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METABÓLITOS VOLÁTEIS DE *Phormidium autumnale* EM DIFERENTES SISTEMAS DE CULTIVO

Santa Maria, RS
2018

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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciência e Tecnologia dos Alimentos, Área de Concentração em Ciência e Tecnologia dos Alimentos, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de **Doutor em Ciência e Tecnologia dos Alimentos**

Orientadora Prof[a]. Dr[a]. Leila Queiroz Zepka

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“O mundo não se divide em pessoas boas e más.

Todos temos Luz e Trevas dentro de nós.

O que importa é o lado o qual decidimos agir.

Isso é o que realmente somos.”

- J. K. Rowling

RESUMO

METABÓLITOS VOLÁTEIS DE *Phormidium autumnale* EM DIFERENTES SISTEMAS DE CULTIVO

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As microalgas são uma potencial fonte de biomoléculas de interesse comercial devido ao seu perfil metabólico diversificado capaz de sintetizar diferentes classes de compostos orgânicos. O gênero *Phormidium autumnale* é uma cianobactéria com habilidade de crescimento nos 3 principais tipos de cultivo: fotoautotrófico, mixotrófico e heterotrófico. Em face disso, a tese teve por objetivo investigar o perfil de compostos orgânicos voláteis (COVs) microalgais em diferentes condições de cultivo. Os resultados obtidos nos experimentos, possibilitaram a identificação de 53 compostos orgânicos voláteis no total. No cultivo heterotrófico utilizando amido como fonte de carbono, foram identificados e quantificados um total de 22 COVs, sendo o experimento menos produtivo na fração volátil; já o cultivo heterotrófico suplementado com sacarose obteve uma maior produção com 34 compostos identificados. Diferentes classes químicas (aldeídos, álcoois, cetonas e hidrocarbonetos) foram encontradas nos experimentos, os majoritários foram os aldeídos e álcoois. A presença significativa dessas classes químicas mostra que a aplicabilidade desses compostos voláteis produzidos é extensa. Pesquisas sobre o perfil de compostos orgânicos voláteis são necessárias para investigar as microalgas, a fim de identificar os candidatos potenciais para uso em uma nova geração de formulações de alimentos, rações, biocombustíveis, produtos farmacêuticos e matéria-prima para a indústria de química fina. Em paralelo à esta pesquisa, foi produzido o artigo de revisão *Microalgal Biorefineries for Bioenergy Production: Can We Move from Concept to Industrial Reality?* que foi publicado na Revista *BioEnergy Research* e encontra-se em anexo a este documento.

Palavras-chave: Cianobactéria, microalga, compostos orgânicos voláteis, GC-MS, biotransformação.

ABSTRACT

Phormidium autumnale VOLATILE METABOLITES IN DIFFERENT CULTIVATION SYSTEMS

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Microalgae are a potential source of biomolecules of commercial interest due to their diversified metabolic profile capable of synthesizing different organic classes of compounds. The *Phormidium autumnale* is a cyanobacteria with growth ability in the three main cultivation types: photoautotrophic, mixotrophic and heterotrophic. The aim of this thesis was to investigate the profile of microalgal volatile organic compounds (VOCs) under different culture conditions. The results obtained in the experiments allowed the identification of 53 volatile organic compounds in total. In the heterotrophic cultivation using starch as carbon source, a total of 22 VOCs was identified and quantified, the least productive experiment being in the volatile fraction; already the heterotrophic culture supplemented with sucrose obtained a greater production with 34 identified compounds. Different chemical classes (aldehydes, alcohols, ketones and hydrocarbons) were found in the experiments, the majority were aldehydes and alcohols. The significant presence of these chemical classes shows that the applicability of these volatile compounds produced is extensive. Research on the profile of volatile organic compounds is required to investigate microalgae in order to identify potential candidates for use in a new generation of formulations of food, feed, biofuels, pharmaceuticals and raw material for the fine chemicals industry. In parallel to this research, the review “Microalgal Biorefineries for Bioenergy Production: Can We Move from Concept to Industrial Reality? which was published in the Journal BioEnergy Research and is attached to this document.

Key words: Cyanobacteria, microalgae, volatile organic compounds, GC-MS, biotransformation.

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CAPÍTULO 1

INTRODUÇÃO

A biotecnologia de microalgas é uma área emergente da tecnologia industrial, que vem se consolidando em função da potencialidade de exploração dos bioprodutos resultantes dos processos de produção. A manufatura de insumos intermediários e/ou produtos finais para a indústria de alimentos ocorre a partir da exploração de metabólitos intracelulares e extracelulares, produzidos por estes microrganismos durante os processos biotecnológicos.

Um sistema baseado em microalgas para a produção de fontes de produtos de alto valor é uma área emergente, representando, portanto, uma grande promessa para aplicações industriais (ROSO et al., 2015). A ocorrência de compostos orgânicos voláteis na microalga é uma consequência do seu metabolismo, embora dependente da espécie, sua produção pode ser modificada por vários fatores, como luz, teor de sal, carbono e fontes de nitrogênio (MILOVANOVIC et al., 2015; HOSOGLU, 2018). Em geral, existem duas vias principais de fixação de carbono em microalgas: (i) fotoautotrófico, que corresponde ao crescimento fotossintético e fixação de carbono inorgânico (dióxido de carbono) através do ciclo de Calvin-Benson, e (ii) heterotrófico, correspondente a assimilação de carbono orgânico na ausência de luz (PEREZ-GARCIA et al., 2011).

Na ausência de luz, o processo fotossintético é suprimido e as algas ganham energia a partir de processos orgânicos alternativos. As microalgas usam moléculas orgânicas como fonte primária de energia e carbono por meio do modo nutricional heterotrófico e facilitam as altas produtividades de biomassa que fornecem uma viabilidade econômica para a produção em grande escala (PÉREZ-GARCIA et al., 2011). Pode-se ainda elaborar um cultivo misto, esse chamamos de mixotrófico, emprega luz e uma fonte de carbono exógena (CHEIRSILP & TORPEE, 2012).

A cianobactéria escolhida para realizar os experimentos, possui a capacidade de sintetizar moléculas orgânicas também na ausência de luz. A rota heterotrófica, quando possível, além de contornar a dependência da luz, pode ser a alternativa mais barata, e é mais simples construir instalações e mais fáceis de manter em escala real (FRANCISCO et al., 2014). Para melhorar o desempenho de reatores microalgais heterotróficos, é necessário um melhor entendimento do uso do carbono orgânico exógeno adicionado à cultura, conhecer a eficiência de conversão, e consequentemente a eficiência do processo (JACOB-LOPES &

FRANCO, 2013; BARROS et al, 2017). A exploração do conhecimento e o aproveitamento integral de todas as frações de reatores microalgais pode representar uma melhoria em relação ao fornecimento de insumos para diferentes tipos de indústrias, como a aplicação desses metabólitos como insumo de produtos químicos finos (SANTOS et al, 2016a).

Em face disso, o objetivo do estudo foi investigar o perfil de metabólitos voláteis a partir de diferentes condições de cultivos microalgais. O estudo teve como foco a avaliação do perfil de compostos orgânicos voláteis a partir das condições cultivos fotoautotrófico, mixotrófico e heterotrófico, suplementado com sacarose em culturas de *P. autumnale*, bem como a análise do perfil volátil decorrente da biotransformação do amido de mandioca utilizado como fonte de carbono exógeno em cultivo heterotrófico.

REVISÃO BIBLIOGRÁFICA

1.1 Microalgas

O termo microalga não apresenta nenhum valor taxonômico. Microalgas são microrganismos heterogêneos, microscópicos, unicelulares, coloniais ou filamentosos, fototróficos, podendo ser procarióticos ou eucarióticos (OLAIZOLA, 2003; LOURENÇO, 2006). Desenvolvem-se principalmente em ambientes aquáticos, além de estarem presentes em solos, rochas, e em ambientes extremos, como geleiras e fossas termais, bem como, associados simbioticamente a outros organismos (líquens, pteridófitas, protozoários) auxiliando-os na fixação de nitrogênio (HERRERO et al., 2001).

Microalgas compreendem um grupo diverso de microrganismos com um número de 72.500 espécies catalogadas (GUIRY & GUIRY, 2018). Os padrões taxonômicos atuais incluem 16 classes desses organismos (*Cyanophyceae*, *Rhodophyceae*, *Chlorophyceae*, *Charophyceae*, *Euglenophyceae*, *Raphidophyceae*, *Xanthophyceae*, *Bacillariophyceae*, *Chrysophyceae*, *Haptophyceae*, *Phaeophyceae*, *Dinophyceae*, *Cryptophyceae*, *Synurophyceae*, *Eustigmatophyceae*, *Glaucophyceae*) (GRAHAM & WILCOX, 2000).

A presença desses organismos em ambientes tão diversos deve-se às várias estratégias metabólicas que utilizam e que podem explicar sua capacidade em responder rapidamente a alterações no meio onde vivem (ACHYUTHAN et al., 2017). As cianobactérias são assim denominadas pela ausência de organização celular e estruturas definidas, fato que as assemelha às bactérias (REVIERS, 2002).

Considerando a grande biodiversidade e os recentes desenvolvimentos na engenharia genética, as cianobactérias representam uma das fontes mais promissoras para novos bioprodutos, como proteínas, amido, celulose, lipídeos, aminoácidos, antioxidantes, carotenoides, glicerol, ácidos graxos poli-insaturados, esteróis, vitaminas B, C e E (RODRIGUES et al., 2014; SATHASIVAM et al., 2017). Além destes bioprodutos, há uma importante vertente que consiste no estudo da capacidade desses organismos em produzir metabólitos secundários como os compostos orgânicos voláteis (COV's) (DURME et al., 2013; SANTOS et al., 2016a; HOSOGLU, 2018).

Vários gêneros de cianobactérias e microalgas apresentam capacidade de produzir metabólitos secundários, os quais podem ser intracelulares e/ou extracelulares. A emissão de

compostos voláteis por estes microrganismos é influenciada pelas condições ambientais (LOPEZ-PÉREZ et al., 2017).

O gênero *Phormidium* sp. é uma cianobactéria filamentosa não ramificada que vive em aglomerados, com conteúdo celular geralmente azul esverdeado, raramente marrom. Pode ser encontrada em solos, rochas úmidas, lama, plantas aquáticas, córregos, algumas em ambientes litorâneos. Outras espécies são encontradas em ambientes extremos como nascentes termais e solos de desertos (THOMAZEU et al., 2010; GUIRY & GUIRY, 2018).

Pesquisas realizadas com a *Phormidium autumnale* demonstraram uma ampla produtividade de biomassa a partir de resíduos agroindustrias utilizados como substrato em cultivos heterotróficos, destacando assim uma forma sustentável de produção de bioprodutos como, óleos unicelulares, carotenoides, biodiesel (FRANCISCO et al., 2014; RODRIGUES et al., 2014). Santos et al (2016a), demonstraram em sua pesquisa a capacidade deste gênero em produzir uma variedade de compostos orgânicos voláteis a partir de diferentes fontes de carbono exógeno.

Fatores ambientais, como temperatura, iluminação, pH, conteúdo mineral, densidade populacional, fase de crescimento e estado fisiológico podem modificar sua composição química. Assim, as condições de crescimento podem ser otimizadas e mapeadas para maximizar a produção de biomoléculas de interesse (HU, 2013; BATISTA et al., 2013).

1.2 Cultivos microalgais

A tecnologia de cultivo de microalgas é um dos principais fatores que restringem a produção de novos bioprodutos. Extensas pesquisas têm sido realizadas com foco nos tipos de cultivos para aumentar a produtividade de biomassa microalgal (MARONEZE et al., 2016; SIQUEIRA et al., 2016; ZHAN, RONG & WANG, 2016). Os cultivos mais abordados, tem sido o fotoautotrófico, heterotrófico e o mixotrófico.

As microalgas são microrganismos fotossintéticos no qual usam o oxigênio para a fixação de CO_2 (KUMAR et al., 2011; SANTOS et al., 2016b). São consideradas como um dos mais eficientes sistemas biológicos de captação de energia solar para a produção de compostos orgânicos através do mecanismo fotossintético (ABU-GHOSH et al., 2015).

As distintas vias metabólicas referem-se à disponibilidade e à forma de nutrientes, particularmente carbono, nos meios em que as microalgas estão sendo cultivadas. O carbono

é de especial importância, pois é essencial para a formação e desenvolvimento de qualquer organismo vivo. Mais especificamente, a forma de carbono disponível determina a via metabólica pela qual as microalgas assimilam (LOWREY, BROOKS & MCGINN, 2015).

Em geral, existem duas vias principais de fixação de carbono em microalgas. O cultivo fotoautotrófico corresponde ao crescimento fotossintético e fixação de carbono inorgânico através do ciclo de Calvin-Benson, este processo compreende três etapas: carboxilação, redução e regeneração (WILLIANS & LAUREN, 2010). O fim do ciclo gera uma molécula de gliceraldeído-3-fosfato que, por meio da ação de enzimas, forma o fosfoenolpiruvato e, finalmente, o piruvato. No entanto, o cultivo de microalgas usando o modo fotoautotrófico tem algumas limitações, como crescimento lento de células microalgais, maior custo de colheita e baixa produtividade de biomassa (ZHAN, RONG & WANG, 2016).

O cultivo heterotrófico correspondente à assimilação de carbono orgânico na ausência de luz pela via oxidativa das pentoses fosfato (LOWREY, BROOKS & MCGINN, 2015). Para usar estes compostos orgânicos, o transporte ocorre através da membrana. Este substrato será convertido em glicose 6-fosfato para que possa iniciar a rota. Durante o metabolismo há a formação de duas moléculas de ATP (adenosina trifosfato). O produto final também é piruvato (FAY, 1983).

O cultivo heterotrófico permite o crescimento de microalgas em meios suplementados com fontes de carbono como, glicose, frutose, sacarose, acetato. Este cultivo tem a vantagem de acelerar a taxa de crescimento celular, aumentar o acúmulo de biomassa em comparação com a cultura autotrófica e até mesmo pode utilizar substratos de baixo custo como resíduos agroindustriais, reduzindo o custo global do processo (FRANCISCO et al., 2014; SIQUEIRA et al., 2016).

Para o cultivo mixotrófico, as microalgas crescem com luz e matéria orgânica como fonte de energia, com carbono orgânico e CO_2 como fontes de carbono, ou seja, têm a capacidade de realizar fotossíntese e adquirir nutrientes orgânicos exógenos (ZHAN, RONG & WANG, 2016). A mixotrofia combina as vantagens da autotrofia e heterotrofia e supera as desvantagens da autotrofia. Sob condição de cultivo mixotróficos, as microalgas não só podem crescer heterotroficamente com o carbono orgânico, aumentando a taxa de crescimento, mas também consomem carbono inorgânico (CO_2) e a produção de oxigênio

por fotossíntese, o que torna a emissão total de CO_2 mais baixa sob o modo de cultivo mixotrófico do que sob o modo de cultivo heterotrófico (IMAMAKI & HIRANO, 2002; BONINI & BASTOS, 2012).

O estudo de diferentes cultivos microalgais é importante para elucidar características como a taxa de crescimento, produtividade de biomassa e consequentemente a geração de bioprodutos para aplicação industrial, assim como da influência das condições de cultivo, que podem ser manipuladas para obtenção de compostos de interesse por via biotecnológica.

1.3 Bioprodutos de microalgas

A biodiversidade e consequente variabilidade na composição bioquímica das microalgas, e ao estabelecimento de tecnologia de cultivo em grande escala, vêm permitindo que as microalgas sejam utilizadas em diversas aplicações (BOROWITZKA, 2013). A produtividade das microalgas pode superar o de qualquer outra matéria-prima terrestre utilizada em processos de biorrefinarias (GERARDO et al., 2015).

Microalgas possuem um elevado crescimento celular, e em paralelo, a biomassa produzida é uma excelente fonte diversificada de moléculas como lipídios, proteínas, polissacarídeos, antioxidantes, esteróis insaponificáveis, agentes antimicrobianos e minerais. Adicionalmente, esses microrganismos são uma fonte potencial de triacilglicerídeos que podem conter quantidades elevadas de ácidos graxos de cadeia longa poli insaturados, tais como ácidos graxos ômega 3, ácido docosa-hexaenoico (DHA) e ácido eicosapentaenoico (EPA) (HERRERO et al., 2015).

Estes microrganismos contêm altos níveis de óleos, carboidratos e proteínas que os tornam matérias-primas versáteis para a produção de combustíveis em paralelo com produtos químicos valiosos e a alimentação humana e animal no contexto biorrefinaria (SILVA et al., 2015). A combinação da produção de biocombustíveis de microalgas com as aplicações convencionais é excelente para prosperar a indústria de biorrefinaria microalgal de forma sustentável (MA et al., 2015).

Também são reconhecidas como uma fonte diversificada de moléculas bioativas. As conversões de biomassa de microalgas para biocombustíveis, produtos e compostos de alto valor agregado estão globalmente ganhando um destaque significativo (HERRERO & IBÁÑEZ, 2015). Entre estes compostos, os pigmentos naturais compreendem um dos

componentes mais interessantes produzidos em sistemas baseados em microalgas (QUEIROZ et al., 2013).

Dentre os pigmentos com possíveis propriedades bioativas, inclui-se duas classes: carotenoides e clorofilas, pigmentos naturalmente presentes na biomassa microalgal que recebem atenção especial devido a sua capacidade antioxidante, ação anticancerígena, anti-inflamatória, anti-obesidade e atividades neuroprotetoras (RODRIGUES et al., 2015; D'ALESSANDRO & FILHO, 2016).

Devido a estes diversos fatores, as microalgas têm sido direcionadas como foco de processos de biorrefinaria economicamente viáveis, visando o fato de, todos os componentes gerados a partir da biomassa desta, possuírem alto valor para a geração de produtos aplicáveis em diversos setores industriais (HERRERO et al., 2015; MORENO-GARCIA et al.,2017). Adicionalmente, observa-se que essa espécie tem sido destacada como uma potencial fonte de carotenoides entre outros compostos (GERARDO et al., 2015).

Embora venha sendo estudado largamente as frações de metabolitos não voláteis, observa-se também que o rendimento em microalga não satisfaz completamente o balanço de carbono total do sistema sugerindo que parte do balanço está direcionado para produção de compostos orgânicos voláteis (JACOB-LOPES et al., 2010).

1.4 Compostos voláteis

Compostos orgânicos voláteis (COV's) são comumente produzidos por microrganismos e emitidos para o ambiente. São caracterizados por possuir baixo peso molecular e alta pressão de vapor, porém esta área do conhecimento ainda é pouco explorada em microalgas (ZUO et al., 2012; POPOVA et al., 2014).

Alguns compostos podem ser produzidos metabolicamente (por enzimas presentes em microalgas) e também por compostos de degradação primária, tais como lipídeos e proteínas (SANTOS et al., 2016a). Os COV's mais comumente encontrados em microalgas são o 2-metil-isoborneol (MIB) e a geosmina, conhecidos por terem um descritor de odor de terra / mofo em água potável. Estes compostos são álcoois terciários com função biológica ainda não definida, embora possam ser subprodutos ou produtos intermediários do metabolismo de pigmentos (LEE et al., 2017). No entanto estes compostos não foram detectados por Santos et al (2016a) ao pesquisar compostos orgânicos voláteis em cultivo

heterotrófico da *Phormidium autumnale*. Outros compostos como os norcarotenóides iononas, β-ciclocitral e geranilacetona são oriundos da degradação de carotenoides e são reportados como produtos de excreção de cianobactérias e microalgas (OZAKI et al., 2008; JUTTNER et al., 2010; FUJISE et al., 2010).

Segundo Nuccio et al (1995), as taxas de produção de COV's produzidos por microalgas apresentam significativo aumento durante a fase de crescimento exponencial e declive na fase de senescência, apresentando um comportamento parabólico com vértice negativo. Alguns desses compostos têm papel importante em processos químicos atmosféricos (JACOB-LOPES et al., 2010). A biossíntese destes compostos depende da disponibilidade de carbono, nitrogênio e enxofre, bem como a energia fornecida pelo metabolismo primário. Por tanto, a disponibilidade destes "blocos de construção" tem um grande impacto na concentração de um metabólito secundário, incluindo compostos voláteis, demonstrando elevado grau de conectividade entre o metabolismo primário e secundário (DUDAREVA et al., 2013).

Uma gama de compostos, incluindo classes, tais como aldeídos, cetonas e álcoois podem ser formados a partir da degradação de lipídeos (RZAMA et al., 1995). As microalgas são relativamente ricas em ácidos graxos poli-insaturados (PUFA's) de cadeia longa tais como o ácido eicosapentaenoico e ácido docosa-hexaenoico (ZHANG et al., 2009). Os aldeídos lineares de cadeia curta, muitas vezes são quimicamente derivados da oxidação lipídica. Aldeídos ramificados e aromáticos, são tipicamente formados devido a oxidação lipídica e enzimática da proteína (DURME et al., 2013). Alguns compostos voláteis derivados de PUFA's atuam como feromônios sexuais ou em defesa química contra outros microrganismos (LEE et al., 2017).

Determinadas reações químicas podem converter os compostos orgânicos voláteis em outros compostos. Os álcoois podem ser oxidados para aldeídos e, em seguida para ácidos carboxílicos, e cetonas podem reagir com os radicais hidroxila no ar para formar aldeídos (SANTOS et al., 2016b). Aldeídos e cetonas podem ser reduzidos para os álcoois por redutases (KORPI et al., 2009). As cetonas podem ser formadas de muitas maneiras; cetonas alifáticas podem ser produtos de oxidação ou degradação de lipídeos e podem ser formadas a partir da clivagem oxidativa de carotenoides (SANTOS et al., 2016a).

A identificação detalhada dos compostos voláteis é muito importante devido aos seus impactos diretos nas propriedades odoríferas do produto final enriquecido com biomassa de microalgas (ROBERTSON et al., 2016; SANTOS et al., 2016a; ZHOU et al., 2016). Segundo Hosoglu (2018), compostos identificados como sendo responsáveis por tais características aromáticas são diferenciados em categorias tais como hidrocarbonetos, aldeídos, álcoois, ésteres, cetonas, lactonas, ácidos graxos livres de cadeia curta a média, compostos fenólicos e enxofre.

OBJETIVOS

2.1 Objetivo geral

Determinar o perfil de compostos orgânicos voláteis microalgais em diferentes condições de cultivo.

2.2 Objetivos específicos

- Demonstrar as principais rotas bioquímicas para a biogeração de compostos orgânicos voláteis e possibilidades de aplicação industrial.
- Elucidar o perfil volátil da *Phormidium autumnale* sob diferentes tipos de cultivo.
- Avaliar a produção de compostos voláteis em cultivo mixotrófico de *P. autumnale* para a obtenção de *flavours*.
- Identificar e quantificar os compostos orgânicos voláteis do cultivo heterotrófico microalgal utilizando amido de mandioca como fonte de carbono exógena.

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CAPÍTULO 2

CHAPTER: Biogeneration of volatile organic compounds by microalgae: occurrence, behavior, ecological implications and industrial applications.

Volatile Organic Compounds: Occurrence, Behavior and Ecological Implications

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Chapter

BIOGENERATION OF VOLATILE ORGANIC COMPOUNDS BY MICROALGAE: OCCURRENCE, BEHAVIOR, ECOLOGICAL IMPLICATIONS AND INDUSTRIAL APPLICATIONS

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ABSTRACT

Microalgae are a source of potential commercial interest biomolecules due to their diverse metabolic profile, able to synthesize different classes of organic compounds. The continual growth of the commercial application of primary and secondary biotechnology metabolites and more strict environmental legislations have led to interest in developing renewable forms to produce these compounds and apply in bulk and fine chemistry. The growing interest in natural products directs the development of technologies that employ microorganisms, including microalgae, which are able to synthesize specific volatile organic compounds (VOCs). The different VOCs can belong to different classes of compounds such as alcohols, esters, hydrocarbons, terpenes, ketones, carboxylic acids and sulfurized compounds. Volatile organic compounds are secondary metabolites obtained from microalgae that could be used as an important alternative source of chemicals. The use of the volatile fraction of microalgal systems may represent an improvement in the supply of a large volume of inputs to many different types of industry. Clearly, there is a need for further studies on the volatile fraction of microalgal systems, as well as on the elucidation of the formation metabolic pathways of these compounds. Exploring the volatile profile of microalgae is a possibility, and it is scientifically challenging to apply these metabolites as chemical feedstocks. Divided into three discrete parts, the chapter covers topics that refer to the occurrence and behavior of volatile organic compounds in microalgae systems, the ecological implications and industrial applications, summarizing a range of useful technological and economic opportunities regarding such compounds.

Keywords: biomolecule, biosynthesis, flavor, off-flavor, fuel

INTRODUCTION

Microalgae are a group of photosynthetic microorganisms typically unicellular and eukaryotic. Although cyanobacteria belong to the domain of bacteria, and are photosynthetic prokaryotes, often they are considered microalgae [1].

Microalgae-based systems for chemicals production are an emergent area, representing a great promise for industrial application. However, there is little information available on the volatile

organic compounds biogeneration of these microorganisms. The characterization of the volatile fraction of microalgal bioreactors can contribute to establishing routes for the bioconversion of substrates, and enable the identification of potential applications of the volatile bioproducts formed [2].

The growing interest in natural products guides the development of technologies that employ microorganisms, including microalgae, which are able to synthesize specific volatile organic compounds (VOCs). Jacob-Lopes [3] reported that the VOCs are the main bioproducts formed during microalgae cultivation. The carbon balance analysis indicates that these compounds represent up to 90% of the total substrate converted in the bioreactor. The different VOCs can belong to different classes of compounds such as alcohol, esters, hydrocarbons, terpenes, ketones, carboxylic acids and sulfurized compounds [4].

Microalgae were always regarded to be typical photosynthetic microorganism in which the light-dependent fixation of CO_2 is the dominant mode of nutrition [5]. Microalgae can also be cultivated heterotrophically without light and with addition of an exogenous source of carbon by using the oxidative pentose phosphate pathway. This metabolic route serves as the exclusive source of energy for maintenance and biosynthesis, besides providing the carbon required as building blocks for biosynthesis [6]. The biosynthesis of volatile compounds depends mainly of the availability of carbon and nitrogen as well as energy provided by primary metabolism. Therefore, the availability of these building blocks has a major impact on the concentration of any secondary metabolites, including VOCs [2].

Based on their biosynthetic origin, these VOCs can be divided into terpenoids, phenylpropanoids/benzenoids, carbohydrate derivates, fatty acids derivates and amino acid derivates, in addition to specific compounds not represented in those major classes [7; 8]. These compounds could therefore be a source of useful chemical products, based on a nonconventional technological route. Chemicals obtained from bioprocesses are sold at prices 1000 times higher than those synthetic chemicals, which show great potential for the exploitation of these processes. In view of the commercial significance, efforts should be made to elucidate the pathways of the formation of these compounds. Thus, the aim of this chapter was to evaluate the biogeneration of volatile organic compounds produced by microalgae, with focus on the occurrence, behavior, ecological implications and industrial applications of these metabolites.

OCCURRENCE AND BEHAVIOR OF VOLATILE ORGANIC COMPOUNDS FROM MICROALGAE

The occurrence of volatile organic compounds in microalgae is a consequence of their metabolism. Microalga species use oxygenic photosynthesis for the fixation of CO_2 [9]. They have pigments such as chlorophyll and carotenoids, which are involved in capturing luminous energy to perform photosynthesis. For the CO_2 be converted into carbohydrates, catalyzed by the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), is referred to as the Calvin cycle. The Calvin cycle is the metabolic mechanism for fixing CO_2 in microalgae. This process comprises three stages; carboxylation, reduction and regeneration [10]. The end of the cycle to form one molecule of glyceraldehyde-3-phosphate that through the action of enzymes form phosphoenolpyruvate, and finally pyruvate.

Additionally, some species of these microorganisms have the versatility to maintain their structures in the absence of light, being able to grow heterotrophically through the assimilation of one or more organic substrates as a carbon source in the oxidative pentose phosphate pathway. To use these organic compounds, transport occurs through the membrane. This substrate will be converted into glucose-6-phosphate so you can start the route. During metabolism there is the formation of two molecules of ATP (adenosine triphosphate). The final product is also pyruvate [5].

Regardless of metabolism, the biosynthesis of volatile organic compounds occurs through the formation of pyruvate molecule. To further illustrate, Figure 1 shows the main pathways of formation of these compounds which may be enzymatically or by reaction degradation. Based on this knowledge, can be suggested applicable routes for the synthesis of volatile organic compounds both for their better ecological understanding as to their potential commercial applications.

The volatile organic compounds from microalgae can belong to different classes of compounds such as esters, alcohols, hydrocarbons, ketones, terpenes, carboxylic acids and sulfur compounds [11]. In order to understand this diversity of compounds, Table 1 shows the main compounds and their cultivations previously found in studies. The biosynthesis of these volatile organic compounds will depend on the availability of building blocks, such as carbon, nitrogen, and energy supply from the primary metabolism. Therefore, the availability of these building blocks has great impact on the concentration of secondary metabolites, including VOCs, demonstrating the high level of connectivity between the primary and secondary metabolism [12, 13].

The formation of compounds from pyruvate can follow the route of terpenoids or also via the keto acids via intermediate 2-ketoisovalerate. With the formation of Acetyl-CoA has the biosynthesis of fatty acids. On arriving at the tricarboxylic acid cycle, follows the route of keto acids by intermediates 2-ketobutyrate and 2-ketovalerate. Started by way 2-keto acids, amino acids, which are

intermediates in the synthesis pathways. These 2-keto acids are formed by deamination followed by decarboxylation catalyzed by transaminase branched chain amino acids such as L-leucine, which has its synthesis from pyruvate, and L-isoleucine from the tricarboxylic acid or also by intermediate amino acid synthesis [14]. These 2-keto acids can be further subjected to decarboxylation, followed by reduction, oxidation and/or esterification, can be formed in addition to alcohols, aldehydes, acids and esters [12].

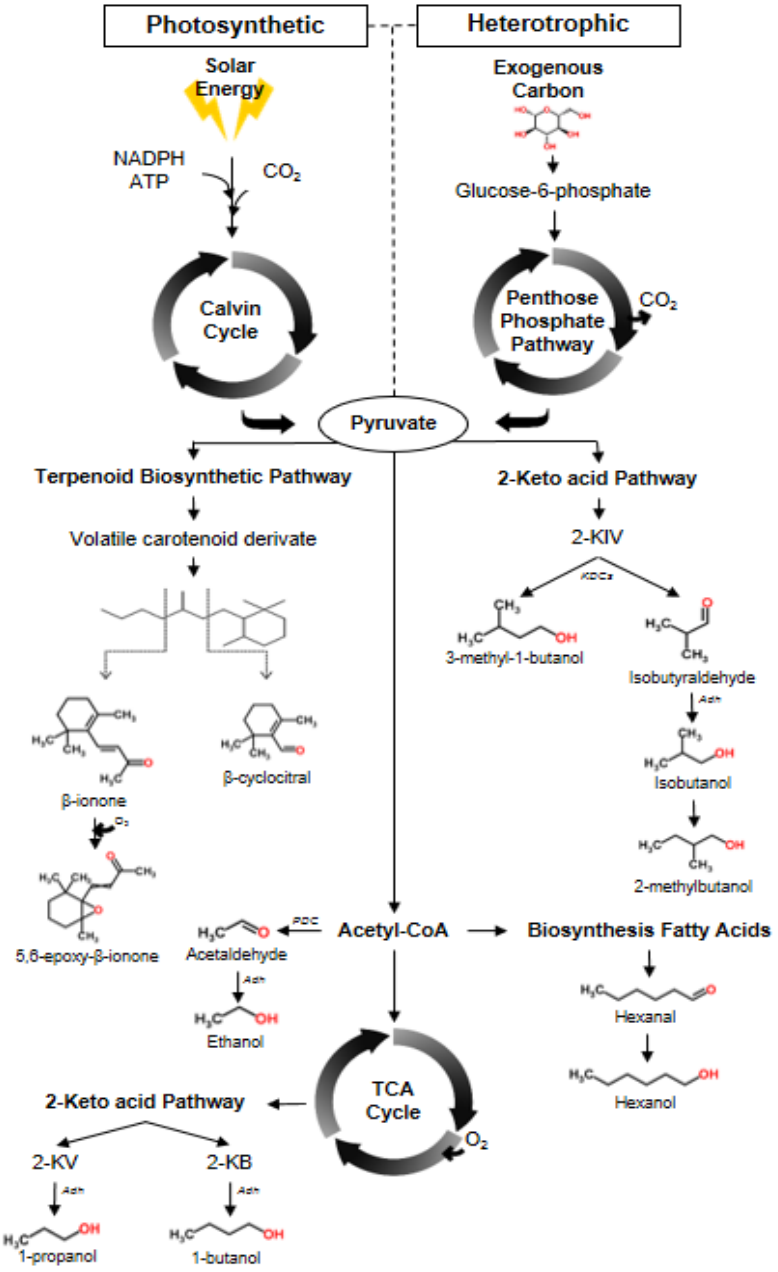

Figure 1. Overview of microalgae metabolism and their potential routes emission of volatile organic compounds.

Table 1. Major VOCs found in microalgae-based systems

Organic classes	Compounds	Microalgae	Cultivation	Reference
Acids	Acetic acid	*Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]
	Butanoic acid	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methylbutanoic acid	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Isovaleric acid	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
Sulfuric compounds	Dimethyl sulfide	*Tetraselmis* sp.	Photoautotrofic	Durme et al., 2013 [8]
	Dimethyl disulfide	*Tetraselmis* sp.*; Rhodomonas* sp.	Photoautotrofic	Durme et al., 2013 [8]
	Methional	*Rhodomonas* sp.	Photoautotrofic	Durme et al., 2013 [8]
	Dimethyl trisulfide	*Tetraselmis* sp.	Photoautotrofic	Durme et al., 2013 [8]
Furans	2-Ethylfuran	*Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	2-Pentylfuran	*Botryococcus braunii; Chlorella vulgaris; Spirulina platensis*	Photoautotrofic	Durme et al., 2013 [8]
Esters	Ethyl acetate	*Nannochloropsis oculata*	Photoautotrofic	Durme et al., 2013 [8]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]
	Methyl hexanoate	*Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	Methyl phenylacetate	*Nannochloropsis oculata*	Photoautotrofic	Durme et al., 2013 [8]
	Methyl octanoate	*Nannochloropsis oculata; Rhodomonas* sp.*; Chlorella vulgaris; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Isoamyl acetate	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]

	Isobutyl acetate	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Methyl decanoate	*Nannochloropsis oculata*	Photoautotrofic	Durme et al., 2013 [8]
	2-Butyl acetate	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
Terpenes	β-Cyclocitral	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcusbraunii; Chlorella vulgaris; Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Durme et al., 2013[8]; Milovanovic et al., 2015 [38]
		Phormidium autumnale;	Heterotrophic	Santos et al., 2015 [13]
	β-Ionone	*Tetraselmis* sp.*: Rhodomonas* sp.; *Chlorella vulgaris;*	Photoautotrofic	Durme et al., 2013 [8]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]

Table 1. (Continued)

Organic classes	Compounds	Microalgae	Cultivation	Reference
Ketones	2,3-Butanedione	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]

		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]
	2-Butanone	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methyl-2-butanone	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	4-Methyl-2-pentanone	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	1-Penten-3-one	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	2,3-Pentanedione	*Tetraselmis* sp.*; Rhodomonas* sp.	Photoautotrofic	Durme et al., 2013 [8]
	Acetophenone	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	2,3-hexanedione	*Phormidium autumnale*	Heterotrophic	Santos et al.,2015 [13]
	3-Pentanone	*Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	2-Heptanone	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Hydroxy-2-butanone	*Rhodomonas* sp.*; Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	2,3-Octanedione	*Rhodomonas* sp.	Photoautotrofic	Durme et al., 2013 [8]
	6-Methyl-5-hepten-2-one	*Tetraselmis* sp.	Photoautotrofic	Durme et al., 2013 [8]
	Tr,tr-3,5-octadien-2-one	*Rhodomonas*	Photoautotrofic	Durme et al., 2013 [8]
	6-Methyl-2-heptanone	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
Alcohols	Ethanol	*Tetraselmis* sp.*; Chlorella vulgaris; Nannochloropsis oculata*	Photoautotrofic	Durme et al., 2013 [8]

	1-Penten-3-ol	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Chlorella vulgaris; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	3-Methylbutanol	*Tetraselmis* sp.*; Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]
	2-Methylbutanol	*Tetraselmis* sp.*; Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	2-Methyl-1-propanol	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	2-Phenylethanol	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	1-Pentanol	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Chlorella vulgaris; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]

Table 1. (Continued)

Organic classes	Compounds	Microalgae	Cultivation	Reference
	Cis-2-penten-1-ol	*Tetraselmis* sp.*; Rhodomonas* sp.*; Botryococcus; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	3-Hexen-1-ol	*Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]

	1-Hexanol	*Tetraselmis* sp.*; Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]; Santos et al., 2015 [13]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]
	2,6-Dimethylcyclohexanol	*Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	2-Ethyl-1-hexanol	*Spirulina platensis; Nostoc* sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	1-Octen-3-ol	*Rhodomonas; Nannochloropsis oculata;*	Photoautotrofic	Durme et al., 2013 [8]
	2-Pentanol	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	2-Propanol	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Butanol	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methyl-1-butanol	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Nonanol	*Phormidium autumnale*	Heterotrophic	Santos et al, 2015 [13]
	2-Ethylhexanol	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
		Spirulina platensis; Nostoc sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	1-heptanol	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methylbutanoic acid	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
Aldehydes	Pentanal	*Rhodomonas* sp.*; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Ethyl-3-hydroxybutanoate	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Decanal	*Phormidium autumnale*	Heterotrophic	Santos et al.,2015 [13]
	Cis-2-pentenal	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella*	Photoautotrofic	Durme et al., 2013 [8]
	2-Methylpropanal	*Phormidium autumnale*	Heterotrophic	Santos et al, 2015 [13]

	Hexanal	*Tetraselmis* sp.*; Rhodomonas* sp.*; Botryococcus braunii; Nannochloropsis oculata; Chlorella;*	Photoautotrofic	Durme et al., 2013 [8]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]
	2-Methylbutanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Trans-2-hexenal	*Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]

Table 1. (Continued)

Organic classes	Compounds	Microalgae	Cultivation	Reference
	Cis-4-heptenal	*Rhodomonas* sp.*; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Heptanal	*Rhodomonas* sp.*; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Tr,tr-2,4-heptadienal	*Rhodomonas* sp.*; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]

	2-Octenal	*Nannochloropsis oculata; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Nonanal	*Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	2-Methylbutanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methylbutanal	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	Furfural	*Rhodomonas*	Photoautotrofic	Durme et al., 2013 [8]
	Benzaldehyde	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	Phenylacetaldehyde	*Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	4-Ethylbenzaldehyde	*Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Acetaldehyde	*Phormidium autumnale*	Heterotrophic	Santos et al, 2015 [13]
	Butanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	2-Methylbutanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methylbutanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Isobutyraldehyde	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
Hydrocarbons	Octane	*Phormidium autumnale*	Heterotrophic	Santos et al. 2015 [13]

	1-Heptene	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Hexadecane	*Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	Tetradecane	*Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	8-Methylheptadecane	*Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	3-Octadecene	*Nostoc* sp	Photoautotrofic	Milovanovic et al., 2015 [38]

Microalgae contain high concentrations of carotenoids, that by having a structure instable double bonds conjugated is easily degraded, for example, they can use the β-carotene which can degrade forming β-ionone and β-cyclocitral. In later stages of degradation of the β-ionone they can oxidize, forming a degradation product called 5,6-epoxy-β-ionone [15, 16].

As for the fatty acid synthesis, it occurs from the Acetyl-CoA molecule by Acetyl-CoA reductase enzyme. Using saturated fatty acids C18 as linoleic and linolenic acids, via the lipoxygenase 9-hydroperoxy form and intermediate 13-hydroperoxide. The branch hydroperoxide lyase converts both hydroperoxides C6 and C9 aldehydes such as 1-hexanal, hexanol and nonanal, which are reduced to alcohols by dehydrogenases [13]. In this synthesis may also be produced unbranched hydrocarbons by two families of enzymes: an acyl-acyl carrier protein reductase (AAR) and an aldehyde decarbonylase (AAD) that operate in the conversion of fatty acids [17].

A range of compounds, including classes, such as aldehydes, alcohols and ketones can be formed from the lipid degradation [7]. Microalgae are relatively rich in polyunsaturated fatty acids (PUFAs). Marine microalgae contain mostly very long chain PUFAs such as eicosapentaenoic acid and docosahexaenoic acid, for example, Chlorella contains principally shorter PUFA, such as α-linolenic acid. Species with low concentrations of PUFA contain a significantly smaller number of linear aldehydes compared with the species having high concentrations of PUFAs (e.g., *Chlorella*, *Botryococcus*, *Rhodomonas*) [18]. Considering that short chain linear aldehydes are often chemically derived lipid oxidation, branched aldehydes and aromatics are typically formed because of lipid oxidation and enzymatic protein [8].

Some chemical reactions may convert the volatile organic compounds into other compounds. For example, alcohols can be oxidized to aldehydes and then to carboxylic acids, and ketones may be reacted with the hydroxyl radicals in the air to form aldehydes [19, 20]. Aldehydes and ketones can be reduced to the alcohols by reductases aldehyde/ketone alcohols can be oxidized to aldehydes by alcohol dehydrogenase, and then further oxidized to the acid by aldehyde dehydrogenase [20]. Ketones can be formed in many ways; aliphatic ketones can be lipid oxidation products or ketone and methyl degradation (C3-C17) could be formed from the oxidative cleavage of carotenoids [13, 21].

Given the above, it is possible to note that some compounds can be produced metabolically (by enzymes present in microalgae) and also by primary degradation compounds such as lipids and proteins. The establishment of biochemical pathways can target specific biomolecules production of microalgae metabolism to compounds of commercial interest and also to better knowledge of their ecological function.

ECOLOGICAL IMPLICATIONS

The term "*off-flavor*" is used to describe the accumulation of odorous compounds within water or tissue produced from biological origins. This is one of the undesirable environmental implications, taste and odor outbreaks were associated with volatile organic compounds such as 2-methylisoborneol (2-MIB) and geosmin, produced by microalgae, are typical of the flavor compounds [14].

Geosmin is a bicyclic tertiary alcohol presenting earth odor even in very dilute aqueous solutions and it can be found naturally in beet and some plant roots. The metilisoborneol or 2-MIB also belong to the same chemical class of geosmin. Both are considered as semivolatile compounds terpenoids, being highly odorous in water or fish [22]. The 2-MIB and geosmin biosynthesis by microorganisms occurs by two common pathways: the mevalonic acid (MEV) and the deoxixilulose (DOXP/MEP) [23].

Geosmin and 2-MIB (Figure 2) are produced by aquatic microorganisms found in source waters such as lakes, reservoirs, and running waters. In addition, there are several other biological sources that are often overlooked, notably those which originate from terrestrial ecosystems, industrial waste treatment facilities, and drinking water treatment plants [24].

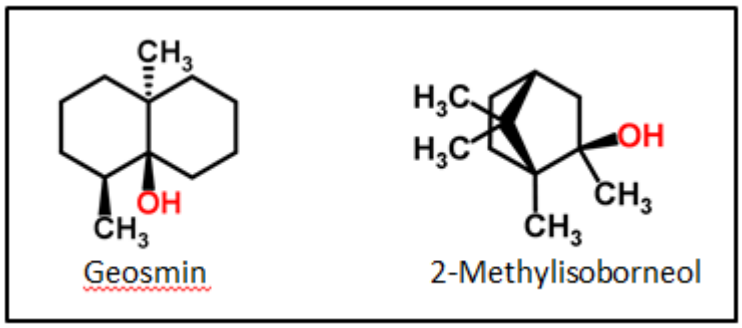

Figure 2. Chemical structure of geosmin and 2-methylisoborneol obtained from microalgae [50].

Microalgae are considered the main sources of geosmin and 2-MIB in aquatic environments where the photosynthetic growth is possible. These are present in freshwater lakes often form dense plankton populations or water blooms in eutrophic waters. In tropical regions, the growth of microalgae can be continuous throughout the year. Unsightly and highly visible surface blooms are usually considered to be primary sources of source water odor [24].

The formation of water blooms results from the redistribution, and often, rapid accumulation of buoyant planktonic populations. When such populations are subjected to optimal conditions, they respond by increasing their buoyancy and move upward nearer to the water surface, causing change in color of the water and often also in taste and odor.

The main reasons for the increased incidence of microalgae in water sources are: The increase in nitrogen nutrient loading and phosphate in water, which cause eutrophication of aquatic environments leading to an artificial enrichment of ecosystems. When this occurs in a relatively contained waterbody, there is an excessive proliferation of algae, due to decomposition, leading to an increased number of microorganisms, and thus, deterioration of water quality; In anaerobic medium inorganic forms of N and P predominate and facilitate uptake by microalgae, causing their blooms; The increase in organic matter load released springs directly or indirectly causes an increase in the amount of decomposing microorganisms and other sediment that eventually consuming the available oxygen in the water; Most microalgae blooms that appear in the springs consists of a few genres and usually produce toxins.

Apart from geosmin and 2-MIB, microalgae release other volatile organic compounds, which are also considered off-flavors, i.e., hydroxyketones formed by fermentation pathways, and carotenoids (e.g. β-cyclocitral) resulting from the degradation of carotenoids [25]. β-Cyclocitral is a well-known odour compound that affects drinking water supplies, and gives *Microcystis* blooms a characteristic hay tobacco odor, but its role in aquatic chemical defense against grazers has only recently been examined [26].

Table 2 describes some of off-flavors produced by some known species of microalgae. Microalgae, particularly filamentous, produce more than 25% of all known off-flavor compounds [27].

In the other hand, there is the possibility of synthesis of biogenic organic compounds by microalgae. In general, the term biogenic volatile organic compounds include organic atmospheric trace gases other than carbon dioxide and monoxide [28]. Consequently, large numbers of compounds saturated, unsaturated, and oxygenated are included within VOCs. And these are the isoprenoids (isoprene and monoterpenes), as well as alkanes, alkenes, carbonyls, alcohols, esters, ethers, and acids.

Table 2. Microalgae species known to produce off-flavor compounds

Source	Odorous metabolite(s)	Reference
Anabaena crassa	Geosmin	Watson (2003) [27]
Anabaena lemmermanii	Geosmin	Watson (2003) [27]
Aphanizomenon flos-aquae	Geosmin	Jüttner et al., (1986) [41]
Aphanizomenon gracile Lemmermann	Geosmin	Jüttner et al., (1986) [41]
Hyella sp.	MIB	Izaguirre and Taylor (1995) [42]
Leibleinia subtilis	Geosmin	Schrader and Blevins (1993) [43]
Lyngbya cryptovaginata	Geosmin	Jüttner and Watson (2007) [24]

Oscillatoria amphibia	Geosmin	Jüttner and Watson (2007) [24]
Oscillatoria limosa	MIB	Izaguirre and Taylor (1995) [42]
Odontamblyopus tenuis	MIB	Izaguirre et al., (1982) [44]
Phormidium amoeneum	Geosmin	Tsuchiya et al., (1981) [45]
Phormidium breve	Geosmin, MIB	Naes et al., (1988) [46]
Phormidium calcícola	Geosmin, MIB	Jüttner and Watson (2007) [24]
Phormidium. formosum	Geosmin	Persson (1988) [47]
Phormidium têнue	MIB	Persson (1988) [47]
Phormidium sp.	Geosmin, MIB	Zimmerman et al., (1995) [48]
Porphyrosiphon martensianus	MIB	Izaguirre and Taylor (1995) [42]
Rivularia sp.	Ketones, ionones	Höckelmann and Jüttner (2005) [49]
Tolypothrix distorta	Ketones, ionones	Höckelmann and Jüttner (2005) [49]

Isoprene and monoterpenes, in particular, as well as their reaction products are involved in tropospheric chemistry, fueling (directly or indirectly) the production of air pollutants and greenhouse gases, such as ozone, carbon monoxide, and methane, and increasing acidity as well as the production of aerossol [28, 29]. Usually these compounds are strong smelling, hardly water soluble, and found in plants as well as in animals, microorganisms as well as animals, microorganisms and microalgae [29]. These biogenic compounds serve as defense mechanisms of these microorganisms.

The group of monoterpenes comprises acyclic, and mono-, bi-, and tricyclic structures; they may exist as hydrocarbons with or without the inclusion of oxygen in compounds such as menthol, camphor, linalool, and geraniol. Oxygenated monoterpenes and their derivatives are often summarized as monoterpenoids [28]. Some examples of the dominant biogenic isoprenoids are given in Figure 3.

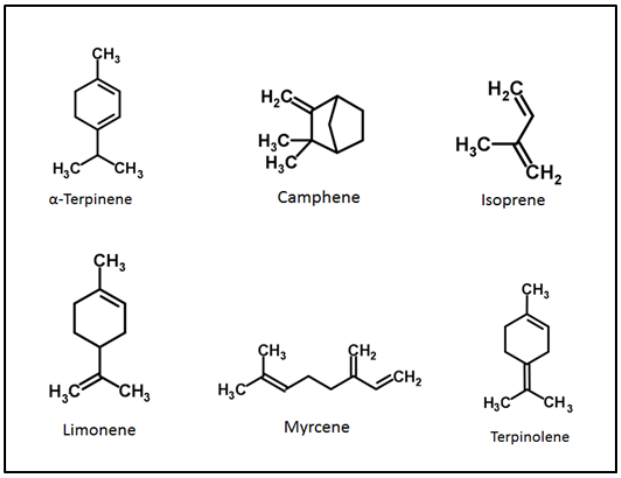

Figure 3. Dominant biogenic isoprenoids in microalgae [50].

In addition to metabolites that result in undesirable flavors and odors (odorous metabolites), there are those who are biochemically active (bioactive metabolites) in fresh and marine waters [28].

Finally, microalgae can also produce a wide range of volatile organic compounds (VOCs) and these compounds have diverse origins biosynthetic. Therefore, some of these odorous compounds apparently are the result of cell decay and decomposition. First, the algal cultures under investigation produced odors reminiscence of mercaptans and other organic sulfur compounds. Second, in the study of decaying cultures, one may logically argue that organic sulfur compounds may be present as a result of anaerobic decomposition of cellular material [30]. Decomposition products of any group are highly dependent on environmental conditions, especially temperature and oxygen available [31].

INDUSTRIAL APPLICATIONS

The most important product of microalgae biotechnology in relation to amount of production and economic value is its biomass. However, it has been noted an emerging trend towards knowledge production of low molecular weight compounds from renewable sources [32, 33].

Typical applications of microalgae correspond to a variety of metabolites (enzymes, lipids, biomass, pigments) with potential application in products such as cosmetics, food ingredients, and bioenergy. They can also be used as environmental indicators and for the treatment of wastewater [34, 35]. Beside many beneficial properties, microalgae also produce numerous volatile organic compounds, which could be used as an important alternative source of bulk and fine chemicals.

Volatile organic compounds generated by microorganisms have long been regarded as a breakthrough in laboratory research. Compounds with commercial appeal include propanol, butanol, 3-methyl-butanol, hexanol, hexanal, β-cyclocitral, β-ionone, and 5,6-epoxy-β-damascenone [36, 37].

Berger [36] reported that flavours from microorganisms can compete with traditional sources. The screening for overproducers, elucidation of metabolic pathways and precursors and application of conventional bioengineering has resulted in a set of more than 100 commercial aroma chemicals derived via biotechnology. Figure 4 shows the chemical structures of the commercialized compounds obtained by microorganisms and which are synthesized by microalgae showing a potential commercial application.

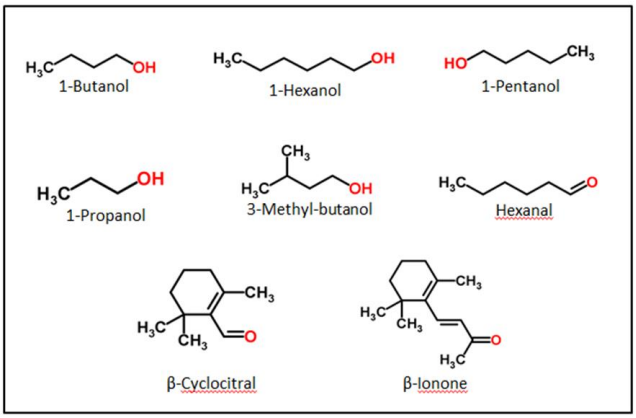

Figure 4. Volatile organic compounds with commercial application obtained from microalgae [50].

Generally, for each microalga species, aldehydes proved to be the most prevalent and, due to their low odor threshold values, might be important headspace volatiles compounds contributing to desirable aromas as well as rancid odors and flavors. Saturated aldehydes have a green-like, hay-like, paper-like odor, whereas unsaturated aldehydes have a fatty, oily, frying odor. Whereas the shorter chain linear aldehydes are often derived from chemical lipid oxidation, branched and aromatic aldehydes are typically formed due to enzymatic lipid and protein oxidation.

Many microalgae show the presence of ketones and alcohols as volatile compounds [8]. The volatile compounds determination shows that medium length alkanes and alkenes represent the main volatile components of the investigated strains of microalgae [38].

The full use of the volatile fraction of microalgal biomass may represent an improvement in the supply of a large volume of inputs to many different types of industry [13]. It can also occur using energy biomolecules of interest, such as hydrocarbons and short chain alcohols. There is increasing interest in the production of biofuels from renewable sources offering sustainable solutions to the energy sector as a promising alternative to traditional petrochemical industry [39].

The production of hydrocarbons is of particular interest due to their potential for use as advanced biofuels. Long-chain compounds can replace diesel, as the short-chain might do to instead of gasoline [33].

Aliphatic alcohols with higher carbon chain length or equal to five are attractive targets for biofuels have a high energy density and low water solubility (e.g., 1-pentanol 23 g/L; 1-hexanol 6.2 g/L; 1-heptanol 1.2 g/L). The enzyme responsible for the production of such compounds is the Acetyl-CoA-reductase that may be present in the reactions of the tricarboxylic acid cycle, mevalonate, and leucine biosynthesis. Other alcohol having substantial energy interest is the 1-butanol to have a comparable gasoline energy (29.2 MJ/L and 32.5 MJ/L, respectively), this can be a substitute fuel or added in the place of ethanol It has a lower energy (21.2 MJ/L) [40].

In summary, microalgae can produce a variety of industrially relevant volatile compounds, and the knowledge about the biosynthesis of these structures from microalgae might prove useful to help elucidated ways to the application of these biobased feedstocks for both food and non-food industries. In view of this commercial significance, efforts should be made to consolidate the technological routes of the production of these compounds.

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CAPÍTULO 3

CHAPTER: Flavour generation from microalgae in mixotrophic cultivation.

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Flavour generation from microalgae in mixotrophic cultivation

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Abstract

Microalgae are known to produce several volatile organic compounds that can be obtained from the biomass or released extracellularly into the medium. The aim of this study was to evaluate the generation of volatile organic compounds with flavour potential from the microalga *Phormidium autumnale* in mixotrophic cultivation. The experiment was conducted in a New Brunswick Scientific BioFlo®310 bioreactor operating under a batch system, with a 1.5 L working volume. The experimental conditions were as follows: initial inoculum concentration 100 $mg.L^{-1}$, temperature 25°C, pH adjusted to 7.6 and aeration of 1.0 volume air per culture volume per minute, supplemented with 5 $g.L^{-1}$ of sucrose and constant light intensity of 4 klux. The volatile compounds were isolated by solid phase micro-extraction applied in headspace of residence time (144 hours), separated by gas chromatography and identified by mass spectrometry (HS-SPME-GC/MS), co-injection of standards and kovatz index. The major products in the bioreactor were 2,4-decadienal (46.03%), 3-methyl-1-butanol (12.39%), hexanol (4.17%) and 2-ethyl-1-hexanol (3,51%). The descriptor flavour of the compounds detected in experiments was mainly classified as fried food, fruity, spice, and floral compounds. In conclusion, the results have shown that the mixotrophic cultivation of the *Phormidium autumnale* could be a potential biotechnological to produce natural flavours.

Introduction

Microalgae are a group of photosynthetic microorganisms typically unicellular and eukaryotic. Although cyanobacteria belong to the domain of bacteria, and are photosynthetic prokaryotes, they are often considered microalgae [1]. Microalgae and cyanobacteria are considered some of the most promising feedstocks for the supply of food and nonfood industries [2; 3]. Because they present a high content of macronutrients (proteins, carbohydrates, and lipids), microalgae have the potential to enhance the

nutritional value of foods [4]. They may also be used as a feed source for many aquatic organisms and livestock [5]. Microalgae-based systems for chemicals production are an emergent area, representing a great promise for industrial application.

The growing interest in natural products guides the development of the technologies that employ microorganisms, including microalgae, which are able to synthesize specific volatile organic compounds. Therefore, the selection of a mode of cultivation of microalgae is of vital importance. Four major modes of microalgae cultivation can be adopted, namely photo-autotrophic, heterotrophic, photo-heterotrophic, and mixotrophic [6]. Mixotrophic microalgae use different energy and carbon sources so that they may use organic or inorganic sources and light in different combinations. Mixotrophy makes microalgae more flexible because it may gather both the carbon and energy demand from organic or inorganic sources and light simultaneously [7].

The occurrence of volatile organic compounds in microalgae is a consequence of their versatile metabolism. The compounds produced may belong to different classes of compounds such as esters, alcohols, hydrocarbons, ketones, terpenes, carboxylic acids and sulfur compounds [8; 9]. Many of these volatiles present odour descriptors such as floral, fruity, spice, sweet, roasted, and can, therefore, be used as a flavouring agent in the food industry and others used in the pharmaceutical and fine chemicals industries.

Thus, the objective of this study was to evaluate the generation of volatile organic compounds with flavour potential from the microalga *Phormidium autumnale* in mixotrophic cultivation.

Experimental

Microorganism and culture conditions

Axenic cultures of *Phormidium autumnale* were originally isolated from the Cuatro Cienegas desert (26°59′ N, 102°03′, W. Mexico). Stock cultures were propagated and maintained in solidified agar-agar (20 $g.L^{-1}$) containing BG11 medium [10]. The cultures were illuminated with 20 W fluorescent day light-type tubes (Osram Sylvania, Brazil), located in a photo period chamber at a photon flux density of 15 μmol photons $m^{-2}s^{-1}$ and a photoperiod of 12/12 h light/dark at 25°C. The photon flux density was adjusted and controlled by using a digital photometer (Spectronics, model XRP3000). To obtain the inoculum in liquid form, 1mL of sterile medium was transferred to slants, and the colonies

were scraped off and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

The experiment was conducted in a New Brunswick Scientific BioFlo®310 bioreactor operating under a batch system, with a 1.5 L working volume. The bioreactor including filtration units was sterilized by autoclaving at 121ºC for 20 min. The experimental conditions were as follows: initial concentration of inoculum of 100 $mg.L^{-1}$, temperature of 26°C, pH adjusted to 7.6, aeration of 1.0 VVM (volume of air per volume of culture per minute per minute). The culture medium consisted of a BG11 synthetic medium supplemented with $5g.L^{-1}$ of sucrose and a constant light intensity of 4 klux.

Isolation of the volatile organic compounds

The volatile organic compounds were analyzed at 144 h of the residence time using headspace solid-phase micro-extraction (HS-SPME) with a 50/30μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, USA). Sample preparation was performed using 20 mL of culture medium, equally separated into two portions. Each of these portions were analyzed by HS-SPME coupled with GC/MS for the quantitative determination of the volatile compounds. The aliquot was placed in a headspace septum vial containing 3 g of NaCl. The SPME fiber was inserted into the headspace of the vial containing the sample (previously kept at 40ºC for equilibration temperature) for 45min at 40°C, with agitation provided by a magnetic stir bar. After this period, the fiber was removed from the vial and immediately desorbed into the injector of the GC. The analytical procedure was performed twice and in duplicate. Therefore, the data refer to the mean value of two repetitions.

GC/MS analysis

The volatile organic compounds were analyzed in a GC system (Agilent 7890A) coupled to a mass spectrometer detector (Agilent 5975) using a DB-Wax fused silica capillary column (60 m in length, 0.25 mm id and 0.25 μm film thickness). The initial oven temperature was held at 35°C for 5 min., followed by a linear increase at 5°C/min to 220°C, and held at this temperature for 5 min. For the identification of the compounds was based on GC-MS, electron-impact ionization voltage of 70 eV was applied, and helium was used as the carrier gas. The volatile compounds were identified by a comparison of their MS spectra with those provided by the computerized library (NIST MS Search). In addition, to assist with identification, each volatile linear retention index (LRI) was calculated using the retention times of a standard mixture of paraffin

homologues prepared in hexane and compared with the LRI values published in the literature for columns with the same polarity (www.flavornet.net). Co-injection of the sample and the standard mixture provided experimental LRIs for the compounds, which were compared with those of standards analyzed under similar conditions.

Results and discussion

The volatile organic compounds produced by *Phormidium autumnale* cultivated in mixotrophic conditions are presented in Table 1. A total of 16 compounds (aldehydes, alcohols, ketones, and hydrocarbons) with different odour descriptors were found. Among the chemical classes identified, 2,4-decadienal (46.03%), 3-methyl-1-butanol (12.39%) and 1-hexanol (4.17%) were the major compounds identified.

Table 1. Volatile organic compounds produced by *Phormidium autumnale* cultivated in a mixotrophic microalgal reactor. The odor description presented was extracted from the literature in comparison to the compound name, chromatographic column and Kovatz index (www.flavornet.org).

Compound	Kovatz Index	Description of odor	Relative peak area (%)
acetaldehyde	714	pungent, ether	2.37
hexanal	1084	grass, tallow, fat	1.96
2-methyl-1-propanol	1099	wine, solvent, bitter	0.73
3-methyl-1-butanol	1205	whiskey, malt, burned	12.39
1-pentanol	1255	balsamic	0.75
1-hexanol	1360	resin, flower, green	4.17
2-octenal (E)	1408	green	1.62
(E,E)-2,4-heptadienal	1463	nut, fat	3.02
2-ethyl-1-hexanol	1487	rose, green	3.51
benzaldehyde	1495	almond, burnt sugar	0.57
hexadecane	1600	alkane	3.28
2-octen-1-ol (E)	1608	soap, plastic	0.72
acetophenone	1645	must, flower, almond	1.43
2,4-decadienal (E,E)	1710	fried, wax, fat	46.03
trans-geranylacetone	1840	green	1.83
β-ionone	1912	seaweed, flower, raspberry	0.82
Other Compounds			14.80
Total			100

Mixotrophic cultivation occurs when the microalga uses photosynthesis and oxidation of organic compounds concomitantly: the oxygen produced in the

photosynthesis is consumed in the heterotrophic route. At the same time, the carbonic gas generated in the oxidation of the organic compound is exploited in photosynthesis. This cultivation is already widely exploited in terms of biomass production [6;7]. The volatile organic compounds biosynthesis mainly depends on the availability of carbon and nitrogen as well as energy provided by primary metabolism. The formation of volatile organic compounds can occur during both primary and secondary metabolism of microorganisms as secondary products, thereby we can suggest that the presence of these compounds is due to the secondary metabolism of these microorganisms.

According to Santos [8], aldehydes proved to be the most prevalent volatile organic compounds and, due to their low odour threshold values, might be important headspace volatiles compounds contributing to desirable aromas as well as rancid odours and flavours. Saturated aldehydes have a green-like, hay-like, paper-like odour, whereas unsaturated aldehydes have a fatty, oily and frying odour. Whereas the shorter chain linear aldehydes are often derived from chemical lipid oxidation, branched and aromatic aldehydes are typically formed due to enzymatic lipid and protein oxidation.

Microalgae can produce a variety of industrially relevant volatile compounds that can represent an improvement in the supply of a large volume of inputs for different types of industry (odour, flavours, energy).

In conclusion, the results show that the mixotrophic cultivation of the *Phormidium autumnal* could be an alternative to obtain flavors by this biotechnological route. More knowledge about the biochemical routes should be taken into account, thereby increasing the production of compounds of interest and the use of all the products generated during the bioprocess.

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CAPÍTULO 4

ARTIGO SUBMETIDO - Biofuel/Bioenergy Production by Microalgae: A Heterotrophic Culture Strategy

Submetido para Frontiers in Energy Research

Biofuel/Bioenergy Production by Microalgae: A Heterotrophic Culture Strategy

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ABSTRACT: The aim of this work was to evaluate the biofuel/bioenergy production from heterotrophic cultures of *Phormidium autumnale*, using cassava starch as exogenous carbon source. The kinetics parameters of cyanobacterial growth, carbon balance, lipid production, biodiesel properties, volatile organic compounds (VOCs) production, and energy production from the heterotrophic bioreactor were evaluated. The results demonstrated that the cyanobacteria reached values of biomass productivity of 1024.3 mg/L/d, maximum specific growth rate of 1.1/d, in a residence time of 5 d. The carbon mass balance shows that 81% of the exogenous carbon was converted into biomass (63.9%), volatile organic compounds (8.5%), carbon dioxide (6.7%), extracellular polymeric substances (0.03%), and mineralization (0.008%). The biomass presented lipid content of 17.85%, which resulted in a lipid productivity of 182.83 mg/L/d. The composition of this oil was predominantly monounsaturated (41.9 %), followed by saturated, (37.0 %) and polyunsaturated ones (21.10%), resulting in a biodiesel that complies with U.S., European and Brazilian standards. Were identified and quantified a total of 22 volatile organic compounds of different chemical classes (aldehydes, 52.1%; alcohols, 42.1%; ketones, 3.5%; and hydrocarbons, 2.3%). The VOCs produced demonstrated an energy potential of 122,205.00 kJ/kg and, consequently, a power generation rate of 10,580.5 kg/m^3/d.

Keywords: algae, cyanobacteria, *Phormidium autumnale*, carbon balance, volatile organic compounds, cassava starch.

1 Introduction

Increasing concerns over global warming associated with the overuse of fossil fuels, the exhaustion of fossil fuels reserves, and the increase of the world population have incited the global interest in the development and production of biofuels. A variety of prokaryotic (cyanobacteria) and eukaryotic microalgae have long been recognized as a potential source for biofuel/bioenergy production because of high calorific value,

high growth rate, and non-requirement of arable land for cultivation (Kasai et al. 2018, Cecchin et al., 2018).

Even though microalgae and cyanobacteria can be a potential source of bioenergy and high-value products, microalgal cultivation with optimal light supply is a major concern to be overcome (Hu et al., 2018). Since, the most commonly cultivated microalgae are photoautotrophic, utilizing sunlight and atmospheric CO_2 as their energy and carbon source, respectively. On the other hand, based on their cell structure, microalgae, especially cyanobacteria also can growth heterotrophically in the dark, supported by an exogenous carbon source. The heterotrophic route, where possible, in addition to circumvent the dependency on light which significantly complicates the process, also can be cheaper, and it is simpler to construct facilities and easier to maintain on a full-scale (Francisco et al., 2014).

The critical point of heterotrophic cultures is the cost of the culture medium, which represents about 50% of the bioprocess cost. Currently, glucose is the most common substrate used for microalgae heterotrophic cultures. However, is estimated that this carbon source accounts for 80% of the total costs of the culture medium. Therefore, using low cost materials instead of glucose is a very potential alternative to make these cultivations economically viable (Wei et al., 2009). Recently, some researchers (Francisco et al., 2014; Santos et al., 2017) have pointed to cassava starch as an inexpensive and efficient substrate for microalgal biomass production. Moreover, it is highly available in tropical and sub-tropical areas.

Since established by Lavoisier, the law of conservation of mass is an indispensable tool for balancing biobased processes. To analyze cultivations, for instance using microorganisms, particularly carbon balancing turned out to be a powerful tool to study and evaluate the fate of carbon in the system. Accurate carbon balancing is essential for successful bioprocess development to qualify cultivation results properly and, in this way, to reach conclusions about optimizing strains and processes can be drawn, finally establishing high product yields and conversion rates (Buchholz et al., 2014).

In this sense, the aim of this work was to evaluate the biofuel/bioenergy production from heterotrophic cultures of *Phormidium autumnale,* using cassava starch as exogenous carbon source. The study focused on evaluating the kinetics parameters of cyanobacterial growth, carbon balance, lipid production, biodiesel quality, and volatile organic compounds production.

2 Material and Methods

2.1 Microorganism and culture conditions

Axenic cultures of *Phormidium autumnale* were originally isolated from the Cuatro Cienegas desert (26º59'N, 102º03'W-Mexico). Stock cultures were propagated and maintained in solidified agar–agar (20 g/L) containing synthetic BG11 medium (Rippka et al., 1979). The incubation conditions used were 25 ºC, a photon flux density of 15 µmol/m^2/s and a photoperiod of 12 h. To obtain the inoculums in liquid form, 1 mL of sterile synthetic medium was transferred to slants, the colonies were scraped and then homogenized with the aid of a mixer tubes. The entire procedure was performed aseptically.

2.2 Bioreactor

Measurements were made in a bubble column bioreactor (Francisco et al., 2014), operating under a batch regime, fed on 2.0 L of culture medium. The bioreactor, including filtering units was previously sterilized by autoclaving at 121 °C for 40 min and then for 30 min containing the synthetic medium. The experimental conditions were as follows: initial concentration of inoculum of 100 mg/L, temperature of 26 °C, pH adjusted to 7.6, aeration of 0.1 volume of air per volume of culture per minute and absence of light. The culture medium consisted of BG11 synthetic medium supplemented with cassava starch (Santos et al., 2016). The concentration of cassava starch (7 g/L) was adjusted using a calibration curve, based on dilutions of a known amount of dry substrate.

2.3 Sampling and analytical methods

To perform the analyzes, were used samples of the liquid phase (culture medium), gaseous phase (headspace of the bioreactor), and solid phase (biomass). Liquid samples were collected aseptically in a previously sterilized laminar flow hood every 24 h during the microorganism growth phase, and were evaluated for cell concentration, the pH dynamics, the volatile organic compounds (VOCs) and the consumption of total (TC), and inorganic carbon (IC). For measurements of TC and IC from the gaseous phase, samples were collected in the headspace of the heterotrophic bioreactor. The biomass was separated from the culture medium by centrifugation, followed by lyophilization, to evaluate the carbon content.

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

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 pH values were determined by potentiometer (Mettler-Toledo, São Paulo-SP, Brazil).

Measurements of total carbon in the liquid and gas phases were conducted in a carbon analyzer TOC-VCSN (Shimadzu, Kyoto, Japan) with normal sensitivity catalyst. In order to determine TC, the samples were injected into the combustion tube at 680 °C, where the catalytic oxidation to CO_2 occurs. For the determination of IC, the injected sample reacts with hydrochloric acid 2 mol/L, and that all inorganic carbon is converted to CO_2. The CO_2 produced in both catalytic oxidations from IC and TC, is quantified by non-dispersive infrared absorption. The concentrations of TC and IC are obtained by interpolation using calibration curves (peak area as a function of standard concentration) previously constructed by injection of standards.

The carbon content of the *Phormidium autumnale* biomass was determined using a Perkin Elmer 2400 CHNS/O element analyzer (PerkinElmer, Waltham, USA).

Total lipid concentration of the biomass was determined gravimetrically by the modified Bligh and Dyer method (1959), using the ratio between methanol, chloroform and distilled water of 2:1:0.8 (v/v/v). 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. Fatty acid composition was determined by using a VARIAN 3400CX gas chromatograph (Varian, Palo Alto-CA, USA. Fatty acid methyl esters 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.

The fuel properties of biodiesel (ester content, EC; cetane number, CN; iodine value, II; degree of unsaturation, DU; saponification value, SV; long-chain saturated factor, LCSF; cold filter plugging point, CFPP; cloud point, CP; allylic position equivalents, APE; bis-allylic position equivalents, BAPE; oxidation stability, OS; higher heating value, HVV; kinematic viscosity, μ and kinematic density, ρ) were determined through the software BiodieselAnalyzer© 1.1, which estimates biodiesel properties based on the fatty acid profile of the parent oil, through a system of empirical equations (Talebi et al., 2014).

2.4 Isolation of the volatile organic compounds

The volatile compounds were isolated using headspace solid phase micro-extraction (HS-SPME) with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, USA). Sample preparation was

performed using 20 mL of culture medium, equally separated into two portions. These two portions were analyzed by HS-SPME coupled with GC/MS for the quantitative determination of the volatile compounds. The aliquot was placed in a headspace septum vial containing 3 g of NaCl and 10 µL of 3-octanol standard solution (82.2 µg/mL). The SPME fiber was inserted into the headspace of the vial containing the sample for 45 min at 40 °C, with agitation provided by a magnetic stir bar. After this period, the fiber was removed from the vial and immediately desorbed into the injector of the GC (Santos et al., 2016). The analytical procedure was performed twice and in duplicate. Therefore, data refer to the mean value of two repetitions.

2.5 GC/MS analysis

The volatile compounds were separated on a DB-Wax fused silica capillary column, 60 m in length, 0.25 mm id, and 0.25 µm film thickness (Chrompack Wax 52-CB) in a Shimadzu QP 2010 Plus gas chromatograph mass spectrometer. The initial oven temperature for the DB-Wax column was set at 35 °C for 5 min, followed by a linear increase at 5 °C.min^{-1} to 220 °C, and this temperature was held for 5 min. For identification, an electron-impact ionization voltage of +70 eV was applied, a scan ranges from *m*/*z* 35 a 350 and helium was used as the carrier gas. The volatile compounds were identified by a comparison of their MS spectra with those provided by the computerized library (NIST MS Search). In addition, to assist with identification, each volatile linear retention index (LRI) was calculated using the retention times of a standard mixture of paraffin homologues prepared in hexane and compared with the LRI values published in the literature for columns with the same polarity (Acree and Arn, 2018). Co-injection of the sample and the standard mixture provided experimental LRIs for the compounds, which were compared with those of standards analyzed under similar conditions.

2.6 Carbon mass balance

The carbon mass balance was calculated from Eq. (1) according to the inputs and outputs in the system (Lee et al., 2015):

$$C_{in} - C_{out} = V_L M_C \left(\frac{d[C_{biomass}]}{dt} + \frac{d[DIC]}{dt} \right) + V_g M_C \left(\frac{d[C_{headspace}]}{dt} \right) \quad (1)$$

where C_{in} (kg/d): mass flow rate of carbon; C_{out} (kg/d): mass flow rate of carbon out of the PBR; V_L (m³): volume of the media; V_g (m³): volume of the headspace; *Mc* (kg): atomic weight of carbon; $(d[C_{biomass}])/dt$ (kg/m^3/d): rate of change in concentration of carbon in the biomass; $(d[DIC])/dt$ (kg/m^3/d): rate of change in dissolved inorganic

carbon concentration; *(d[C$_{headspace}$])/dt* (kg/m^3/d): rate of change in carbon concentration in headspace.

2.7 Estimation of production and power generation rate of volatile organic compounds

The rate of production of VOCs (kg/m^3/d) was estimated corroborating the total organic carbon (TOC) data measured in the gaseous phase of the photobioreactor with the total compounds identified and quantified in the GC/MS using the Eq. 2 (Severo et al., 2018):

$$r_{VOCs} = \frac{\left(\frac{(OC_i - OC_O) \times Cc}{100}\right) \times Q}{V_R} \quad (2)$$

where OC_i and OC_O corresponds to the inlet and outlet carbon organic concentration, respectively, and *Cc* is the concentration of the volatile organic compound. Additionally, the power generation rate was estimated based on the calorific power of the carbon (40,000 kJ/kg) and hydrogen (120,000 kJ/kg) atoms of organic molecule (Hanby, 1994).

2.8 Kinetics parameters

Biomass data were used to calculate the biomass productivity (mg/L/d), the maximum specific growth rate (/d) and generation time from Eq. 3-5, respectively:

$$Px = \frac{(X_i - X_{i-1})}{(t_i - t_{i-1})} \quad (3)$$

$$\ln\left(\frac{X_i}{X_0}\right) = \mu_{max} \times t \quad (4)$$

$$tg = \frac{0.693}{\mu_{max}} \quad (5)$$

where X_0 is the initial biomass concentration, X_i is biomass concentration at time t_i (mg/L) and X_{i-1} is biomass concentration at time t_{i-1} (mg/L), *t* is residence time (d) and μ_{max} is the maximum specific growth rate. Residence time was defined as the elapsed time to reach maximum cell biomass.

The different organic carbon sources were used to calculate the substrate consumption rate (mg/L/day) were calculated according to Eq. 6:

$$r_S = \frac{ds}{dt} \quad (6)$$

where *S* is the organic carbon concentration (mg/L), and *t* is the time (d).

Conversion efficiency (%) and the biomass yield coefficient ($mg_{biomass}/mg_{substrate}$), were calculated from Eq. 7-8:

$$CE = \frac{S_0 - S}{S_0} \times 100 \quad (7)$$

$$Y_{\frac{X}{S}} = \frac{dx}{ds} \quad (8)$$

where S_0 is the initial organic carbon concentration (mg/L), *S* is the organic carbon concentration (mg/L), and *t* is the time (d).

3 Results and Discussion

3.1 Growth kinetics

The use of cassava starch as exogenous source of carbon is considered a promising alternative to replace glucose in heterotrophic cultivations, because it is both an inexpensive substrate and can efficiently support cyanobacterial heterotrophic cultures. In this sense, to evaluate the performance of this substrate in *Phormidium autumnale* cultures, Table 1 shows the kinetic parameters of the process.

Table 2: Kinetic parameters obtained from heterotrophic cultivation from *Phormidium autumnale* using cassava starch as a carbon source.

Kinetic parameters	Values
X_{max} (mg/L)	5,280.0±99.7
μ_{max} (/d)	1.1±0.0
tg (d)	0.6±0.0
P_X (mg/L/d)	1,024.3±17.4
r_s (mg/L/d)	1,710.0±42.7
CE (%)	80.2±2.1
$Y_{X/S}$ (mg/mg)	0.60±0.0
RT (d)	5±0.0

μ_{max} maximum specific growth rate, tg generation time, X_{max} maximum cell biomass, P_x biomass productivity, r_s average rate of substrate conversion, CE conversion of substrate, $Y_{X/S}$ substrate yield coefficient, RT residence time.

A rapid growth rate (μ_{max}=1.1 /d) and a low generation time (0.6 d) was verified in the cultivations, resulting in an average biomass productivity of 1,024.3 mg/L/d. In addition to a substrate conversion of 80.2% and a biomass yield coefficient of 0.60 $mg_{biomass}/mg_{substrate}$. Similar results were found by Francisco et al. (2015) with heterotrophic cultivation of *Phormidium* sp. in cassava wastewater, who reported biomass productivity of 1,272.0 mg/L/d, biomass yield coefficient of 0.52 mg/mg and carbon conversion of 66.1%. Santos et al. (2017) evaluated the growth of the cyanobacteria *Aphanothece microscopica Nägeli* in corn and cassava starch in different ratios of carbon

and nitrogen. These authors concluded that cassava starch with C/N ratio of 5,000/250 was the best condition for the growth of this cyanobacteria.

According to Santos et al. (2017), the adequacy of cassava starch as exogenous source of carbon in cyanobacterial cultures is related to the chemical characteristics of the molecule, which have high amylopectin content (76-83%) and low amylose content (17-24%) (Peres et al., 2016). The endogenous reserves of oxidizable substrates in cyanobacteria are mostly glycogen and cyanophycean starch, which is composed of α-1,4-glucan. In the absence of exogenous carbon source, these reserves are used to maintain the level of ATP in the cell in a process coupled to an aerobic respiratory phosphorylating electron transport chain. As the chemical structure of cyanophycean starch is very similar to amylopectin e, consequently to cassava starch, the cells are fully adapted to this substrate, and its use as exogenous carbon source is suitable for heterotrophic growth of cyanobacteria (Francisco et al., 2014).

3.2 Carbon balance

Table 2 presents the representative carbon mass balance in the heterotrophic bioreactor for a hydraulic retention time of 5 d. As seen previously, the cyanobacteria *P. autumnale* was able to convert 81% (4536 mg) of the available carbon and, approximately 19% (1064 mg) remained in the culture medium. The organic carbon (5600 mg) bioconverted in the system was distributed in biomass (3581.05 mg), volatile organic compounds (479.47 mg), carbon dioxide (377.52 mg), extracellular polymeric substances (2.09 mg), and mineralization (0.5 mg). About 1.7% of the converted carbon was not identified, which was probably used for cell maintenance.

Table 2: Carbon mass balance in the bioreactor.

Carbon balance	Mass (mg)	Percent (%)
Input carbon	5600 ± 0.00	100.00
Converted carbon	4536.00 ± 0.01	81.00
Biomass	3581.05±3.3	63.94
Extracellular polymeric substances	2.09±0.0	0.03
Mineralization	0.5	0.008
CO_2	377.02±0.2	6.74
Volatile organic compounds	479.47±0.7	8.56
Not identified	95.97 ± 0.01	1.70

Most of the carbon (63.9%) available to the cells was used for biomass production, which is regarded as the primary product of the process. In cyanobacterial biomass, carbon is distributed in carbohydrates (mono- and oligosaccharides, starch, cellulose, and other polysaccharides), lipids (free fatty acids, triacylglycerides, and sterols), proteins (including amino acids and peptides), and other minor compounds (chlorophyll, carotenoids, phycobiliproteins, nucleic acids, and inorganic material). However, in terms of bioenergy, currently, the lipid fraction has attracted more interest from researchers and companies around the world due to the physical and chemical characteristics of the biodiesel obtained from single-cell oils (SCO) (Chiu et al., 2015).

In addition to the intracellular compounds of interest, cyanobacteria can also excrete various extracellular polymeric substances (EPS) into the medium. As shown in Table 2, the cyanobacteria *Phormidium autumnale* converted 0.03% of the organic carbon into EPS. According to Liu et al. (2016), among the EPS produced by microalgae and cyanobacteria can be the exopolysaccharides, extracellular protein, organic acids, extracellular lipids, and extracellular phytohormones. Some of these extracellular metabolites can be potentially used as drugs, antioxidants, growth regulators or metal chelators.

Although the commercial cultivation of microalgae became increasingly popular, only microalgal biomass is processed to current products, while large amounts of volatile compounds are unexploited. During the respiration in heterotrophic cultures, oxygen is consumed, and CO_2 is produced. As shown in the Table 2, at the residence time of 5 d 377.52 mg of CO_2 was produced, which means that 6.74% of the organic carbon was converted to CO_2. Still in terms of volatile fraction, 8.56 % of the organic carbon was converted into VOCs.

The carbon balance drives to two main technological routes for biofuel/ bioenergy production in this culture strategy. The first is biodiesel production from the microalgal biomass, where 63.9% of the carbon was directed. Another route is through the exploitation of the volatile organic compounds, where 8.5% carbon was used for the biogeneration.

3.3 Single-cell oil and biodiesel production

The lipid production and fatty acids profile are shown in Table 3, in order to evaluate the viability of biodiesel production through biomass obtained from experiments. The biomass presented a lipid content of 17.85%, which resulted in a lipid productivity of 182.83 mg/L/d. Additionally, the composition of this oil indicated nine

major compounds, with a predominantly monounsaturated profile (41.9%), followed by saturated (37.0%), and polyunsaturated (21.1%) fatty acids.

Table 3: Lipid and fatty acids production the heterotrophic bioreactor.

Parameter	
Lipid content (%)	17.85±0.49
Lipid productivity (mg/L.d)	182.83±2.86
Fatty acids (%)	
C11:0	7.2 ± 0.09
C12:0	5.90 ± 0.07
C16:0	16.40 ± 0.16
C16:1	19.80 ± 0.28
C18:1n9c	21. 80 ± 0.31
C18:2n6c	3.10 ± 0.05
C18:3n6t	6.50 ± 0.08
C22:0	4.40 ± 0.01
C22:2	7.02 ± 0.11
Minority	8.60 ± 0.13
Saturated (SFA)	37.00 ± 0.61
Monounsaturated (MUFA)	41.90 ± 0.40
Polyunsaturated (PUFA)	21.10 ± 0.09

To assess the potential of biodiesel as a complement or substitute traditional diesel fuel in engines, the properties of biodiesel, such as ester content (EC), cetane number (CN), iodine value (II), degree of unsaturation (DU), saponification value (SV), long-chain saturated factor (LCSF), cold filter plugging point (CFPP), cloud point (CP), allylic position equivalents (APE), bis-allylic position equivalents (BAPE), oxidation stability (OS), higher heating value (HVV), kinematic viscosity (μ) and kinematic density (ρ), were determined. A comparison of these properties of biodiesel from microalgal oil, soybean oil, and U.S (ASTM, 2002), European (EN, 2003), and Brazilian (ANP, 2003) biodiesel standards are shown in Table 4.

Table 4: Properties of microalgal biodiesel produced in the heterotrophic bioreactor and its comparison with soybean and the standards used in the US (ASTM 6751), Europe (EN 14214) and Brazil (ANP 255).

Properties	Microalgae	Soybean[a]	ANP 255	ASTM 6751	EN 14214
EC (%)	99.8	96.9	-	-	min. 96.5
CN	52.86	49.0	min. 45	min. 47	min. 51
IV ($gI_2/100g$)	82.12	128	-	-	max. 120
DU (%)	81.44	143.8	-	-	-
SV	218.01	-	-	-	-
LCSF (%)	8.58	1.6	-	-	-
CFPP (ºC)	10.48	-5.0	max. 19	-	-
CP (°C)	5.42	-	-	-	-
APE	52.02	-	-	-	-
BAPE	22.10	-	-	-	-
OS (h)	11.95	1.3	-	min. 3	min. 6
HVV	38.93	-	-	-	-
μ (mm^2/s)	1.22	4.2	-	1.9-6.0	3.5-5.0
ρ (g/cm^3)	0.87	-	-	-	-

[a]Knothe (2005)

According to Knothe (2005), oils with predominantly monounsaturated profile are the most suitable for biodiesel synthesis, such as that obtained from the heterotrophic cultivation of *P. autumnalle*. The Table 3 demonstrated that except for kinematic viscosity, the cyanobacterial biodiesel complies with the limits established by the international standards, in addition to being comparable to soybean biodiesel. The kinematic viscosity of biodiesel can be increased by using additives or by blending with biodiesel from oilseeds, which are characterized by high viscosity (Mandrota et al., 2016). Thus, these results indicate the potential for the exploitation of this feedstock for biofuel production.

3.4 Volatile organic compounds production

The VOCs from microalgae-based processes have long been neglected in the development of production methods for valuable secondary metabolites. With the advances in the last years, more and more structures of this compounds have been identified, and the potential utilization over wide fields is attracting attention. In this sense, the Figure 1 shows the VOCs production as a function of residence time. Figure 1 also shows (upper left corner) the cell growth curve. Together with carbon dioxide, volatile organic compounds are products of heterotrophic metabolism. When analyzing Figure 1, it is possible to realize the relationship between the production of biomass and VOCs, where the highest production of VOCs was in 5 d of experiment, as well as biomass production.

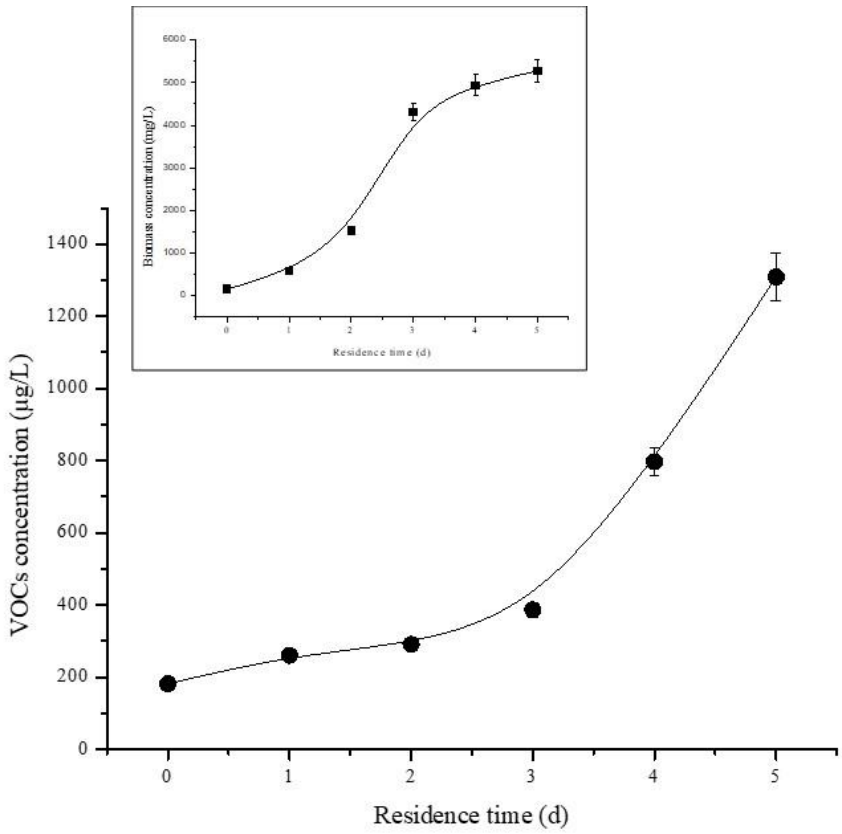

Figure 1. Concentration of volatile organic compounds as a function of residence time (main figure), and cell growth curve (upper left corner).

The Figure 2 and Table 5 show the generated VOCs in 5 d of the experiment. Despite the selective character of HS-SPME, a total of 22 different compounds were identified in the gaseous phase of the bioreactor, which are alcohols (12), aldehydes (5), ketones (4) and hydrocarbon (1). Among the identified chemical classes, (E,E)-2,4-decadienal (350 µg/L), (E,Z)-2,4-decadienal (268.1 µg/L), hexanol (115.6 µg/L), and 2-ethyl-1-hexanol (104.5 µg/L) has been identified as the major.

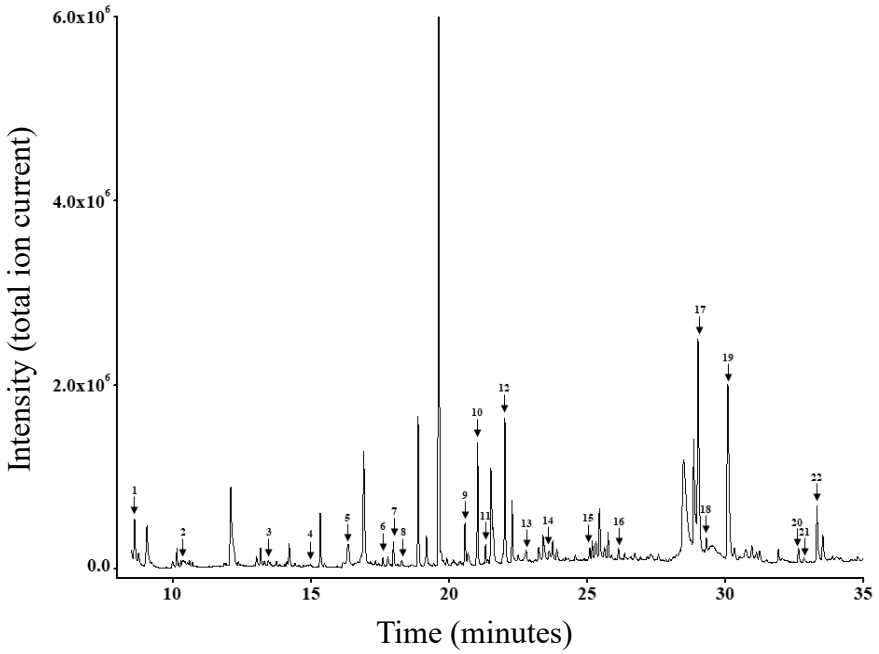

Figure 2. Chromatogram of the volatile organic compounds obtained from the microalgal bioreactor at the residence time of 5 d.

Table 5: Production and power generation rate of volatile organic compounds produced in the heterotrophic bioreactor.

Peak	Compound	Linear retention indices	Concentration* (µg/L)	Production rate ($kg/m^3/d$)	Energy potential (kJ/kg)	Power generation rate ($kg/m^3/d$)
1	4,7-dimethylundecane	1015	29.8±0.1	0.043	8664	372.5
2	hexanal	1084	10.6±0.0	0.015	3888	58.3
3	2-heptanone	1178	4.7±0.4	0.007	4536	31.7
4	3,3-dimethylhexane	1229	5.2±0.1	0.008	5424	43.3
5	1-pentanol	1255	38.3±0.7	0.055	3480	191.4
6	2-heptenal	1336	12.4±0.1	0.018	4296	77.3
7	6-methyl-5-hepten-2-one	1346	22.2±0.9	0.032	4944	158.2
8	hexanol	1360	115.6±0.9	0.166	4128	685.2
9	2-octenal	1438	41.1±0.5	0.059	4944	291.6
10	1-octen-5-ol	1451	89.7±0.5	0.129	5184	668.7
11	1-heptanol	1467	22.1±0.7	0.032	4776	152.8
12	2-ethyl-1-hexanol	1487	104.5±0.9	0.150	5424	813.6
13	2-nonanol	1535	2.8±0.0	0.004	6072	24.2
14	1-octanol	1553	27.1±0.5	0.039	5424	211.5
15	2-octen-1-ol	1610	32.1±0.8	0.046	5184	238.4
16	1-nonanol	1612	9.6±0.0	0.014	6072	85.0
17	(E,E)-2,4-decadienal	1632	350.0±2.9	0.503	6000	3018.0
18	(Z)-4-decen-1-ol	1710	28.5±0.7	0.041	6480	265.7

19	(E,Z)-2,4-decadienal	1768	268.1±1.8	0.385	6000	2310.0
20	β-ionone	1953	13.3±0.1	0.019	7704	146.3
21	1-undecanol	1976	4.1±0.0	0.006	7368	44.2
22	2,4-decadien-1-ol	1987	77.0±0.2	0.111	6240	692.6
	TOTAL		1309.0±0.6	1.8800	122,205.00	10,580.5

ND: not detected, *Mean values corresponding to the residence time of 5 d.

The Table 5 also shows the energy potential of VOCs. The compounds have estimated values ranging from 3480 to 8664 kJ/kg, resulting in a total energy content 122,205.00 kJ/kg. Volumetrically, a power generation rate 10,580.5 kg/m^3/d is estimated in this experimental condition. These values are higher than those other conventional fuels used in combustion processes, e.g. gasoline (47,300 kJ/kg), liquefied petroleum gas (46,100 kJ/kg), diesel (44,800 kJ/kg), and natural gas (39,360 kJ/kg). (Metz et al., 2007; Severo et al., 2018). Therefore, the VOCs produced in this cyanobacterial-based process can be considered a potential energy source, due to its high energy value.

4. Conclusion

The cassava starch was suitable to support the heterotrophic cultivation of *Phormidium autumnale*. According to carbon balance analysis, the organic carbon from the substrate was converted mainly into biomass (63.9%) and volatile organic compounds (8.5%) in these conditions. The high intracellular lipids (17.8%) obtained is interesting for the generation of quality biodiesel that meets or surpasses the most stringent US, European and Brazilian fuel standard requirements. In these cultures, volatile organic compounds presented energy potential of 122,205.00 kJ/kg and power generation rate of 10,580.5 kg/m^3/d. These compounds are considered valuable and profitable bioenergy products when efficiently stored by suitable recovery operations.

Conflicts of interest

The authors declare no conflicts of interest.

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CAPÍTULO 5

MANUSCRITO: Assessment of profile the volatile organic compounds (VOCs) from different cultures of *Phormidium autumnale*.

Em fase de conclusão. Será submetido a Revista Ciência Rural.

Assessment of profile the volatile organic compounds (VOCs) from different cultures of *Phormidium autumnale*

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ABSTRACT

Microalgae are known to produce several volatile organic compounds (VOCs) that can be obtained from the biomass or released extracellularly into the medium. Depending on species, culture medium, and environmental conditions, microalgae are capable of producing a wide variety of VOCs and are originated from different chemical classes of compounds such as alcohol, aldehydes, ketones, hydrocarbons, esters, terpenes, and sulfurized compounds. Thus, the aim of this study was to evaluate the profile of volatile compounds produced by *Phormidium autumnale* during photoautotrophic, mixotrophic and heterotrophic cultures. The volatile compounds were isolated by solid phase microextraction applied in headspace every 24 hours during the experiment, separated by gas chromatography and identified by mass spectrometry (HS-SPME-GC/MS). Regardless the fermentation time, the major products in the headspace were 2,4-decadienal, 3-methyl-1-butanol, hexanol and hexanal. Exploring the volatile profile of microalgae is a possibility and represent an improvement in the supply of a large volume of inputs to many different types of industry.

Key words: cyanobacteria, microalgae, microalgae-based process

Avaliação do perfil dos compostos orgânicos voláteis (COVs) a partir de diferentes culturas de *Phormidium autumnale*

RESUMO

Sabe-se que as microalgas produzem vários compostos orgânicos voláteis (COVs) que podem ser obtidos a partir da biomassa ou liberados extracelularmente no meio. Dependendo das espécies, meio de cultura e condições ambientais, as microalgas são capazes de produzir uma ampla variedade de compostos orgânicos voláteis e são originárias de diferentes classes químicas de compostos como álcool, aldeídos, cetonas, hidrocarbonetos, ésteres, terpenos e compostos sulfurados. Assim, o objetivo deste trabalho foi avaliar o perfil de compostos voláteis produzidos por *Phormidium autumnale* durante culturas fotoautotróficas, mixotróficas e heterotróficas. Os compostos voláteis foram isolados por microextração em fase sólida aplicada em *headspace* a cada 24 horas durante o experimento, separados por cromatografia gasosa e identificados por espectrometria de massas (HS-SPME-GC / MS). Independentemente do tempo de fermentação, os principais produtos no *headspace* foram o 2,4-decadienal, o 3-metil-1-butanol, o hexanol e o hexanal. A exploração do perfil volátil de microalgas é uma possibilidade e representa uma melhoria na oferta de um grande volume de insumos para muitos tipos diferentes de indústria.

Palavras-chave: cianobactérias, microalgas, processo baseado em microalgas.

INTRODUCTION

Microalgae-based systems produce a vast array of secondary metabolites of major socio-economic importance, representing a great promise for industrial application (WATSON et al., 2016). Current literature on the cultivation of *Phormidium autumnale*

has demonstrated the potential of these microalgae in synthesizing several high-value compounds. They have pigments such as carotenoids and chlorophyll (FERNANDES et al., 2017), oils with properties for biodiesel production (FRANCISCO et al., 2014; SIQUEIRA et al., 2015), as well as volatile organic compounds (VOCs) of interest for the food and non-food industries (SANTOS et al., 2016).

The growing interest in natural products directs the development of technologies that employ microorganisms, which are able to synthesize volatile organic compounds (VOCs). Many of these volatiles present odor descriptors such as floral, fruity, spice, sweet, roasted, and can, therefore, be used as a flavoring agent in the food industry and others used in the pharmaceutical and fine chemicals industries (HOSOGLU, 2018). Depending on species, culture and environmental conditions, microalgae are capable of producing a variety of volatile organic compounds. In addition, other biomolecules such as hydrocarbons and alcohols, it can use for bioenergy. There is a growing interest in the production of biofuels from renewable sources, offering sustainable solutions for the energy sector as a promising alternative to the traditional petrochemical industry (SEVERO et al., 2018).

The occurrence of VOCs in microalgae is a consequence of their metabolism, though dependent on the species, their production can be modified by various factors, such as light, salt content, carbon and nitrogen sources (MILOVANOVIC et al., 2015; HOSOGLU, 2018). More specifically, the form of available carbon dictates the metabolic pathway by which the microalgae assimilate carbon. In general, there are two primary avenues of carbon fixation in microalgae: (i) photoautotrophic, which corresponds to photosynthetic growth and fixation of inorganic carbon (i.e., carbon dioxide) through the Calvin-Benson cycle, and (ii) heterotrophic, corresponding to assimilation of organic carbon in the absence of light (PEREZ-GARCIA et al., 2011). Furthermore, some

microalgae can commonly exist in either metabolic condition, therefore being referred to as mixotrophic. The mixotrophic culture regime is a variant of the heterotrophic culture, where CO_2 and organic carbons are simultaneously assimilated and both respiratory and photosynthetic metabolism operates concurrently (CHEIRSILP & TORPEE, 2012).

Many studies have demonstrated that heterotrophic and mixotrophic growth for various microalgae species yields greater biomass and lipid content as compared to photoautotrophic cultivation. In view of this outline, the aim of this study was to evaluate the volatile organic compound profile produced from microalgae *Phormidium autumnale* employing photoautotrophic, heterotrophic, and mixotrophic cultures.

MATERIAL AND METHODS

Microorganism and culture conditions

Axenic cultures of *Phormidium autumnale* were originally isolated from the Cuatro Cienegas desert (26°59’ N, 102°03’ W - Mexico). Stock cultures were propagated and maintained in solidified agar-agar (20 g L^{-1}) containing synthetic BG11 medium (RIPPKA et al., 1979). The cultures were illuminated with 20 W fluorescent day light-type tubes (Osram Sylvania, Brazil), located in a photoperiod chamber. The incubation conditions used were 25°C, a photon flux density of 15 µmol m^{-2} s^{-1} and a photoperiod of 12/12 hour light/dark. The photon flux density was adjusted and controlled by using a digital photometer (Spectronics, model XRP3000). To obtain the inoculums in the liquid form, 1 mL of a sterile synthetic medium was transferred to slants, and the colonies were scraped and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

Bioreactor

The bioprocess was produced in a bioreactor with a working volume of 2 L (Bioflo 310, Bioflo & Celligen, New Brunswick, USA). Temperature, agitation and aeration were kept at 26°C, 50 rpm, and 0.5 slpm, respectively. The inlet of air and outlet of gases were filtered through filtering units made up of polypropylene membrane with a pore diameter of 0.22 µm and total diameter of 50 mm (Millex FG®, Billerica-MA, USA). The bioreactor including filtering units was previously sterilized by autoclaving at 121°C for 40 min and then for 30 min containing the synthetic medium.

Obtaining the kinetic data

Experiments were performed in a bioreactor operating under a batch regime, fed on 1.5 L of culture medium. The general experimental conditions were as follow: initial concentration of inoculum of 100 mg L^{-1} and pH adjusted to 7.6. The culture medium consisted of BG11 synthetic medium for all cultures. The heterotrophic experiment was performed in the absence of light and the culture medium supplemented with sucrose (11.9 g L^{-1}) as an exogenous carbon source to obtain 12 g L^{-1} of organic carbon concentration. In the photoautotrophic was performed with a photon flux density of 150 µmol m^{-2} s^{-1} and 0.5% carbon dioxide in the air. The mixotrophic was performed under the same photoautotrophic conditions but with 5 g L^{-1} of sucrose added in the culture medium.

Determination of cellular concentration

Cell concentration was determined gravimetrically by filtering a known volume of culture through a 0.45 µm membrane filter (Billerica-MA, USA), and drying at 60°C for 24 hours. Sampling of the experiments was performed daily until the end. The experiments were performed in duplicate. Therefore, data refer to the mean value of three repetitions.

Isolation of the volatile organic compounds

The volatile compounds were isolated using headspace solid phase micro-extraction (HS-SPME) with a 50/30µm divinylbenzene/carboxen/polydimethylsiloxane

(DVB/Car/PDMS) fiber (Supelco, Bellefonte, USA). Sample preparation was performed using cultured broth (20 mL) was equally separated into two portions. These two portions were subjected to a HS-SPME coupled with GC/MS for the quantitative determination of the volatile compounds. The aliquot was placed in a headspace septum vial containing 3 g of NaCl and 10 μL of an internal standard solution. The SPME fiber was inserted into the headspace of the vial containing the sample for 45 min at temperature for 40°C, with agitation provided by a magnetic stir bar. After this period, the fiber was removed from the vial and immediately desorbed into the injector of the GC. Therefore, data refer to the mean value of two repetitions.

GC/MS analysis

The analysis and identification of volatile compounds was performed on a GC/MS system with a gas chromatograph HP-7890 coupled to a mass spectrometer HP- 5975C (Agilent Technologies, Santa Clara, CA, USA). A fused silica capillary column DB-WAX (J&W Scientific, Folsom, California, USA) with 60 m length x 0.25 mm i.d. x 0.25 μm of film thickness was used to separate the volatile organic compounds. The initial oven temperature for the column was set at 35°C for 5 min, followed by a linear increase at 5°C min^{-1} to 220°C, and this temperature was held for 5 min. For identification, an electron-impact ionization voltage of 70 eV was applied, and helium was used as the carrier gas. The volatile compounds were identified by a comparison of their MS spectra with those provided by the computerized library (NIST MS Search). In addition, to assist with identification, each volatile linear retention index (LRI) was calculated using the retention times of a standard mixture of paraffin homologues prepared in hexane and compared with the LRI values published in the literature for columns with the same polarity (ACREE & ARN, 2018). Co-injection of the sample and the standard mixture provided experimental

LRIs for the compounds, which were compared with those of standards analyzed under similar conditions.

Standards

The standards hexanol, 3-methyl-1-butanol, 6-methyl-5-hepten-2-one, acetophenone, β-cyclocitral and β-ionone, as well as 3-octanol which was employed as an internal standard, were from Sigma-Aldrich (USA). The identities of volatile compounds were confirmed with the MS spectral database.

RESULTS AND DISCUSSION

According to GC/MS analysis, the presence of forty-one VOCs belonging to four major classes, including aldehydes, alcohols, ketones, and hydrocarbons was revealed in three types of *Phormidium autumnale* culture (Table 1). Photoautotrophic, mixotrophic and heterotrophic cultures were identified: 23, 33 and 34 volatile compounds, respectively, these compounds 18 were found in both cultures. The compounds include 13 aldehydes (peaks 1, 2, 4, 9, 13, 15, 20, 22, 24, 26, 30, 33 and 36), 12 alcohols (peaks 5, 8, 10, 12, 17, 19, 23, 25, 27, 31,34 and 40), 9 ketones (peaks 11,14, 16, 18, 28, 32, 37, 38 and 41), 5 hydrocarbons (3, 6, 21, 29 and 35), 1 ester (peak 7), and 1 nitrogenous (peak 39). Generally, for all cultures, the most prevalent compounds in relatively high amounts were aldehydes, alcohols, and ketones. This information is relevant with recent studies of microalgae and cyanobacteria (SANTOS et al., 2016; ZHOU et al., 2017; HOSOGLU, 2018).

The photoautotrophic culture obtained the highest production of volatile compounds in 48 hours of culture (Figure 1A). During this period nine compounds were formed, obtaining all 23 compounds from the experiment. One of the most abundant volatile groups produced in the experiment were alcohols, and also the two majorities peaks 3-methyl-1-butanol and 2-ethylhexanol.

The volatile profile of *Phormidium autumnale* under mixotrophic cultivation showed a significant increase in 120 hours of experiment (Figure 1B). In this time a total of 27 compounds were found, and 17 of these were formed. 2,4-decadienal and 2,4-decadienol were the peaks with the highest arbitrary area, representing the two main classes of compounds produced, aldehydes and alcohols, respectively.

Figure 1C shows the volatile profile of heterotrophic culture using sucrose as exogenous carbon. Aldehydes, alcohols and ketones were the major groups formed in descending order, respectively. Qualitatively the heterotrophic culture was similar to mixotrophic, obtained 30 compounds found, of these 15 were formed during 120 hours of experiment. 2,4-decadienal and 6-methyl-5-hepten-2-one were the highest peaks obtained in the chromatogram.

The compounds found in the experiment, 24 have already been mentioned in the literature and of these 12 have already been found in *Phormidium autumnale* in heterotrophic culture supplemented with monosaccharides (SANTOS et al., 2016). Seven compounds (hexanol, hexanal, heptanal, 6-methyl-5-hepten-2-one, β-ionone, β-cyclocitral and 3-methyl-1-butanol) were found in the photoautotrophic culture of *Tetraselmis* sp., *Rhodomonas* sp., *Chlorella vulgaris, Nannochloropsis* sp., *Crypthecodinium cohnii* and *Botryococcus braunii* (DURME et al., 2013; HOSOGLU, 2018).

Compounds such as 6-methyl-5-hepten-2-one, isophorone, acetophenone and β-ionone were found in the all cultures. The presence of these ketones could be formed from the oxidative cleavage of carotenoids, as a consequence the high concentration of β-carotene and echinenone in the cyanobacteria, thus, corroborating with the results (RODRIGUES et al., 2014; SANTOS et al., 2016).

Volatile organic compounds generated by microalgae with commercial appeal include 3-methyl-butanol, hexanol, hexanal, β-cyclocitral, and β-ionone (SMITH et al.,

2010; SANTOS et al., 2016). Microalgae also produces energy-related biomolecules, such as alcohols and hydrocarbons, with potential for use as biofuels (CHOI & LEE, 2013). Severo et al. (2018) obtained 86,320.00 kJ/kg of energetic potential of *Snedesmus obliquus* CPCC05 in photoautotrophic regime, compounds identical to those found in the *Phormidium autumnale* cultures of this study, such as 2-ethyl-1-hexanol, hexanal, 6-methyl-5-hepten-2-one, acetophenone and β-ionone were detected, thus showing potential for obtaining biofuels from these microorganisms.

Volatile organic compounds from microalgae and cyanobacteria associated with undesirable taste and odor, such as geosmin and methylisoborneol, and the toxic compound dibutyl-phthalate, were not found in this study. The absence of these undesirable compounds increases the commercial potential of this technological route, increasing the possibility of applying these metabolites as chemical fine feedstocks (HAYES & BURCH, 1989; FUJISE et al., 2010; SUN et al., 2012).

The importance of obtaining aromas through this biotechnological route, the alcohols and aldehydes contribute the most in the volatile profile of the microalgal biomass, both chemical classes obtained relevance in the experiments. The most alcohols have a strong pungent odor, which can make them substantial contributors to the microalgae odor (HOSOGLU, 2018). 3-methyl-1-butanol is the main volatile compounds found in fermented beverages, this compound was found in all three types of cultures. This volatile compound was found in *Chorella vulgaris* (DURME et al., 2013) and *Tetraselmis chuii* (HOSOGLU, 2018) in photoautotrophic culture, and as a major volatile compound formed in the heterotrophic culture of *Phormidium autumnale* (SANTOS et al., 2016).

Aldehydes, as major lipid degradation products, were the majority found. With their low odor threshold values, aldehydes could be important VOCs in microalgae, contributing to their desirable or undesirable aromas (CZERNY et al., 2008). All aldehydes identified

by GC–MS are compounds which have odor descriptor. Due to the significant contribution of lipid-derived volatiles (aldehydes and alcohols) to the perceived aroma characteristics of microalgae, prevention or control of lipid oxidation is recommended to ensure good flavor quality of the product (HOSOGLU, 2018).

2,4-decadienal is derived from the lipoxygenase/hydroperoxidase lyase dependent degradation of arachidonic or eicosapentaenoic acid however, the fatty acids linoleic or linolenic acid, are precursor of aldehydes such as nonanal, hexanal. and 2-pentanal, as well as alcohol hexanol (YU et al., 2014; JERKOVIC et al., 2018). These volatile compounds confirm the high lipid concentration of the microalga already mentioned in other studies. Several pigments and long-chain polyunsaturated fatty acids, such as omega-3 fatty acids, are valuable products, with great importance for the food and pharmaceutical market (CHALIMA et al., 2017).

Chemicals obtained from microalgae-based systems are sold at prices 1000 times higher than those synthetic chemicals, which show great potential for the exploitation of these processes. Typical applications of microalgae correspond to a variety of metabolites with potential application in products such as cosmetics, food ingredients, and bioenergy. They can also be used as environmental indicators (JACOB-LOPES et al., 2008; ABDEHL-RAOUF et al., 2012).

Advances in microalgal biotechnology are the beginning to microbial production as a viable means of biochemical synthesis, emerging as an alternative means for flexible, efficient and low impact production to can produce a variety of industrially relevant volatile compounds.

CONCLUSION

The elucidation of the gas fraction with the support of a gas chromatograph coupled to a mass spectrometer allowed to observe the diversity of the compounds produced and were identified alcohols, aldehydes, ketones and hydrocarbons. Thus, the information supplied by this and previous studies presents a strong argument for the volatile organic compounds “fingerprint” of microalgae species. This is important tool for evaluation of their potential benefits in different applications. In conclusion, additional research into the profile of volatile organic compounds is required to further investigate samples of microalgae species in order to identify the best prospective candidates for use in a new generation of food and feed formulations, biofuels, pharmaceuticals and chemical fine feedstock.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare no conflict of interest.

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Table 1: Volatile compounds detected by GC/MS in the samples from bioreactors with retention index (LRI) and odor descriptors.

Peak	LRI DB-Wax[a]	Compound	Cultivation [b]	Odor Descriptors [c]
01	737	acetaldehyde	p,h,m	pungent, ether
02	976	pentanal	h,m	almond, malt, pungent
03	998	decane	p,h	alkane
04	1083	hexanal	p,h,m	grass, tallow, fat
05	1112	2-methyl-1-propanol	p,h,m	wine, solvent, bitter
06	1118	ethylbenzene	h,m	*nd
07	1123	isoamyl acetate	p	banana
08	1173	1-penten-3-ol	h,m	green, vegetable, fruit
09	1181	heptanal	p,h,m	fat, citrus, rancid
10	1217	3-methyl-1-butanol	p,h,m	whiskey, malt, burnt
11	1253	3-octanone	h,m	herb, butter, resin
12	1260	1-pentanol	p,h,m	balsamic
13	1285	octanal	p,h,m	fat, soap, lemon, green
14	1297	1-octen-3-one	h,m	mushroom, metal
15	1320	2-heptenal	h	soap, fat, almond
16	1337	6-methyl-5-hepten-2-one	p,h,m	green, must, vegetable
17	1361	1-hexanol	p,h,m	resin, flower, green
18	1387	2-nonanone	h	hot milk, soap, green
19	1389	3-hexen-1-ol	m	grass
20	1391	nonanal	p,h,m	fat, citrus, green
21	1395	tetradecane	p,h,m	alkane
22	1427	2-octenal	h,m	green
23	1457	1-octen-3-ol	h,m	mushroom
24	1465	2,4-heptadienal	m	nut, fat
25	1496	2-ethyl-1-hexanol	p,h,m	rose, green
26	1523	benzaldehyde	m	almond, burnt sugar
27	1565	1-octanol	p	chemical, metal, burnt
28	1594	isophorone	p,h,m	green, wood, citrus
29	1600	hexadecane	p,h,m	alkane
30	1622	β-cyclocitral	p	mint

31	1623	2-octen-1-ol	h,m	soap, plastic
32	1652	acetophenone	p,h,m	must, flower, almond
33	1664	2,4-nonadienal	h	watermelon
34	1666	1-nonanol	m	fat, green
35	1700	heptadecane	p,h	alkane
36	1769	2,4-decadienal	h,m	fried, wax, fat
37	1858	geranylacetone	p,h,m	green
38	1945	β-ionone	p,h,m	seaweed, violet, flower
39	1961	benzothiazole	p,h,m	gasoline, rubber
40	1994	2,4-decadien-1-ol	h,m	fat, chicken, wax
41	2160	β-methyl ionone	h,m	wood, flower

a= Linear retention indices in the DB-Wax column; b= detected in photoautotrophic (p), heterotrophic (h) and/or mixotrophic (m) cultivation; c= according to Acree and Arn (2018).

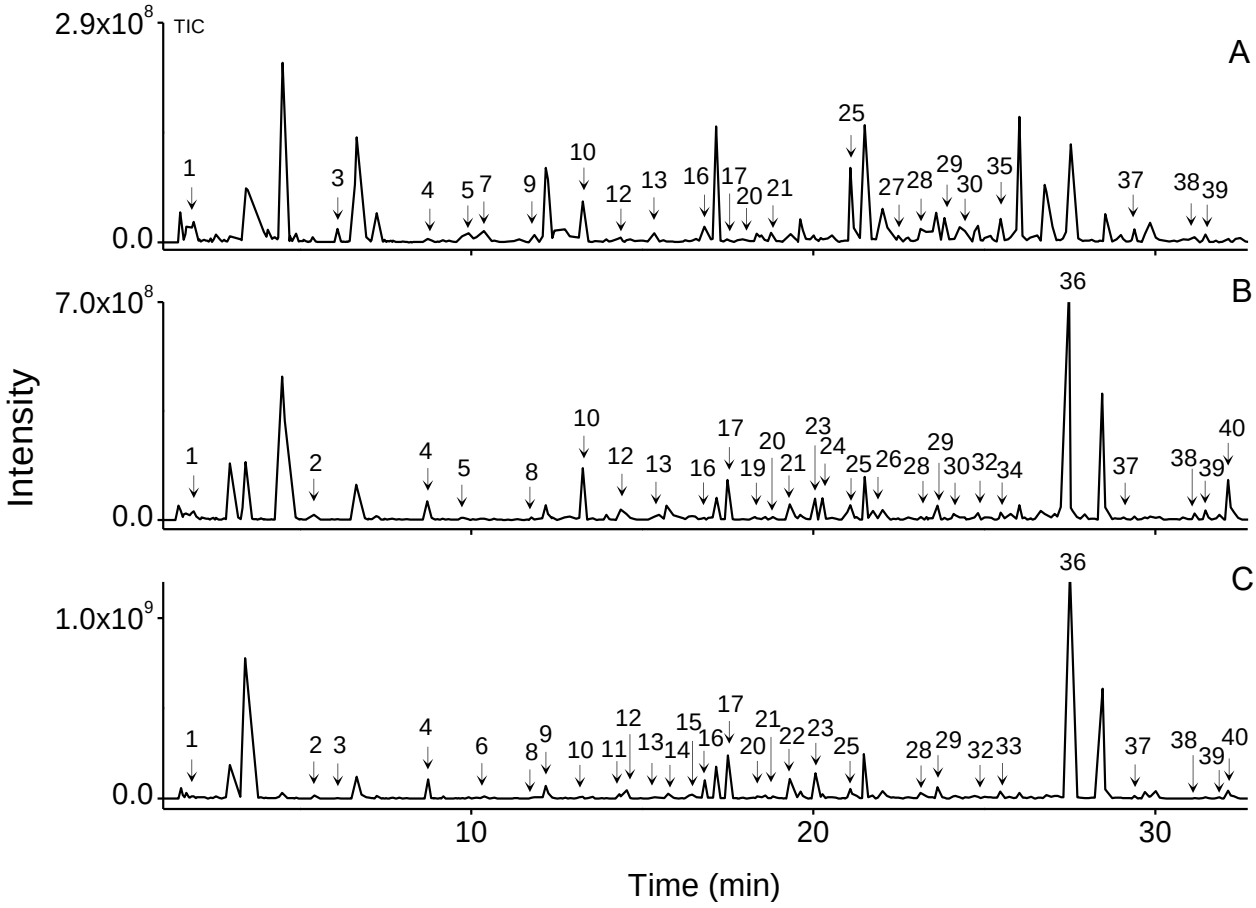

Figure 1: Chromatogram (total ion current) of the volatile organic compounds from microalgal bioreactor. The letters correspond to the different microalgae culture with which the chromatograms were obtained: A=photoautotrophic, B= mixotrophic, C=heterotrophic.

CONCLUSÃO GERAL

O presente estudo demonstrou a importância sobre o conhecimento da biossíntese dos compostos orgânicos voláteis produzidos pelas microalgas, sendo útil na elucidação das rotas biotecnológicas aplicáveis para a obtenção destas matérias-primas de base para a aplicação industrial.

Quanto à elucidação da fração gasosa permitiu observar a diversidade dos compostos produzidos. Essa etapa foi importante para a definição de qual cultivo teve a maior capacidade de produzir mais compostos orgânicos voláteis, podendo prever o desempenho e otimizar as condições operacionais.

Os resultados obtidos no cultivo mixotrófico da *P. autumnale*, mostram que os COVs podem ser uma alternativa para a biossíntese de aromas e sabores por essa via biotecnológica. Mais conhecimento sobre as rotas bioquímicas deve ser levado em conta, aumentando assim a produção de compostos de interesse e o uso de todos os produtos gerados durante o bioprocesso.

Ao analisar o cultivo heterotrófico com a adição de amido de mandioca como fonte de carbono exógena, foi constatado uma conversão de 81% do carbono corroborando com a eficiência do processo. A alta concentração de lipídios e a identificação de compostos voláteis com grande potencial energético, mostram que esse microrganismo pode ser cotada com uma fonte de bioenergia.

Assim, as informações fornecidas por este e estudos anteriores apresentam um forte argumento para a “impressão digital” de compostos orgânicos voláteis das espécies de microalgas. Esta é uma ferramenta importante para avaliação de seus benefícios potenciais em diferentes aplicações industriais.

ANEXO

Artigo de Revisão realizado em paralelo à pesquisa, intitulado "*Microalgal Biorefineries for Bioenergy Production: Can We Move from Concept to Industrial Reality?*". Foi publicado na revista *BioEnergy Research*.

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Microalgal Biorefineries for Bioenergy Production: Can We Move from Concept to Industrial Reality?

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Abstract
Biorefineries are commercial facilities that transform raw materials into commodities of considerable interest to the world bioeconomy. In addition, biorefineries have the potential to achieve favorable environmental characteristics, such as minimal greenhouse gas (GHG) emissions and a lower water footprint, compared to homologous fossil fuels. However, for this concept to become efficient and viable, the use of potentially abundant and specific renewable biological feedstocks should be considered, such as microalgae biomass and other generated products. However, there is an emerging need to consolidate industrial plants that are not only affected by market fluctuations but also aim to transform biological materials into industrially usable products. Thus, for a microalgae biorefinery to compete with the resilient oil refineries in the future, process integration in the supply chain is a promising engineering approach, associating all the components from the cultivation to obtain multiple products that are economically and environmentally sustainable. Therefore, the objective of this review is to compile issues related to microalgal biorefineries applied to bioenergy and biofuel production.

Keywords Biorefinery · Algae · Energy · Fuel · Co-products

Abbreviations

ABE	Acetone-butanol-ethanol
BASF	Badische Anilin & Soda Fabrik
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
FAO	Food Agriculture Organization
GHG	Greenhouse gas
GWP	Global warming potential
LCA	Life cycle assessment
LCI	Life cycle inventory
LCIA	Life cycle impact analysis
LEA	Lipid extracted algae
MAAs	Mycosporine-like amino acids
NER	Net energy ratio
PUFA	Polyunsaturated fatty acids
TAG	Triacylglycerol
VOC	Volatile organic compounds
VCSS	Vapor compression steam stripping
WF	Water footprint
WHO	World Health Organization

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Introduction

The energy crisis and global warming demonstrate two major challenges for sustainable development around the world, thus providing substantial interest in the production of bioenergy. According to estimates by the International Energy Agency [1], the demands for fossil fuels, trade flows, and greenhouse gas (GHG) emissions will not be sustainable until 2030. Government policies were also formally launched at COP21 to boost the global innovation of clean energy both publicly and privately with the aim of making it widely accessible [2].

Given this scenario, intensive research focuses on replacing a conventional fossil fuel to solve this problem with less environmental impact. However, the poor supply of raw materials is a major obstacle in the current development of commercialization [3]. In this context, microalgae have emerged to be a potential alternative resource to reduce the dependence on

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fossil fuels. Microalgae have a large metabolic capacity for bioconversion and low nutrient requirements; can accumulate carbohydrates, lipids, and proteins; and are considered to be a promising biomass to produce renewable energy in addition to the production of high-added-value products [4, 5].

In addition, production strategies based on biorefinery systems are used to reduce plant costs [6]. Thus, a biorefinery is conceptualized as a facility similar to the traditional petroleum refinery, where all the compounds available in biomass are converted into a spectrum of marketable products and energy without producing any residue [7]. Process integration aims to optimize the use of energy and materials in the total chain, from biomass planting to end-use, while improving economic viability, societal acceptance, and chain sustainability [8, 9].

In this context, although some researchers have investigated the production of biofuels from algae, the most effective operating conditions of the cultivation systems and the commercialization of the resulting co-products, aimed at competing with resilient petroleum refineries, have not yet been established [10]. Thus, the primary objective of this review is to compile issues related to microalgae biorefineries applied to the production of bioenergy and biofuels. The study focuses on evaluating the configurations of the microalgae culture system related to biorefineries and process integration supported by the bioeconomy and life cycle assessment (LCA).

Technological Routes for Microalgal Biomass Production

Microalgae biofuels can be obtained through heterotrophic, autotrophic, or mixotrophic metabolism. However, they are preferably derived from photosynthesis. Therefore, the metabolic capacity of several species of microalgae to produce large amounts of lipids as a storage product using an inorganic carbon source and light energy makes microalgal biomass an attractive resource for biodiesel production [11]. However, large-scale production becomes a primary requirement for biomass production, and key considerations include productivity, ease of cultivation, harvesting, and economic viability [12].

Commonly, microalgae are cultured in widespread industrial scale in two systems: open systems (i) and closed systems (ii). Open systems, such as circular and raceway ponds, are the simplest and least expensive. Although they are low cost and extend their useful life, they have disadvantages such as the need for large areas of land, problems with high evaporation rate, contamination, and low productivity [13, 14].

Recently, open systems, such as raceway ponds, have also been used for the mass cultivation of microalgae to produce biofuels. Some studies suggest that, although there are many steps involved from the raw material harvesting process to the extraction and transesterification in the lipid conversion process in final biodiesel, the cultivation stage is the focus of intensive research. Although the raceway ponds are direct systems, the productivities examined in these systems generate a great variation in the data reported in the literature [15].

Comparatively, closed systems, such as tubular photobioreactors, allow a certain level of control over operating conditions and prevent contamination, and it is possible to obtain greater biomass production. Alternatively, the construction of the closed systems is more costly, since they require transparent materials, are more complex operationally, and are difficult to scale [16].

The tubular photobioreactors offer stricter control among the chemical, physical, and biological factors and can improve the conditions for the growth of microalgae, optimizing the absorption of light due to turbulent conditions in the culture [17]. However, the two most critical issues of closed photobioreactors are the risk of overheating and their potential for oxygen (O_2) accumulation and subsequent inhibition of growth [18].

Hybrid reactors were introduced as a promising alternative to attempt to compensate for the disadvantages of one system and to enhance the virtues of the other. These systems are based on an adequate height/diameter ratio and allow a greater surface/volume ratio, thus enabling a higher volume of work, in order to achieve higher productivity, reduce energy consumption, increase photosynthetic efficiency, and offer a lower cost [19, 20].

Faced with these configurations, algal biofilm culture systems emerge as an alternative to traditional suspension culture systems. Algal biofilm culture systems can reduce the costs associated with harvesting algae biomass and involve the immobilization of algal cells within a biofilm of algae that can be separated from the liquid medium. Consequently, the microalgae biomass content of the system can be maintained at a high level [21].

In parallel, industrial fermenters are generally used to drive the growth of heterotrophic cultures. This technological route tends to achieve higher yields when compared to photoautotrophic metabolism. In addition, since cell growth is independent of a light source, the increase in turbidity of the medium during cultivation will not interfere with high cell densities, enabling an improvement in the biomass harvesting. However, issues related to the high cost of the substrate, the high risks of contamination, and the emission of carbon dioxide (CO_2) resulting from cellular respiration currently limit the large-scale heterotrophic cultivation of microalgae [22, 23].

In contrast, although the highest percentage of industrial processes involving microalgae is carried out in open systems, success in the large-scale commercial production of microalgae has not yet been sustainable when the focus is on commodities. In this context, substantial research and development efforts have been conducted in recent years to overcome these barriers.

Biorefinery of Microalgal Biomass

Once the biochemical process in the bioreactor is finished, the upstream processing ends and gives way to the downstream processing [24]. The choice of the downstream processes used greatly influences the economic viability, efficiency, operating costs, and energy requirements of the microalgae biorefineries [25]. The technological pathway traditionally envisioned for the downstream processing of microalgal biomass involves biomass harvesting and drying followed by the extraction of the lipidic fraction and conversion to biofuels. The remaining lipid-extracted algae (LEA) fraction would be directed to the production of fertilizers, animal feed, or other bioproducts [26]. This model, however, has been challenged by recent advances in the extraction/conversion processes that can handle wet feedstocks and convert whole algal biomass into oil [25, 27, 28].

Dewatering is a major cost contributor while producing microalgal biomass. Although microalgal biomass can be energy rich, the growth of microalgae in dilute suspensions poses considerable challenges to achieve a viable energy balance in microalgal biofuel process operations [29]. Additional challenges of microalgae harvesting come from the small size of the microalgal cells (3–30 μm diameter), the similarity of the density of the microalgal cells to the growth medium, the negative surface charge on the microalgae, and the microalgal growth rates which require frequent harvesting [30–32]. For this reason, biomass dewatering sometimes requires one or more solid-liquid separation steps [33]. The most common dewatering techniques used are flocculation, filtration, centrifugation, gravity sedimentation, and flotation. However, no single harvest method may be suitable for every case [34–36].

Processing represents a major economic limitation to the production of low-cost commodities, such as biofuels and to higher value products, such as pigments. It is difficult to discuss processing, since it is highly specific and strongly depends on the products desired. The harvested biomass slurry (typical 5–15% dry solid content) is perishable and must be processed rapidly after harvest; dehydration or drying is commonly used to extend the viability depending on the final product required [37]. Methods that have been used include sun drying [38], spray drying [39], drum drying [38], and fluidized bed drying [40]. Sun drying is the cheapest dehydration method, but its main disadvantages include long drying times, the requirement for large drying surfaces, and the risk of material loss [38]. Spray drying is commonly used to extract high-value products, but it is relatively expensive and can cause significant deterioration of some algal pigments [39].

After drying, the microalgae cells are disrupted to release the metabolites of interest. Several methods can be used depending on the microalgae wall and the nature of the desired product either based on mechanical action (e.g., cell homogenizers, bead mills, ultrasound, autoclave, and spray drying) or non-mechanical action (e.g., freezing, organic solvents and osmotic shock, and acid, base, and enzyme reactions) [41].

Solvents, such as hexane, have been used to extract and purify soybean seed oils, high-value fatty acids, and specialty nutraceutical products. These types of solvent-based processes are most effective with dried feedstocks or those with minimal free water. The cost of drying the feedstock significantly adds to the overall production cost and requires significant energy. A limited number of solvents have been evaluated for the large-scale extraction of algal biomass with some success, but no effort was made to determine the process economics or material and energy balances of such processes [42]. The drying of algal wet pastes for large-scale organic solvent extraction may not be economically feasible in terms of embodied energy for biofuels. However, it is frequently used in the economic assessments of algal biofuel production, since it is an established technology and at least for oilseeds, is practiced on a large scale with well-established economics [43].

Some research suggests that oil can be recovered from the humid biomass [44, 45]. Algenol's "Direct to Ethanol" process involves the direct conversion from cyanobacteria to ethanol, without drying and dehydration, making algae-based fuel production commercially viable. Vapor compression steam stripping (VCSS) is utilized to extract and purify the ethanol, reducing the cost of ethanol. During this process, the blue-green algae are cultivated in seawater media in the low-cost closed proprietary flexible plastic film photobioreactor. In the presence of CO_2 and light, the blue-green algae can produce ethanol and then secrete it onto the culture medium. In the sunlight, condensed ethanol evaporates into the top area of the photobioreactor, while at night, evaporated ethanol and water can be collected into the collector. The collected water and ethanol are separated subsequently. Algenol's goal is to reduce its cost to $1.5–1.7 gal [3].

To extract biofuels, it is important to establish a balance between the drying efficiency and cost-effectiveness in order to maximize the net energy output of the fuels [46]. The drying temperature during lipid extraction affects both the lipid composition and the lipid yield from the algal biomass. For example, drying at 60 °C still retains a high concentration of triacylglycerol (TAG) in the lipids and only slightly decreases the lipid yield with higher temperatures decreasing both the concentration of the TAG and the lipid yield [47]. OriginOil, a biofuel company based in Los Angeles, developed a wet extraction process that combines ultrasound and electromagnetic pulse induction to breakdown the algae cell walls. CO_2 is added to the algae solution, lowering the pH and separating the biomass from the oil. This method has the advantages of dewatering, low energy demand, a lack of toxic chemicals involved, and wide utilization. When applied to industrial-scale production, it can reduce 90% of the total energy, whose cost is reduced to 200 dollars per ton of seaweed oil [3, 48].

The conversion technologies of algal biomass-to-energy can be separated into two basic categories of thermochemical and biochemical conversion (Fig. 1). Factors that influence a choice of conversion process include the type and quantity of the biomass feedstock, the desired form of the energy, economic consideration, project specifics, and the desired end form of the product [49].

Thermochemical conversion encompasses the thermal decomposition of organic components in biomass to yield fuel products and is achievable by different processes, such as direct combustion, gasification, thermochemical liquefaction, pyrolysis, carbonization, and torrefaction [50–52]. In a direct combustion process, biomass is burned in the presence of air to convert the stored chemical energy in the biomass into hot gases, usually in a furnace, boiler, or steam turbine, at temperatures above 800 °C [53, 54]. It is possible to burn any type of biomass, but combustion is only feasible for biomass with a moisture content < 50% of the dry weight. The heat produced must be used immediately since storage is not a viable option [55].

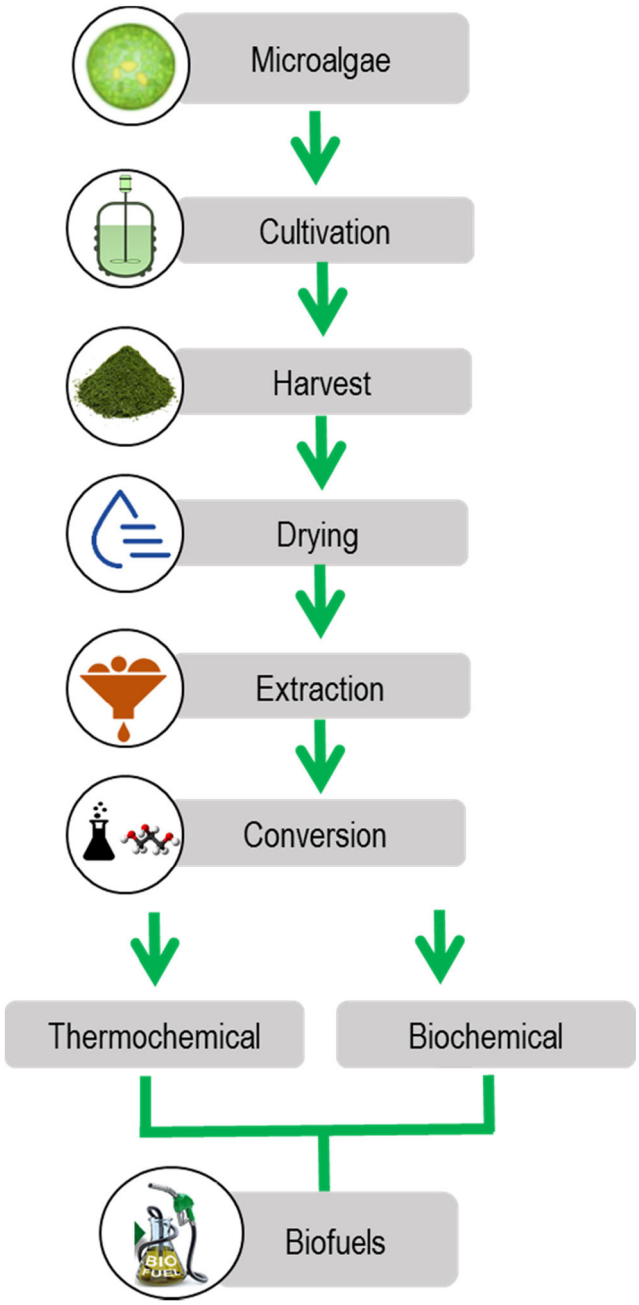

Fig. 1 Process of obtaining biofuels from microalgae

Gasification is a term that describes a chemical process by which carbonaceous material hydrocarbons are converted to a synthesis gas, syngas, using partial oxidation with air, O_2, and/or steam at high temperatures, typically in the range of 800–900 °C. During the normal gasification process, the biomass reacts with O_2 and water steam to generate syngas, a mixture of carbon monoxide (CO), hydrogen (H_2), CO_2, N_2, and methane (CH_4). The key advantage of gasification as a biomass-to-energy pathway is that it can produce syngas from a wide variety of potential feedstocks [55, 56].

Thermochemical liquefaction is a low-temperature (300–350 °C) and high-pressure (5–20 MPa) process [54] that can be utilized to convert wet algal biomass material into liquid fuel [57]. Reactors for thermochemical liquefaction and fuel-feed systems are complex and therefore expensive [53] but have advantages in their ability to convert wet biomass into energy [55]. The process utilizes the high-water activity in sub-critical conditions to decompose biomass materials to shorter and smaller molecular materials with a higher energy density [57].

Pyrolysis is the conversion of biomass to a biofuel, charcoal, and its gaseous fraction by heating the biomass in the absence of air to approximately 500 °C [53, 58] or by heating in the presence of a catalyst at a high heating rate (10^3–10^4 K/s) and with short gas residence time to crack the biomass into short-chain molecules that are then rapidly cooled to liquid [59]. Previous studies used slow pyrolysis processes that were performed at a low heating rate and with a long residence time. The longer residence times can cause secondary cracking of the primary products, reducing yield and adversely affecting the biofuel properties. In addition, a low heating rate and long residence time may increase the energy input. In recent years, fast pyrolysis processes for biomass have attracted a great deal of attention to maximize liquid yields, and many types of research have been performed. The advantage of fast pyrolysis is that it can directly produce a liquid fuel [53].

Carbonization is a slow pyrolysis process in which biomass is converted into a highly carbonaceous, charcoal-like material. Typically, carbonization consists of heating the biomass in an oxygen-free or oxygen-limited environment, and reaction conditions are tailored to maximize the production of char. During heating, biomass materials start to decompose evolving low and high molecular weight volatiles and form a carbon-rich charcoal residue. The volatile products can either condense at room temperature as bio-oils or remain in the

vapor form as biogases. The composition and concentration of the liquid and gas production depend largely on the heating conditions, heating rate, and pressure. Generally, faster heating rates promote biomass thermal cracking and, subsequently, higher volatile evolution rates, while carbonization temperature conditions favor charcoal formation [52, 60].

Torrefaction is the thermochemical treatment of biomass at lower temperatures, such as less than 300 °C, compared with pyrolysis and gasification. Biomass is pre-treated to produce a high-quality solid biofuel that can be used for combustion and gasification. In this process, the biomass partly decomposes and releases various types of volatiles and some non-condensable gases. The remaining torrefied biomass (solid) has approximately 30% more energy per unit of mass and is usually pelleted to allow for higher densification (620–650 kg/m^3) than regular biomass (820–850 kg/m^3). Pellets are homogeneous, hydrophobic, free of biological activity, and produce less smoke upon incineration. Torrefied pellets also contain substantially more energy per unit of volume due to their higher energy (18–20 vs. 10–11 GJ/m^3) content [51, 61–63].

The process of the energy conversion of biomass into other fuels includes anaerobic digestion, alcoholic fermentation, and transesterification [64]. Anaerobic digestion is the conversion of organic wastes into a biogas, which consists primarily of CH_4 and CO_2, with traces of other gases such as hydrogen sulfide (H_2S) [65]. It involves the breakdown of organic matter to produce a gas with an energy content of approximately 20–40% of the lower heating value of the feedstock. The anaerobic digestion process is appropriate for high moisture content (80–90% moisture) organic wastes, which can be useful for wet algal biomass [53].

Alcoholic fermentation is the conversion of biomass materials, which contain sugars, starch, or cellulose, into ethanol [53]. The biomass is ground down, and the starch is converted to sugars that are then mixed with water and yeast and kept warm in large tanks called fermenters. The yeast breaks down the sugar and converts it to ethanol. A purification process (distillation) is required to remove the water and other impurities in the diluted alcohol product (10–15% ethanol). The concentrated ethanol (95% volume for one distillation) is drawn off and condensed into liquid form, which can be used as a supplement or substitute for petrol in cars. The solid residue from the process can be used for cattle feed or for gasification. This helps offset feedstock costs, which typically make up 55–80% of the final sale price of the alcohol [66].

Transesterification is a process of exchanging the alkoxy group of an ester compound with another alcohol. The reactions are often catalyzed by an acid or a base using a homogeneous or heterogeneous catalytic process [67]. Transesterification is the reaction of a fat or oil with an alcohol to form esters and glycerol. The alcohol combines with the triglycerides to form glycerol and esters. The result of the transesterification process is biodiesel. To get the high yield of the ester, the alcohol has to be used in excess [68].

Process Integration Applied to Microalgae Biorefineries

Process integration has been studied extensively in recent years for resource conservation and is widely implemented in the processing and power generation industries. This methodology combines several parts of processes or whole processes that play an important role in energy saving and minimize raw material use and harmful emissions to the environment [69].

As an alternative to traditional processing industries that rely on the use of fossil inputs, bio-based processes have been a mainstay of industrial technological development, yet the current trend toward sustainability further emphasizes the role that bioprocesses can play in the production of a wide array of products and energy [70]. The bioprocess integration mediated by microalgae is an appropriate and innovative approach to complying with green engineering requirements [71].

In this context, for improved sustainability, microalgae biorefinery systems require integration into the total chain. At a facility level, two possibilities are considered for reuse, recovery, and possibly recycling of (i) mass and (ii) energy surpluses, whose focus is on the microalgal biomass production [72]. Table 1 shows the integration approaches in microalgae-mediated processes.

Mass integration is an approach that provides an understanding of the global flow of mass within the process, including effluent minimization and water consumption. Different methodologies of mass integration can be implemented to consider the possible effects on economic and environmental impacts [85]. This can be reflected in the use of gaseous effluent streams from industrial combustion processes, rich in CO_2 and NO_x, allowing the input of inorganic carbon and nitrogen (N_2) in microalgae photosynthetic cultures [86]. Since the microalgae can also be grown heterotrophically, the liquid effluent integration allows the supply of essential nutrients, such as organic carbon, nitrogen, and phosphorus, which are efficiently assimilated by these microorganisms. Therefore, these two bioprocesses offer a pathway for biomass production using low-cost substrates and reducing the carbon and water footprint (WF) [8, 71]. In addition, both effluent integration strategies permit the reuse of the thermal energy contained in the materials derived from the waste, which can be used to maintain the medium temperature culture in the mesophilic range, thus increasing the microalgal biomass productivity rate. This type of integration is highly dependent on the type of industrial waste [87].

Additionally, it is also possible to integrate water through its reuse, either directly or partially treated for use in other processes. Wastewater reduction and water conservation are increasingly important issues from an environmental point of view because it is a basic resource in manufacturing processes and is widely used for various purposes [88]. Therefore, the

Table 1 Different possibilities of process integration mediated by microalgae

Type of integration	Substance integration	Process description	Cultivation system	Goal	Process bottlenecks	Reference
Mass	Effluents	Integration of cassava wastewater as the culture medium of cyanobacteria for bulk oil and biodiesel production	Heterotrophic	Biodiesel	Presence of inhibitory compounds Competition with native effluent microorganisms	[70]
		Integration of crude glycerol as an exogenous carbon source for the cultivation of different species of microalgae in a two-phase process	Phototrophic	Biohydrogen and lipids	Large amount of impurities Suitable bioreactor configuration	[73]
		Integration of aquaculture wastewater for microalgae growth, the evaluation of biomass composition and nutrient removal	Heterotrophic	Biomass	Accumulation of toxic substances	[74]
		Wastewater integration from poultry and swine slaughterhouses	Heterotrophic	Biomass	Presence of inhibitory compounds Competition with native effluent microorganisms Suitable bioreactor configuration	[75]
		Integration of different proportions of municipal wastewater and assessment of the carbohydrate production performance	Phototrophic	Bioethanol	Presence of inhibitory compounds Competition with native effluent microorganisms	[76]
		Integration of wastewater (C, N, and P as organic sources) and flue gas (CO_2, CO, NO_x and SO_x as inorganic sources) from municipal effluents for microalgae growth and treatment efficiency	Mixotrophic	Carbohydrate and lipid	Elevated temperature Few microalgae species tolerate high levels of impurities in the flue gases Presence of particulate matter Scale up	[77]
		Integration of flue gas containing CO_2, NO_x, and SO_2 from a coke oven, hot stove, and power plant for microalgae cultivation, lipid production, and fatty acid composition	Phototrophic	Biodiesel	Elevated temperature Few microalgae species tolerate high levels of impurities in the flue gases Presence of particulate matter	[78]
	Water	Water integration and harvesting methods for microalgae cultivation	Phototrophic	Biodiesel	Energy intensive and expensive method	[79]
		Seawater integration to optimize the cultivation conditions for biomass production and the accumulation of microalgal lipids	Heterotrophic, mixotrophic, and phototrophic	Lipid	Depletion of abiotic natural resources Environmental licensing	[80]
		Reuse of effluent water from a municipal wastewater treatment plant in the cultivation of microalgae for the production of biofuels	Heterotrophic	Biodiesel	Presence of inhibitory compounds Competition with native effluent microorganisms	[81]
Energy	Heat	Energy integration of microalgae biomass drying for gasification and power generation	Phototrophic	Increased energy efficiency	High operating temperatures Product degradation risk Scale up	[82]
		Recovery of waste heat from the microalgae biodiesel production process	Phototrophic	Biodiesel	High operating temperatures Product degradation risk Scale up Complex operational structure	[83]
	Volatile organic compounds	Integration of volatile organic compounds and oxygen released from the exhaust gases of microalgal photobioreactors in oxycombustion systems	Phototrophic	Gaseous fuels, oxidizers, and nitrogen diluent	Concentration of the volatile organic compounds and oxygen Scale up	[9, 84]

microalgae can play a valuable role in the treatment of waste and contribute to the reuse of water and nutrient cycling [89].

Finally, energy integration considers all the forms of energy, such as heating, power generation/consumption, and fuels. This methodology has been driven by the increasing demand for expensive equipment within industries, aiming to minimize energy consumption and to maximize internal heat recovery. In this way, solid biomass can be converted to energy using thermochemical processes to obtain electric energy, syngas, or biofuels. More specifically, energy integration can occur during the recovery of the waste heat produced in these thermal processes to generate other gaseous or liquid products, thus maximizing energy efficiency [71, 90]. Alternatively, through the photosynthetic cultures, it is also possible to recover and integrate mass and energy from photobioreactor exhaust gases. Volatile organic compounds (VOCs), O_2, and unconverted CO_2 are released and reused to improve the energy efficiency of the combustion systems [84]. However, so that the different process integration strategies are developed in practice, it is necessary to couple a microalgae-based process in other industrial facilities already established. This is because the projection of an integrated microalgae process stand-alone greatly increases operating expenses.

Bioenergy and Biofuels from Microalgae

First- to fourth-generation biofuels have been extensively studied with the goal of possibly replacing fossil fuels, which are vulnerable to depletion. However, while most options demonstrate several barriers associated with their sustainability, production, and technical-economic viability, biofuels based on microalgae are considered to be the most promising alternative to obtain renewable energy [91].

In this context, microalgae have some advantages when compared to terrestrial cultures, such as relatively simple cultivation techniques and the quicker growth rate of many species, a fact that enables higher biomass productivities and therefore demonstrates the possibility of application for biofuel production [92, 93]. In addition, depending on the production route step chosen, microalgae are a potential feedstock for the production of different types of biofuels, such as biodiesel, bioethanol, biohydrogen, biomethane, syngas, bio-oil, and, more recently, VOCs [94, 84] (Fig. 2). There are different methods of microalgae cultivation for biofuels and bioenergy production, as summarized in Table 2.

Biodiesel from microalgae is one of the most efficient alternative sources of biofuels. It is characterized as an environmentally friendly alternative to petroleum-based diesel, producing lower levels of CO, sulfur dioxide (SO_2), and unburned hydrocarbons than petroleum-based diesel fuel and is biodegradable, renewable, and non-toxic because it is obtained from the fatty acids produced by the microalgae [110–112]. Another important factor is the amount and lipid composition because they determine the oxidative stability of biodiesel together with the performance properties in the engines [112].

Due to their simple cellular structure, microalgae have great versatility in their total lipid content, ranging from 20 to 50% of their dry weight [113]. For example, *Botryococcus braunii* demonstrates a remarkable ability to synthesize and store a high content of hydrocarbons, which can exceed 80% of its dry biomass [114, 115]. However, the primary limitation related to this microalgae is its low growth rate, resulting in low biomass and lipid productivities [116]. The values found in the literature are very low, in the range of 0.1 to 0.3 g L^{-1} day^{-1}, when compared to other strains of microalgae [117]. In a study conducted by Francisco et al. [99], the lipid content of different microalgae species ranged from 6.3 to 27%, and *Chlorella vulgaris* demonstrated the best performance in terms of lipid productivity (5.3 mg L^{-1} h^{-1}), intermediate cell growth (20.1 mg L^{-1} h^{-1}), and fatty acid profiles with quality characteristics for biodiesel production. Normally, lipid content and productivity are inversely proportional, a fact explained by the high metabolic cost of lipid biosynthesis. For example, if a strain obtained high productivity of biomass with an average oil content of 30% by dry weight, this would be equivalent to a microalgae biodiesel yield of approximately 98 m^3/ha [110]. This scenario seems feasible. However, few species are effectively investigated and considered to be potential sources for single cell oil exploration.

A critical analysis of the current status of microalgae biodiesel indicates that its production is not feasible commercially, both from a technical and economic point of view. First, to achieve the success of practical oil production from microalgae, it is necessary to design an efficient cultivation system. In theory, photobioreactors are suitable for this purpose; however, to date, no bioreactor has been found that meets the industrial production needs [118]. In addition, bottlenecks associated with high capital and operational expenditures and the high costs of biomass processing (cultivation and harvesting) need to be overcome. There is a clear need to establish a rigorous economic analysis of microalgal biodiesel production to compete with petroleum-derived fuels that currently have relatively low prices [119].

Another attractive biofuel is bioethanol, since many microalgae species can produce ethanol from starch, cellulose, and intracellular glycogen under anaerobic conditions in the dark [120]. Microalgae-based carbohydrates have a low lignin content, facilitating the fermentation of sugars, thus offering a promising and sustainable biomass source to produce this type of fuel [121, 122]. In addition, the percentage of carbohydrates in the biomass depends on the microalgae species and the cultivation and

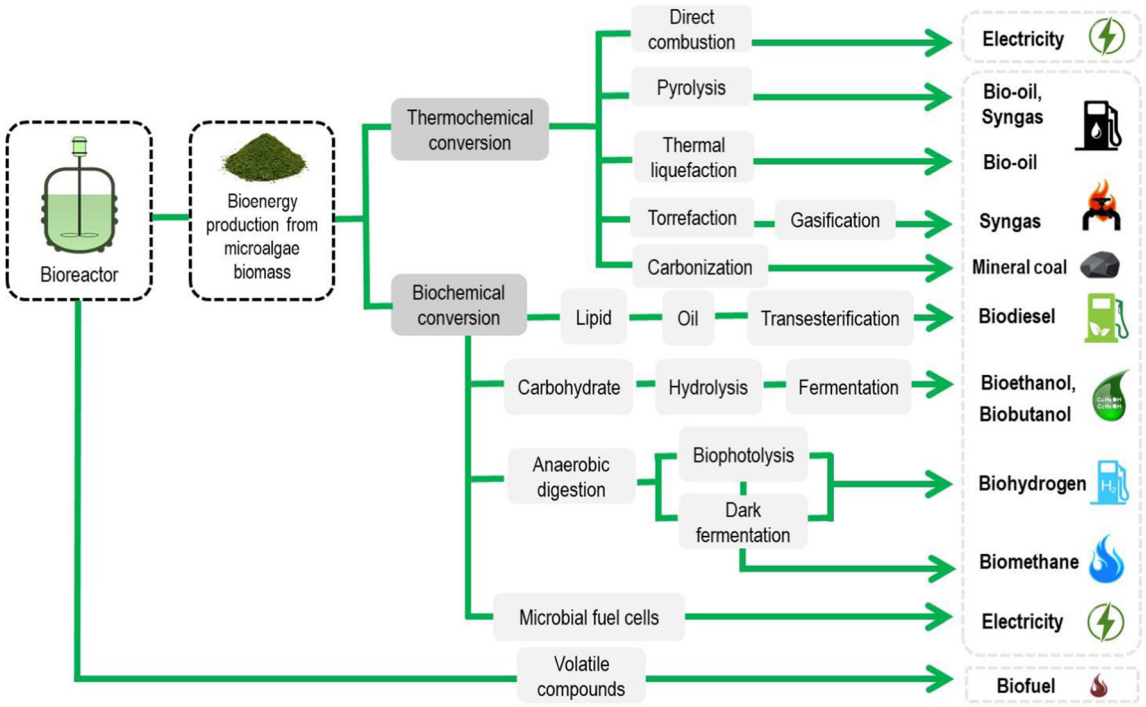

Fig. 2 Potential processes for energy production from microalgae biomass

environmental conditions [123]. Some studies report that the genus *Chlorella* has a high carbohydrate content, especially *Chlorella vulgaris*, with values of up to 55% of its dry weight [124, 125]. In terms of yield, some microalgae containing glucose-based carbohydrates can reach values of approximately 0.08 to 0.12 g of ethanol per dry biomass weight [122]. However, although these microorganisms are considered suitable substrates to produce bioethanol, and because they consume low amounts of energy compared to biodiesel production, the extraction efficiency of this

Table 2 Production of different types of biofuels and bioenergy from different cultivation methods

Design	Cultivation method	Microalgae/cyanobacteria	Biofuel/bioenergy	Productivity/production	References
Open	Raceway pond	*Chlorella vulgaris/Monoraphidium dybowskii* Y2	Biodiesel	13.91/14.45 ton ha^{-1} $year^{-1}$	[95]
	Circular ponds	*Graesiella* sp.	Biodiesel	5.44 g	[96]
	Biofilm	*Chlorella pyrenoidosa*	Biodiesel	1.80 g m^{-2} day^{-1}	[97]
	Thin-layer	*Chlorella vulgaris*	Syngas	18.10 MJ kg^{-1}	[98]
Closed	Bubble column heterotrophic bioreactor	*Phormidium* sp.	Biodiesel	43.8 mg L^{-1} h^{-1}	[70]
	Bubble column	*Chlorella vulgaris*	Biodiesel	5.3 mg L^{-1} h^{-1}	[99]
	Bubble column	*Aphanothece microscopica Nägeli*	Biodiesel	0.08 g L^{-1} day^{-1}	[100]
	Bubble column	*Scenedesmus obliquus*	Volatile organic compounds	86.32 MJ kg^{-1}	[84]
	Bubble column	*Arthrospira platensis*	Biomethane	0.40 m^3 kg^{-1}	[101]
	Airlift	*Chlamydomonas reinhardtii*	Biodiesel	117.62 mg L^{-1} h^{-1}	[102]
	Tubular	*Chlorella vulgaris*	Bio-oil	0.12 g L^{-1} day^{-1}	[103]
	Tubular	*Scenedesmus bijugatus*	Biodiesel/bioethanol	63 mg L^{-1} day^{-1}/0.158 g/g	[104]
	Flat-plate	*Chlamydomonas reinhardtii*	Biohydrogen	1.3 mL L^{-1} h^{-1}	[105]
	Bubble column	*Chlorella vulgaris*	Microbial fuel cell	126 mW m^{-3}	[106]
	Bubble column	*Chlorella vulgaris*	Biobutanol	0.93 g h^{-1} L^{-1}	[107]
	Hybrid	*Chlorella vulgaris*	Biodiesel/bioethanol	36.9/32.9 mg L^{-1} day^{-1}	[108]
	Plastic bag	*Chlorococcum humicola*	Bioethanol	10 g L^{-1}	[109]

biofuel is low, since bioethanol contains approximately 5% of water, which makes the process unfeasible [94, 126, 127].

Biobutanol can also be obtained from carbohydrate-based microalgae as an alternative fuel. Traditionally, biobutanol is produced from microalgae by fermentation with acetone-butanol-ethanol (ABE) using anaerobic bacteria, such as *Clostridium* sp., that also produce other organic compounds, such as acetone, ethanol, gases, and organic acids [128]. It is known to have more advantages than bioethanol with a high energy content, water solubility, and low volatility, making this compound well adapted to the existing infrastructure of petroleum-based fuels [129]. The energy density of butanol is 29.2 MJ/L, comparable to gasoline (32.3 MJ/L), but greater than that of anhydrous ethanol (19.6 MJ/L) [121]. However, the fermentation process of biobutanol is less efficient and less productive, causing low yields due to the severe inhibition of biobutanol to the host cells in the medium [130]. In addition, the theoretical maximum biobutanol yield is 0.41 g/g glucose, while the yield of bioethanol is 0.5 g/g glucose [122].

Gaseous fuels generated by microalgae, such as biohydrogen [131] and biomethane [132], are the most efficient in terms of net energy gain among all the biofuel conversion technologies. This is due to the use of all biodegradable components by microalgae using anaerobic digestion, unlike other biofuels [93]. Biohydrogen is the biofuel with the highest energy content compared to other fuels (142 MJ/kg) and can be used in combustion cells to produce electricity with high efficiency [121, 133]. In addition, each 1 kg of biologically generated H_2 contains the same energy of 2.8 kg of gasoline and can be used as clean energy in alternative combustion engines and continuously generate electricity in fuel cells [134]. Metabolically, microalgae can synthesize specific enzymes, such as hydrogenases and nitrogenases, which receive electrons donated by ferredoxin in the final stage of photosynthesis for the water biophotolysis process and the subsequent production of significant amounts of H_2. Biophotolysis can take place by a direct path, in which H_2 comes from the protons and electrons formed in the chloroplasts of microalgae to reduce molecules, or by an indirect path, where the protons and electrons are supplied by the degradation of intracellular carbon compounds. In addition, through distinct metabolic pathways and genetic engineering approaches, some microalgae strains have been developed to efficiently potentiate H_2 production [135]. According to Rashid et al. [136], the ability of H_2 synthesis is directly related to certain specific growth conditions, such as light intensity, wavelength, and illumination pattern. Their photobiological production can be potentiated by increasing the light intensity and the carbon content in the biomass. However, biohydrogen production by microalgae on a commercial scale is only in the early stages of studies due to its conversion rate, which is unsatisfactory. In the future, biohydrogen is expected to become a viable bioenergy option.

Alternatively, biomethane can be obtained in parallel to biohydrogen using anaerobic digestion followed by the dark fermentation of microalgae waste after the oil is extracted. It can be collected to generate electricity, as in landfill sites and to manufacture fertilizers. More than 60% of biomethane can be produced from the microalgal biomass [93]. Because it demonstrates low energy requirements during its processing, biomethane production by microalgae appears to be a viable source of bioenergy [137]. A study by Harun et al. [120] revises the different microalgae strains for biomethane production, and the genus *Macrocystis* has a yield of 0.39 to 0.41 m^3/kg. Although microalgae offer a good potential for biogas production, commercial production has still not been implemented.

In addition, microalgae biomass can also be thermally processed to produce syngas and bio-oil through gasification, pyrolysis, or thermal liquefaction [138]. However, due to the great heterogeneity of microalgae, the execution of these thermochemical processes depends highly on the fuel composition and a complete characterization of the feedstock thermal behavior [139], which often requires a longer time of analysis, high cost, and low energy efficiency, making its operation unfeasible [140].

Finally, it should be considered that microalgae could be used as microbial fuel cells (MFCs) directly converting chemical energy into electricity through their metabolic reactions. In addition, MFCs can also simultaneously convert atmospheric CO_2 into bioelectricity and remove the organic matter contained in wastewater [94]. In this process, CO_2 is used as a carbon source from the oxidation of organic materials at the anode chamber. Similarly, O_2 is required at the cathode to receive the excess protons from the anode and electrons from the external circuit [141]. In recent years, many types of research have been conducted to improve the performance of MFCs to expand bioenergy production [142]. However, to produce economically viable microalgae MFCs, technical obstacles need to be removed, such as the difficult design of the chambers to receive light at the cathodic chamber and the costs of the cell culture processing.

Finally, recent research has demonstrated VOC production for application as gaseous biofuels [143]. Substantial concentrations of VOCs, O_2, and unconverted CO_2 are released from the exhaust gases of the photobioreactors and simultaneously reused as fuels, oxidizer, and nitrogen diluent in biocombustion processes. VOCs have an energy potential of 86.32 MJ/kg (Table 3), which is higher than other traditional fuels, such as gasoline (47.30 MJ/kg) and diesel (44.80 MJ/kg). However, to meet the energy demands that a combustion system requires, it would be necessary to have a photobioreactor that produces these compounds in high volumes, which does not currently exist. Although microalgae appear to be promising sources for the production of various known types of bioenergy, they become unattractive in the

Table 3 Volatile organic compounds generated by microalgae and their energy potential. Adapted from [84, 9]

Compound	Energy potential (MJ/kg)
Alcohols	
2-ethyl-1-hexanol	5.42
2-propil-1-heptanol	6.72
Aldehydes	
2-methylbutanal	3.24
Hexanal	3.88
2,4-heptadienal	2.97
2,4-decadienal	6.0
Hydrocarbons	
2-methoxy-2-methyl-propane	3.48
3,3-dimethyl-hexane	5.42
2,4-dimethyl-heptane	6.07
4,7-dimethyl-undecane	8.39
Ketones	
2-propanone	1.94
2,4-dimethyl-3-pentanone	4.53
4-octen-3-one	5.18
6-methyl-5-hepten-2-one	4.94
Acetophenone	4.22
β-ionone	7.70
Terpenes	
2-phenylpropene	4.87

market due to the high cost of cultivation methods and processing. In addition, emerging bioenergy processes are far from the level of development necessary for scaling at the industrial level [9, 84].

Co-Products Derived from Manufacturing Processes

Microalgae biofuels are not economically viable due to high capital and operating costs. In an effort to reduce the high capital investment requirements, many researchers have suggested biorefinery approaches [144, 145]. Biorefineries of bioenergy include low value but high-volume biofuels, such as biodiesel, bioethanol, or biogas. In contrast, high-value co-products are designed to increase the profitability of biorefineries (Fig. 3), while high-volume biofuels generate lower energy costs for domestic use and provide additional revenue [146].

However, not all high-value co-products are suitable for biorefineries. The basic requirements for co-products include the feasibility of large-scale production and the high extension of the coupling between the co-product and the bioenergy processing. In addition, the following criteria should be considered for the selection of co-products: (i) the cost of the raw material required, (ii) the cost of processing the raw material, (iii) the current and expected market price of the future co-product, (iv) the capacity, and (v) the technical characteristics appropriate to the market needs [147].

The biomass of microalgae and cyanobacteria contains lipids, carbohydrates, proteins, and other valuable compounds (Table 4). Lipids can be used as a source for biofuels, as building blocks in the chemical industry, and edible oils for the food and health market. The carbohydrates can be used for the production of chemicals, such as those used in pharmaceuticals and cosmetics, or transitioned into bioethanol through fermentation or into CH_4 via anaerobic digestion [152]. Purified proteins can be used in the food, feed, and bulk chemical markets [153, 154].

The lipid content in microalgae can reach up to 40% in dry biomass. The interest in algal lipid arises primarily from the fact that these organisms can synthesize considerable quantities of polyunsaturated fatty acids (PUFAs) that either reach humans via the food chain or are used as food supplements. In addition, microalgae have been attracting interest as sources of fatty acids, especially the long-chain polyunsaturated fatty acids such as γ-linolenic acid, arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) [155, 156].

The lower the lipid content, the greater the necessity to produce co-products in an attempt to enhance the economics of the algal biofuel production chain [152]. Fatty acid methyl esters (FAME) are the chemical ingredients of biodiesel. However, not all lipids can be converted into FAME. One example is chlorophyll. The combination of chlorophyll and derivative, such as sodium copper chlorophyllin, production from the waste of the biodiesel can greatly improve the sustainability of the algal biofuel industry.

Microalgae typically have a high carbohydrate content, which is approximately 50% higher than its dry weight since it has a relatively high conversion efficiency and can easily store carbohydrates [157]. Algal carbohydrates are primarily composed of glucose, starch, cellulose, and various types of polysaccharides. Microalgal polysaccharides can modulate the immune system and inflammatory reactions, making them highly favorable as sources of biologically active molecules, such as cosmetic additives, food ingredients, and natural therapeutic agents. Carbohydrates are normally extracted from microalgae via chemical hydrolysis [158].

The high protein content of various microalgal species is one of the primary reasons to consider them as an unconventional source of protein. In addition, the amino acid pattern of almost all algae compares favorably with other food proteins. Since the cells can synthesize all the amino acids, they can provide the essential ones to humans and animals. To completely characterize the protein and determine the amino acid content of microalgae, information on the nutritive value

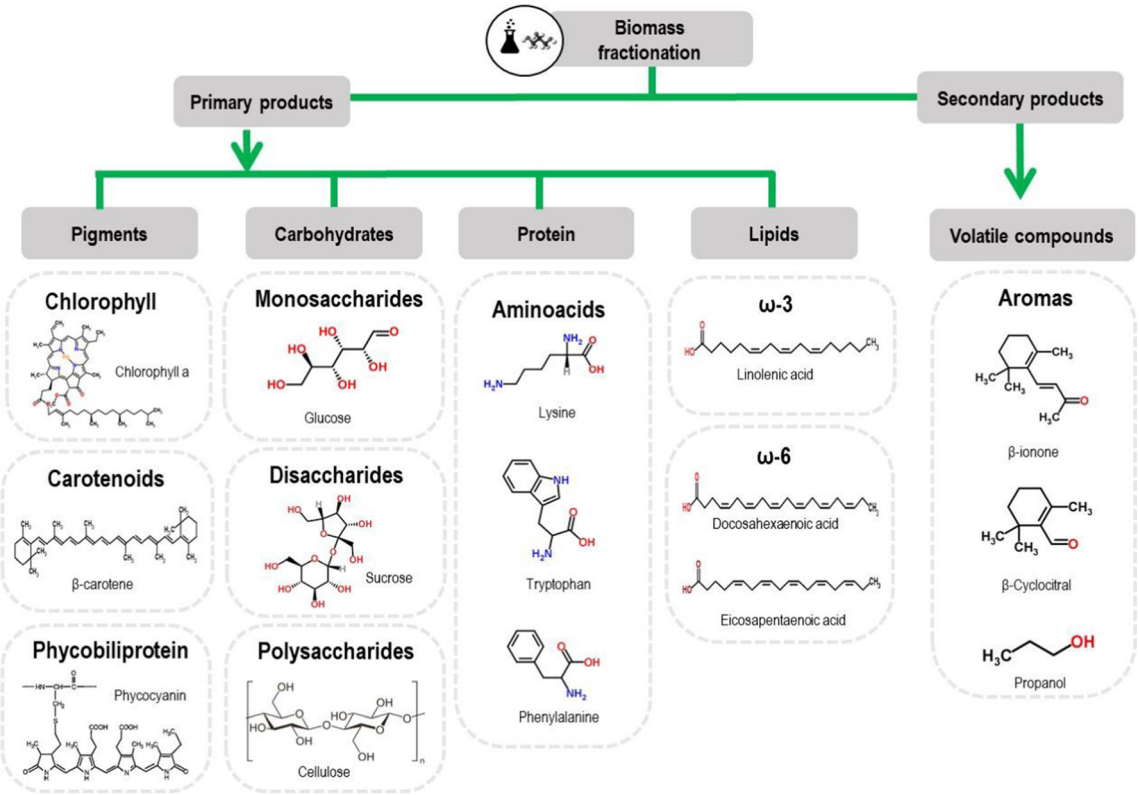

Fig. 3 Fractionation of microalgal biomass and co-products

of the protein and the degree of availability of amino acids should be made available [157].

Microalgae have been suggested to be commercial sources of vitamins, such as vitamins C, E, and B_{12} [159, 160]. The bioavailability studies on these components are often more essential compared to the feed quality, and the effects of these components when introduced into the body may be evaluated. The production of vitamins from microalgae depends on the availability of N_2 [161].

Faced with so many chemical classes present in the microalgal biomass, pigments receive substantial attention. Thus, the use of strategies to integrate the sustainable processing of biomass to obtain chlorophylls, phycobiliproteins, and carotenoids makes a microalgae biorefinery an attractive market [162, 163].

The phycobiliproteins are proteins that act as photosynthetic accessory pigments and are found in cyanobacteria, in a class of biflagellate unicellular eukaryotic algae, and in Rhodophyta. The primary commercial producers of phycobiliproteins (i.e., phycoerythrin and phycocyanin) are the cyanobacterium *Arthrospira* and the rhodophyte *Porphyridium* [164, 165]. The primary potential of these molecules seems to be as natural dyes, but an increasing number of studies have shown their health-promoting properties and broad range of pharmaceutical applications. The prices of phycobiliprotein products are USD 3–25/mg for native pigments, but they can reach USD 1500/mg for certain cross-linked pigments [166].

The chlorophyll derivatives are valuable natural colorants used by the textile, food, and paper industries [167, 168]. In addition to containing chlorophyll a and b molecules, microalgae biomass may contain chlorophyll c, d, e, and f, which are exclusive sources from microalgae and cyanobacteria, thus differing from the conventional sources of these pigments [169]. Due to this advantage, the market price of chlorophyllin is approximately € 150/kg, and can therefore offset a considerable amount of the cost of algal biofuel production [170].

Among the over 400 known carotenoids, only very few are used commercially, including β-carotene, astaxanthin, and, of lesser importance, lutein, zeaxanthin, and lycopene [166, 171, 172]. The production of carotenoids using microalgae as feedstock has become one of the most successful activities in the biotechnology industry. When industrially applied, the carotenoids are widely used as color enhancers in natural foods including egg yolk, chicken, meat, or fish. It appears that the use of natural colorants has been steadily increasing primarily because of changes in consumer preference toward more natural products known to exhibit specific functional properties [173].

Table 4 Comparison between the centesimal composition of two species of microalgae

Microalgae	*Chlorella vulgaris*[a]	*Arthrospira platensis*[b]
Protein	51–58	60–71
Lipids	14–22	6–7
Carbohydrates	12–17	13–16
Rhamnose (%)	45	30.1
Glucose (%)	4	30.9
Galactose (%)	26	21.4
Mannose (%)	7	1.42
Xylose (%)	19	2.23
Fructose (%)	–	1.21
Amino acids		
Aspartic acid (g)	10.94	5.80
Threonine	6.09	2.97
Serine	7.77	3.0
Glutamic acid	9.08	8.38
Glycine	8.60	3.10
Alanine	10.9	–
Cysteine	0.19	0.66
Valine	3.09	3.51
Methionine	0.65	1.15
Isoleucine	0.09	3.21
Leucine	7.49	4.95
Tyrosine	8.44	2.58
Phenylanine	5.81	2.77
Histidine	1.25	1.08
Lysine	6.83	3.02
Arginine	7.38	4.15
Tryptophan	2.21	0.93
Ornithine	0.13	2.38
Proline	2.97	–
Minerals		
Calcium (mg)	0.27	1.20
Iron (mg)	0.68	0.28
Magnesium (mg)	0.44	1.95
Phosphorus (mg)	0.96	1.18
Potassium (mg)	2.15	13.63
Sodium (mg)	1.35	10.48
Zinc (mg)	0.55	0.02
Copper (mg)	0.19	0.06
Manganese (mg)	0.40	0.01
Selenium (μg)	–	0.07
Vitamins		
C (Ascorbic acid) (mg)	15.6	10.1
B_6 (Pyridoxine) (mg)	1.7	0.36
A (Retinol) (IU)	13.2	570.0
E (Tocopherol) (mg)	20.0	5.0
K (μg)	–	25.5
B_1 (Thiamine) (mg)	2.4	2.4
B_2 (Riboflavin) (mg)	4.8	3.7
B_3 (Niacin) (mg)	23.8	12.8
B_5 (Pantothenic acid) (mg)	1.3	3.5
B_9 (Folic acid) (μg)	26.9	94.0
Fatty acids		
Myristic acid (%)	–	7.5
Palmitic acid (%)	4.36	25.0
Stearic acid (%)	1.20	7.7
Palmitolytic acid (%)	23.47	32.8
Oleic acid (%)	21.81	34.7
Linoleic acid (%)	6.26	12.5
α-linolenic acid (%)	20.0	17.84
Pigments		
β-Carotene (μg/g)	12	211.5
Astaxanthin (μg/g)	550	–
Cantaxanthin (μg/g)	362	–
Lutein (μg/g)	3830	–
Chlorophyll-a (μg/g)	9630	200.8
Chlorophyll-b (μg/g)	5770	
Violoxanthin (μg/g)	37	
Phycoerythrin (μg/g)	–	31.46
Phycocyanin (μg/g)	–	43.75

[a,b] [148–151]

In many markets, microalgal carotenoids compete with the synthetic forms of the pigments. Although the synthetic forms are much less expensive than the natural ones, microalgal carotenoids have the advantage of supplying natural isomers in their natural ratio [174, 175]. It is currently accepted that the natural isomer of β-carotene is superior to the synthetic all-trans form [166, 176].

Currently, the German chemical company Badische Anilin & Soda Fabrik (BASF) is the undisputed world leader in β-carotene production from *Dunaliella salina*, with over a thousand hectares of production ponds in two plants in Australia [177]. Consequently, due to this increased demand for pigments such as β-carotene, the global market of carotenoids was estimated to be valued at USD 1.24 billion in 2017. In addition, projections indicate that in 2021, their value will reach USD 1.53 billion [178].

As the basis of the natural food chain, microalgae play a key role in aquaculture, especially mariculture, since they are the food source for the larvae of many species of mollusks, crustaceans, and fish [179]. Astaxanthin is principally consumed by the salmon feed industry. The annual worldwide aquaculture market of this pigment is estimated at USD 200 million with an average price of USD 2500/kg. This co-product has a high biotechnological potential, and culture

techniques for *Haematococcus pluvialis* are well developed for this purpose [170, 180].

In addition to many beneficial properties, microalgae also produce numerous VOCs, which can be used as an important alternative source for bulk and fine chemicals. The compounds of commercial interest include propanol, butanol, 3-methyl-butanol, hexanol, hexanal, β-cyclocytral, β-ionone, and 5,6-epoxy-p-damascenone [181, 100]. Thus, according to Berger [182], compounds synthesized by microorganisms are potential competitors when compared to traditional sources.

Additionally, microalgae synthesize specific compounds with a high potential to produce fine chemicals (Fig. 4). Rodrigues et al. [183] consider keto-carotenoids and glycosylated carotenoids, such as echinenone, mixoxanthophyll, and canthaxanthin, to be produced exclusively by microalgae. In addition, some species of microalgae produce unconventional sterols, such as brassicasterol, campesterol, stigmasterol, and sitosterol. These algal phytosterols may have applications in the pharmaceutical industry or for functional foods. Additionally, important sun blockers derived from microalgae have emerged as alternatives to synthetic molecules and/or molecules of botanical origin. Compounds with photoprotective action include two major classes: the mycosporine-like amino acids (MAAs) and the *scytonemin*. These compounds exhibit high blocking efficiency, photostability, and low toxicity [35]. Finally, although they do not fit in any of the previous chemical groups, compounds such as ciguatoxin, karatungiol, okadaic, gambieric, and gamma-aminobutyric acids have been identified in microalgal extracts and have antimicrobial properties [184].

Life Cycle Assessment Applied to Microalgal Biofuels

In the microalgae system for biofuels, there are a variety of conversion technologies being exploited prior to commercialization [185]. Thus, to evaluate the sustainability and economic viability of any biofuel production system, it is necessary to apply a robust tool that can measure the inputs and outputs of the system [186].

Life cycle assessment (LCA) is defined as an emerging methodology used to quantify the input and output flows of materials and energy throughout the production chain [187, 188]. Conducting an LCA is a first step in setting goals and scope, i.e., including the intended application, the reasons for the study, the target audience, and the use of the results [189].

The life cycle inventory (LCI) stage involves the compilation and quantification of inputs and outputs for each process included within the system boundary. The impact assessment categories are chosen to have an overview of the inventory data: energy balance, WF, and global warming potential (GWP). Therefore, the final stage of the interpretation evaluates the results of the inventory analysis and impact analysis (LCIA) to select the preferred product or process with a clear understanding of the uncertainties and assumptions used to generate the results [187].

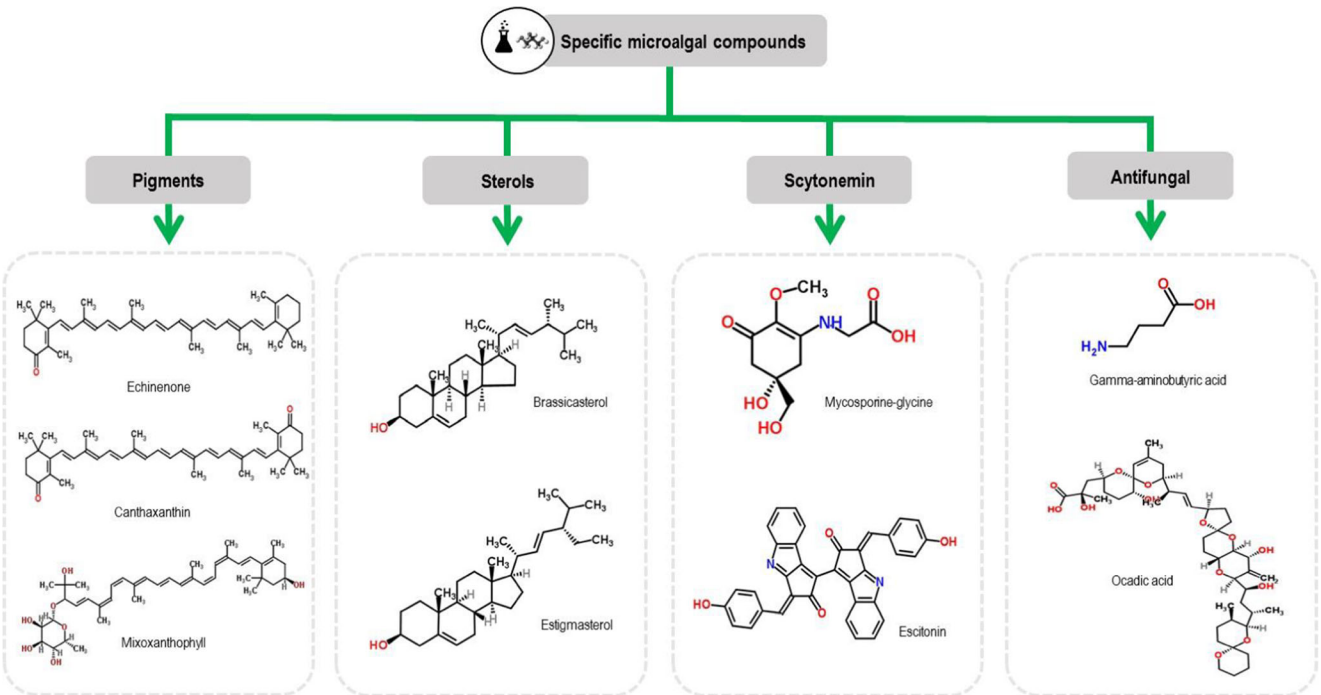

Fig. 4 Specific components synthesized by microalgae

Currently, there have only been a few LCA studies conducted on microalgae biofuels due to limited data. However, all the activity involved in the production of biofuels from microalgae is energy intensive, and the production-related parameters usually refer to biomass productivity, lipid content, and energy efficiency downstream. Given this scenario, any proposed biofuels process should have a positive net energy ratio (NER > 1), i.e., to produce more energy than is consumed [190, 118].

WF is used to assess the water use of supply chains, the sustainability of water use within river basins, water use efficiency, water allocation equitability, and dependence on water supply water or the dependence of water on the supply chain. This value is generated by quantifying the fresh water consumption of a process or product per functional unit [191].

In processes of biofuels modeled by microalgae, the direct withdrawal of WF reflects the water that is consumed in each step in the process, including the water for microalgae cultivation, water required to compensate for evaporation of the pond, water loss during the filtration process, and water that reacts during the conversion of the fuel. The internal recycling of water from the microalgae process to biofuels, e.g., centralized recycling, displaces direct water consumption [192, 193].

In addition, the environmental impact generated by the GHG is measured by the potentials of acidification and eutrophication, which, in total, are determined by the sum of the masses of the gases (CO_2, CH_4, NO_x) intensified by the characterization factors of the gases [194]. To incorporate LCA as an effective tool to assist in making sustainable performance decisions in a process of microalgae biofuels, Table 5 reports a comparative scenario between microalgal biofuel production systems and their environmental assessment.

Currently, only two forms of microalgae cultures are commercially available, including raceway ponds and tubular photobioreactors. However, It is known that raceway ponds are characterized by a lower energetic amount when compared to photobioreactors, providing an NER between 0.71 and 3.78 [196]. This is the extent to which open systems do not require a high energy supply and use light energy to perform photosynthesis. In addition, photobioreactor systems require higher energy support requirements, both for the operation of the reactor itself and for illumination simulation, with an NER < 1. However, in order to implement strategies to promote NER > 1 in photobioreactors, resources such as the intensification of research in metabolic engineering should be used to raise the rates of lipid synthesis in microorganisms, to allow the biomass to be dried in systems that have a lower value, such as natural drying and increasing crop volumes, are potential alternatives to supply the required energy and to compensate for the energy generated by the systems [200, 201].

Against this background, the separation of the biomass from the culture medium is usually identified as one of the major bottlenecks in the process. Historically, raceway ponds have been used for algae growth; however, projections show that the harvest contributes approximately 23% of the cost of cultivation (1.2 €/kg). Alternatively, only tubular photobioreactors are considered suitable for large-scale biomass production [202]. This justifies the high production rates of the microalgae biomass, since inefficient mixing and light distribution are solved in these configurations. In addition, values between 5 and 7% of the total cost for closed systems (0.2 to 0.3 €/kg) are considered to be due to higher concentrations of biomass [203].

Water balance calculations take into account the fact that the open systems have high evaporation rates. This justifies the high-water expenditure in these processes. However, the photobioreactors, because they are closed, only need the volume necessary for biomass cultivation. This volume is 0.75 L/kg on average, which refers to the amount of water used by the biomass for photosynthesis [198].

Atmospheric emissions are measured by equivalent amounts of CO_2, i.e., the sum of CO_2 emissions emitted by parameters such as non-conversion and the loss of CO_2 in the culture system, the electricity requirement, and carbon emitted by cell respiration in heterotrophic cultures. Raceway ponds have a wide range of emissions of 0.1–4.4 kg CO_2 eq/kg of algae [203]. Comparatively, tubular photobioreactors emit between 1.44 and 1.53 kg CO_2 eq/kg of algae [199]. In addition, the GWP for algae biodiesel can vary between 2830 and 836 kg CO_2 eq/ton of biodiesel. In comparison, the GWP produced throughout the gasoline supply chain is 3884.5 kg CO_2 eq/ton of gasoline [204].

Bottlenecks of Microalgae Biorefineries for Biofuels and Co-Products Production

Over the years, the processes involving microalgae have undergone several critiques about their productivity and

Table 5 Comparative scenario of life cycle analysis between commercial microalgae culture systems

Growth systems	Raceway pond	Tubular photobioreactor	References
Biomass (g/L)	~0.5	~5.0	[195]
NER	0.71–3.78	0.7–1.2	[196]
WF (L/kg)	0.74–83.14[1]	~0.75[2]	[1] [197]; [2] [198]
GWP (kg CO_{2eq})	0.1–4.4[1]	1.44–1.53[3]	[3] [199]

The superscript numbers are corresponding to the respective references.

industrial scalability. Therefore, strategies were developed through metabolic engineering and the development of production systems in order to prove their efficiency. In addition, one of the bottlenecks for commercialization that still persists in these processes is the high-energy consumption for the production and recovery of the microalgae biomass [205]. Commonly, studies report that 13.8 $MJ.kg^{-1}$ of dry mass are spent only to centrifuge the microalgal biomass [206]. Thus, process estimates show that an energy expenditure of 24,062,619.72 MJ/year is required for the resultant centrifugation and drying process. However, the total return energy when compared to other sources of bioenergy can neutralize this energy expenditure.

In this context, as shown in Table 6, Chisti [110] calculated a projection analysis on the raw materials commonly used industrially for biodiesel compared to the productivity that can be obtained from soybeans and palm trees. Thus, one can consider a scenario in which the average lipid yield of soybeans and palm trees are 0.46 and 0.56 g/m^2/day, respectively, in a production cycle of 120 days. Each hectare of arable land produces an average of 2700 kg of soybean containing up to 20% oil and 1500 kg of palm containing up to 45% oil; therefore, the total annual energy yield was 56,025.67 MJ/year for soybean and 70,031.99 MJ/year for palm. Comparatively, the data obtained for microalgae cultivation containing 20% oil indicate that in 1 L/m^2 of work volume, operating in a cycle of 120 days/year, and assuming an open and closed system scenario where the average cellular productivity would be 0.5 and 5.0 g/L, respectively, the process would yield between 1,246,400.00 MJ/year and 12,470,806.00 MJ/year. This comparison indicates that lipid productivity can be increased by several folds. Therefore, in conjunction with the optimal design of the photobioreactor, a continuous operation should be expanded by up to 330 days/year, providing an energy return of up to three times greater compared to other sources. Additionally, this provides a strong argument that microalgae are more advantageous than the other options available for the sustainable supply of biofuels such as biodiesel [99].

Microalgae have a wide potential to replace fossil fuels by generating energy, as they can remedy environmental problems. However, while a scenario involving processes that balance a positive energy balance using some devices and strategies that utilize environmentally friendly fuels needs to be structured, one dilemma in question, which needs to be overcome, is economic barriers. Studies have reported that the production costs of microalgae biomass are estimated to be USD 0.70–4.16 kg DW for the tubular photobioreactor and USD 1.28–4.95 kg DW for open crop systems. In addition, the cost of producing the heterotrophic fermenters is close to USD 12.0/kg. Alternatively, the current demand for oil was approximately 90 million barrels per day (Mb/day) in 2014. The International Energy Agency forecasts global demand growth to increase world production by almost 120 Mb/day through 2040 [207]. Therefore, with high supply and demand, it was observed that the oil price dropped 50% since these projections were made. As a result, the techno-economic hurdle has increased to achieve a cost-competitive biofuel production. Thus, according to Wijffels and Barbosa [208], the cost of producing microalgae biomass cannot exceed 0.55 cents (the ideal theoretical price) for the manufacture of bulk products, such as biofuels, that would make the microalgae feasible from a commercial point of view [75].

Additionally, the cost of producing microalgae and bioconversion in biofuels could be substantially reduced when combined with strategies using biorefineries. To exploit the full potential of the defatted biomass, many bulk chemical products can be obtained simultaneously, and, therefore, the market value can be higher than the production costs [209]. In terms of amino acid content, the nutritional value of the proteins from various microalgae are favorable compared to egg, soybean, and wheat proteins, as well as those specified in the WHO and FAO requirements [210]. In addition, assuming that the microalgal biomass has a protein fraction between 40 and 70%, the proteins can be treated in a single water-soluble fraction of 20% and a water-insoluble fraction of 80%; it can be concluded that it is possible to obtain a value of 5.6 USD/kg for a soluble fraction and 0.84 USD/kg for an insoluble fraction when destined for food [211]. However, the use for human consumption has not gained significant importance as food or substitutes for food, but biomass powders continue to be marketed as food supplements. Finally, the production costs for microalgae are still very high when competing for protein sources. An alternative, defatted microalgae biomass, could potentially save the use of soybean meal and corn in pig and poultry diets. Considering these estimates, the application of microalgae to animal feed is the most solid market estimated at 30% of the world's production sold [212, 213].

Table 6 Estimates of energy production between scenarios of microalgae and feedstock used for biodiesel

Biomass	Productivity biomass (kg/ha year)	Total annual energy yield (MJ/year)
Soybean	7391.25	56,025.67
Palm	4106.25	70,031.99
Microalgae (raceway pond)	152,000.00	1,246,400.00
Microalgae (tubular photobioreactor)	1,520,833.00	12,470,806.00

When considering the fine chemical compounds, the focus of microalgae biorefinery is the pigments. Thus, a key issue is to design a suitable integrated platform able to not only efficiently extract/fractionate the target compounds but also to comply with the green chemistry principles and sustainability [214]. Industrially, three different technologies are used to extract the pigments: extraction with refined oil, extraction with organic solvents, and extraction with an alkaline solution. The extraction with organic solvents, such as hexane, presents an efficiency that is 3.5–5.2% higher compared to the other types of extractions. Alternatively, there is an increase in unit operations (solvent recovery and purification), which consequently generates the energetic increase in the process. In addition, techniques such as the extraction of supercritical fluid emerge with the promise of reducing the volume of reagents, dispensing with purification and capable of being easily scalable at the industrial level [215]. However, regardless of the form of extraction, the pigment in powder form is resuspended in oil and the resin oil or gel capsules are marketed for food supplements with an average value of USD 12,000/kg [162]. Finally, it should be emphasized that for each pigment to be extracted, the different parameters and methods of extraction, each with its own advantages and limitations, must be considered to ensure better viability and financial return during the implementation of the process [216].

In this scenario, although there are visible bottlenecks in the microalgae-based processes, the barriers have been constrained since, through extensive research using emerging new technologies, a foundation can be laid for microalgae industries and production, extraction, and large-scale processing. In this way, microalgae-based processes could play an important role in global development.

Final Considerations

The race for renewable energy is underway, and microalgae based-processes and products play a central role in this issue.

In the current state, the bioeconomy of the processes and products based on microalgae faces serious restrictions. This is amplified in light of the persistence of low fossil fuel prices. In this context, the energy microalgae-based industry will be forced to shift its focus from lower-value commodity biofuels and bioenergy products to higher-value (non-energy) products that can be profitable today.

However, it is expected that with the application of some process engineering approaches, including the integration and intensification of processes associated with the concept of biorefinery, it will be possible to establish the economic viability of microalgae biofuels.

However, the large-scale production of algae biofuels is unlikely to be feasible, since the technological routes are still immature against the significant substitution of oil-based petroleum fuels. Therefore, it is necessary to fully implement studies on this development with the goal of making microalgae biotechnology competitive and commercially attractive in the future.

Finally, it should be considered that microalgae are the only biomass source in nature that offer a high application potential, since they have a rich and versatile chemical composition, and thus could become an effective feedstock to produce many high-value-added products.

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