are the main feedstock for biodiesel production.

In microalgae, the formation of fatty acids occurs in the cytoplasm with the formation of acetyl coenzyme A (acetyl-coA), and the elongation of carbon chain of fatty acids will mainly depend on the reaction of two enzyme systems including acetyl-coA carboxylic enzyme (ACCE) and the fatty acid synthase (FAS). The triglyceride biosynthesis has as main initiators L-α-phosphoglycerol and acetyl coenzyme A. Typically, short chain fatty acids, which are the main components of biodiesel, are the majority of the fatty acids found in *Chlorella* sp. (Huang et al. 2010). However, long chain fatty acids exist in several microalgae and the content varies from species to species.

Despite many strains naturally have a high lipid content, it is possible to increase their concentration through crop selection and adaptation, optimizing determinants factors of their as growth as temperature, photon flux density, CO_2 concentration and nutrient and substrate availability (Silva Ferreira and Sant'Anna 2017).

For instance, in *Chlorella protothecoides*, the shift of light intensity from 35 to 420 μmol photons $m^{-2} s^{-1}$ reduce the cell population doubling time and increase the growth rate and the total lipid content (Krzemińska et al. 2015). In contrast, light intensity reduction in *Scenedesmus dimorphus* culture from 123.5 to 16.9 μmol photons $m^{-2} s^{-1}$ results in a very small quantity of lipid bodies, together with a decrease in the cell growth and volume (Ferreira, Pinto and Sant'Anna 2016). In this context, it's evident that, under certain conditions, the oil content may increase considerably and technological advances suggest that the industrial production of biodiesel from microalgal oils may be feasible in the near future.

Occasionally, there are three possible strategies for increased lipid overproduction in microalgae: the biochemical engineering (BE) approaches, the genetic engineering (GE) approaches, and the transcription factor engineering (TFE) approaches (Singh et al. 2016). The BE strategy relies on creating a physiological stress such as nutrient-starvation or high salinity to channel metabolic fluxes to lipid accumulation. In this scenario, nitrogen starvation increases the accumulation of neutral lipids in many species of microalgae, including *Chlorococcum littorale*, *Neochloris oleoabundans* and *Tetraselmis suecica* (Benvenuti et al. 2015).

In addition to nutrient-starvation, other stress conditions may also cause enhanced accumulation of oil, as exposure to high light. The increase lipid accumulation triggered by exposure to light of high intensity is probably due to the production of photoassimilators, which are produced by converting excess light into chemicals, for protection of the cells of from photo-oxidative damage (Silva Ferreira and Sant'Anna 2017).

Assiduously, all changes occurred in the cell are detected by specialized sensory proteins, or sensors that alter their properties under stress. The sensors transfer the signal over the changes to other polypeptides (transducers), which in turn regulate the expression of the genes sensitive to stress. Transducer proteins can recognize the special regions of DNA, interact with them and regulate transcription. Finally, the protective proteins or metabolites are synthesized which help cells and organisms to adapt to the new environment (Paliwal et al. 2017). The accumulation of lipids occurs in response to the adaptation suffered by the microalga.

Conventional process biochemistry approaches involving nutrient deprivation and/or application of physiological stress might enhancing lipid content, but often fail to enhance lipid productivity (Bhowmick, Koduru and Sen 2015). For this reason, a commonly suggested countermeasure is to use a two-stage cultivation strategy, dedicating the first stage for cell growth in nutrient-sufficient medium, to achieve

maximum biomass productivity, and the second stage for lipid accumulation under nutrient-starvation or other physiological stress (Paliwal et al. 2017).

Conversely, metabolic engineering strategies (GE and TFE) address the problem of increasing lipid content and quality without compromising biomass yield. Alteration of metabolic pathways involve several strategies such as over-expressing a rate limiting enzyme involved in the synthesis of a desired product, blocking competing pathways and lipid catabolism via RNAi mediated silencing, over-expressing transcription factors, and site-directed mutagenesis to improve the performance of key proteins (Bhowmick, Koduru and Sen 2015).

In general, the GE approach targets a single gene, and the TFE approach affects a large number of genes involving multiple metabolic pathways, resulting in an integrated regulation of these pathways.

Functionally, TFE approaches in microalgae are still in their embryo. In essence, the potential ecological, economic, and health impacts of transgenic algae that persist and alter natural ecosystems should be deeply studied and reported (Snow and Smiyh 2012). In this context, despite the many advantages, the limitation of the use of metabolic engineering is your its high cost, because it involves expensive techniques and universal techniques not yet established throughout the microalgae realm. For this reason, BE approaches are, today, the most established in the production of microalgal lipids, because they do not present complicated and costly techniques.

3.2. Extraction Processes

Biomass processing after the end of the growing cycle is based on the collection and extraction of lipids. There are numerous harvesting methods for microalgae biomass, among which we can mention centrifugation, sedimentation, flocculation, flotation, filtration and any combination of these (Tiron et al. 2017). For extraction of the lipids usually is carried out drying of the biomass which can be carried out by different techniques such as spray drying, drying drum (transfer of heat to the sample through the inner walls of the drum cylinder), lyophilization and drying in the sun (Mondal et al. 2017). Posteriorly, the processing of the biomass for oil extraction is performed according to the cell wall characteristics of each microalgae species.

The selection of appropriate methods of cell disruption and lipid extraction largely revolves around the distinct biology of microalgae species and cell wall characteristics, the main barrier to the recovery of intracellular lipids. The cell-wall components include a microfibrillar network within a gel-like protein matrix (Yap et al. 2016). However, some microalgae are protected by an inorganic rigid wall (Bolton et al. 2016).

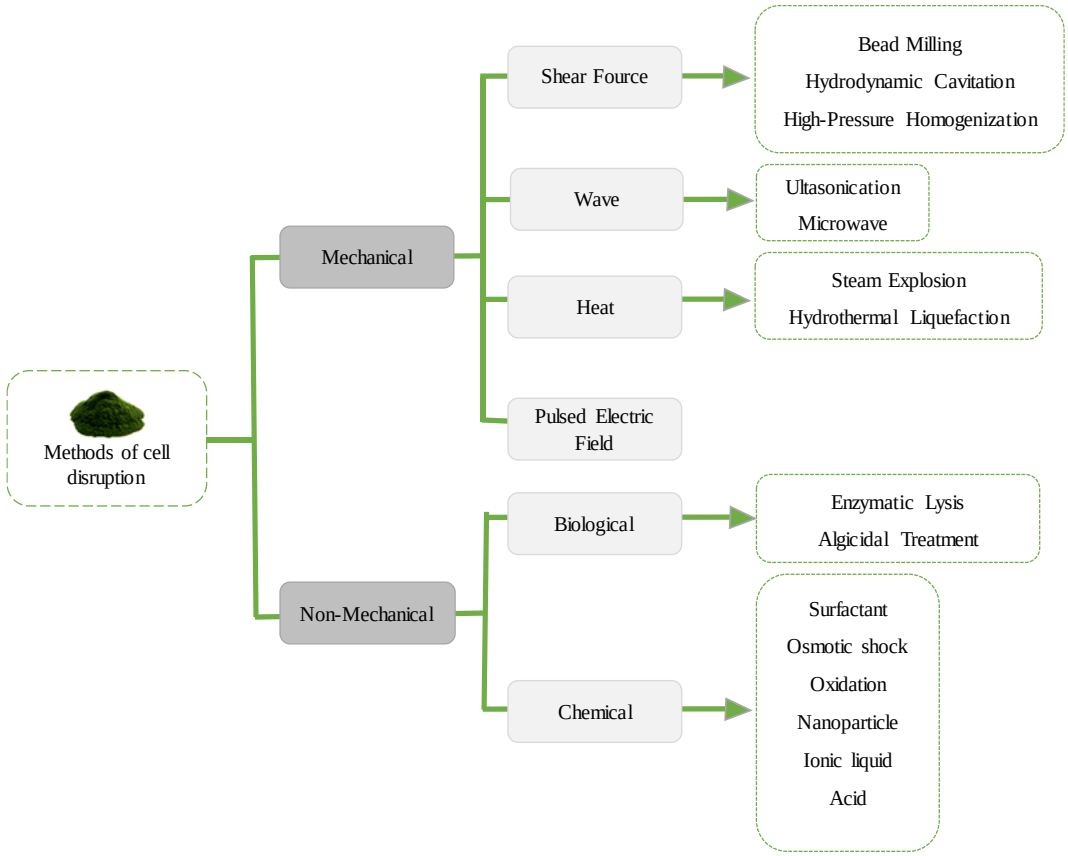

Figure 1. Mechanical and non-mechanical methods of cell disruption.

The thickness and chemical composition of microalgae cell walls change significantly in response to the growth environment (Praveenkumar et al. 2015). Microalgae with highly resistant cell walls are the main obstacle for microalgal lipid extraction. They might need pre-treatment at to disrupt the cell walls, and enhance the extraction of the lipid content, which consist of fatty acids (Hernández et al. 2015).

Currently, there are several methods of cellular disruption for the extraction of valuable components, as lipids. These techniques can be broadly divided into two types, mechanical and non-mechanical (Figure 1). Mechanical disruption requires energy inputs in the forms of shear forces, electrical pulses, waves or heat (Lee et al. 2017). Non-mechanical cell-disruption methods use chemical or biological materials that directly interact with the cell wall or membrane to allow passage of intracellular components to the surrounding medium (Dong et al. 2016). Sometimes the methods are combined to reduce power consumption and increase interruption efficiency.

Cellular disruption is often required for the recovery of intracellular microalgae products. In essence, the presence of a cell wall may prevent direct contact between the solvent and the cell membrane and prevent extraction (Lee et al. 2017). However, cell disruption is not always necessary for oil extraction, but can improve considerably

extraction. For example, the lipids extracted from *Nannochloropsis oculata* with petroleum ether range from 8.2 to 16.2% under Soxhlet extraction conditions. But, a combination of petroleum ether and high-pressure homogenization (230 MPa/6 passes) results in a 34% increase over the Soxhlet method (Shene et al. 2016). Suggesting that cell disruption substantially increases lipid extraction.

Fortuitously, there are three well-known methods to extract the oil from algae: (1) Expeller/oil pressing, (2) solvent extraction (chemical) and (3) supercritical fluid extraction. Expeller/oil pressing is a mechanical method for the extraction of oil using pressure. Microalgal biomass needs to be dried at high pressure for the optimal performance of the process. Expeller/oil pressing can extract up to 75% of oil (Topare et al. 2011). Alternatively, commercial manufacturers also use a combination of oil pressing with chemical solvents for efficient more extraction.

In chemical extraction, the lipids are normally extracted from lyophilized biomass. This fast and efficient method slightly reduces degradation. Various solvents, such as hexane, ethanol or a hexane-ethanol mixture, may be used, being possible to obtain a quantitative extraction larger than the Expeller/oil extraction.

Occasionally, the most popular chemical for solvent extraction and the hexane, which is relatively inexpensive. The Soxhlet method is the most commonly used method for the extraction of oils from dry microalgal biomass, in which organic solvents such as hexane, benzene, cyclohexane, acetone and chloroform are used in repeated washings of microalgae under reflux in special glassware called a Soxhlet extractor. This method offers several advantages, such as low cost and the use of less solvent. Major limitations associated with chemical extraction are the poor extraction of polar lipids and hazards of boiling solvents (Kiran, Kumar and Deshmukh 2014).

In supercritical fluid extraction, high pressures and temperatures are used for the rupturing of cells. Supercritical fluids are selective, thus providing the high purity (Abrahamsson, Jumaah and Turner 2018). This technique may extract almost 100% of the oils all by itself. Carbon dioxide is the most commonly used supercritical solvent because it does not lead to contamination or thermal degradation of the compounds.

In the supercritical fluid carbon dioxide extraction, carbon dioxide is liquefied under pressure and heated to the point that it has the properties of both a liquid and gas. This liquefied fluid then acts as the solvent in extracting the oil (Demirbas and Demirbas 2011). This method has several advantages being far more efficient than traditional solvent separation methods. However, high capital investment and high-pressure requirements are the main factors limiting the use of this process (Kiran, Kumar and Deshmukh 2014).

4. THE BIODIESEL SYNTHESIS

Conversion of microalgal lipids to biodiesel is commonly accomplished by transesterification or alcoholysis of triglycerides with acyl acceptor employing a suitable catalyst that yields fatty acid alkyl esters (FAAE) and glycerol (Brennan and Owende 2010; Guldhe et al. 2016a). Commonly employed acyl acceptors are short-chain alcohols like methanol and ethanol, while the catalysts used are either homogeneous and heterogeneous chemical catalysts or enzyme biocatalysts (lipase) (Mata, Martins and Caetano 2010; Milano et al. 2016; Guldhe et al. 2016a).

This three-step process first converts triglycerides to diglycerides, then diglycerides to monoglycerides, and finally monoglycerides to glycerol with yield of a monoalkyl ester of fatty acid in each of the three steps. Stoichiometrically, three moles of alcohol are required for converting one mole of triglyceride into biodiesel, as shown in Figure 2 (Guldhe et al. 2016a; Milano et al. 2016; Mardhiah et al. 2017).

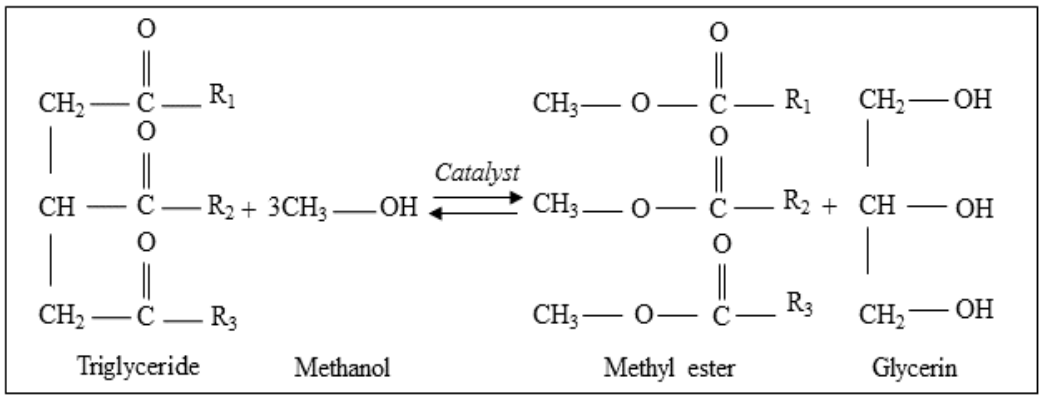

Figure 2. Triglyceride to glycerol via transesterification.

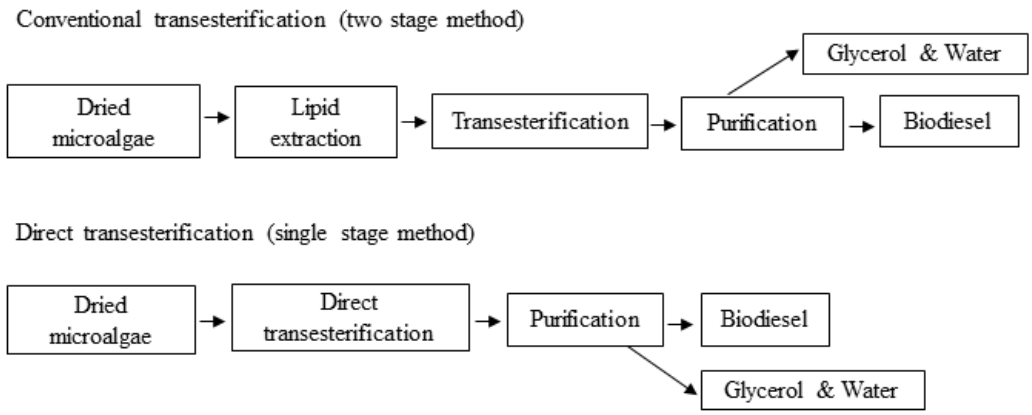

Figure 3. Conventional transesterification and direct transesterification method.

There are two ways to perform transesterification, which are conventional (two-stage methods) and direct transesterification (single-stage method). By using conventional transesterification, dried biomass will go through lipid extraction process via mechanical or chemical methods, before diverted to transesterification and purification steps. The other method which is known as in-situ or direct transesterification involved lipid extraction and transesterification simultaneously as shown in Figure 3 (Milano et al. 2016).

Currently, biodiesel production plants are applying homogeneous chemical catalysts for the conversion of raw materials lipids (Helwani et al. 2009; Guldhe et al. 2017a). The catalytic conversion of microalgae lipids is not a well-explored area. The microalgae lipids contain a high content of free fatty acids, thus making it impossible to use a chemical homogenous catalyst (Guldhe et al. 2017b).

The homogeneous chemical catalyst offers high yields however, it is associated with several disadvantages such as further product separation, recovery of the liquid catalyst and washing of the product is required through the acid neutralization step, which can generate a high amount of wastewater (Mardhiah et al. 2017; Guldhe et al. 2017a). In addition to the formation of soap in the presence of high free fatty acids content, which impairs the biodiesel yield (Mata, Martins and Caetano 2010; Guldhe et al. 2017a). Thus, a two-step process or acid catalyst is suggested for the conversion of high-lipid feedstock oils (Guldhe et al. 2017a).

Homogeneous acid catalysts or two-step process (using first acid esterification and then alkali transesterification) are widely used for conversion of microalgal lipids. However, these catalysts are associated with several disadvantages including corrosion of reaction vessels and piping, slows reaction rates, the requirement for repeated product washing steps, and generation of wastewater. To increase yield, higher temperatures and methanol oil rates are required (Helwani et al. 2009; Guldhe et al. 2017b).

Heterogeneous catalysts have advantage of easy separation from reaction mixture, reuse and minimal wastewater generation can improve the microalgal biodiesel production economics (Guldhe et al. 2017a; Mardhiah et al. 2017). Miao and Wu (2006) studied conversion of *Chlorella protothecoides* lipids using sulfuric acid catalyst and the biodiesel yield obtained was 60%. When heterogeneous Al_2O_3 supported CaO catalyst was employed for the conversion of lipids extracted from *Nannochloropsis oculata* biodiesel, yield of 97.5% was observed (Umdu, Tuncer and Seker 2009). Guldhe et al. (2017b) studied catalytic conversion microalgal lipids to biodiesel using chromium-aluminum mixed oxide catalyst shown promising potential for synthesis of microalgal biodiesel with 94.58% conversion and reuse potential of up to 3 batches without much loss in conversion efficiency.

The biocatalytic conversion has the advantages of less effluent generation and lower energy consumption. Enzyme catalyzed reactions are less energy intensive than chemical catalyzed reactions, as they can be performed at moderate temperatures. Lipases are the

enzymes that can be employed as catalysts for the transesterification of microalgae lipids. Lipases can be used in two ways, i.e., extracellular and intracellular (whole-cell catalyst) (Guldhe et al. 2016b). The high cost is the main disadvantage associated to the enzymatic catalysts for its application in the production of biodiesel in commercial scale. However, immobilization of the enzyme makes it possible to reuse it thus improving the overall economy (Guldhe et al. 2017b). Enzymatic catalysis and raw materials from microalgae have promising potential for sustainable biodiesel production.

Guldhe et al. (2016b) studied the application whole cell lipase from *Aspergillus* sp. and *Candida* sp. for conversion of microalgal lipids to biodiesel and Scenedesmus obliquus grown in open circular ponds was selected as the microalgal feedstock. Immobilized *Aspergillus niger* whole cell lipase showed biodiesel conversion of 80.97% and yield of 90.82%. Whole cell lipase application for conversion microalgal lipids could alleviate cost concerns associated with commercial lipases. Whole cell lipase catalyst and microalgal feedstock make biodiesel synthesis process sustainable.

Microalgae produce a renewable biodiesel that can potentially replace liquid fuels derived from petroleum. However, challenges to commercializing the production at large scale need to be addressed.

5. THE QUALITY CHARACTERISTICS OF THE BIODIESEL

The rise in consumption of biodiesel has led to the need for standardising the quality requirements for alkyl ester-based fuels. Biodiesel has to comply with the specifications set by agencies like ASTM (American Society for Testing and Materials), EN (European norms, European Committee for Standardization) and ANP (National Agency of Petroleum)) for its use in compression ignition engines. Fuel characteristics of biodiesel are influenced by feedstock quality, fatty acid composition of feedstock oil, conversion techniques and purification steps (Guldhe et al. 2017a).

The fatty acid composition of lipids is an important criterion for selection of suitable microalgal strains for biodiesel synthesis. Fuel properties of the biodiesel are primarily influenced by the carbon chain length, degree of unsaturation, and percentage composition of saturated and unsaturated fatty acid in microalgal lipids. Microalgal lipids are composed of saturated, monounsaturated, and polyunsaturated fatty acids. Many of the lipid-accumulating microalgae have been shown to comprise C14:0, C16:0, C18:1, C18:2, and C18:3 as major contributing fatty acids of their lipids, which are considered to be suitable for good quality biodiesel (Song et al. 2013). Table 3 shows fatty acid profile and lipid content of various microalgal strains studied for biodiesel production (Guldhe et al. 2016a).

Table 3. Fatty acid profile and lipid content of different microalgae

Microalgal strain	Lipid contend (%)	Fatty acid composition (%)									Reference
		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	SFA	PUFA	
Neochloris oleoabundans	29.00	0.43	19.35	1.85	0.98	20.29	12.99	17.43	20.76	64.6	Gouveia and Oliveira (2009)
Scenedesmus sp.	16.00	-	15.62	4.06	2.97	15.23	7.00	22.99	18.59	56.86	Talebi et al. (2013)
Chlorella vulgaris	17.30	-	14.55	1.18	10.51	23.62	13.80	32.10	25.06	70.70	Talebi et al. (2013)
Chlorella vulgaris	20.80	0.78	36.97	5.10	0.50	4.96	4.38	8.42	-	-	Song et al. (2013)
Staurastrum sp.	10.00	4.97	40.0	16.50	-	-	-	-	-	-	Song et al. (2013)
Monoraphidium sp. KMN5	20.80	-	41.03	-	17.67	10.16	3.03	1.53	58.7	16.26	Tale et al. (2014)
Scenedesmus sp. KMN4	28.60	-	41.27	-	20.53	9.21	1.91	0.57	61.82	12.77	Tale et al. (2014)
Nannochloropsis gaditan	-	3.00	16.0	17.10	-	3.90	8.00	9.00	19.40	68.30	Hita Peña et al. (2015)
Scenedesmus sp. ISTGA1	20.00	-	14.60	12.10	21.20	24.90	8.80	4.20	35.80	54.30	Tripathi, Singh and Thakur (2015)
Ankistrodesmus falcatus	23.30	-	-	21.15	-	28.18	19.25	14.33	-	-	Singh et al. (2015)
Scenedesmus obliquos	23.08	0.95	33.83	9.98	2.47	12.26	17.50	20.49	38.41	44.84	Maroneze et al. (2016)
Phormidium autumnale	20.70	7.30	22.50	8.50	10.50	26.20	17.80	2.10	-	-	Siqueira et al. (2016)

A systematic analysis of the fatty acid methyl ester (FAME) composition and comparative fuel properties is crucial for species selection for biodiesel production. Schenk et al. (2008) recommended a good quality biodiesel should have 5:4:1 fatty acid ratio of C16:1, C18:1 and C14:0. The composition of fatty acids in microalgae biodiesel has direct influence of their biodiesel property including cetane number, iodine value, oxidation stability, cold filter plugging point, among others, as described below (Islam, Heimann and Brown 2017).

Cetane number is one of the most significant indicators of fuel combustion ability. The cetane number relates to the autoignition quality and cetane number decreases with decreasing chain length and increased branching or higher unsaturation of the fatty acid chain. The ASTM D-6751 standard specifies the minimum allowable cetane number as 47, whereas EN 14214 specifies a higher value of 51 and the ANP 255 a minimum of 45. A lower cetane number indicates longer ignition times causing engine knocking and incomplete combustion; increasing exhaust pollutants (Islam, Heimann and Brown 2017).

Calorific value is important fuel property determining the energy content and suitability as transport fuel (Ferrari, Pighinelli and Park 2011). Density is a measure of

the mass per unit volume of a substance. In terms of engine performance, fuels with greater densities have the capability to provide more energy per litre than fuels with lower densities as injector pumps meter fuel to the engine volumetrically. The higher the density, the greater the amount of energy supplied. Biodiesel has a higher density than petroleum diesel and can potentially provide more power but at the cost of fuel consumption (Ferrari, Pighinelli and Park 2011; Islam, Heimann and Brown 2017).

Methyl ester content is parameter an important tool for determining the presence of other substances. Low values of pure biodiesel samples may originate from inappropriate reaction conditions (Ferrari, Pighinelli and Park 2011). Acid value or neutralization number is a measure of free fatty acids contained in a fresh fuel sample and of free fatty acids and acids from degradation in aged samples. It is influenced by the type of feedstock used for fuel production and its degree of refinement and too by acidity generated during the production process (Ferrari, Pighinelli and Park 2011).

Iodine number is a measure of the total unsaturation within a mixture of fatty acids and is expressed in grams of iodine which react with 100 grams of biodiesel. The engine that uses fuels with higher iodine number tend to polymerize and form deposits on injector nozzles, piston rings and piston ring grooves when heayed (Ferrari, Pighinelli and Park 2011). The kinematic viscosities of extracted microalgae oilsare high and require conversion into biodiesel to reduce the level of viscosity to a level similar that of diesel fuel. To ensure adequate supply to injector biodiesel must have an appropriate kinematic viscosity (Ramírez-Verduzco, Rodríguez-Rodríguez and Jaramillo-Jacob 2012). Kinematic viscosity limits are set to 1.9-6.0 $mm^2\ s^{-1}$ and 3.5-5.0 $mm^2\ s^{-1}$ as per ASTM 6751-02 and EN 14214 for 100% biodiesel. A higher viscosity affects the fuel atomisation and can lead to deposits forming inside the engine. The viscosity is directly proportional to the chain length of fatty acids but is inversely proportional to the amount of double bonds. Biodiesel standard EN 14214 has limitation for the maximum amount of 4 double bond content to 1% of total fatty acids. Microalgae species naturally contain higher amounts of polyunsaturated fatty acid (PUFA) compared to other seeds oils. Therefore, selecting a microalgae species for biodiesel production should consider species producing lower amounts of PUFAs (Islam, Heimann and Brown 2017).

The higher heating value (HHV), one of the most important properties of fuel is its energy content, which is quantified by the higher heating value, also known as the heat of combustion. The higher heating value is determined by the amount of heat released during complete combustion of a unit quantity of fuel under standard atmospheric conditions (101 kPa, 25°C). An increase in chain length and degree of saturation in the fatty acid composition increases the HHV for microalgae biodiesel whereas 10-12% oxygen content in it reduces the higher heating value. Therefore, microalgae species with higher amounts of long chain saturated fatty acids would be ideal for biodiesel production with better higher heating value (Islam, Heimann and Brown 2017).

Oxidation stability is one of the crucial fuel properties for storage time and distribution of any liquid fuel in large-scale production. A Rancimat test is undertaken to quantify the time it takes for fuel degradation producing volatile acids. If the "induction" time is short, the sample is said to be unstable. Therefore the ASTM D-6751, EN14214 and ANP 255 have set the minimum threshold of three and six hours. The oxidation stability of microalgae biodiesel depends on the chemical structure of the fatty acid methyl esters, especially degree of unsaturation and the presence of air, heat, light, traces of metal, antioxidants and peroxides (Francisco et al. 2010). The presence of double bonds in fatty acid chains and their position determine the rates of oxidation of the compound. It is reported that, Palmitic (C16:0) and Oleic (C18:1) acid in microalgae biodiesel have a positive effect on oxidation stability, whereas Linoleic (C18:2) and Linolenic acid (C18:3) have an adverse effect. Therefore, the EN14214 specifies a limit of ≤12% mass for linolenic acid content in biodiesel (Islam, Heimann and Brown 2017).

Another critical fuel property is the cold filter plug point, which is directly depends on the amount of unsaturated fatty acids in the fuel. Cold filter plugging point is the lowest temperature, expressed in degrees Celsius (°C), at which a given volume of fuel still passes through a standardized filter and limits have been set to≤5/≤-20°C in the EN 14214 for summer and winter respectively (EN 14214, 2008). The higher the amounts of unsaturated fatty acids or low concentration of saturated fatty acids, lower the temperature range for Cold filter plugging point. In general microalgae biofuel contains higher amounts of unsaturated fatty acids which are desirable for Cold filter plugging point, but this adversely affects the Iodine value for which limits of 120 gI2 100 g^{-1} biodiesel have been set in the EN14214. Therefore, an optimum ratio of saturated and unsaturated fatty acids in microalgae biodiesel should be determined so that quality complies biodiesel standards (Islam, Heimann and Brown 2017).

Fuels with high sulfur contents have been associated with negative impacts on human health and on the environment. Moreover, fuels rich in sulfur cause engine wear and reduce the efficiency and life-span of catalytic systems. Low sulfur fuels are an important enabler for the introduction of advanced emissions control systems (Ferrari, Pighinelli and Park 2011). Most of the microalgae have fatty acid compounds ranging from C11:0 to C26:0 with the combination of polyunsaturated fatty acid (PUFA), monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) in a balanced quantity to provide enhanced biodiesel properties (Guldhe et al. 2014). Table 4 depicts the fuel properties of biodiesel produced from different microalgal strains.

The contents listed in specifications of ASTM D6751-02 and EN14214 are also important but strongly influenced by biomass harvesting, processing, biomass actual oil content, extraction, conversion and purification efficiencies (Islam et al. 2013). To be an ideal source of sustainable biodiesel, selected microalgal species should contain sufficient lipid with suitable fatty acids for good biodiesel properties.

Table 4. Fuel properties of biodiesel produced from different microalgae

Biodiesel characteristics	Units	ASTM 6751	EN 14214	ANP 255	*Chlorella protothecoides*	*Scene-desmus* sp.	*Scenedesmus obliquus*
Cetane number	-	Min 47	Min 51	Min 45	-	-	51.74
Calorific value	MJ kg^{-1}	-	-	-	41	-	38.93
Density	kg m^{-3}	860-900	860-900	Max 900	864	852	869
Methyl ester content	%	-	Min 96.5	-	-	91.0	80.97
Acid value	mgKOH g^{-1}	Max 0.8	Max 0.5	-	0.374	0.52	0.48
Iodine number	g100 g^{-1}	-	Max 120	-	-	-	98.86
Cold filter plugging point	°C	-	-	-	-11	-	3.5
Oxidative stability	h	Min 3.0	Min 6.0	Min 6.0	-	5.42	3.53
Sulfur	wt%	Max 0.05	-	-	-	0.02	<0.001
Reference	-	-	-	-	Miao and Wu (2006)	Chen et al. (2012)	Guldhe et al. (2016)a

6. THE ENVIRONMENTAL ASPECTS

Biofuel production is being widely advocated as a renewable and environmentally friendly way of reducing the use of fossil fuels. However, there are concerns about the environmental impacts that a widespread adoption of biofuels could exert at a global scale, which could lead to further environmental degradation depending on the production system (Tilman et al. 2009; Immerzeel et al. 2014; Correa et al. 2017).

Microalgal production systems has emerged as a promising biofuel source, as microalgae-based biofuels are biodegradable, renewable, and eco-friendly in comparison to fossil driven fuels (Qari, Rehana and Nizamia 2017). However, this assessing is particularly more challenging since there are few experimental plants or industrial facilities in operation (Carneiro et al. 2017). To undertake an evaluate environmental sustainability of a biofuel production system it is necessary to use life cycle analysis (LCA) (Collotta et al. 2017).

Life cycle assessment is a methodology used to quantify the environmental impacts of all the associated steps over the entire life cycle, commonly incorporating a “cradle to grave” approach of a product or process within a defined system boundary (Togarcheti et al. 2017). According to the international organization for standardization (ISO) guidelines for conducting a life cycle assessment (LCA) within the series ISO 14040 and 14044 (Zivi, Galovi and Virag 2014; Wu et al. 2017), there are four phases in an LCA study: (i) goal and scope definition, (ii) life cycle inventory (LCI) analysis, (iii) life cycle impact assessment (LCIA), and (iv) life cycle interpretation (Corominas et al. 2013; Deprá, Zepka and Jacob-Lopes 2017). Moreover, the procedure of LCA is depicted in Figure 4.

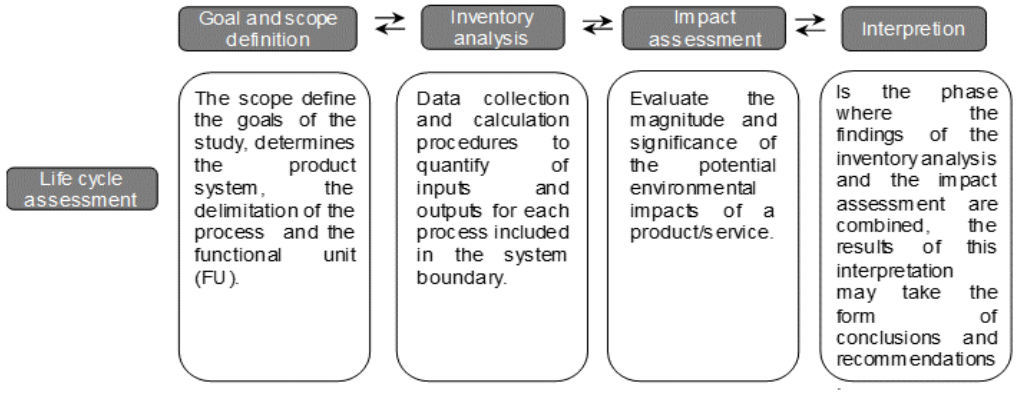

Figure 4. Driving steps for life cycle assessment.

Although the LCA methodology is standardized and consistent, the sub-procedures used in the analysis may strongly differ. It starts with the fact that all LCA phases present an opportunity in applied in different ways and, therefore, often leads to divergent results (Carneiro et al. 2017), as pointed out in Table 5.

At the impact assessment stage, as factors such as choice, modeling, and evaluation of impact categories present a main source of variability (Carneiro et al. 2017). The impacts most commonly considered in these assessments include global warming potential (GWP), land use (LU), water footprint (WF) and the energy balance (NER) (Béchet et al. 2017).

The global warming potential it is an indicator of the biofuels' contribution to climate, is based on International Panel on Climate Change (IPCC) characterization factors, calculated through sum the masses of substances that contribute to the impact (Mi), whether masses of gases (CO_2, CH_4, NOx) according to Equation 1 (Laratte et al. 2014; Deprá, Jacob-Lopes and Zepka 2018).

$$E = \sum_i Mi \times Pi \quad (1)$$

With:

E Environmental impact over time;

Mi Masses of substance i contributing to impact E over time;

Pi Characterization factors of substance i contributing to impact E.

Microalgal systems can interpret a significant role in the reduction of primary greenhouse gas (GHG) emissions (Qari, Rehana and Nizamia 2017), primarily carbon dioxide (CO_2) emissions by 78% (Ali et al. 2017) and the reduces net NOx emission of 16.54 g MJ^{-1} of produced energy, compared to petroleum diesel (Batan et al. 2010; Živković et al. 2017). Thus, the emission profile of biodiesel is mostly ascribed to the lower content of aromatic substances and the oxygen presence in ester molecules that

improves the combustion process and reduces CO_2 emission (Kegl 2011; Živković et al. 2017).

One widely-cited benefit of microalgal production systems as a biofuel is the expectation to not compete with food production (Langholtz et al. 2016). Due the ability for microalgae of be cultivated on non-arable land, what reduces its impact on the environment relative to other biofuels and fossil fuels.

Another important criterion for evaluating a process is the water consumption what can significantly impact the environmental sustainability (Derlue et al. 2012; Baudry et al. 2017). Microalgae cultivation is a water-intensive process when compared to other system for biodiesel production, since for the production of 1L of microalgal biodiesel requires approximately 3000L of water (Farooq et al. 2015).

Averaged between all processes of production microalgae biofuel, the upstream water accounts for 29.3% of life cycle, the evaporation and process use accounts for 74.2%, while fuel conversion and precipitation water gain account for 10.3% and 13.9%, respectively and, transportation and distribution account for less than 0.002% of the lifecycle (Batan et al. 2013).

Table 5. Summaries of case studies in the life cycle assessment of microalgae biofuels

Descrip tion of LCA system	NER	GWP	WF	LU	Reference
Cultivation the microalgae, harvesting by centrifuge and filtration, homogenization, lipid extraction, and trans-esterification.	92.77 MJ MJ^{-1}	1.32 $kgCO_2eq$ MJ^{-1}	nd	nd	Dutta, Neto and Coelho (2016)
Hypothetical production of *Nannochloropsis* sp cultivated in integrated photobioreactor-raceway system (Singapore).	4.44 MJ MJ^{-1}	nd	nd	nd	Khoo et al. (2011)
Cultivation the microalgae in open ponds, harvesting by flocculation and centrifugation.	$30x10^4$ MJ 317^{-1} MJ^{-1}	$1.8x10^4$ $kgCO_2eq$ 317^{-1} MJ^{-1}	$12x10^4$ m^3 317^{-1} MJ	0.4 ha 317^{-1} MJ^{-1}	Clarens et al. (2010)
Cultivation of the microalga *Nannochloris* sp. e *Nannochloropsis* sp. in raceway ponds.	33.4 MJ MJ^{-1}	2.9 $kgCO_2eq$ 317^{-1} MJ^{-1}	nd	nd	Passel et al. (2013)
Cultivation of the microalga in open ponds.	1.4 MJ m^{-3}	0.19 $kgCO_2eq$ m^{-3}	nd	nd	Liu, Clarens and Colosi (2012)
Hypothetical integrated hybrid cultivation system for the production of microalgae *Chlorella vulgaris*. Conversion of microalgal lipids to biodiesel via trans-esterification.	31 MJ ton^{-1}	2137 $kgCO_2eq$ ton^{-1}	16.3 m^3 ton^{-1}	nd	Adesanya et al. (2014)

* nd: not defined.

Water footprint (WF) of an enclosed area or system is determined by the sum of the water footprints of all processes (Deprá, Zepka and Jacob-Lopes 2017), according to Equation 2. The green WF which refers to the consumption of rainwater, blue WF which refers to the consumption of surface- or groundwater and the so-called grey WF, represents the volume of water required to assimilate pollutants entering freshwater bodies (Hoekstra et al. 2011; Hoekstra 2016).

$$WF = \sum WFgreen + WFblue + WFgray \quad (2)$$

With:

WF Water footprint;
WFgreen Green water footprint;
WFblue Blue water footprint;
WFgray Gray water footprint.

However, microalgae grow in any kind of water, fresh water, salt water, or wastewater (Elrayies 2018), water recycling also is desirable, since it to reduce the water demand and also improves the economic feasibility of algal biofuels as due to nutrients and energy savings (Farooq et al. 2015).

One of the main criticisms of possible commercial scale microalgae-based biofuels development is from the energy perspective (Acheampong et al. 2017), as production of microalgal biodiesel can be an energy intensive process (Togarcheti et al. 2017), the results indicate that the largest impacts across all categories come from the energy use shows that among the various stages, microalgae culture and harvesting consumes the highest energy, i.e., 94%, followed by oil and biomass processing (Pragya and Pandey 2016).

The energy ratio (NER) is calculated through a ratio of the biofuel's energy content (MJ of bioenergy output) over the energy required (total or fossil) to produce the biofuel (MJ input) (Tredici et al. 2015), according to Equation 3.

$$NER = \frac{\sum energy\ produced}{\sum energy\ requeriments} \quad (3)$$

Thus, in terms of energy efficiency, microalgae biofuels cannot compete with other biofuels or fossil fuels. But, they present very low performances, demanding more energy for its production then the energy they can deliver (NER<1) (Carneiro et al. 2017).

Utilization of microalgae as the raw material for producing biodiesel is beneficial to the production of renewable fuels and improvement of the ecological environment. However, production microalgae biofuels suffer from several bottlenecks at the current

level of technology, such as the need for process optimization (Khan, Shin, and Kimcorresponding 2018). Thus, life cycle assessment as a is tool a way to evaluate environmental sustainability at early phase of process design is process simulation through a life cycle assessment (Guo et al. 2016).

7. The Economic Analysis of Microalgae Biodiesel

Due to the increasing concerns over climate change and energy security, more attentions have been focused on clean and renewable energy in today's society, debates over the future in microalgae biofuels have focused on quantitative methods of assessing the environmental benefits and the economic feasibility (Chisti 2007; Batan et al. 2016). Although the technical feasibility of microalgae biofuels has been demonstrated, at present, the process appears to be uneconomic (Griffiths et al. 2016).

Regarding, economic estimation of microalgae-based biofuels, it is important to note at the outset that microalgae biofuel technology is still in an early stage of development, and there are numerous opinions on the optimal processing steps associated with the production of feedstock through to fuel conversion (Quinn and Davis 2015), disparities among these studies can largely be attributed such as products, cultivation systems, biomass productivities, oil contents, production capacities and conversion technologies (Xin et al. 2016).

The estimated cost of production varies in other studies according to the table 6. It is clear that, in the present state of technology the production of biodiesel from microalgae is not competitive with petrodiesel (Faried et al. 2017) which the average price from july 2013-july 2017 was \$3.09 $gallon^{-1}$ (U.S. energy and information administration; Xin et al. 2018). These results reiterate that the economics of microalgal biofuel production would not be competitive with traditional fossil fuels if a large scale facility were to be built today (Quinn and Davis 2015).

Thus, there is an urgent need to improve the economics of microalgal biofuels by optimizing the whole process of biofuels production (Faried et al. 2017), the cost of microalgae biofuels mainly depends on its cultivation and during cultivation carbon supply is the most important nutrient source which controls the growth rate (recycling of by-product as a carbon source for its cultivation can reduce its production cost) and increase its lipid productivity (Borowitzka 2005; Faried et al. 2017).

Efforts to advance the commercial feasibility of microalgae derived biofuels have focused on improvements to the various processing steps associated with the configuration and conditions at each stage of the production process (Quinn and Davis 2015).

Table 6. Minimum sale prices for microalgae biofuels according from literature and technological processes used

Cultivation System	Harvesting	Conversion technology	Biofuel selling price (US$ gal^{-1})	References
Open ponds	Filter press dewatering	Wet solvent lipid extraction and oil hydro-treating	8.79	Delrue et al. (2012); Brasil, Silva and Siqueira (2016)
Open ponds	Coagulation-flocculation and decantation	Hydro-treating	10.64	Delrue et al. (2012); Xin et al. (2016)
Open ponds	Coagulation-flocculation and decantation	Transesterification	6.28-9.87	Delrue et al. (2012); Klein, Bonomi and Maciel Filho (2018)
Open ponds	Flotation and centrifugation	Simultaneous oil extraction and transesterification with methanol	1.60-3.72	Nagarajan et al. (2013); Klein, Bonomi and Maciel Filho (2018)
Hybrid (Closed PBR/Open ponds)	Flotation and centrifugation	Hydrolysis, in situ transesterification and flash separation	13.94	Amer, Adhikari and Pellegrino (2011); Brasil, Silva and Siqueira (2016)
Hybrid (Closed PBR/Open ponds)	Filter press dewatering	Wet solvent lipid extraction and oil hydro-treating	14.12	Delrue et al. (2012); Brasil, Silva and Siqueira (2016)
Closed PBR	Flotation and centrifugation	Hydrolysis, in situ transesterification and distillation	87.14	Amer, Adhikari and Pellegrino (2011); Brasil, Silva and Siqueira (2016)

In this context, a biorefinery based production strategy is used, in order to lower the cost, producing high added value products from components not used in the biofuels (Carneiro et al. 2017). Biorefinery comprises a number of specialized methods used to extract the most out of primary and/or secondary metabolic products. Microalgae biorefineries must use methods and technology for isolating compounds and obtaining principal constituents from biomass, without damaging one or more of the product fractions, thereby adding value to the bioproduct formed (Goettel et al. 2013; Jacob-Lopes et al. 2015).

It can be concluded that despite microalgae system being a potential biofuel feedstock, thus some improvements are necessary to create a more economically viable and sustainable system (Chew et al. 2017).

FINAL CONSIDERATIONS

The production of biodiesel from microalgae is seen as one of the most efficient ways to produce biodiesel. Meantime, the production, the harvesting and the extraction should

be optimized, as well as improvements biology through metabolic engineering for generation of strains with high lipid productivity.

The economics of microalgae biodiesel production still need to improve to make it competitive with fossil fuels, but the level of improvement needed seems to be attainable. Great efforts are already underway to achieve biodiesel production on commercial scale and the combined use with the extraction of high value compounds can reduce the cost of production and make it profitable.

Achieving the ability to economically produce biodiesel from microalgae is of strategic significance for an environmentally sustainable society.

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APÊNDICE C - MICROALGAE-BASED SYSTEMS APPLIED TO BIOELECTROCATALYSIS

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Microalgae-Based Systems Applied to Bioelectrocatalysis

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Abstract The increasing demand by energy and the current need of the replace fossil resources it is leading the research and development (R&D) sector to search by renewable feedstock and renewable processes. Thus, major emphasis is being put into sustainable technologies and environmentally benign. In this context, microalgae have been extensively exploited for their versatility and capacity of the produce a broad spectrum of bioproducts. In particular, the viability of these microorganisms to generate electrical energy from organic and inorganic residues is an attractive technological route. The use of microalgae in electrochemical systems has the potential to produce bioelectricity associated with bioremediation and wastewater treatment. This integration could be advantageously exploited to the development of a self-sustaining biobased system. In this sense, this chapter is intended to provide a overview of various aspects associated with the bioelectricity production from microalgae.

Keywords Microalgae · Photosynthesis · Microbial fuel cells · Bioelectricity

1 Introduction

In the last years, significant advances have been made towards robust technologies, cost-effective and eco-friendly, aiming to overcome the excessive dependence of fossil resources [14]. Among recently developed technologies, the microbial fuel cell (MFC) gained great scientific and technological importance [48]. These bioelectrochemical systems are an attractive technology because of their sustainability at harvest of energy from readily available substrates [41, 83]. Biological entities as catalysts of electrochemical processes offer a means of produce clean energy associated with bioremediation of wastes [15, 47, 56].

In MFCs, microorganisms convert a diversity of substrates into electricity from a series of oxireduction reactions [67]. The system consists of a set of electrodes

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linked by an outer electric circuit, physically divided by a membrane or not [77]. The distinguishing feature, central these biotechnological devices, is the application of living microorganisms. Besides that, in contrast to traditional fuel cells, the MFC has the advantage of using wastewater treating it and producing bioelectricity simultaneously [76].

Since the turn of the century, MFCs research has advanced, and new projects have been launched towards in direction the synchronization of technologies and bioenergy sources sustainable. In this context, considerable researches are being realized to investigate the potential biotechnological of microalgae at the distinct bioelectrochemical processes. The microalgae present high photosynthetic efficiency and can be applied in MFCs to produce an electron acceptor in the cathodic compartment (photosynthetic microalgae), electron donor in the anode and/or remove organic matter also in the anode (heterotrophic microalgae) [23].

Microalgae as efficient solar energy converters have been utilized in the cathode of MFCs to O_2 generate and capture CO_2, with parallel biomass production and biocompounds of high value-added [17]. The application of bio-cathodes based in these microorganisms allows noble catalysts, used for oxygen reduction, to be substituted by natural materials, improving sustainability and cost-effectiveness [3]. On the other hand, microalgae also can be applied in the anode as fuel/substrate for exoelectrogenic bacteria, owing to the adequately elevated content of carbohydrate, protein and lipid [71]. Besides, recently discovered, some species of microalgae can be utilized as the sole electron-donating source. The utilization of electrogenic microalgae in the anode compartment is an extraordinary opportunity [75].

The use of microalgae in electrochemical processes as the MFCs has been recognized as a versatile technology and potentially promising. Thereby, this chapter intends to furnish a comprehensive review of the fundamental elements of microalgae-based microbial fuel cells (mMFCs), their configurations, limitations, and applications.

2 Biological Fuel Cells

A fuel cell is an electrochemical device that involves the transfer of electrons and conversion of chemical energy of a fuel into electricity. In these systems, anodic (electron addition) reactions are fed continuously by a fuel, while cathodic reactions (electron consumption) are fed continuously by an oxidizer. Natural gas and hydrogen are the more commonly used fuels in these systems, while the oxygen of the air is utilized as the oxidant [72]. All these reactions occur through a medium (electrolyte) containing dissolved ions capable of withstanding the current flow and allowing electric charge transfer [24].

The concept of electricity generation from biological sources arose even in the eighteenth century. However, it was only in 1911 that the disaggregation of substrates by organisms accompanied by the clearance of electricity was demonstrated [59,

73]. Despite extensive research in the last century on routes and bioelectrochemical generation, few prototypes were reported [43].

The traditional generation of energy for methods such as chemical fuel cells is not able to supply the need for sustainable energy of high demand and recent advances in microbiological research have led to the bioelectricity production through fuel cells by microorganisms. These current systems have become a promising, innovative, economical and environmentally favourable technology for sustainable electricity production [75].

The microorganisms growth in MFCs can be termed as a self-sustaining cycle. They may develop in suspension to shape a biofilm at the electrode and superficies of the anode chamber. This biofilm can directly exchange electrons through physical contact with the cell. Also, in this compartment is carried out the reaction of oxidation of the organic matter, producing protons and finally an electric current of biomass [58, 69].

In the so-called direct electron transfer, the microorganisms present have active and efficient electrochemical redox enzymes in their membranes that cross the MFC circuit without the need for exogenous chemicals to accompany the transfer of electrons to the electrode [34, 62]. However, when they are unable to perform this process, intermediary mediators are used to capturing the electrons in the cell, to reduce and promote the extracellular transfer [31].

In general, the oxidation of substrate in the anode chamber generates CO_2, protons, and electrons. The electrons are transported to the electrode, then flowing to the cathode, and the protons are directed to the cathode by means of a permeable membrane. These, when consumed in the cathodic chamber, reduce O_2 to H_2O and produce electrical energy [72, 89].

The consideration of MFCs as a issue negligence has been following another notions of bioconversion in last times. Substantial research is being conducted, new projects have evolved, and the operation has advanced towards microalgae-based fuel cells for bioelectricity production through the reaction of photosynthesis [77].

In comparison with conventional MFCs, microalgae-based fuel cells (mMFCs) represent a more developed technology since are able of capture carbon dioxide with parallel wastewater treatment and energy production [67]. This integration has the potential to generate fuel and oxygen for cells, providing the development of a system that satisfies with sustainable and economic metrics [71].

3 Factors Influencing the Bioelectricity Production from Microalgae

Several factors such as pH, temperature, light intensity, substrate, carbon dioxide, electrode material, and membrane can severely affect the power output and overall performance of the mMFCs. These parameters are discussed below.

The pH as an environmental parameter affects the microbial activity and, consequently, the anodic and cathodic performance. In the anode and cathode chambers, the pH should be maintained between 6 and 8, which is considered ideal for metabolic activity of the microorganisms [75]. Undesirable pH variations reduce the bioelectricity production and perturb the physiological reactions that occur inside the cell. In traditional mMFCs, can maintain distinct pH conditions in each compartment to optimize their reactions, which is impossible for single-chamber configurations, because merely one electrolyte is present [25].

In the cathodic chamber, the O_2 reduction reaction and the accumulation of cation species (for example, Na^+ and Ca_2^+) that permeate the membrane result in alkaline pH (8–10), decreasing the cathodic potential. For mMFCs of air cathode or single chamber, this is favourable the cathodic reactions and for the general performance, but can inhibit the activity of the microorganisms. Addition of tampons such as carbonate, phosphate and carbon dioxide helps the maintain the pH [61]. In single-chambered mMFCs, the CO_2 generated by bacteria from the oxidation of the fuel can assist in the growth of microalgae and reducing the pH of the electrolyte and, in two-chamber mMFCs, the carbon dioxide of the anodic compartment can be diverted for the cathodic compartment for the same purpose [77].

In the anodic chamber, the electrochemical oxidation of fuels elevates the generation of protons [98]. This production is superior to its transport through the membrane. The accumulation of protons in the anodic compartment causes changes in pH, reducing the microbial activity, the electron transfer, and the cathodic reactions due to the limited supply of protons. Therefore, it is suggested, that the anodic pH be controlled keeping next to neutrality for optimizing the energy output.

The temperature can significantly affect mMFCs performance through the metabolism microbial. Control the temperature fluctuations in mMFCs allows the formation of anodic biofilms more quickly, resulting in shorter startup times. In general, the mMFCs can be classified into thermophiles and mesophiles depending on the microorganism employed. In the thermophilic mMFCs, the microorganisms require high temperatures for their development; therefore, the mMFC operate at elevated temperature. On the other hand, in mesophilic mMFCs, the microorganisms perform better under moderate temperature conditions [78]. Most of the mMFCs operate in the mesophilic range, and the optimal temperatures vary according to the microorganism employed. It is evident, from studies [90, 99], what taller potency densities can be obtained by maintaining a suitable temperature range for the microorganisms. Thus, similar to other biological processes, environmental parameters as temperature and pH must be adjusted to reach a high-performance system.

The photosynthesis exercises a critic function in mMFCs, and its performance can be optimized to potentiate the generation of bioelectricity. Both the intensity of light such as its duration can affect the photosynthetic efficiency of the microalgae and, consequently, the oxygen supply in the cathode [13]. Therefore, several investigations have been realized to evaluate the different conditions of intensity and regime of light in the efficiency of mMFCs [93].

It is reported that the augmentation in light energy increases proportionally the dissolved O_2 and the potency density of the system [92]. However, exist a range

of light intensity considered optimal which needs to be applied. Variations in light intensity affect both the performance of the mMFC, such as biomass production and its composition. The feasibility of parallel generation of value-added biomass enables the development of economically viable systems. Therefore, its influence on the productivity and composition of biomass is also evaluated in mMFCs [22].

Studies show that as light intensity increases, a higher concentration of biomass is obtained, but at the same time, occurs the shortening the life useful of microalgae. This is attributed to the damage caused to the photosynthetic apparatus by photoinhibition [45]. Regarding the effect of light/dark cycles, higher power densities are obtained under continuous illumination. Besides that, in this condition, it favours the supply of dissolved oxygen positively on the cathode, with a reduction during the dark period. However, it is pointed out that continuous lighting also reduces the life useful of microalgae. In this sense, intermittent lighting is indicated to prolong the life cycle of microalgae cultures [4]. Concomitant, the light source is also reported by influencing the photosynthetic rate of microalgae and, therefore, the system performance [38].

In this context, it is observed that higher bioelectric generation can be obtained from the optimizing the photosynthetic rate, applying a light intensity, duration (light/dark cycle) and adequate light source.

The mMFCs utilize a variety of substrates, including, for example, wastewater. Still, detention time and organic loading rates (OLRs) can also influence the performance of these systems, which is notably reliant on the substrate utilized [16, 54].

According to the system configuration and the kind of wastewater to be treated, it is necessary to use an adequate load of OLR for utmost chemical oxygen demand removal. Typically, in between 0.05 and 2.0 kg COD/m^3 d of OLR is used to obtain an increase in the power density of the mMFC [57, 72]. The potency density is proportional to the degradation of the substrate rate, but it is still necessary to use pretreatment systems on more complex substrates to achieve better results [21, 32, 46].

Domestic and industrial wastewater and substrates highly digestible as acetate and proteins, have been quite studied and utilized in mMFCs for effluent treatment as well as for energy generation [35, 50, 74, 97].

The supply of carbon dioxide in mMFCs is essential to promote healthy growth of microalgae. The photosynthetic microalgae use carbon dioxide to perform the photosynthesis and produce oxygen and biomass [86]. The ideal growth of microalgae in the cathode of mMFCs can be obtained by the continuous bubbling of CO_2 or by the supply of carbon dioxide diverted of the anodic chamber. The carbon dioxide diverted of the anodic chamber is suggested by optimum produce growth and, therefore, the bubbling of carbon dioxide in the cathodic chamber is not necessary [10, 40].

The existence of carbon dioxide dissolved in the cathodic chamber decline the pH of the electrolyte, requiring the control of the same for that the inoculum of the microalga is not affected and ensures the operation of the processes. The microalgae growth kinetics increase as the carbon dioxide concentration increases, however, can

reduce significantly in high or very low concentrations of the same. Furthermore, the carbon dioxide concentration also influences the chemical composition of microalgae biomass [80].

The success of an mMFC is associated with the selection of the electrode material. Both electrodes follow different selection criteria, but specific properties must be presented equally. Are necessary electrodes with a great superficial area, high electrical conductivity, durability and stability, biocompatible and low cost [70]. By decreasing the resistance, using electrode materials of high electrical conductivity, it is possible to augmentation the superficial area and, consequently, the efficiency of the system. In terms of durability, stability, the materials should be able to withstand both acid and basic medium. The durability can be augmented with increased surface roughness, however, this can result in contamination and, in the long run, the performance of the mMFC would be reduced.

The metallic materials have higher conductivity than carbon materials, but the corrosion propensity limited the choice of these. The low costs, support of microbial adherence to form steady biofilms and electrical conductivity, make the carbon the material more used and versatile, available as graphite plates, rods, and granules. The biofilm helps in the capture of the electron, therefore, have been used coatings like platinum and Teflon to potentiate the formation of these structured communities. In the cathode, ferricyanide, and oxygen are the electron acceptors most popular. Due to the oxidation potential, free cost and water as the final product, oxygen is the best option for mMFCs and, Based on the application, the cathodic material is selected. In general, most anodic materials can be used as cathodes. Graphite and carbon screen are the more commonly used cathodic materials [79].

The functions of a membrane are to segregate the reactions occurring in the cathodic and anodic compartment; reduce the passage of O_2 of the cathodic compartment to the anodic, and ensure the sustainable operation and lasting [52]. Before being used in any system, the choice of a membrane requires considering divers relevant aspects. The ideal characteristics of selection be an excellent ionic conductor, electronic insulating, ion selective, durable, chemically steady, biocompatible, insensible to biofouling and mainly of viable economic cost [72].

Currently, the main membranes utilized in mMFCs are cation exchange membrane (CEM) and anion exchange membrane (AEM) [63, 68]. A CEM allows only the passage of cations, per carrying negatively charged groups fixed to membrane skeleton. The most widely used materials are perfluorosulfonic acid polymers (PFSA). Within the family of PFSA membranes, the Nafion (DuPont) is the most applied. However, commercialization of Nafion-based membranes has been complicated by the high cost and environmental incompatibility of perfluorinated material processing [95].

In contrast, AEM has the potential to reduce costs, since a more comprehensive selection of cheap materials can be realized. In addition, the utilization of low-cost porous membranes has also been evaluated, for example, nylon and glass fibers. These membranes are not selective of ions, allowing the transfer of anions and cations in opposite directions. Despite being cheap, these separators present limitations such as high internal resistance [77].

4 Interactions Between Microalgae and Electrodes

4.1 Microalgae at the Cathode

The limitations that are put forward with the utilize traditional catalysts (chemical and metal) for the cathode have boosted researchers cogitate other alternatives. The microalgae biocathodes can supply oxygen jointly with the bioconversion of nutrients and CO_2, through the process of photosynthesis. As consequence of photosynthesis, biomass is produced and can be utilized to generate value-added compounds or serve as anodic fuel of the mMFC [93].

The photosynthetic microalgae use sunlight and the CO_2 diverted from the anodic compartment for their development and oxygen production. The in situ production oxygen plays as an electron acceptor and help in replacing the traditional mechanical agitation techniques. Studies of Gajda et al. [19] and Wu et al. [91] evidenced the use of microalgae as efficient in situ oxygenators. In comparative studies, it was possible to demonstrate that the aeration of microalgae at the cathode significantly favours acquisition a higher potency density, when compared to mechanical agitation [33, 88].

The utilize of microalgae as cathodic reaction catalysts have the capacity to improve electricity production. In addition, expenditures can be substantially reduced by using them, because they can replace the use of expensive catalysts. Another advantage of biocathodes, in contrast to abiotic cathodes, is biomass production and the possibility of removing nutrients using microalgae metabolism [63].

Beyond the already existent basic systems, other projects are developed for the application of microalgae biocathodes such as the mMFCs connected to photobioreactors [20]. These systems are operated to provide sustainably dissolved oxygen by recirculating of the catholyte connected to the photobioreactors. It is worth mentioning that the application of the different species of microalgae requires distinct inoculation media. Consequently, they exhibit different growth parameters. Therefore, the application of each configuration, substrate, as well as the utilized of the different species microalgae, either in the anodic or cathodic compartment, take to variations in power density. Table 1 summarizes some microalgae-based MFCs systems and their power density.

4.2 Microalgae at the Anode

Different sources of the substrate are evaluated for their utilization in the anode of mMFCs. The more common are formate, glucose, and acetate. Other sources include microalgae biomass (either living cells or dry biomass/powder) and wastewater [15]. The substrate a nutrient source for bacteria is a determining factor in mMFCs According to Parkash et al. [55], the anolyte type used and its internal resistance affect the power production, which also depends on following factors: membrane resistance,

Table 1 Microalgae-based MFCs systems and their respective potency densities

Cathodic content	Anodic content	Power output (mW/m^2)	References
C. vulgaris	Activated sludge	13.50	del Campo et al. [12]
C. vulgaris	*S. cerevisiae* and glucose	0.95	Powell et al. [60]
C. pyrenoidosa	Synthetic wastewater and sodium acetate	60.60	Jadhav et al. [28]
C. pyrenoidosa	Potassium ferricyanide	6030.00	Xu et al. [94]
Bacterial community	*C. vulgaris*	327.67	Huarachi-Olivera et al. [27]
Desmodesmus sp.	Synthetic wastewater	99.09	Wu et al. [92]
C. vulgaris	bacteria	24.40	Wu et al. [91]
C. vulgaris	Enriched bacterial consortium	62.70	Gouveia et al. [22]
S. quadricauda	Domestic wastewater	62.93	Yang et al. [96]
C. reinhardtii	*G. sulferreducens*	41.00	Nishio et al. [51]
C. reinhardtii	*G. sulferreducens* and Formate	140.00	Nishio et al. [51]
C. reinhardtii	*G. sulferreducens* and Acetate	630.00	Nishio et al. [51]
S. obliquus	GM media	153.00	Kakarla and Min [33]
Ferricyanide	*S. obliquus* and wastewater	102.00	Kondaveeti et al. [36]
Potassium hexacynoferrate (III) and KH_2PO_4 buffer	*C. reinhardtii transformation*	12.94	Lan et al. [38]
Ferricyanide	*C. vulgaris* and anaerobic consortium	15.00	Lakaniemi et al. [37]
Ferricyanide	*D. tertiolecta* and anaerobic consortium	5.30	Lakaniemi et al. [37]
C. vulgaris	Sediment	38.00	Wang et al. [88]
C. vulgaris	*Scenedesmus* and microbes	1926.00	Cui et al. [10]
Mixed microalgae	Photosynthetic bacteria	103.00	Chandra et al. [8]

high generation of ions in the anode compartment, and crossing of oxygen through the membrane. The substrates oxidation by bacteria is directly related to electron transfer and sequentially to the electric current production. Therefore, the substrate influences the microbial community and the performance general of mMFCs.

The microalgae as anodic substrate are passable to be oxidized by bacteria to produce electrons. The degradation of microalgae biomass in an mMFC can produce

by-products such as acetate and lactate, which are used to generate bioelectricity. However, the conversion efficiency of the biomass is identified as being low, which, consequently, not generate a tall potency density [36]. Therefore, a pre-treatment of biomass is proposed as a method to rupture the cell wall and do it more digestible for bacteria. Another critical factor is the biomass concentration employed, as shown by [65], which can considerably intervene in the efficiency of the mMFC.

Researches have also been done with microalgae used at the anode of mMFCs as the only electron donor source. de Caprariis et al. [11] and Xu et al. [94] highlighted in their studies the interesting potential of such an application. By regulating the O_2 concentration, light intensity and the density of microalgae cells in the anode, microalgae generate bioelectricity without the need for substrates and mediators.

It is essential that in mMFCs with exoelectrogenic microalgae the unfavourable effect of the microalgae on the anode be minimized, this is, there should be control of the oxygen content since the presence of O_2 in the anodic compartment plays like a competitive electron acceptor. In this case, oxygen inhibitors, like activated carbon and iron powder, can be utilized. In contrast, suitable dosages should be administered to avoid disturbances in redox reactions.

5 Microalgae-Based Microbial Fuel Cells Configurations

Normally, the microalgae are cultivated in closed photobioreactors or raceway ponds, where they can utilize sunlight, CO_2 and nutrients for their growth [14, 44]. Thus, projects have been developed to allow microalgae to would generate electricity in an MFC. The overall process involving microalgae and the configurations of the anode chamber and the cathodic chamber is shown in Fig. 1. To create cost-effective processes, several configurations are proposed. Among the main stand out the mMFCs of single-chambered, two-chambered, sediment and coupled types [66]. New configurations with biotechnological development are still emerging with the goal of will improve the power output, the coulombic efficiency, the stability, the longevity and the cost-effective of the mMFCs [55].

In single-chambered mMFCs, bacteria and microalgae coexist parallel in the same chamber and may not contain membrane. They are generally configured with an air cathode. Microalgae absorb the CO_2 produced by the microbes, and both cultures are grown synergistically without the presence of a membrane (Fig. 2). The main advantage of these systems is the relatively simple architecture and the low cost of construction. However, have limitations, such as the high diffusion of oxygen, which may lead to a diminution in coulombic efficiency [6].

In a single-chambered mMFC configured with an air cathode, the oxygen supply to the cathode is given by microalgae and atmospheric air [26]. Similar projects include the integration of a biofilm from microalgae at the mMFC [96]. In these, the presence of a microalgae biofilm fixed about support, that establishes an interface among the anode and the cathode, permit minimizing the adverse effects of the microalgae on the

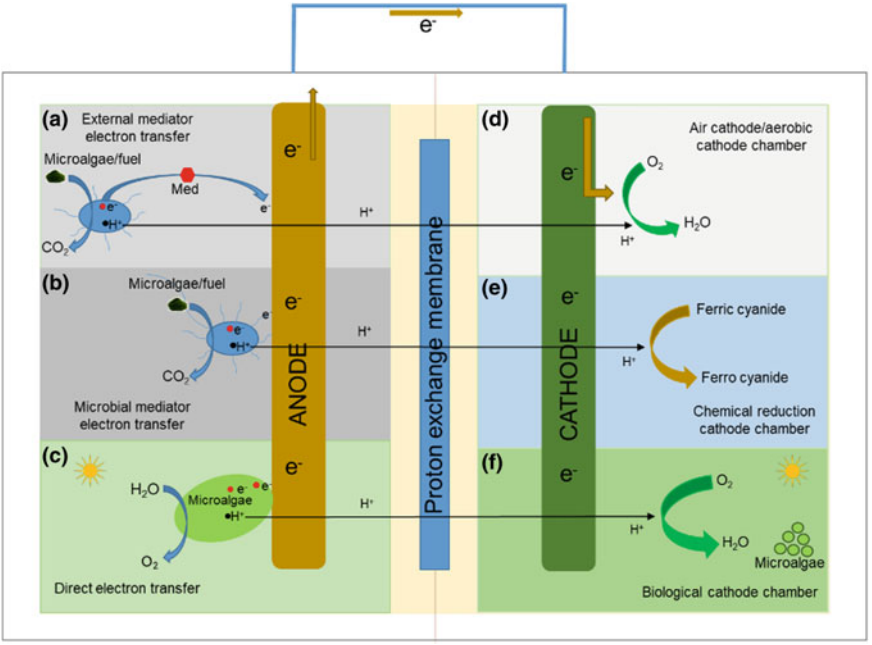

Fig. 1 Representation of various configurations of the anode chamber and the cathodic chamber of mMFCs. **a**, **b**, **c** Anodic chamber dependent on the mediator and dependent on bacterial mediator fed with microalgae substrate, and, of direct electron transfer. d, **e**, **f** cathodic chamber of air, of chemical acceptance of electrons and biological acceptance of electrons, respectively. Adapted from [78]

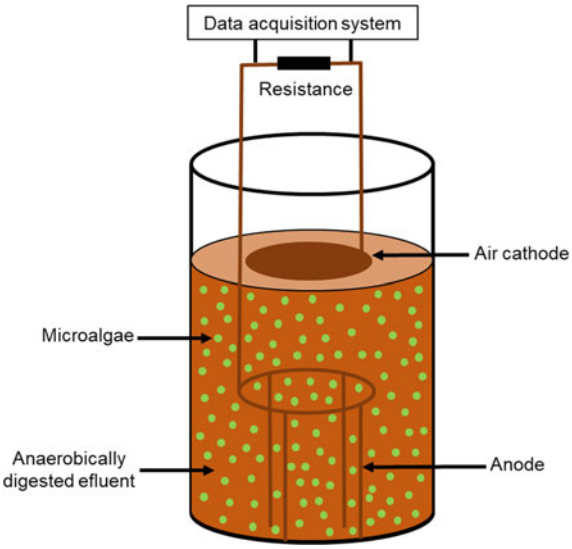

Fig. 2 Schematic of one single-chambered mMFC. *Source* Adapted from [26]

anode (this is, O_2 as a concurrent electron acceptor). This approach aims to achieve a better performance, as well as facilitating the removal of nutrients of the system.

On the same configuration, projects are developed using microalgae exoelectrogenic [11]. Microalgae are employed in the anodic compartment without the need for mediators. To date, there are few reports of microalgae being used to produce electrons in the anode compartment [71]. The use of such microalgae represents a potential possibility and, therefore, new studies are being realized out in this new field [17].

In two-chambered mMFCs, the anode and cathode chamber is physically divided by a membrane. The employ of the membrane helps to reduce the dissemination of oxygen into the anode, which easily occurs in the single-chamber mMFCs. The use of microalgae to generate O_2 in the cathodic compartment is the more promising project of this configuration [79]. Generally, a light source is positioned in the cathodic compartment to supply photons for photosynthetic reactions of microalgae and the anode compartment is covered. The compartments usually present forms cylindrical, rectangular, miniature and can be operated in batch and fed-batch mode [15].

A typical two-chamber mMFC, as mentioned above, is shown in Fig. 3 and exemplified by Gouveia et al. [22] and del Campo et al. [12]. About these mMFCs different projects have been proposed as, for example, the two-chamber mMFC in H-shaped [27]. However, it has been observed that these configurations have, as a disadvantage, a very small membrane area between the catholyte and the anolyte. Therefore, ionic exchange among the two compartments is much low, and membrane encrustation and internal resistance are some of the limitations presented by the H-shaped mMFCs [69].

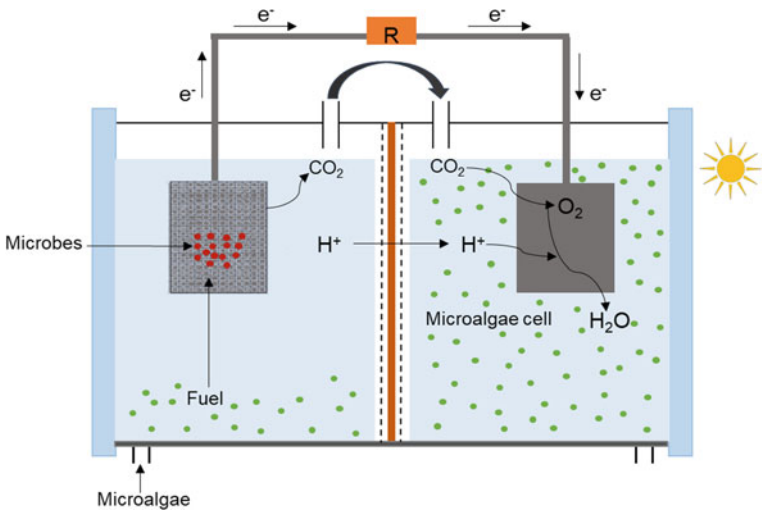

Fig. 3 Schematic of one two-chambered mMFC. *Source* Adapted from [10]

Similarly, some projects two-chamber mMFC are enriched with microalgae in the anodic and cathodic compartment [10]. The anode compartment, which contains bacteria, is fed with microalgae biomass a substrate. The CO_2 generated in the degradation of biomass is carried through a tube, arrested on the compartments, and can be utilized by microalgae cells at the cathodic compartment to stimulate their development and generate oxygen. These projects prove that microalgae-based fuel cells can be economically viable with the self-sustaining generation of bioelectricity and biomass of microalgae.

In the three-chamber mMFC configurations, the third compartment is among the anode and the cathode. The partial desalination is detected in the intermediate compartment, where the cations displace to the cathode and anion to the anode. However, the power density of that configuration has been observed to be minor than two-chamber mMFCs [77].

In sediment mMFCs, energy can be generated by the application of an anode disposed into sediment and a cathode filled with microalgae which are above the same [78]. Typically, the sedimentary mMFC is composed of an anode and a cathode positioned in contrary sides of a chamber. Electrodes are connected externally, and the anode is deposited in the centre of the sediment. Posteriorly, the sediment is sheeted with sterile sand [9, 29]. Figure 4 presents a model of sedimentary mMFC, membrane-less, composed of a microalgae biocathode.

Lastly, the coupled mMFCs are systems developed for the concomitant generation of electricity and distinct bioprocesses. The application of coupled systems provides a means of treat wastewater, produce bioelectricity and biomass simultaneously [20, 30]. Figure 5 features a coupled mMFC model.

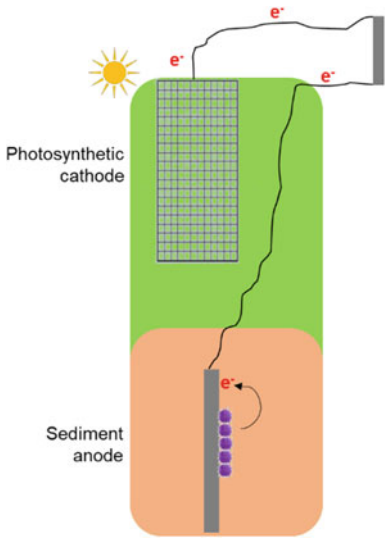

Fig. 4 Schematic of one sediment-type mMFC. *Source* Adapted from [9]

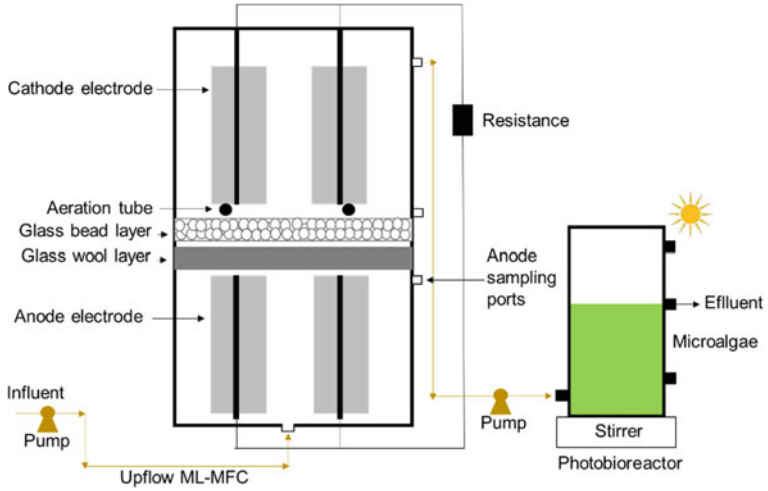

Fig. 5 Schematic of one coupled type mMFC. *Source* Adapted from [30]

6 Applicability of the Process

The use of microalgae has attracted considerable interest and has been gaining much prominence in the field of bioeconomics mainly to its practical value in the development of alternative biofuels and the implementation of mMFCs in several other applications.

The incorporation of microalgae as bioenergy sources into an mMFC serves as a promising alternative due to its inherent advantages in its rapid and versatile growth, energy conversion efficiency, carbon dioxide fixation, wastewater recovery and the parallel biomass production of high added value [67].

Several authors have presented the efficiency of these processes and researches have been intensively conducted through the light energy conversion into bioelectricity by the metabolic and electrochemical reactions of photosynthetic organisms [5, 7, 39, 64, 71, 75].

The integration of mMFCs technology based on microalgae has the advantage of reusing the nutrients in the same system, do not require special catalysts and still have intermediate organic material which helps them in the conversion of bioelectricity in the dark. This makes these systems achieve high performance with a low maintenance cost [17, 77].

Another term in evidence in microalgae mMFCs is the CO_2 sequestration with simultaneous treatment of wastewater and bioenergy generation. The mMFCs use the anode and the cathode to accomplish the process of photosynthesis and by-products production through the O_2 generated in situ. This has led many to evaluate aspects of biocathodes as a practical solution to the oxygen-limiting processes of conventional processes while enabling low-cost aeration with the parallel biomass production [17, 49, 67]. Still, one of the most promising applications of mMFCs is

to recuperate energy from low-quality substrates, which make a process sustainable and economically viable [39].

Although the concept of mMFCs shows a promising option, there are still some problems to be solved mainly due to their limited energy production. The challenge to be overcome today is to convert the technologies developed into laboratory scales into a much larger projection field. High costs with membranes, electrode materials and other problems that are sometimes not observed on small scales could affect the industrial practice of this system [2].

Different from already consolidated traditional projects, the progress in diverse renewable energy technologies yet need considerable investment. Based on statistics and analysis from U.S. Energy Information Administration the bioenergy generation costs for alternative sources as hydro, solar, wind and biomass can reach average values of $0.062, $0.063, $0.059 and $0.095 per kW/h, respectively. While theoretically, the operating cost of an mMFC equals $0.120 per kW/h [1, 42, 82].

Economically, recent advancement in the overall efficiency of mMFCs and the integration of new technologies are presented as a promising aspect shortly [81]. A clear benefit of this technology is the opportunity to unite and rearrangement of sundry processes in one place. Separation techniques, CO_2 conversion, waste treatment, and power generation is probably the best option and certainly has a profound socio-environmental impact [54, 84].

The cooperation of regulatory policies, interdisciplinary aids and life cycle assessments (LCAs) are essential to enhance production and economics for microalgae-based bioelectricity generation [85]. Life cycle assessment is a crucial tool for analyzing all the environmental and economic impingement of a process or product, particularly before being applied on a large commercial scale [55].

Few economic analyzes have been detailed so far for mMFCs. To date, the R&D has focused on laboratory-scale projections, however it is expected that commercialization of larger scale configurations will be possible in the coming years [53, 87]. Also, the cost comparison of these processes with conventional chemical systems is not feasible because of the substantial costs of substrates used in these systems.

Future advancements should take into account the elevated efficiency and low cost for scheduling mMFCs configurations, as well as new advances should contain energy management systems to improve performance and system capacity for practical use and real [18].

Finally, the configuration and integration of microalgae-based fuel cell systems play a significant role, and today many advances are being made toward the applicability of production processes. Ongoing projects of new modularities and programming, material longevity, inclusion of bioenergy collection and storage processes and the understanding built on pilot researches are driving innovation and consolidation into broader market acceptance of mMFCs.

7 Conclusions and Future Prospects

The strategy of integrating microalgae into microbial fuel cells is a technology that benefits sustainable energy production. Nonetheless, some limitations are persisting scale-up. A steady voltage is necessary to operate, however, the voltage generated in the mMFCs is small, and the robustness of generation is also inconstant. The solution would be to make adjustments to the operating parameters regularly, including microbial load and oxidant concentration. Among other limitations, the high internal resistance found in mMFCs can be surpassed by decrease the space among the electrodes; the low electron transfer can be solved by recognizing good mediators without toxicity and; the low potency density of the systems can be improved by using microbial consortia or by identifying microalgae with effective electrogenic potential. Another bottleneck of the mMFC system is the reduction of proton transfer efficiency due to biological encrustation occurred at the membrane. Therefore, a regular exchange of the membrane is required to operate continuously.

Most mMFCs configurations feature advantages and disadvantages, but all have the potential for bioelectricity production. What needs to be done is to select the most sensitive parameters and perfect them. It is expected that the utilization of a single configuration or the integration of mMFCs projects will be simplified towards field scale application. Integrated systems must be developed to simultaneously solve different problems, such as waste treatment and CO_2 sequestration. Moreover, life-cycle analysis and technical-economic of the mMFCs is necessary to propose the feasibility of this technology on an industrial scale.

Despite the existing bottlenecks, currently, the mMFCs have proven to be a potential technology for the generation of electricity associated with bioremediation of wastes, CO_2 fixation, and production of value-added biomass. The application of microalgae in these systems has the possibility of making the fuel cells a doable technology. In comparison to conventional fuel cells, mMFCs exhibit attractive resources, which will influence future applications and the development of this technology. Recent research results suggest that of the mMFCs will become, in the next future, of practical use and the best choice among the sustainable bioenergy processes.

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