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# PRODUÇÃO DE COMPOSTOS ORGÂNICOS VOLÁTEIS POR *SCENEDESMUS OBLIQUUS* EM FOTOBIORREATORES

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

Orientador: Prof. Dr. Eduardo Jacob Lopes

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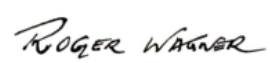

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Santa Maria, RS
2019

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## RESUMO

## PRODUÇÃO DE COMPOSTOS ORGÂNICOS VOLÁTEIS POR *SCENEDESMUS OBLIQUUS* EM FOTOBIORREATORES

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ORIENTADOR: Eduardo Jacob Lopes

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 voláteis. Tanto em ambientes naturais quanto artificiais, as microalgas podem ser expostas a uma variedade de condições ambientais e disponibilidade de nutrientes que afetam tanto a taxa de crescimento como a sua composição celular. Em face disto, este trabalho teve como objetivo produzir compostos orgânicos voláteis por S*cenedesmus obliquus* em fotobiorreatores. Na primeira etapa foi avaliado o efeito de diferentes climas e estações do ano de regiões extremas do Brasil sobre a produção de compostos orgânicos voláteis. Um total combinado de 47 compostos foram identificados por *Scenedesmus obliquus* em todas as amostras estudadas e destes, 6 voláteis foram comuns em todos os experimentos. Os principais grupos voláteis encontrados foram hidrocarbonetos, aldeídos, cetonas e álcoois, sendo o heptadecano o principal composto volátil formado. No segundo trabalho foi avaliado a influência dos ciclos claro e escuro na produção de compostos orgânicos voláteis. Nesse estudo0 37 compostos foram identificados, com os grupos álcoois, aldeídos, cetonas e terpenos variando ao longo do tempo. Os resultados mostram que os principais compostos voláteis encontrados para o ciclo claro foram os compostos 3-Metil butanol e 6-Metil-5-hepten-2-ona e para o ciclo escuro, os compostos mais relevantes foram os álcoois 2-Etil hexanol e 1-Pentanol. Os resultados obtidos colaboraram tanto para elucidação metabólica destes compostos, como a identificação de uma fração de biocompostos microalgais com grande potencial de exploração comercial.

**Palavras-Chave:** compostos orgânicos voláteis, microalgas, climas e estações, ciclos claro/escuro.

## ABSTRACT

### PRODUCTION OF VOLATILE ORGANIC COMPOUNDS BY *SCENEDESMUS OBLIQUUS* IN PHOTOBIOREACTORS

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Microalgae are a potential source of biomolecules of commercial interest due to their diversified metabolic profile capable of synthesizing different classes of volatile organic compounds. In both natural and artificial environments, microalgae can be exposed to a variety of environmental conditions and nutrient availability that affect both the growth rate and their cell composition. In the face of this, this work had as objective to produce volatile organic compounds by *Scenedesmus obliquus* in photobioreactors. In the first stage, we evaluated the effect of different climates and seasons of the year in extreme regions of Brazil on the production of volatile organic compounds. A combined total of 47 compounds was identified by *Scenedesmus obliquus* in all samples studied, and of these, 6 volatiles were common in all experiments. The main volatile groups found were hydrocarbons, aldehydes, ketones, and alcohols, with heptadecane being the main volatile compound formed. In the second work, the influence of light and dark cycles on the production of volatile organic compounds was evaluated. A combined total of 37 compounds were identified, with the alcohols, aldehydes, ketones and terpenes groups varying over time. The results show that the main volatile compounds found for the light cycle were 3-Methyl butanol and 6-Methyl-5-hepten-2-one compounds and for the dark cycle, the most relevant compounds were 2-Ethylhexanol alcohols and 1-Pentanol. The results obtained contribute to both the metabolic elucidation of these compounds and to the identification a fraction of biocompounds microalgal with great potential for commercial exploitation.


**Keywords:** volatile organic compounds, microalgae, climates and seasons, light/dark cycles.

## SUMÁRIO

# INTRODUÇÃO

O potencial comercial e biotecnológico das microalgas representa um recurso quase que inexplorado, uma vez que das possíveis espécies existentes, relativamente poucas foram estudadas em detalhe, do ponto de vista bioquímico e fisiológico. Esta grande biodiversidade, que resulta na variabilidade da composição química e das características metabólicas, aliadas em determinados casos ao melhoramento genético e desenvolvimento de novas tecnologias de cultivo, faz destes microrganismos um importante campo de pesquisa o qual vem permitindo o uso das microalgas em diversos segmentos.

Tanto em ambientes naturais quanto artificiais, as microalgas podem ser expostas a uma variedade de condições ambientais e disponibilidade de nutrientes que afetam a sua taxa de crescimento e sua composição celular (JUNEJA et al., 2013). Essas condições influenciam a composição da célula em termos de conteúdo de proteínas, carboidratos, lipídios, aminoácidos, pigmentos, entre outros compostos (SHARMA et al., 2012; VIDOTTI, 2015).

Algumas espécies de microalgas podem alterar até mesmo o tipo de metabolismo em resposta às mudanças nas condições ambientais (MENINA et al., 2015). Metabolicamente, as microalgas apresentam sistema biológico bastante eficiente na transformação da energia luminosa em compostos orgânicos, através da fotossíntese. É sabido que o metabolismo fotossintético é constituído de duas fases, a fase clara e a fase escura. Na primeira fase, a energia luminosa é convertida em energia química, que é armazenada em compostos de alto valor energético. Na fase escura, a energia química é utilizada para a fixação do $CO_2$ e paralela formação de carboidratos (WILLIANS & LAUREN, 2010). Essas características metabólicas que os microrganismos fotossintéticos possuem tornam possível a elucidação de diferentes condições ambientais e o uso de ciclos claro e escuro em bioprocessos baseados em microalgas (JACOB-LOPES et al., 2009; VIDOTTI, 2015).

As microalgas produzem muitos metabólitos com estruturas diversificadas que desempenham papéis importantes no seu desenvolvimento. Embora os metabólitos primários sejam indispensáveis para o crescimento de microrganismos, os metabólitos secundários não são essenciais, mas são cruciais para que as microalgas sobrevivam em condições adversas,

mantendo um delicado equilíbrio com o meio ambiente (DUDAREVA et al., 2013, YANG et al., 2018).

Dependendo da espécie, cultura e condições ambientais, as microalgas são capazes de produzir uma ampla variedade de compostos orgânicos voláteis. A exploração das alterações metabólicas dinâmicas que ocorrem durante o crescimento e desenvolvimento de microalgas podem fornecer uma nova visão sobre os mecanismos de compostos extracelulares e, consequentemente, a produção de compostos orgânicos voláteis em nível metabólico (HOSOGLU, 2018; LIANG et al., 2018).

Devido ao fato de a composição bioquímica da biomassa de microalgas estar diretamente relacionada às condições externas aplicadas, seu crescimento e produtividade dependem de vários fatores, incluindo a disponibilidade de nutrientes do meio, temperatura, intensidade luminosa, fotoperíodo e tipo de cultivo (MATOS et al., 2017).

Assim, baseado em que a elucidação de rotas de formação dos compostos orgânicos voláteis é de fundamental importância para o aprimoramento da biotecnologia microalgal, o presente trabalho fundamenta-se em um estudo exploratório sobre o efeito de diferentes climas e estações do ano de regiões extremas do Brasil e a influência dos ciclos claro e escuro sobre a produção de compostos orgânicos voláteis pela microalga *Scenedesmus obliquus*.

# OBJETIVOS

## Objetivo geral

Avaliar a produção compostos orgânicos voláteis da microalga *Scenedesmus obliquus* em fotobiorreatores.

## Objetivos específicos

- Identificar os compostos orgânicos voláteis da biomassa de *Scenedesmus obliquus*;

- Avaliar o efeito de diferentes climas e estações do ano de regiões extremas do Brasil na produção de compostos orgânicos voláteis;

- Avaliar a influência dos ciclos claro e escuro na produção de compostos orgânicos voláteis;

- Estabelecer possíveis rotas de biossíntese desses compostos.

# CAPÍTULO 1

# REVISÃO BIBLIOGRÁFICA

## 1. Microalgas

O termo alga é completamente desprovido de valor taxonômico e refere-se a uma assembleia polifilética artificial de organismos (APT & BEHRENS, 1999). São constituídas por células eucarióticas e procarióticas, contendo núcleos e organelas (BENEMANN et al., 1978; KHAN et al., 2018). Envolve seres unicelulares e multicelulares, com hábitos planctônicos e bentônicos. A partir dessas características derivam os termos de uso consagrado, tais como microalgas, que define seres microscópicos diversos presentes em sistemas aquáticos, em sua maioria com hábitos planctônicos (LOURENÇO, 2006).

As microalgas são microrganismos fotossintéticos que crescem em uma ampla gama de habitats aquáticos, incluindo lagos, lagoas, rios, oceanos e até mesmo em águas residuais (KHAN et al., 2018). Ao contrário das plantas superiores, as microalgas não possuem raízes, hastes e folhas (SUGANYA et al., 2016). Compreende um diversificado grupo de microrganismos com cerca de 72.500 espécies catalogadas de forma consistente, porém as estimativas sugerem que o número de espécies não descritas varia de centenas de milhares a milhões de espécies espalhadas pela biosfera (MOBIN & ALAM, 2017; JACOB-LOPES et al., 2019).

As microalgas emergiram como um potencial recurso de biomassa sustentável devido à sua neutralidade em relação ao cultivo natural e ao meio sintético (KAREMORE et al., 2013; CHEN et al., 2015). São essenciais para a vida na terra, capazes de converter a energia solar em formas químicas através da fotossíntese e apresentam maior taxa de crescimento do que as plantas terrestres (NDIMBA et al., 2013; MILANO et al., 2016). Além disso, elas podem viver em um ambiente diverso com requisitos simples de nutrientes, crescem extremamente rápido, ao longo do ano e podem ser cultivadas de forma contínua (CHEN et al., 2011; BILAD et al., 2014; MILANO et al., 2016).

Na taxonomia atual, os padrões incluem 16 classes desses organismos. Entre essas classes, as mais abundantes são as diatomáceas (*Bacillariophyceae*), as algas verdes (*Chlorophyceae*) e as algas douradas (*Chrysophyceae*). No entanto, as cianobactérias (*Cyanophyceae*), as diatomáceas (*Ochrophyta*) e as algas verdes (*Chlorophyta*) são as mais significativas em termos de exploração e uso biotecnológico (BOROWITZKA, 2018).

As cianobactérias são classificadas no reino das eubactérias, são frequentemente unicelulares, enquanto algumas espécies formam filamentos ou agregados. A organização interna de uma célula cianobacteriana é procariótica, com uma região central (nucleoplasma) rica em DNA e uma região periférica (cromoplasto) contendo membranas fotossintéticas.

Apresentam em sua estrutura a clorofila a e os fotossistemas I e II, ao contrário de outras bactérias fotossintetizantes, o que as permite realizar a fotossíntese na presença de oxigênio (MASOJÍDEK et al., 2013; WIJFFELS et al., 2013). Já as diatomáceas, apresentam em sua estrutura uma frustula silicosa, clorofila a e c, os plastídios contém fucoxantina e outras xantofilas. De um modo geral, são desprovidas de flagelos e quase sempre autotróficas, mas podem ser encontradas espécies heterotróficas incolores ou simbiontes (SINGH & SAXENA, 2015).

Por outro lado, as algas verdes ou clorofíceas, apresentam grande variedade nos níveis de organização, desde unicelulares, microalgas flageladas ou não, até talos morfologicamente complexos. Seu aparelho fotossintético é organizado em organelas especiais, os cloroplastos, que contêm alternância camadas de membranas lipoproteicas (tilacóides) e fases aquosas, o estroma (MASOJÍDEK et al., 2013). Assim como as cianobactérias, as clorofíceas podem ser encontradas em quase todos os ambientes, contudo, cerca de 90% do total de espécies ocorrem em água doce (RIVIERS, 2006). As espécies de microalgas do gênero *Scenedesmus*, pertencentes a esta classe, são microalgas bastante comuns e, devido a sua robustez são relativamente fáceis de cultivar. A *Scenedesmus obliquus*, em particular, é caracterizada pelas altas taxas de crescimento e elevado teor lipídico (RIVIERS, 2006; PATIAS et al., 2017).

Microalgas também podem ser classificadas quanto ao fornecimento de carbono e são muito versáteis em seus modos de nutrição. A maioria das algas são fotoautotróficas, isto é, usam apenas energia da luz, $CO_2$ e água para produzir compostos orgânicos através da fotossíntese. Várias microalgas também podem crescer no escuro usando moléculas orgânicas simples, como acetato ou glicose, como fonte de carbono e energia, este modo de nutrição é chamado heterotrofia ou quimiorganotrofia. Algumas microalgas são também conhecidas como mixotróficas, ou seja, elas têm a capacidade de crescer usando uma combinação de processos metabólicos que são necessários para a autotrofia e para a heterotrofia, realizando, por exemplo, a fotossíntese com aquisição simultânea de nutrientes orgânicos exógenos (BOROWITZKA, 2018).

A fotossíntese é o principal processo autotrófico e é realizada pelos seres clorofilados, caracterizando-se por um mecanismo de duas etapas: reação fotoquímica, em que é indispensável à presença de luz para que aconteçam as reações, e a fase escura ou reação de fixação de carbono, em que as reações independem da ação luminosa, além disso, os sistemas de cultivo de microalgas em grande escala (tais como lagoas abertas) são geralmente operados sob essa condição de cultivo (JACOB-LOPES et al., 2009, WILLIANS & LAUREN, 2010; MOHAN et al., 2015; SIQUEIRA et al., 2018).

Na fase fotoquímica, as moléculas de clorofila a são excitadas pela energia luminosa quando esta é absorvida por estruturas altamente organizadas dos pigmentos fotossintéticos e por transportadores de elétrons, chamados fotossistemas. Seus elétrons são então transferidos para uma molécula aceptora de elétrons que fluem através de uma série de transportadores ligados à membrana, a fim de gerar um potencial eletroquímico. A energia resultante dessas reações é guardada na forma de NADPH (nicotinamida adenina dinucleotídeo fosfato) e em moléculas de ATP (adenosina trifosfato) no processo de fosforilação. A última fonte de elétrons para a fotossíntese é a $H_2O$, o qual cede no processo de fotólise (reação de Hill), átomos de hidrogênio, elétrons e libera $O_2$, produto da fotossíntese das microalgas e das plantas verdes (FAY, 1983; ZHAO & SU, 2014; MARONEZE, 2016).

Microalgas contêm em seus cloroplastos uma maquinaria enzimática singular, que catalisa a conversão de $CO_2$ em compostos orgânicos simples (reduzidos), um processo denominado fixação de carbono. Nesse processo o $CO_2$ é incorporado (fixado) em um composto orgânico de três carbonos, a triose-fosfato-3-fosfoglicerato. Esse produto simples da fotossíntese é o precursor de biomoléculas mais complexas, como açucares, polissacarídeos e os metabólitos derivados deles, onde o $CO_2$ é assimilado por uma via cíclica e comumente chamada de ciclo de Calvin-Benson. O ciclo inicia-se com a incorporação do $CO_2$ na ribulose 1,5-bisfosfato (Ru5BP), que é catalisada pela enzima ribulose 1,5-difosfato carboxilase/oxigenase (rubisco), com o $CO_2$. O produto da reação é quebrado em duas moléculas de três carbonos, ácido fosfoglicérico (PGA), e a redução do PGA na presença de ATP e NADPH. A maior parte do PGA reduzido é utilizado para regenerar Ru5BP, de modo que o ciclo possa ser fechado. Uma das seis moléculas de Ru5BP não é reciclada e, então se condensa para formar açucares fosforados intermediários e, posteriormente glicose (CALVIN & BENSON, 1948; WILLIANS & LAUREN, 2010; NELSON & COX, 2014).

Nos organismos vivos, várias reações químicas e enzimáticas ocorrem no metabolismo para manter sua estrutura. Essas reações produzem compostos que se referem a metabólitos primários e secundários. A distinção entre metabólitos primários e secundários é que os primeiros sendo constitutivos, são produzidos continuamente e são essenciais para manter a vida. Metabólitos secundários, por outro lado, podem ser derivados de metabólitos primários e não estão diretamente envolvidos no crescimento, desenvolvimento e reprodução (ACHYUTHAN et al., 2017). Enquanto o metabolismo primário se refere aos processos de síntese de moléculas essenciais a células microalgais (como proteínas, carboidratos e lipídios), o metabolismo secundário são as moléculas a serem dispensadas para o metabolismo e crescimento das microalgas, e sua grande variedade e alta diversidade de produtos secundários

são componentes-chave para adaptação e permite que o organismo sobreviva em condições ambientais adversas (DUDAREVA et al., 2013; YANG et al., 2018).

Metabólitos secundários são geralmente classificados de acordo com sua estrutura química e vários grandes grupos de moléculas fazem parte desta classificação, incluindo os ácidos fenólicos, flavonóides, esteróis, alcaloides e os compostos orgânicos voláteis (HARBORNE, 1999; YANG et al., 2018). A ampla gama desses compostos orgânicos voláteis são originários das classes de terpenóides, fenilpropanóides/benzenoides, derivados de ácidos graxos e aminoácidos (SANTOS et al., 2016). Ainda, as composições químicas das misturas de voláteis emitidos pelas microalgas e as suas intensidades podem traduzir o status fisiológico em que elas se encontram ou até mesmo o estresse a que elas estão sendo submetidas.

## 2. Processos baseados em microalgas

As microalgas apresentam grande potencial para produzir uma ampla gama de produtos tecnológicos em uma vasta variedade de aplicações. As microalgas têm um grande potencial biotecnológico em produzir substâncias valiosas para as indústrias de rações, alimentos, nutracêuticos e farmacêuticos. Além disso, outras aplicações podem ser atribuídas ao processo fotossintético realizado por esses microrganismos, como mitigação de $CO_2$, tratamento de águas residuais e produção de biocombustíveis (CHISTI, 2007; ACIÉN et al., 2017).

O interesse na microalga como fonte renovável e sustentável para a produção de biocombustíveis inspirou um novo foco em biorrefinaria. Biorrefinaria é um processo totalmente integrativo e multifuncional que utiliza matéria-prima para gerar um espectro de diferentes produtos em um caminho sustentável. Melhoria de técnicas de crescimento e engenharia genética podem ser usadas para melhorar o seu potencial como futura fonte de recursos e bioprodutos renováveis (KHAN et al., 2018).

Microalgas são ricas em lipídeos, proteínas, carboidratos e muitos outros compostos de elevado valor agregado. Os lipídios extraídos das células de microalgas podem ser convertidos em biodiesel, enquanto os carboidratos, tal como amido, podem ser convertidos em bioetanol a partir de processos fermentativos. Além dessas rotas, os carboidratos, proteínas e lipídios podem ser convertidos em metano e bio-hidrogênio através do processo de digestão anaeróbia (ULLAH et al., 2015).

Esses biocombustíveis derivados de microalgas são alternativas promissoras como biocombustíveis de terceira geração, devido a características únicas inerentes às microalgas, como a proliferação rápida, alto acúmulo de óleo, baixas taxas de consumo de água e

viabilidade de crescer em terras não aráveis (CHISTI, 2007; KHAN et al., 2018). São vistos como um caminho próspero para os combustíveis fósseis, podendo fornecer até 25% da energia necessária global (SMITH et al., 2010; CHRISTENSON & SIMS, 2011; RAWAT et al., 2011; BARROS et al., 2015).

Além disso, as microalgas apresentam potencial para serem usadas como biocatalisadoras em um bioprocesso integrado (CHARPENTIER, 2005). A ciclagem de nutrientes por microalgas surge como uma promissora tecnologia porque equilibra vetores sustentáveis por poluentes reutilizados, como carbono, nitrogênio e fósforo, presente nas águas residuais geradas pela indústria. Juntos, tratamento de águas residuais e a valiosa produção de biomassa de algas aumenta a sustentabilidade ambiental e econômica benéfica deste processo (GONÇALVES et al., 2017; SANTOS et al., 2018). Segundo Brennan & Owende (2010), a combinação desses processos será a aplicação comercial mais concebível a curto prazo; e é provavelmente uma das formas mais sustentáveis de produzir bioenergia e bioprodutos.

Para a produção de microalgas sob condições fototróficas, é necessário utilizar fotobiorreatores que devem ser adequadamente projetados, construídos e operados para satisfazer as exigências das microalgas. Vários projetos e configurações de fotobiorreatores foram propostos, mas ainda não existe um design ideal. Para qualquer aplicação, o fotobiorreator a ser utilizado deve ser adequadamente selecionado de acordo com os requisitos do processo. Portanto o estabelecimento dos requisitos do sistema biológico a ser utilizado é necessário para projetar adequadamente o fotobiorreator ideal, que constitui o ponto de partida ao projetar um processo baseado em microalgas (ACIÉN et al., 2017).

Além dos fotobiorreatores, os sistemas abertos têm sido amplamente utilizados para o cultivo em larga escala, devido à simplicidade e baixo custo. Embora de construção simples, estes sistemas permitem apenas um controle limitado das condições de operação. Além disso, a produtividade é baixa devido à baixa absorção de luz no fundo do tanque e pela maior probabilidade de contaminação. Outras limitações desse tipo de cultivo incluem a grande necessidade de espaço de terra para o cultivo, perdas por evaporação, alta temperatura e, consequente baixa eficiência de transferência de massa (VASUMATHI et al., 2012).

Seja em tanques abertos ou em fotobiorreatores fechados, uma cultura de microalgas exige a consideração de alguns fatores. Os relatórios sobre o uso de sistemas baseados em microalgas são principalmente focados na influência das condições operacionais, como o fornecimento de luz e nutrientes, a manutenção das condições de cultura adequadas e a mistura para evitar gradientes destes parâmetros que reduz o rendimento do sistema biológico (TREDICI & ZITTELLI, 1997; ACIÉN FERNÁNDEZ et al., 2013).

A intensidade da luz é um dos principais fatores limitantes no cultivo de microalgas. Duração e intensidade de luz afetam diretamente a fotossíntese de microalgas e tem influência sobre a composição bioquímica de microalgas e rendimento de biomassa (PIASECKA et al., 2014). Fenômenos de fotolimitação e fotoinibição são frequentes em culturas iluminadas inadequadamente, ocasionando significativas perdas de desempenho cinético nos biorreatores. Além dos aspectos quantitativos, deve-se considerar a natureza qualitativa da luz incidente nos sistemas. Iluminação natural ou artificial pode ser utilizada em função das características requeridas nos sistemas de cultivo. Aspectos como localidade, variações sazonais, e ocorrência de fotoperíodos de claro/escuro são as principais questões dos sistemas naturalmente iluminados (JACOB-LOPES et al. 2009). Em sistemas iluminados artificialmente, a fonte de energia luminosa e o custo da energia são os principais elementos a serem definidos na implementação dos sistemas de iluminação. Alternativamente, os cultivos heterotróficos eliminam integralmente todos os aspectos relacionados à energia luminosa (PEREZ-GARCIA et al. 2011; QUEIROZ et al., 2018).

A temperatura é outro fator importante no crescimento de microalgas e influencia diretamente os processos bioquímicos, incluindo a produção de células através da fotossíntese. Cada espécie tem sua própria temperatura ótima de crescimento. Em geral a ótima temperatura de cultivo ocorre na região mesófila (25 a 35 °C), embora algumas linhagens termófilas resistam a temperaturas na faixa dos 60 °C. Aumentar a temperatura para o alcance ideal exponencialmente aumenta o crescimento de algas, mas um aumento ou diminuição na temperatura além do ponto ideal retarda ou até impede o crescimento e a atividade das algas (BECHET et al., 2017).

A composição da biomassa microalgal é predominante composta por carbono, nitrogênio e fósforo, em proporções que variam entre 50, 8 e 1% do seu peso seco, respectivamente, e em quantidades menores são encontrados outros elementos como enxofre, potássio, magnésio, ferro e cálcio. De acordo com esta composição de biomassa o meio de cultura deve conter esses nutrientes para permitir o máximo desempenho das culturas. O excesso de nutrientes deve ser definido de acordo com critérios econômicos e de sustentabilidade, porque os nutrientes em excesso geralmente são liberados e perdidos do sistema se o meio de cultivo não for recirculado e ainda, sob condições limitantes, poderá ocorrer redução na produtividade das células microalgais (ACIÉN et al., 2017).

Finalmente, agitação é uma operação necessária no cultivo de microrganismos fotossintéticos, uma vez que assegura a uniformidade espacial dos vasos de reação, favorecendo a exposição das células a luz, a transferência de calor e estratificação térmica, além de melhorar

a troca de gases. Uma mistura adequada minimiza ainda a formação de agregados celulares que aumentam a ineficiência global do biorreator. Embora fundamental para o adequado desenvolvimento do processo, a operação de mistura está relacionada a estresses hidrodinâmicos associados ao cisalhamento celular, que danifica e inibe o crescimento microalgal. Os biorreatores microalgais são normalmente equipados com sistemas de aeração pneumática e agitação mecânica, ou ainda uma combinação entre estes sistemas (BOROWITZKA, 1999; QUEIROZ et al., 2018).

## 3. Bioprodutos microalgais

Os biocatalisadores microbianos têm sido empregados há muito tempo para uma série de novos produtos. À medida que a escassez global de energia e de alimentos aumenta com o respectivo crescimento populacional a atual biotecnologia industrial sofre impactos e demonstra a necessidade de novas propostas sustentáveis. As microalgas possuem características únicas em comparação com biocatalisantes microbianos convencionais, com a capacidade fotossintética e espécies maciças e a diversidade de bioprodutos (GUARNIERI & PIENKOS, 2014). Embora a aquicultura de algas tenha sido utilizada há décadas para produzir nutracêuticos e produtos alimentares de alto valor, os recentes avanços na biologia de sistemas estão desbloqueando a diversidade na biocatálise de algas, revelando uma série de novas capacidades metabólicas e potenciais bioprodutos (GULDHE et al., 2017).

A exploração comercial em larga escala do conteúdo intracelular microalgal teve início na década de 1950, motivada pelo elevado teor de proteínas da biomassa, para utilização como recurso alimentar alternativo (SPOLAORE et al., 2006). Desde então, abriu-se um grande leque de produtos passíveis de serem explorados. Hoje, as microalgas para consumo humano são comercializadas em diferentes formas, tais como comprimidos, cápsulas ou líquidos.

As biorrefinarias de microalgas desenvolveram o progresso da transformação da biomassa na produção em larga escala de alimentos e compostos de alto valor agregado devido a sua capacidade de acumular grandes quantidades de carotenoides favoráveis à saúde como o ß-caroteno e a astaxantina, vitaminas e ácidos graxos poliinsaturados. (GUARNIERI & PIENKOS, 2014; WANG et al., 2015; GULDHE et al., 2017).

Em relação ao uso na alimentação animal, são consideradas importantes fontes de proteína com respostas zootécnicas relacionadas a ganho de peso, melhora na resposta imunológica e melhora na fertilidade (PULZ & GROSS, 2004). Adicionalmente, a biomassa

microalgal é também utilizada para refinar os produtos de aquicultura, como por exemplo a coloração característica de salmonídeos (CHISTI, 2018).

Outro aspecto de relevância na exploração destes bioprodutos está associado aos pigmentos. Além da clorofila a, as microalgas contêm pigmentos auxiliares como as ficobiliproteínas e os carotenoides (WANG & PENG, 2008). Extratos de pigmentos microalgais incluem ß-caroteno, astaxantina e ficocianina que possuem uma vasta gama de aplicações em indústrias de alimentos e farmacêuticas (VISKARI & COLYER, 2003; MATOS et al., 2017).

Por outro lado, segundo Pereira et al. (2012) a biomassa de microalgas apresenta três componentes principais: carboidratos, lipídeos e proteínas. O interesse pela fração lipídica destes organismos soma-se a fração protéica, em virtude principalmente, da qualidade dos ácidos graxos presentes. Neste sentido, os óleos unicelulares tem sido alvo de extensivos esforços em pesquisa e desenvolvimento, para tornar viável comercialmente a produção de ácidos graxos poliinsaturados como o ácido eicosapentaenóico, docoeicosapentaenóico, linoléico e linolênico (RATLEDGE & COHEN, 2008). Dentro deste grupo, para a maioria das espécies, os ácidos graxos poliinsaturados, das famílias ω3 e ω6, correspondem a maior fração, podendo chegar a 60% dos lipídios totais (PEREZ-GARCIA et al., 2011). Já os carboidratos à base de microalgas consistem principalmente em celulose e amido sem lignina, tornando-os úteis como fontes de açucar ou transformados em bioetanol ou em metano (ZHU, 2014; CHEW et al., 2017). Além do biodiesel, bioetanol e biogás, as microalgas também podem ser usadas para produzir biohidrogênio, biobutanol, gás de síntese, combustível para jatos e bio-óleo através de processos de conversão termoquímica, química e bioquímica (ZHU et al., 2016).

As microalgas também podem ser usadas para obtenção de moléculas bioativas, porque podem ser cultivadas em alta escala produzindo em escala industrial (MOLINA-GRIMA et al, 2003; CHU, 2012). Os compostos bioativos são geralmente metabólitos secundários, que incluem várias substâncias como ácidos orgânicos, aminoácidos, vitaminas, antibióticos, enzimas e até compostos tóxicos. No entanto, tem-se observado uma tendência emergente no sentido do conhecimento de produção de compostos de baixo peso molecular a partir de fontes renováveis (WINTERS et al, 1969; SCHIRMER et al, 2010; MATOS et al., 2017).

Dependendo da espécie, cultura e condições ambientais, as microalgas são capazes de produzir uma ampla variedade de compostos orgânicos voláteis. A exploração das modificações metabólicas dinâmicas que ocorrem durante o crescimento e desenvolvimento de microalgas podem fornecer uma nova visão sobre os mecanismos de compostos extracelular e, consequentemente, a produção de compostos orgânicos voláteis a nível metabólico (HOSOGLU, 2018; LIANG et al, 2018).

Compostos orgânicos voláteis (COVs) 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 (ZUO et al, 2012; ACHYUTHAN et al., 2017). A caracterização da fração volátil dos biorreatores pode contribuir para o estabelecimento das rotas de bioconversão dos substratos, além de possibilitar a identificação de aplicações potenciais dos bioprodutos formados (JACOB-LOPES et al, 2010).

Segundo Nuccio et al. (1995), as taxas de produção de COVs 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. Porém, a biossíntese destes compostos depende da disponibilidade de carbono, nitrogênio, e enxofre, bem como a energia fornecida pelo metabolismo primário. Portanto, 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; SANTOS et al., 2016).

Finalmente, a versatilidade das microalgas em se adaptar em uma ampla gama de condições climáticas e produzir produtos de alto valor agregado é um parâmetro promissor para que estes microrganismos sejam usados como um recurso viável de bioprodutos. Com o crescente número de tecnologias de conversão em desenvolvimento, a biomassa de microalgas fornece uma ótima plataforma para fabricação e desenvolvimento de novos produtos, devido à concorrência mínima em terras com culturas aráveis e aos impactos ambientais reduzidos (BHARATHIRAJA et al., 2015; GONG & YOU, 2016).

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# CAPÍTULO 2

# SECONDARY METABOLITES IN MICROALGAE: EFFECT OF DIFFERENT CLIMATES AND SEASONS ON THE PRODUCTION OF VOLATILE ORGANIC COMPOUNDS

# Secondary metabolites in microalgae: effect of different climates and seasons on the production of volatile organic compounds

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**Abstract:** Microalgae produce many metabolites with diversified structures that play important roles in their development. Although primary metabolites are indispensable for the growth of microorganisms, secondary metabolites are not essential but are crucial for microalgae to survive in adverse conditions while maintaining a delicate balance with the environment. In this sense, the objective of this work was to evaluate the effects of different climates and seasons about the production of volatile organic compounds by *Scenedesmus obliquus*. The study focused on the extreme geographic locations of Brazil and evaluated parameters such as average temperature, maximum light intensity and light hours per day of the equatorial, tropical, tropical Atlantic, and subtropical climates referent in the cities of Rio Branco (AC), Boa Vista (RR), João Pessoa (PB) and Rio Grande (RS), respectively. Total of 47 compounds was identified by *Scenedesmus obliquus* in all samples studied, and of these, 4 volatiles were common in all experiments. The main volatile groups found were hydrocarbons, aldehydes, ketones, and alcohols, with heptadecane being the main volatile compound formed. Many of the compounds detected during the cultures were originated and derived from the terpenoid pathways, fatty acids, and amino acids derivates.

## 1. Introduction

Microalgae are a potential source of biomolecules of commercial interest due to its diversified metabolic profile capable of synthesizing different classes of organic compounds (MATOS et al., 2017). Because they are photosynthetic microorganisms, microalgae produce energy through light and use atmospheric $CO_2$, which accumulate in the cell, as an inorganic carbon reservoir for photosynthesis (BADGER & PRICE, 2003; JACOB-LOPES et al., 2010).

Similar to plants, microalgae attempt to find a balance between energy from light reactions in chloroplasts and the energy used for metabolic processes and carbon fixation

(GEIDER et al., 1996). Perturbations in different environmental conditions (for example, light and temperature) can disturb the balance in chloroplasts and force primary producers to adjust or acclimate physiologically, through the dissipation of the photon flux by the accessory pigments of the photosynthetic reactions (HUNER et al, 1998; MACINTYRE et al., 2002; MESKHIDZE et al., 2014).

In living organisms, various chemical and enzymatic reactions occur in metabolism to maintain its structure. These reactions produce compounds that refer to primary and secondary metabolites necessary for the growth and development of cells or allow the organism to survive under adverse environmental conditions (DUDAREVA et al., 2013, YANG et al., 2018). These metabolites can be described as the end products of gene expression and define the phenotype of a cell under defined physiological conditions at a specific biochemical level (TUGIZIMANA et al., 2018).

Secondary metabolites are biosynthesized through intermediates of primary metabolism and often produced by ecological interactions (YANG et al., 2018). Under conditions, of stress or specific environmental conditions the expression of responsive genes is activated, and subsequently, metabolites with variable profiles are biosynthesized to adapt to different environmental conditions (BARSANTI & GUALTIERI, 2018; LIANG et al., 2018). For most enzymes associated with secondary metabolism, their structures can be encoded in the nuclear genome, but some of them are directed to the chloroplast to perform their function (HEYDARIZADEH et al., 2013; GIMPEL et al., 2015).

Several genetic, ontogenic, morphogenetic and environmental factors can influence the biosynthesis and accumulation of secondary metabolites in microalgae (YANG et al., 2018). Depending on the species, culture and environmental conditions, microalgae are capable of producing a wide variety of volatile organic compounds (VOCs). The exploration of the dynamic metabolic changes that occur during the growth and development of microalgae can provide new insight into the mechanisms of extracellular compounds and, consequently, the production of volatile organic compounds at metabolic level (HOSOGLU, 2018; LIANG et al., 2018).

Due to the fact that the biochemical composition of the microalgal biomass is directly related to the applied external conditions, its growth and productivity depend on several factors, including the nutrient availability of the medium, temperature, light intensity, photoperiod and the type of cultivation (MATOS et al., 2017).

To demonstrate the potential of microalgae as a source of high-value metabolites, it is very important to assess their potential productivity in a given geographic location and the local

climatic factors that determine it (BANERJEE & RAMASWAMY, 2017; JESUS et al., 2018). In this sense, the objective of this study was to evaluate the effect of different climates and seasons of extreme regions of Brazil on the production of volatile organic compounds from the *Scenedesmus obliquus* microalgae.

## 2. Material and methods

### 2.1 Microorganism and culture media

Axenic cultures of *Scenedesmus obliquus* CPCC05 were obtained from the Canadian Phycological Culture Centre. Stock cultures were propagated and maintained in synthetic BG-11 medium (RIPPKA et al., 1979) with the following composition ($mg^{-1}$ L): $K_2HPO_4$ (3.0), $MgSO_4$ (75.0), $CaCl_2 \cdot 2H_2O$ (36.0), ammonium citrate and iron (0.6), $Na_2EDTA$ (1.0), NaCl (0.72), $NaNO_3$ (150.0), citric acid (0.6), $Na_2CO_3$ (15.0), $H_3BO_3$ (2.8), $MnCl_2.4H_2O$ (1.8), $ZnSO_4 \cdot 7H_2O$ (0.22), $Na_2MoO_4 \cdot 2H_2O$ (0.39), $CoSO_4 \cdot 6H_2O$ (0.04). The incubation conditions used were 30 ºC, photon flux density of 30 µmol.$m^{-2}$ $s^{-1}$ and a photoperiod of 12 h.

### 2.2. Photobioreactor design

Measurements were made in a bubble column photobioreactor (MARONEZE et al., 2016). The system was built in 4 mm thick glass with an internal diameter of 9.0 cm, a height of 40 cm and a nominal working volume of 2.0 L. The dispersion system for the reactor consisted of a 1.5 cm diameter air diffuser located in the center of the column. The reactor was illuminated with forty-five 0.23W LED lamps (total consumption of 0.01125 kW h), located in a photoperiod chamber. The $CO_2$/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the airstream, through three rotameters that measured the flow rates of carbon dioxide, air and the mixture of gases.

### 2.3 Experimental conditions

The experiments were carried out in bioreactors operating in batch mode, fed with 2.0 L synthetic BG-11 medium. The luminous intensity was determined using a quantum sensor (Apogee Instruments, Logan, USA), measuring the light incident on the external reactor surface. The temperature was controlled by using thermostats. The flow rates of carbon dioxide, air, and $CO_2$ enriched air were determined with rotameters (AFSG 100 Key Instruments,

Trevose, USA). Experimental conditions were as follows: initial cell concentration of 100 mg/L, constant aeration of 1 VVM (volume of air per volume of culture per minute) with an injection of air enriched with 15% carbon dioxide and pH adjusted to 7.6. All experiments were conducted until the declining phase and the tests were carried out in duplicate.

## 2.4 Climate simulation

Climatic conditions related to the average temperature, light hours per day and maximum light intensity of the different geographical locations of Brazil were evaluated to investigate the effect on the formation of volatile organic compounds by *Scenedesmus obliquus*. The study focused on the climates of the extreme regions of Brazil, as can be observed in Figure 1. Still, the climatic parameters used in the study are presented in Table 1.

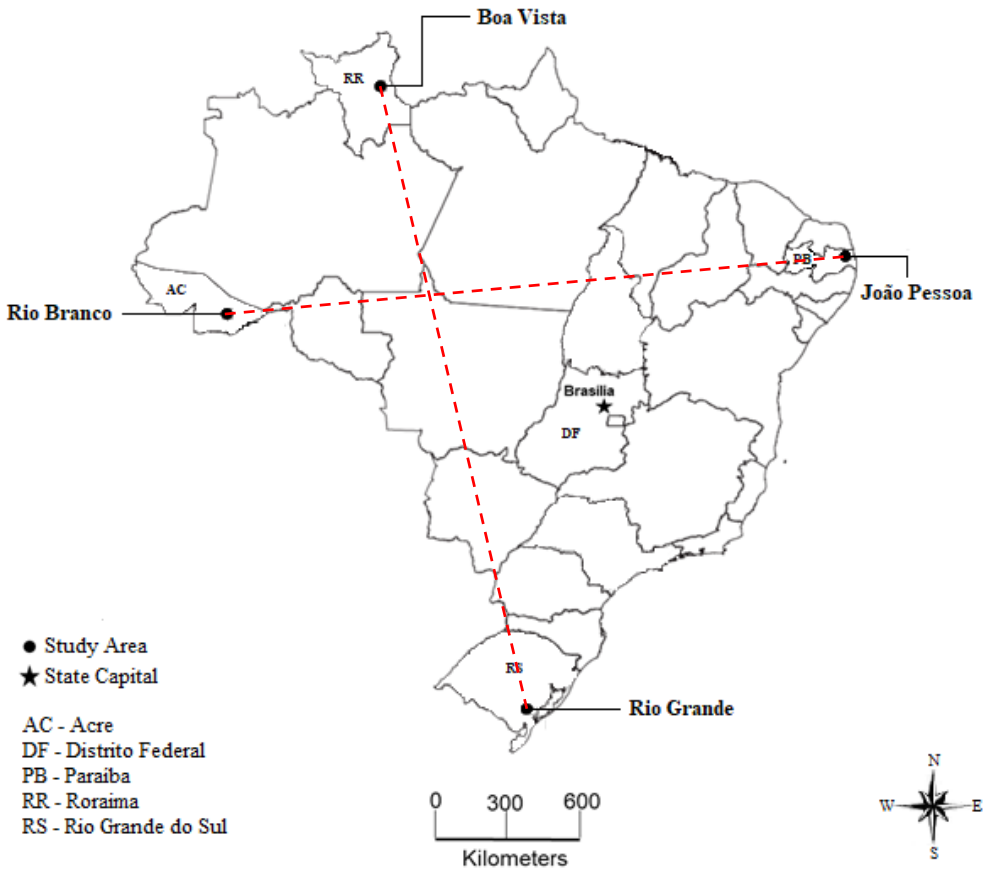


Figure 1. The location of the geographic areas of Brazil under study.

Table 1. Climatic conditions of the geographic locations studied.

| Climate | Geographic location | Latitude | Longitude | Maximum light intensity ($\mu mol.m^{-2}\ s^{-1}$) | Light hours per day (h) | Average temperature (°C) |
|---|---|---|---|---|---|---|
| | Rio Branco (AC) | 09º 58' 29" S | 67º 48' 36" W | | | |
| **Equatorial** | Summer | | | 1553.06$^{g}$ | 12.33$^{b}$ | 26.3$^{c}$ |
| | Winter | | | 1832.1$^{e}$ | 11.37$^{g}$ | 24.6$^{d}$ |
| | Boa Vista (RR) | 02º 49' 12" N | 60º 40' 23" W | | | |
| **Tropical** | Summer | | | 1949.9$^{c}$ | 11.59$^{e}$ | 27.7$^{a}$ |
| | Winter | | | 1725.2$^{f}$ | 12.14$^{d}$ | 26.1$^{c}$ |
| | João Pessoa (PB) | 7º 06' 54" S | 34º 51' 47" W | | | |
| **Tropical Atlantic** | Summer | | | 2162.7$^{a}$ | 12.26c | 27.06$^{b}$ |
| | Winter | | | 1890.5$^{d}$ | 11.46$^{f}$ | 24.56$^{d}$ |
| | Rio Grande (RS) | 32º 02' 06" S | 52º 05' 55" W | | | |
| **Subtropical** | Summer | | | 2058.2$^{b}$ | 13.24$^{a}$ | 22.7$^{e}$ |
| | Winter | | | 1177.3$^{h}$ | 10.45$^{h}$ | 12.8$^{f}$ |

## 2.5 Isolation of the volatile organic compounds

The volatile compounds were isolated from a matrix using headspace solid-phase micro-extraction (HS-SPME) with divinylbenzene/Carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (50/30 µm film thickness ×20 mm; Supelco, Bellefonte, PA). Firstly, the biomass was separated from the cultivation medium by centrifugation for 10 min at 11200 ×g (Hitachi, Tokyo, JP). It was subsequently freeze-dried for 24 h at -50 °C under -175 mmHg. Used 0.2 g of the biomass was added to a 20 mL sealed with a polytetrafluoroethylene (PTFE) faced silicone septum. The SPME fiber was exposed into the headspace of the vial containing the sample for 60 min at 40 °C. After this period, the fiber was removed from the vial and submitted to chromatographic analysis (SANTOS et al., 2016). The analytical procedure was performed twice and in duplicate. Therefore, data refer to the mean value of four repetitions. HS-SPME coupled with GC/MS for the determination of the volatile compounds.

## 2.6 GC/MS analysis

The volatile compounds were analyzed in a Shimadzu QP 2010 Plus gas chromatograph coupled to mass spectrometer (Shimadzu, Kyoto, Japan). Thus, the fiber was thermally desorbed for 10 min in the split/splitless injector, operating in splitless mode (1.0 min splitter off) at 250 °C. Helium was used as a carrier gas at constant 1.6 $mL.min^{-1}$. The analytes 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). The initial column temperature was set at 35 °C for 5 min, followed by a linear increase of 5 °C.min$^{-1}$ to 250 °C, and this temperature was held for 5 min. The MS detector was operated in electron impact ionization mode +70 eV and mass spectra obtained by scan range from m/z 35 to 350. The volatile compounds were identified by a comparison of experimental MS spectra with those provided by the computerized library (NIST MS Search). In addition, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a standard mixture of paraffin homologous series (C6-C24) to aid the identification (ACREE & HEINRICH, 2018). Co-injection and separated injection of samples and standard mixture, providing experimental LRIs and mass spectra for the compounds for identification (TIC: Total Íon Current) by directed comparison.

### 2.7 Statistical analysis

Analysis of variance (one-way ANOVA) and Tukey's test were used to test differences between treatments. Trends were considered significant only where means of compared parameters are different at $p < 0.05$ significance level. The principal component analysis (PCA) was used to identify the relationships samples from between climates and seasons. All the analyses were performed using Statistica 10.0 software (StatSoft, Tulsa, USA).

## 3. Results and discussion

### 3.1 Identification of volatile organic compounds

The volatile compounds identified and their respective areas obtained in each extraction condition of *Scenedesmus obliquus* microalgae from the different climates in the summer and winter seasons are presented in Table 2. Based on your diversity, the volatile compounds can be divided into seven groups (alcohols, aldehydes, hydrocarbons, ketones, pyrazines, terpenes, and other organic compounds).

Table 2. Retention index and the total area expressed in arbitrary units ($\times 10^6$) of the volatile organic compounds found in different climates and seasons by *Scenedesmus obliquus*.

| Compounds | ID | $RI_C$ | $RI_L$ | Equatorial | | Tropical | | Tropical Atlantic | | Subtropical | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Summer | Winter | Summer | Winter | Summer | Winter | Summer | Winter |
| ***Alcohols*** | | | | | | | | | | | |
| Propanol | A | 1049 | 1049 | 1.48 | 1.48 | 1.20 | 0.38 | - | - | - | - |
| 3-Methyl butanol | A | 1216 | 1217 | 1.52 | 6.87 | 4.38 | 3.38 | 1.18 | - | 2.72 | - |
| 1-Pentanol | A | 1259 | 1256 | 2.17 | 11.06 | 3.21 | 4.95 | - | - | 11.45 | - |
| (Z) 2-Pentenol | A | 1327 | 1325 | 1.40 | 1.23 | 2.53 | 25.36 | 0.35 | 22.19 | 6.70 | - |
| Diacetone alcohol | B | 1352 | 1374 | - | - | 1.53 | 37.39 | 3.62 | 33.10 | - | - |
| 1-Nonanol | A | 1668 | 1666 | - | - | 2.56 | 42.97 | - | - | - | - |
| ***Aldehydes*** | | | | | | | | | | | |
| Propanal | A | 823 | 784 | 0.55 | 0.21 | 0.91 | 1.35 | 0.19 | 0.81 | 0.56 | 0.79 |
| Butanal | A | 882 | 867 | 1.28 | 1.01 | 0.27 | 0.96 | 0.28 | 1.08 | 2.05 | 0.00 |
| 2-Methyl butanal | A | 907 | 896 | 0.61 | - | 0.95 | 1.57 | 0.55 | 2.59 | 1.30 | 0.85 |
| 3-Methyl butanal | A | 919 | 900 | 0.84 | 0.60 | 1.22 | 8.54 | 0.50 | 7.00 | - | - |
| Pentanal | A | 983 | 979 | 5.64 | 26.16 | - | - | 8.90 | 23.50 | - | - |
| Hexanal | A | 1084 | 1084 | - | - | 1.85 | 4.51 | - | - | - | - |
| (E) 2-Pentenal | B | 1132 | 1127 | - | - | 0.94 | 7.35 | - | - | - | - |
| (E) 2-Hexenal | B | 1222 | 1220 | - | 1.35 | - | 6.92 | 0.33 | 7.93 | 5.51 | 1.43 |
| (Z) 4-Heptenal | B | 1244 | 1238 | - | - | - | 2.02 | - | 1.73 | - | - |
| ***Hydrocarbons*** | | | | | | | | | | | |
| 2,5-Dimethyl octane | B | 930 | 927 | - | - | - | - | - | - | - | 12.61 |
| Decane | A | 1004 | 1000 | 1.10 | 2.55 | 0.28 | 2.20 | - | 7.03 | 3.28 | - |
| 2,6-Dimethyl Nonane | B | 975 | 1017 | - | - | - | - | - | - | - | 51.69 |
| 3-Ethyl-1,5-Octadiene | B | 1027 | 1018 | - | - | - | 2.88 | - | 9,93 | 34.56 | 32.44 |
| 5-Ethyl-2-methyl-Octane | B | 1047 | 1037 | - | - | - | - | - | - | 10.79 | - |
| Undecane | A | 1097 | 1100 | - | 4.07 | 0.66 | 0.65 | - | 0.82 | - | 1.33 |
| Dodecane | A | 1200 | 1200 | 0.23 | 3.57 | 2.47 | 2.10 | - | 1.71 | 12.23 | 65.81 |
| 1,2,4-Trimethyl benzene | B | 1279 | 1285 | - | 1.37 | - | 0.93 | 0.06 | 1.95 | 16.97 | 1.80 |
| Tridecane | A | 1301 | 1300 | - | - | 3.77 | - | 0.31 | 1.34 | 4.98 | 31.47 |
| Tetradecane | A | 1402 | 1400 | - | - | 1.50 | - | 0.70 | 2.20 | 7.77 | 22.45 |
| Pentadecane | A | 1500 | 1500 | - | 12.82 | 5.41 | 12.58 | 4.46 | 22.95 | - | 14.42 |
| Hexadecane | A | 1601 | 1600 | 3.45 | 6.28 | 2.42 | 6.31 | 3.35 | 9.56 | 12.51 | 5.35 |
| Heptadecane | A | 1703 | 1700 | - | 89.40 | 55.62 | 78.82 | 111.14 | 115.98 | 122.12 | 39.73 |
| 8-Heptadecene | B | 1720 | 1718 | 4.67 | 8.06 | - | - | - | - | 18.55 | 11.36 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Ketones*** | | | | | | | | | | | |
| Acetone | A | 840 | 821 | 5.79 | 7.01 | 41.47 | 38.56 | 40.35 | 36.97 | - | - |
| 2-Pentanone | A | 983 | 980 | - | - | 11.08 | 17.86 | - | - | - | - |
| 2,3-Pentanedione | A | 1065 | 1062 | - | - | - | - | - | 2.92 | - | - |
| 3-Penten-2-one | A | 1132 | 1127 | - | - | - | - | 1.13 | 1.99 | - | 3.04 |
| 3-Hydroxy-2-butanone | B | 1290 | 1287 | 15.97 | 30.25 | 24.72 | 25.74 | 16.75 | 16.16 | 12.49 | - |
| 2,3-Octanedione | B | 1329 | 1325 | 2.20 | 5.11 | - | - | 1.22 | - | - | - |
| 3,5-Octadien-2-one | B | 1529 | 1516 | 3.34 | 0.89 | 5.54 | 18.19 | 1.83 | 11.48 | 6.93 | 12.53 |
| α-Ionone | A | 1868 | 1857 | - | - | 0.98 | 3.79 | - | - | - | - |
| β-Ionone | B | 1955 | 1953 | 0.11 | 0.37 | 0.60 | 5.85 | - | - | 3.04 | 3.36 |
| ***Pyrazines*** | | | | | | | | | | | |
| 2,6-Dimethyl pyrazine | B | 1338 | 1338 | - | 1.19 | 0.83 | 33.86 | - | 18.05 | 1.87 | 0.03 |
| 2,3,5-Trimethyl pyrazine | B | 1419 | 1413 | - | 0.96 | - | - | - | 3.15 | 0.89 | - |
| ***Terpenes*** | | | | | | | | | | | |
| Limonene | A | 1196 | 1189 | - | - | 0.18 | 0.59 | - | - | - | 1.16 |
| β-Cyclocitral | A | 1632 | 1632 | - | - | 0.70 | 7.23 | - | - | - | - |
| Safranal | A | 1657 | 1648 | 0.19 | 0.35 | - | - | 0.63 | 23.36 | 0.90 | 3.29 |
| ***Others*** | | | | | | | | | | | |
| 2-Ethyl Furane | B | 952 | 960 | - | - | 0.01 | 0.19 | - | - | 0.18 | 3.29 |
| 2-Ethylhexyl acetate | B | 1390 | 1420 | 0.32 | 5.66 | - | 3.87 | - | 2.09 | - | 5.22 |
| Dimethyl Sulfoxide | B | 1610 | 1595 | - | - | - | - | - | 3.65 | - | - |
| Isovaleric acid | A | 1672 | 1679 | 0.58 | 0.32 | 0.57 | 8.97 | - | 9.92 | 2.61 | - |
| ***Total*** | | | | 53.44 | 230.20 | 197.83 | 403.14 | 180.36 | 418.82 | 302.96 | 325.45 |

A total of 47 compounds were identified in all the samples studied, and of these, 4 volatiles were common in all experiments (Propanal, Butanal, Hexadecane, and 3,5-Octadien-2-one). The subtropical climate was the one with the highest abundance of total area in the summer with 302.96 $\times 10^6$, followed by tropical climates (197.83 $\times 10^6$), tropical Atlantic (180.36 $\times 10^6$) and equatorial (53.44 $\times 10^6$) climates, while in winter the tropical Atlantic reached a larger abundant area of 418.82 $\times 10^6$, followed by tropical, subtropical and equatorial climate with a total area of 403.14, 325.45 and 230.20 $\times 10^6$, respectively.

Based on the identification of the volatile groups, hydrocarbons were identified as the most prevalent compounds with the highest number of compounds detected (14), followed by aldehydes and ketones (9), alcohols (6), terpenes (3), pyrazines (2), and other compounds (4). Previous works on the microalgae volatile profile reported hydrocarbons as the major VOCs in many species of cyanobacteria (HAYES & BURCH, 1989); and hydrocarbons, aldehydes, ketones and alcohols in microalgae (VAN DURME et al., 2013, ZHOU et al., 2016).

It has been demonstrated that the production of hydrocarbons in microalgae and cyanobacteria is mainly achieved by metabolic pathways linked to fatty acids (MILOVANOVIC et al., 2015). Biochemical studies of alkane biosynthesis have focused on eukaryotic systems, with most evidence supporting a decarbonylation of fatty aldehydes as the primary mechanism (CHEESBROUGH & KOLATTUKUDY, 1984; SCHIRMER et al., 2010). Two families of enzymes are responsible for the production of alkanes, which play a crucial role in the conversion of fatty acid intermediates to alkanes and alkenes (DUCAT et al., 2011). The first step involves the reduction of fatty acyl-ACP to intermediate fatty aldehydes by acyl-ACP reductase, which is followed by the conversion of fatty aldehydes to n-alkanes by aldehyde decarbonylase.

The results show that the unbranched alkanes represent the major volatile compounds in this species. Heptadecane is shown to be present in all analyzed climates (except in summer equatorial), with its content ranging from an area of 39.73 to 122.12 $\times 10^6$. Other alkanes were also detected in all climates, though in smaller amounts compared to heptadecane. Heptadecane has been described as the most abundant alkane in photoautotrophic species resulting from the decarbonylation of even-numbered fatty aldehydes (SCHIRMER et al., 2010) and reported by Jesus et al. (2018) as originating from the decarboxylation of palmitic and stearic acid. They were still the major constituents in *Synechocystis sp.* obtaining about 0.1% of its dry weight (HU et al., 2013).

The occurrence of most of the identified volatiles can be attributed to the particular composition of the microalgae biomass. Microalgae are rich in polyunsaturated fatty acids,

especially long chain PUFA. Species with low concentrations of PUFA contain significantly fewer linear aldehydes when compared to species with high concentrations, such as *Scenedesmus obliquus* (KOST & HEIL, 2008). Abomohra et al. (2014) reported that fatty acid productivity for *Scenedesmus obliquus* can reach up to 18 mg/L/d during the exponential phase, and of this 73.1% are related to polyunsaturated fatty acids. In addition, a significant number of aldehydes identified may be related to the oxidation of polyunsaturated fatty acids attributed to the decomposition of lipid hydroperoxides and peroxyl radicals (PEINADO et al., 2014).

According to data from Table 2, several ketones and alcohols present in the samples corroborate the findings of Van Durme et al. (2013). Ketones, such as acetone and 3-Hydroxy-2-butanone, one of the main compounds found by *Scenedesmus obliquus*, appeared to predominate in all marine microalga samples surveyed (*Botryococcus*, *Rhodomonas*, *Tetraselmis*, and *Nannochloropsis*), and the freshwater microalga (*Chlorella*). A α-Ionone and β-Ionone were also measured in most of these samples. According to the literature, these compounds are characterized by low odor thresholds, making them important aromatic compounds (MILOVANOVIC et al., 2015). Likewise, alcohols such as 1-Pentanol and (Z) 2-Pentenol have also been identified at high concentrations, in particular for *Chlorella vulgaris* (VAN DURME et al., 2013). According to Giri et al. (2010), branched-chain alcohols can be produced by the secondary decomposition of hydroperoxides of fatty acids, but some of them might also come from carbohydrates by the glycolysis and/or from amino acids via the Ehrlich pathway.

The occurrence of volatile organic compounds in microalgae is a consequence of their metabolism. Eukaryotic microalgae capture light energy and assimilate $CO_2$ in chloroplasts through the Calvin cycle with the intention of forming glyceraldehyde 3-phosphate to act in the synthesis of pyruvate via the glycolytic pathway. This process is traditionally divided into two stages, the so-called light reactions, and carbon assimilation reactions. In light reactions, which are bound to photosynthetic membranes, the light energy is converted into chemical energy providing an NADPH reductant and a high energy ATP compound. In the other step, which takes place in the stroma, NADPH and ATP are utilized in the sequential biochemical reduction of $CO_2$ in carbohydrates and other organic compounds (MASOJÍDEK et al., 2013).

The distinction between primary and secondary metabolites is that the former being constitutive, are continuously produced and are essential for maintaining life. Secondary metabolites, on the other hand, are the molecules derived from primary metabolites that have been dispensed and are not directly involved in the growth, development, and reproduction of the organism (ACHYUTHAN et al., 2017). The secondary metabolites can be gradually

generated in response to different environmental conditions, and therefore, can be seen as a behavior that is in part the capacity for adaptation and survival in response to environmental stimuli during life, and serve to establish ecological relationships with other microorganisms (YANG et al., 2018).

Regardless of metabolism, the biosynthesis of volatile organic compounds occurs through the formation of the pyruvate molecule (shown in Figure 2) dividing into several classes, including terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and amino acids.

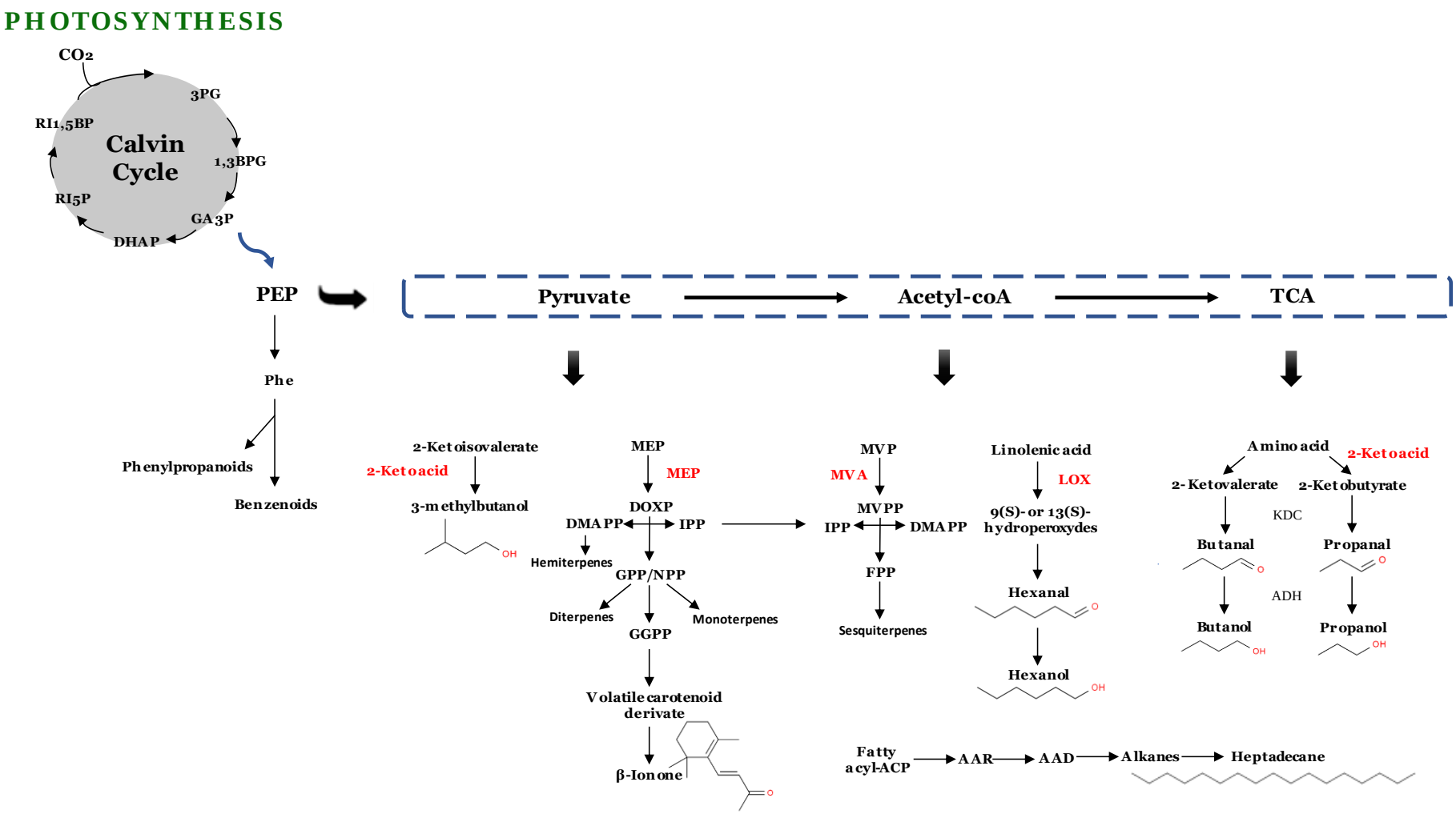


Figure 2. Overview of biosynthesis pathways leading to the emission of volatile organic compounds in photosynthetic microorganisms. Adapted from: Schirmer et al., 2010; Dudareva et al., 2013; Tashiro et al., 2014; Santos et al., 2016; Achyuthan et al., 2017. Abbreviations: ADH, alcohol dehydrogenase; AAR, acyl-ACP reductase; AAD, Aldehyde decarbonylase; DHAP, dihydroxyacetone phosphate; DMAPP, dimethylallyl pyrophosphate; DOXP, 1-deoxy-D-xylulose-5-phosphate; FPP, farnesyl pyrophosphate; GA3P, glyceraldehyde-3-phosphate; GPP, Geranyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; IPP, isopentenyl pyrophosphate; KDC, 2-keto acid decarboxylase; LOX, Lipoxygenase; MEP, Methylerythritol phosphate; MVA, Mevalonic acid; MVP, Mevalonate 5-phosphate; MVPP, Mevalonate 5-pyrophosphate; NPP, Neryl pyrophosphate; Phe, Phenylalanine; PEP, Phosphoenolpyruvate; RI1,5BP, ribulose-1,5-bisphosphate; RI5P, ribulose-5-phosphate; TCA, tricarboxylic acid; 1,3 BPG, 1,3-biphosphoglycerate; 3PG, 3-phosphoglycerate.

The formation of compounds from pyruvate can follow the route of terpenoids or also via the keto acids via intermediate 2-ketoisovalerate. Microalgae can generate isoprenoid (C5) precursors by isopentenyl pyrophosphate (IPP) and its dimethylallyl pyrophosphate isomer (DMAPP), via 2-C-methyl-D-erythritol 4-phosphate (MEP) in plastids or by mevalonic acid (MVA) into the cytosol by acetyl-CoA (LIANG et al., 2018). While, farnesyl pyrophosphate (FPP, C15) is a key precursor in the biosynthesis of sesquiterpenes, triterpenes, and sterols, the geranylgeranyl pyrophosphate (GGPP, C20) is an essential precursor for a variety of products including diterpenes, tetraterpenes, carotenoids and other compounds (FABRIS et al., 2014; LIANG et al., 2015).

As previously reported, many volatiles is derived from fatty acids, which arise through the unsaturated C18, formed through acetyl-CoA. After entering the lipoxygenase (LOX) pathway, the unsaturated fatty undergoes stereospecific oxygenation to form 9-hydroperoxy and 13-hydroperoxy which are further metabolized to produce volatile compounds (DUDAREVA et al., 2013).

In addition, the structural relationship of microalgae metabolism suggests that amino acids may also be the precursors of some important secondary metabolites (GONZALEZ & MORALEZ, 2017). Amino acids such as valine, leucine, and isoleucine are slowly absorbed after undergoing initial deamination or transamination catalyzed by aminotransferases, leading to the formation of the corresponding α-keto acid (HOSOGLU, 2018). These α-keto acids can be subjected to decarboxylation, followed by reductions, oxidations and/or esterifications, forming aldehydes, acids, alcohols, and esters (REINECCIUS, 2006; DUDAREVA et al., 2013).

It is clear the biosynthesis of volatile organic compounds depends mainly on the availability of nutrients and energy of the primary metabolism, demonstrating the high level of connectivity between primary and secondary metabolism (DUDAREVA et al., 2013). Thus, 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 (SANTOS et al., 2016). Furthermore, changes in cultivation conditions and/or specific environmental conditions may provide different varieties of volatile organic compounds.

### 3.2 Effect of different climatic conditions on production VOCs

In photosynthetic cultures, the temperature and amount of light energy received and stored by the cells are directly related to the growth, metabolism, and morphology of microalgae, including the volatility of the secondary metabolites. In this sense, the effects of the climatic conditions of the different climates proposed and their relation in the production of volatile organic compounds were examined. The total percentage of volatile groups analyzed under different climates is summarized in Figure 3.

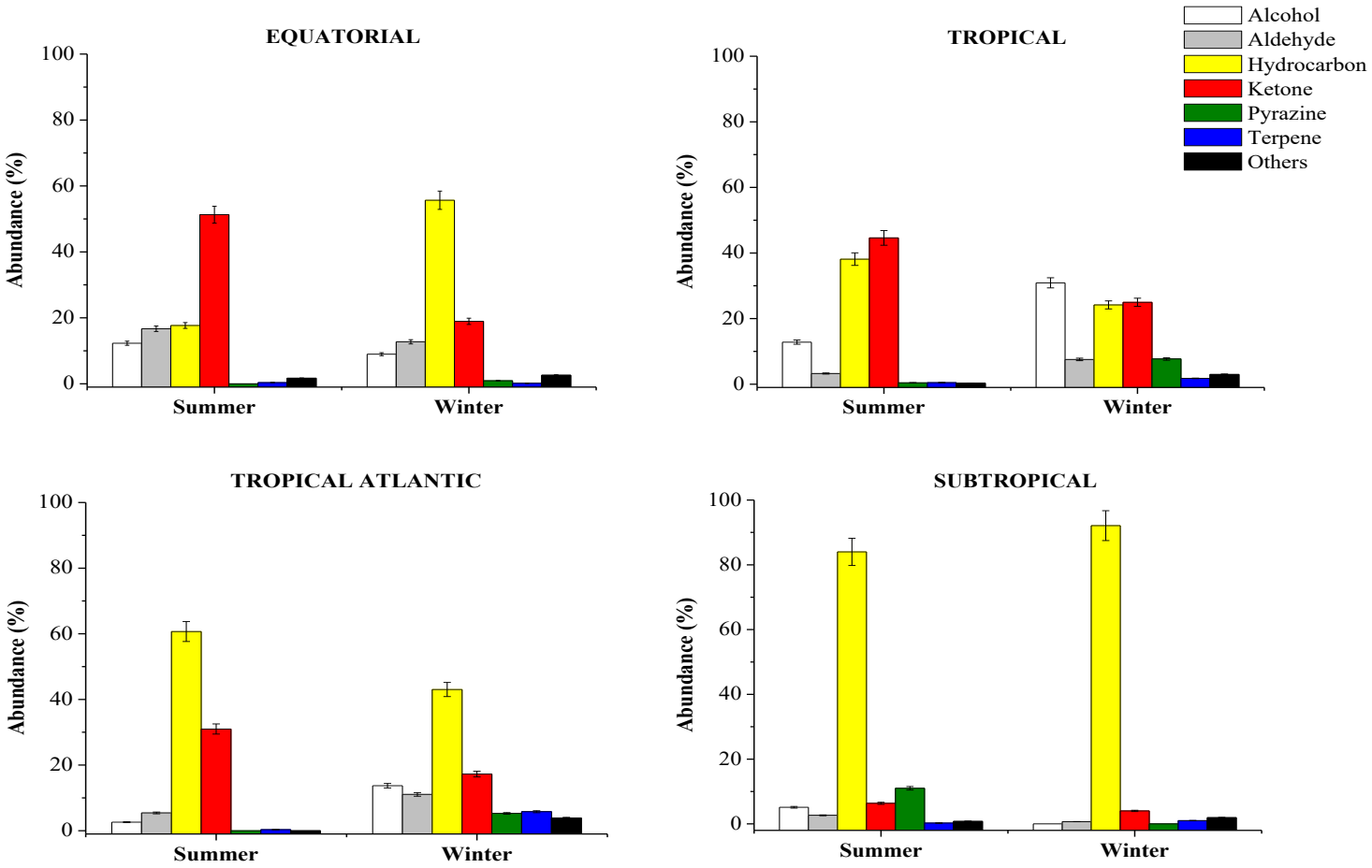


Figure 3. Percentage of the volatile groups measured in relation to their abundance of total area in the summer and winter periods of the different climates.

According to Figure 3, hydrocarbons were identified as the most abundant VOCs in terms of percent composition. This chemical class showed a relative growth in its total content throughout the treatments and was the main biomarker of the subtropical climate reaching a yield of 80.46% and 89.25% in summer and winter, respectively. It can be noted that the climatic conditions of the subtropical climate show a lower average temperature compared to other climates (12.8 °C for the winter and 22.7 °C for the summer), and it was the most varied

in relation to the maximum light intensity and amounts of light hours per day between the different seasons.

As can be seen, the ketone group reached a higher percentage of VOCs in the equatorial climate in the summer with a percentage of 51.29%, but decreased dramatically in winter, obtaining around only 18.95%. Sun et al. (2013) show that ketones can be produced mainly via thermal degradation and therefore are reported in this study as the main compounds found in warmer climates. Furthermore, the other groups of volatile compounds had a relatively constant concentration throughout the study.

In fact, the impact of stress conditions has been documented at genetic and protein level and its reflection are reported in the alteration of the pool of secondary metabolites in affected microorganisms (LORETO & SCHNITZLER, 2010). Consequently, the observed differences between summer and winter samples can be attributed to environmental conditions, which vary considerably with the season during growth, which logically give rise to altered VOCs production (VITA et al., 2015).

Microalgae of the same species, grown in different environments, may have differences in the concentration of particular secondary metabolites. Abiotic factors cause stresses in photosynthetic organisms as a consequence of unfavorable conditions due to these factors (VERMA et al., 2015). For most microalgae, in particular, the modification of photosynthesis by parameters such as light availability and temperature affect significantly some processes associated with its ability to synthesize secondary metabolites (YANG et al., 2018).

While light provides the energy to support the metabolism of autotrophic microorganisms, the effects of temperature are related both to the temperature dependence of the structural components of cells (particularly lipids and proteins) and to the temperature coefficients of the reactions of all chemical reactions that occurrence in microalgae cells (CHALOUB et al., 2015).

As already recognized, the photosynthetic efficiency decreases as the light intensity increases beyond ideal. As the photosynthetic apparatus is not able to process the high amount of photons received, an inhibitory effect can promote energy damage and dissipation through photosaturation and photoinhibition phenomena (SFORZA et al., 2015). In addition, an ideal specific duration of light/dark periods is required for the synthesis of ATP and NADPH to drive the dark reactions of photosynthesis in the production of carbon skeletons (CHEIRSILP & TORPEE, 2012).

Previous studies have shown that the irradiance and duration of light exposure played a predominant role in regulating the levels of various volatiles metabolites in photosynthetic

species. Meskhidze et al. (2014) have confirmed that both isoprene and monoterpene production from algae can be influenced by the variability of irradiation and time of exposure to light. These authors studied the rate of production of six phytoplankton monocultures (including diatom strains and dinoflagellate) at different levels of irradiance and confirmed that emission rates were found to increase when acclimatized low-light was subject to higher irradiance levels (900 $\mu mol.m^{-2}\ s^{-1}$), reaching the maximum rate of isoprene emission and sum of all studied monoterpenes (α-Pinene, β-Pinene, Camphene, and d-Limonene).

These results indicate that the accumulation of compounds volatiles and secondary metabolites depend on the duration and amount of daylight. In addition, high-intensity light can increase the content of secondary metabolites by three times more than the mean light intensity and may even activate the higher antioxidant capacity of secondary metabolites in photosynthetic systems (ARENA et al., 2017).

In the second moment, the temperature is another important factor in the microalgal metabolism and has an important impact on the chemical balance of the species. Most microalgae have the ability to grow over a wide range of temperatures, but the response and adaptation of microalgae to different levels of stress depends on their origin (HE et al., 2018).

While Rubisco carboxylation activity increases exponentially with increasing temperature (URBAN et al., 2018), very high temperatures can lead to a reduction of the Rubisco enzyme by inhibitors such as xylulose 1,5-bisphosphate, the limitation of ATP (adenosine triphosphate), and consequently the reduction of $CO_2$ assimilation rate, resulting in increased rates of photorespiration (FARQUHAR et al., 1989; ROY & ANDREWS, 2000; KILLI et al., 2017; URBAN et al., 2018). On the other hand, according to Verma et al. (2015), the low temperature induces different physiological, biochemical and molecular changes in photosynthetic species. In addition, photosystem II (PSII) shows a decrease in photochemical efficiency, causing increased stress in the organism of these species.

Some authors report that low temperature and high light intensity have a synergistic effect on the expression of genes in the biosynthesis pathway of secondary metabolites (AZUMA et al., 2012; WANG, 2016). Low temperatures and high irradiation were used to promote the increase in the accumulation of secondary compounds such as anthocyanins and phenolic compounds in photosynthetic cultures (LIN-WANG et al., 2011).

Also, at high temperatures combined with high light intensity led to severe photoinhibition of cell growth in microalgae such as *Dunaliella tertiolecta*, since photosynthesis is considered to be one of the most heat sensitive cellular functions, especially the oxygen complex in PSII (JUNEJA et al. al. 2013, SEEPRATOOMROSH et al., 2016).

At a low temperature, some strains of microalgae regulate the lipid composition to maintain the structural integrity and membrane fluidity, increasing the content of unsaturated fatty acids (HE et al., 2018). A recent report showed that cold stress can inhibit glycolysis and the TCA cycle, leading to less energy and supply of NAPDH for anabolism, and upregulated PUFA synthase, resulting in an increase in the content of PUFAs (MA et al., 2017).

As already reported, the production of hydrocarbons in microalgae may be closely linked to metabolic pathways of fatty acids which could be directly correlated with the production of hydrocarbons in our study. In fact, the main mechanisms associated with metabolic synthesis such as temperature stress mainly refers to the instability of the enzymes associated with carbon fixation (ribulose biphosphate carboxylase/oxygenase: Rubisco) and lipid biosynthesis (Acetyl CoA carboxylase) (VALLEDOR et al., 2013).

### 3.3 Exploratory multivariate analysis

Principal component analysis (PCA) was used to better visualize the effect of different climatic conditions on the production of VOCs. Figure 4 shows the seasonally separated PCA graph (summer and winter), and the data matrix was composed of the sum of the total area in each chemical class versus general climate. In this analysis, average temperature (AT), maximum light intensity (MLI) and light hours per day (LHD) were added as variables

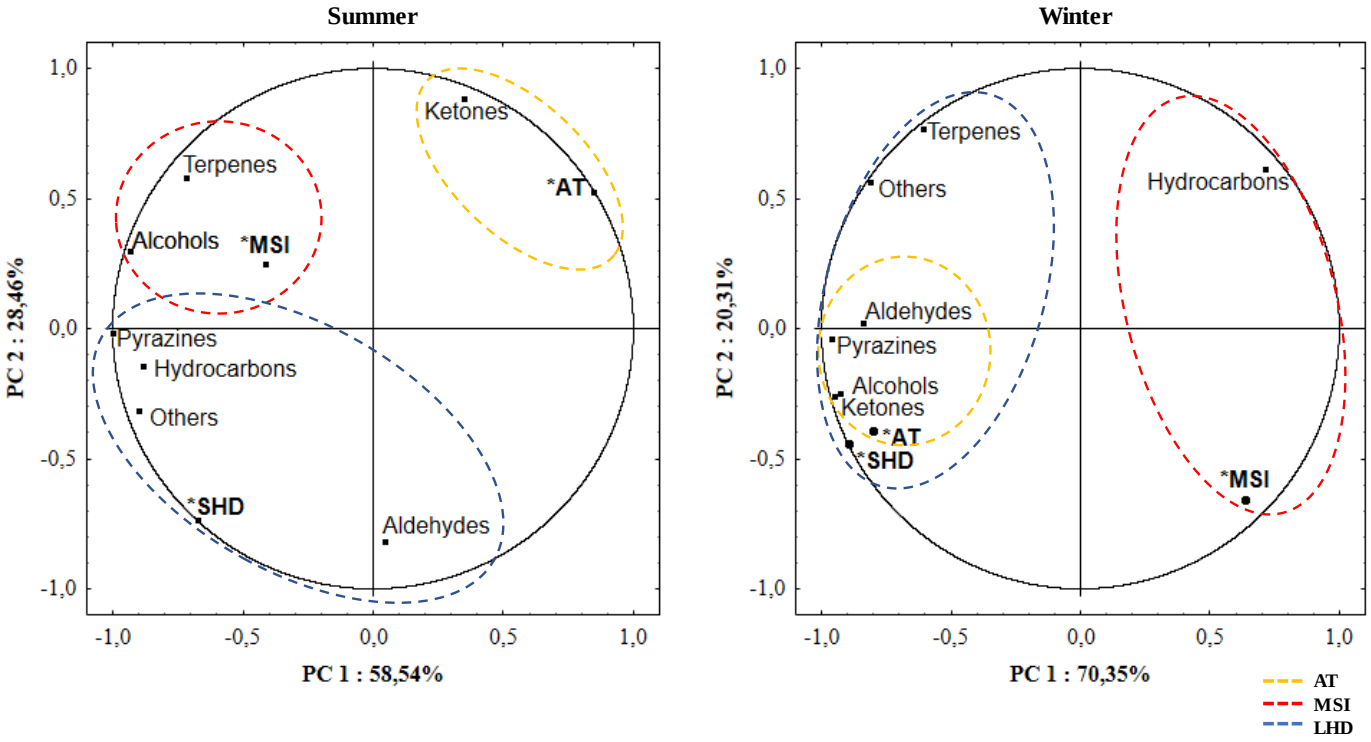


Figure 4. Principal component analysis of the volatile groups found for *Scenedesmus obliquus* in different climatic conditions in summer and winter.

As shown in Figure 4 the first two main components, PC1 and PC2, explained 87% of the total variance (58.54% for PC1 and 28.46% for PC2) in the summer season, whereas for winter PC1 explained 70.35% and PC2 explained only 20.31% in a total variance of 90.66%. Furthermore, it is possible to verify that the two main components showed that there was a separation of treatments in three groups for the summer and two groups for the winter based on the additional factors (AT, MLI, LHD).

In the summer season discrimination was observed in each treatment, indicating that each proposed climatic condition produced different volatile groups. The first factor observed was the light hours per day in which it appeared in both the negative part of PC1 and in PC2, the second factor being the maximum light intensity, appeared in the negative part of PC 1 as in the positive part of PC2, while the average temperature only appeared in the positive area of the two PCs.

At this season, it is possible to clearly identify that ketones are associated mainly by the high temperatures of the hotter climates. In contrast, hydrocarbon production was inversely correlated and reinforces the hypothesis that this volatile chemical group is influenced by lower temperatures. Still, hydrocarbons, pyrazines, and aldehydes were influenced by the light hours per day, while the maximum light intensity affected the group of terpenes and alcohols.

Already, in the winter period, the average temperature and the number of hours of light per day were the factors that most marked the total production of VOCs. These additional factors were negative in both PC1 and PC2 and were clearly separated from the maximum light intensity, which appears on the positive side of the two main components. Therewith, it is possible to identify that lower temperatures and greater light intensity promoted the significant increase of hydrocarbons in the subtropical climate.

The proposed methods allowed *Scenedesmus obliquus* to be differentiated and evaluated through its metabolism in different situations. Consequently, the differences observed between the summer and winter samples might be attributable to the environmental conditions, which vary considerably with the season, which logically give rise to altered VOCs production.

## 4. Conclusion

*Scenedesmus obliquus* can produce a variety of interesting secondary metabolites, and knowledge about the biosynthesis of these structures from microalgae can be quite useful to help elucidate ways of commercial application.

The analysis of VOCs in the climatic conditions proposed for the climates of extreme geographic locations of Brazil made it possible not only to verify the abundance of total area between volatile chemical groups, but also the distinction between summer and winter production. It appears that, for each climate and season, the VOCs of the microalga *Scenedesmus obliquus* is, at least metabolically, quite distinct.

Thus, it is possible to check the formed compounds against different culture conditions and to select the most favorable conditions for the production of precursors or bioproducts of interest.

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# CAPÍTULO 3

## THE INFLUENCE OF LIGHT/DARK CYCLES ON THE PRODUCTION OF VOLATILE ORGANIC COMPOUNDS BY *SCENEDESMUS OBLIQUUS*

# The influence of light/dark cycles on the production of volatile organic compounds by *Scenedesmus obliquus*

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**Abstract:** The objective of this study was to evaluate the influence of light/dark cycles on the production of volatile organic compounds by *Scenedesmus obliquus* through the simulation of the climatic conditions such as average temperature, maximum light intensity, and hours of light/dark per day in the summer seasons of the equatorial climate of Brazil. The volatiles were isolated by headspace solid-phase micro-extraction in different residence times, separated by gas chromatography, and identified by mass spectrometry (SPME-GC/MS). A combined total of 37 compounds was identified, of which 10 compounds were produced only in the light cycle, while 5 compounds were identified only in the dark cycle. The results show that the main volatile compounds found in *Scenedesmus obliquus* for the light cycle were 3-Methyl butanol compound, followed by 6-Methyl-5-hepten-2-one. Already, for the dark cycle, the most relevant compounds were the alcohols 2-Ethyl hexanol, and 1-Pentanol. The main volatile groups found were alcohols, aldehydes, ketones, and terpenes which varied over time through the light/dark cycles.

## 1. Introduction

Microalgae are a highly diversified polyphyletic group of microscopic photosynthetic organisms that have evolved over time to thrive in adverse environments and have gained considerable attention in the last decade because of their wide variety of applications (RIZWAN et al., 2015; MATOS et al., 2017). Microalgae and cyanobacteria produce different compounds, such as pigments, antioxidants, β-carotenes, proteins and vitamins, which can be used to produce high-value products (GONÇALVES et al., 2016).

Depending on the species, crop and environmental conditions, microalgae are capable of producing a variety of volatile organic compounds (VOCs). The identification of these compounds is very important because of their direct impacts on the aromatic properties of the

final product and can still provide an important alternative source of drugs, aromas, and fragrances at low cost (SANTOS et al., 2016; ZHOU et al., 2016; HOSOGLU, 2018).

In the natural environment, all life is exposed to a daily cycle of light and dark fluctuation of light intensities and seasonal oscillation of daylight length as a result of the rotation of the planet. Eukaryotic and prokaryotic cells have evolved to respond to the rhythmic changes in environmental conditions and synchronize their cellular processes to the most appropriate time of the day (DIXON et al., 2014; DUANMU et al., 2014; XU et al., 2016). It is recognized that photosynthetic metabolism consists of two phases, the light phase, and the dark phase. In the first stage, the light energy is converted into chemical energy, which is stored in high energy compounds while in the dark phase, the chemical energy is used in the carbon-fixation reactions (WIJFFELS et al., 2013).

For photosynthetic organisms, synchronization to the light and dark cycle translates into fine-tuning their photosynthetic apparatus to capture sunlight efficiently during the day and to schedule ultraviolet or oxygen sensitive processes (e.g. nitrogen fixation, DNA synthesis or cell division) at night (FÁBREGAS et al., 2002). In addition, the length of the light and dark periods under natural sunlight conditions vary depending on the region and the season, which has an impact on biomass productivity and photosynthetic efficiency depending on the species (JACOB-LOPES et al., 2009; LEÓN-SAIKI et al., 2018).

In industrial algal cultivations, illumination conditions such as continuous light or a light/dark cycle, the length of the photoperiod and the light intensity affect both the growth of microalgae and the biomass composition (WAHIDIN et al., 2013). Continuous illumination in a photobioreactor system is often used to maximize the biomass production; however, excess light energy that cannot be converted into chemical energy induces photoinhibition damage to the algal photosynthetic apparatus and inhibits the growth of algal cells (MULDERS et al., 2014; WINTER et al., 2017).

Thus, the provision of appropriate light and dark periods, and synchronization under day/night cycles are therefore essential for both the growth of microalgae and optimum yields of target products, such as volatile organic compounds. Based on this, the objective of this study was to evaluate the influence of light/dark cycles on the production of volatile organic compounds by *Scenedesmus obliquus* through the simulation of the climatic conditions in the summer seasons of the tropical climate of Brazil.

## 2. Material and methods

## 2.1 Microorganism and culture media

Axenic cultures of *Scenedesmus obliquus* CPCC05 were obtained from the Canadian Phycological Culture Centre. Stock cultures were propagated and maintained in synthetic BG-11 medium (RIPPKA et al., 1979) with the following composition (mg/L): $K_2HPO_4$ (3.0), $MgSO_4$ (75.0), $CaCl_2 \cdot 2H_2O$ (36.0), ammonium citrate and iron (0.6), $Na_2EDTA$ (1.0), NaCl (0.72), $NaNO_3$ (150.0), citric acid (0.6), $Na_2CO_3$ (15.0), $H_3BO_3$ (2.8), $MnCl_2.4H_2O$ (1.8), $ZnSO_4 \cdot 7H_2O$ (0.22), $Na_2MoO_4 \cdot 2H_2O$ (0.39), $CoSO_4 \cdot 6H_2O$ (0.04). The incubation conditions used were 30 ºC, photon flux density of 30 µmol.$m^{-2}$ $s^{-1}$ and a photoperiod of 12 h.

## 2.2 Photobioreactor design

Measurements were made in a bubble column photobioreactor (MARONEZE et al., 2016). The system was built in 4 mm thick glass with an internal diameter of 9.0 cm, a height of 40 cm and a nominal working volume of 2.0 L. The dispersion system for the reactor consisted of a 1.5 cm diameter air diffuser located in the center of the column. The reactor was illuminated with forty-five 0.23W LED lamps (total consumption of 0.01125 kW h), located in a photoperiod chamber. The $CO_2$/air mixture was adjusted to achieve the desired concentration of carbon dioxide in the airstream, through three rotameters that measured the flow rates of carbon dioxide, air and the mixture of gases.

## 2.3 Experimental conditions

The experiments were carried out in bioreactors operating in batch mode, fed with 2.0 L synthetic BG-11 medium. The luminous intensity was determined using a quantum sensor (Apogee Instruments, Logan, USA), measuring the light incident on the external reactor surface. The temperature was controlled by using thermostats. The flow rates of carbon dioxide, air, and $CO_2$ enriched air were determined with rotameters (AFSG 100 Key Instruments, Trevose, USA). Experimental conditions were as follows: initial cell concentration of 100 mg/L, constant aeration of 1 VVM (volume of air per volume of culture per minute) with an injection of air enriched with 15% carbon dioxide and pH adjusted to 7.6. The choice of photoperiod, average temperature, and maximum light intensity were based on the simulation of the average values of the summer season of the city of Boa Vista (RR) referring to the tropical climate in Brazil. The light cycles evaluated were (h:h) 11.59:12.41 (light:dark), average

temperature (27.7 °C) and maximum light intensity (1949.9 μmol.$m^{-2}$ $s^{-1}$). The concentrations of volatile organic compounds were measured daily at each end of the dark cycle (07:00 a.m.) and after reaching the maximum light intensity (01:00 p.m.), obtained through a ramp of luminosity by the photobioreactor.

**2.4 Isolation of the volatile organic compounds**

The volatile compounds were isolated from matrix by the technique headspace solid-phase microextraction (HS-SPME) using a divinylbenzene/Carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (50/30 μm film thickness ×20 mm; Supelco, Bellefonte, PA). Sample preparation was performed with 20 mL of culture medium, equally separated into two portions (10 mL). The aliquot was placed in a 20 mL vial sealed with a politetrafluoretilene (PTFE) faced silicone septum. After, it was added 3 g of NaCl and 10 μL of 3-Octanol solution (82.2 mg $L^{-1}$), that was used as the internal standard. For the VOCs isolation, first the SPME fiber was maintained without exposure, to reach the chemical equilibrium from the sample and headspace, remaining for 15 min at 40 °C. Subsequently, there was acquired the exposure at the headspace for 60 min some temperature, with agitation provided by a magnetic stir bar. After this period, the fiber was retracted, removed from the vial and immediately desorbed into the injector port of the GC/MS. The analytical procedure was performed twice and in duplicate.

**2.5 GC/MS analysis**

The VOCs were analyzed in a gas chromatography coupled to a mass spectrometer GC/MS Shimadzu QP 2010 Plus (Shimadzu, Kyoto, JP). Therefore, the fiber desorption occurred for 10 min in the split/splitless injector port, operating in the splitless mode (1.0 min splitter off) at 250 °C. Helium was used as a carrier gas at constant 1.6 mL.$min^{-1}$. The analytes were separated in 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) (Chrompack, Palo alto, USA). The initial column temperature was 35 °C for 5 min, followed by a linear increase of 5 °C.$min^{-1}$ to 250 °C, held for 5 min. The MS detector was operated in the electron impact ionization mode +70 eV, and mass spectra was performed scanning in a range from m/z 35 to 350. The VOCs were identified by the comparison of experimental MS spectrum with those provided by the library (NIST MS Search). In addition, the linear retention index (LRI) was calculated for each volatile compound by using the retention time of standard mixture of paraffin homologues series (C6-

C24) to aid the identification, after the calculated LRI was compared with those LRI obtained from the literature (ACREE & HEINRICH, 2018; EL-SAYED, 2018). Also, co-injection and separated injection of standard mixtures, were performed to auxiliary the identification.

### 2.6 Statistical analysis

Analysis of variance (one-way ANOVA) and Tukey's test were used to test differences between treatments. Trends were considered significant only where means of compared parameters are different at $p < 0.05$ significance level. The principal component analysis (PCA) was used to identify the relationships samples from between climates and seasons. All the analyses were performed using Statistica 10.0 software (StatSoft, Tulsa, USA).

## 3. Results and discussion

### 3.1 Production of volatile organic compounds under different light/dark cycles

In photosynthetic cultures, the amount of light energy received and stored by cells and the synchronization of light/dark cycles are essential for microalgal growth and are directly related to metabolism as well as the production of metabolites of interest. In this sense, the influence of light/dark cycles on the production of volatile organic compounds was examined. The results over time are summarized in Table 1 and the yield of VOCs of each volatile group identified in the experiment are presented shown in Figure 1.

Table 1. Quantification of volatile compounds (μg $mL^{-1}$) obtained through of the simulation of the light/dark cycles of the equatorial climate of Brazil by *Scenedemus obliquus.*

| Compounds | $RI_L$ | 0h | Light 6 h | Dark 24 h | Light 30 h | Dark 48 h | Light 54 h | Dark 72 h | Light 78 h | Dark 96 h | Light 102 h | Dark 120 h | Light 126 h | Dark 144 h | Light 150 h | Dark 168h | Light 174 h | Dark 192 h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Alcohols*** | | | | | | | | | | | | | | | | | | |
| 2-Propanol | 927 | - | 1.78 | - | 1.16 | 3.44 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2-Methyl propanol | 1114 | - | - | - | - | 2.15 | - | - | - | - | - | - | - | - | - | - | - | - |
| 2-Pentenol | 1138 | - | 0.45 | - | 1.07 | - | 0.65 | - | 0.60 | - | 0.31 | - | 1.09 | - | 3.32 | - | 1.43 | - |
| 1-Butanol | 1160 | 1.04 | 2.64 | 2.67 | - | 5.04 | 0.82 | 1.05 | 1.05 | - | - | - | 1.05 | - | - | - | - | - |
| 4-Methyl-2-Pentanol | 1173 | - | 1.22 | - | 1.39 | 0.93 | 1.40 | 1.66 | 1.63 | 2.05 | 0.33 | - | 0.32 | 1.77 | - | 1.99 | - | 0.76 |
| 3-Methyl butanol | 1217 | 0.72 | 19.82 | 13.85 | 2.37 | 7.07 | 0.56 | - | 7.96 | 6.62 | 14.38 | 11.92 | 20.35 | 7.43 | 30.79 | 16.34 | 24.29 | 6.28 |
| 1-Pentanol | 1261 | - | - | 3.79 | - | 6.84 | - | 8.34 | - | 27.40 | - | 30.24 | - | - | - | 0.24 | - | 19.12 |
| Hexanol | 1363 | 1.00 | 5.19 | 3.29 | 0.19 | 1.70 | 2.11 | 0.56 | - | - | - | - | - | - | 0.60 | 4.35 | 2.30 | - |
| Cyclohexanol | 1410 | - | - | - | - | - | - | - | - | 0.38 | - | - | - | 0.51 | 1.01 | 1.25 | 0.74 | 0.97 |
| 1-Heptanol | 1462 | 1.73 | 3.69 | 2.81 | 6.92 | 1.70 | 0.95 | 1.11 | 1.66 | 1.68 | 1.36 | 1.43 | 1.27 | 1.24 | 1.78 | 2.78 | 1.83 | 1.06 |
| 2-Ethyl hexanol | 1484 | - | - | 21.13 | - | 23.15 | - | 12.50 | - | 19.03 | - | 15.35 | - | 17.48 | - | 17.25 | - | 0.74 |
| 1-Octanol | 1564 | 4.70 | 7.52 | 3.64 | 3.36 | 3.20 | - | - | 3.56 | 4.01 | 3.78 | - | 2.66 | 2.99 | 2.59 | 4.13 | 2.72 | 0.38 |
| 1-Undecanol | 1876 | - | 7.04 | - | - | - | - | - | - | - | - | - | 0.56 | - | 0.48 | - | - | - |
| 1-Tetradecanol | 2174 | 1.66 | 2.41 | - | 0.40 | - | - | - | 0.94 | - | 1.38 | - | - | - | 1.25 | - | 1.62 | - |
| ***Aldehydes*** | | | | | | | | | | | | | | | | | | |
| Pentanal | 979 | 2.75 | 11.11 | 6.28 | - | 4.19 | - | - | 3.27 | 5.46 | - | 40.92 | 10.07 | 8.69 | 10.44 | 10.44 | 8.76 | 8.76 |
| Hexanal | 1084 | 4.34 | 2.57 | 2.19 | 0.81 | 0.52 | 0.92 | 0.50 | 0.49 | 0.83 | 1.24 | 3.15 | 1.78 | 0.98 | 2.29 | 1.79 | 1.78 | 0.86 |
| Heptanal | 1186 | 2.04 | - | - | - | - | - | - | - | - | - | - | - | - | 2.04 | - | - | - |
| Octanal | 1291 | 3.50 | 2.73 | 1.37 | - | 0.46 | - | 0.52 | 0.55 | 0.18 | 0.38 | 0.24 | 0.70 | - | 0.88 | 1.19 | 0.45 | - |
| Nonanal | 1396 | 9.35 | 3.34 | - | - | - | 0.50 | - | 1.30 | - | 0.69 | - | 0.71 | - | 2.20 | - | 1.23 | - |
| (E,E)2,4-heptadienal | 1463 | 0.70 | 2.27 | 1.11 | - | 0.12 | 1.55 | 1.44 | 2.35 | 3.17 | 3.99 | 7.99 | 3.10 | 4.01 | 5.20 | 6.01 | 4.90 | 0.28 |
| Decanal | 1508 | 6.39 | 4.36 | - | 15.82 | - | 1.45 | - | 4.17 | - | 0.94 | - | 1.39 | - | 5.41 | - | 1.92 | - |
| ***Ketones*** | | | | | | | | | | | | | | | | | | |
| 2,3-Pentanedione | 1065 | - | - | - | 0.36 | - | 0.62 | 0.42 | 0.54 | 1.27 | 0.70 | 2.35 | 0.94 | 1.24 | 1.47 | 1.14 | 1.02 | 0.91 |
| 6-Methyl-2-Heptanone | 1228 | 1.17 | 0.86 | - | 1.42 | 0.96 | 0.97 | 1.64 | 1.48 | 1.35 | 1.92 | 1.52 | 1.36 | 0.91 | 0.86 | 0.65 | 0.94 | - |
| 3-Hydroxy-2-butanone | 1290 | 5.03 | - | - | 5.03 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3-Octanone | 1303 | - | 0.28 | 0.49 | - | 1.98 | 0.34 | 5.19 | 0.49 | - | 0.67 | 6.83 | 0.86 | 37.52 | 0.90 | 36.72 | 0.87 | - |
| 2,3-Octanedione | 1329 | - | 2.36 | 1.08 | - | 2.51 | 2.10 | 2.99 | 6.13 | 8.08 | 12.29 | 13.94 | 14.26 | 7.45 | 10.70 | 7.68 | 10.70 | 3.22 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6-Methyl-5-hepten-2-one | 1342 | 5.69 | 17.73 | 3.34 | 10.69 | 3.34 | 6.16 | 3.34 | 10.06 | 3.34 | 6.12 | 3.34 | 8.16 | 3.34 | 9.90 | 0.89 | 6.36 | 0.89 |
| 3,5-Octadien-2-one | 1529 | - | - | 2.65 | - | - | - | - | - | - | - | 2.65 | - | - | - | - | - | - |
| α-Ionone | 1857 | - | - | - | - | - | - | - | - | - | 4.05 | 3.93 | - | - | - | - | - | - |
| β-Ionone | 1953 | 2.04 | 5.93 | - | - | 1.45 | 1.08 | - | - | 2.59 | 1.65 | - | 1.00 | 1.00 | - | - | - | - |
| ***Terpenes*** | | | | | | | | | | | | | | | | | | |
| Limonene | 1196 | - | 1.46 | - | - | - | - | - | - | - | 1.84 | - | - | - | 0.81 | - | 1.75 | - |
| Linalool | 1552 | - | - | 2.02 | - | 3.07 | - | 2.47 | - | - | - | 1.18 | - | 2.08 | - | - | - | - |
| α-Terpineol | 1708 | - | 5.43 | 1.37 | 3.29 | 2.13 | 1.24 | 2.21 | 1.85 | 3.46 | 0.29 | - | 2.01 | 2.08 | - | - | - | - |
| Citronellol | 1750 | - | 36.82 | 1.81 | - | 2.19 | - | 3.53 | 2.55 | 1.28 | - | 4.41 | - | 1.98 | - | 1.53 | - | 15.45 |
| β-Citronellol | 1782 | 0.96 | 22.32 | - | 8.02 | - | - | - | 2.09 | - | 0.99 | - | 1.37 | - | 1.39 | - | 1.62 | - |
| (E)Geranyol | 1851 | - | - | - | 3.11 | - | - | - | 1.02 | - | - | - | - | - | - | - | - | - |
| Geranylacetone | 1864 | 4.37 | 24.35 | 4.36 | 2.56 | 6.44 | 2.13 | 2.04 | 4.28 | 2.11 | - | - | 2.06 | 1.99 | 1.84 | 3.03 | 2.18 | 0.47 |
| ***Total*** | | **59.18**$^{n}$ | **195.65**$^{a}$ | **75.91**$^{j}$ | **67.97**$^{k}$ | **81.24**$^{g}$ | **25.55**$^{p}$ | **48.17**$^{o}$ | **60.02**$^{l}$ | **90.95**$^{f}$ | **59.30**$^{m}$ | **148.05**$^{b}$ | **77.07**$^{i}$ | **101.35**$^{d}$ | **98.15**$^{e}$ | **118.51**$^{c}$ | **79.41**$^{h}$ | **59.22**$^{n}$ |

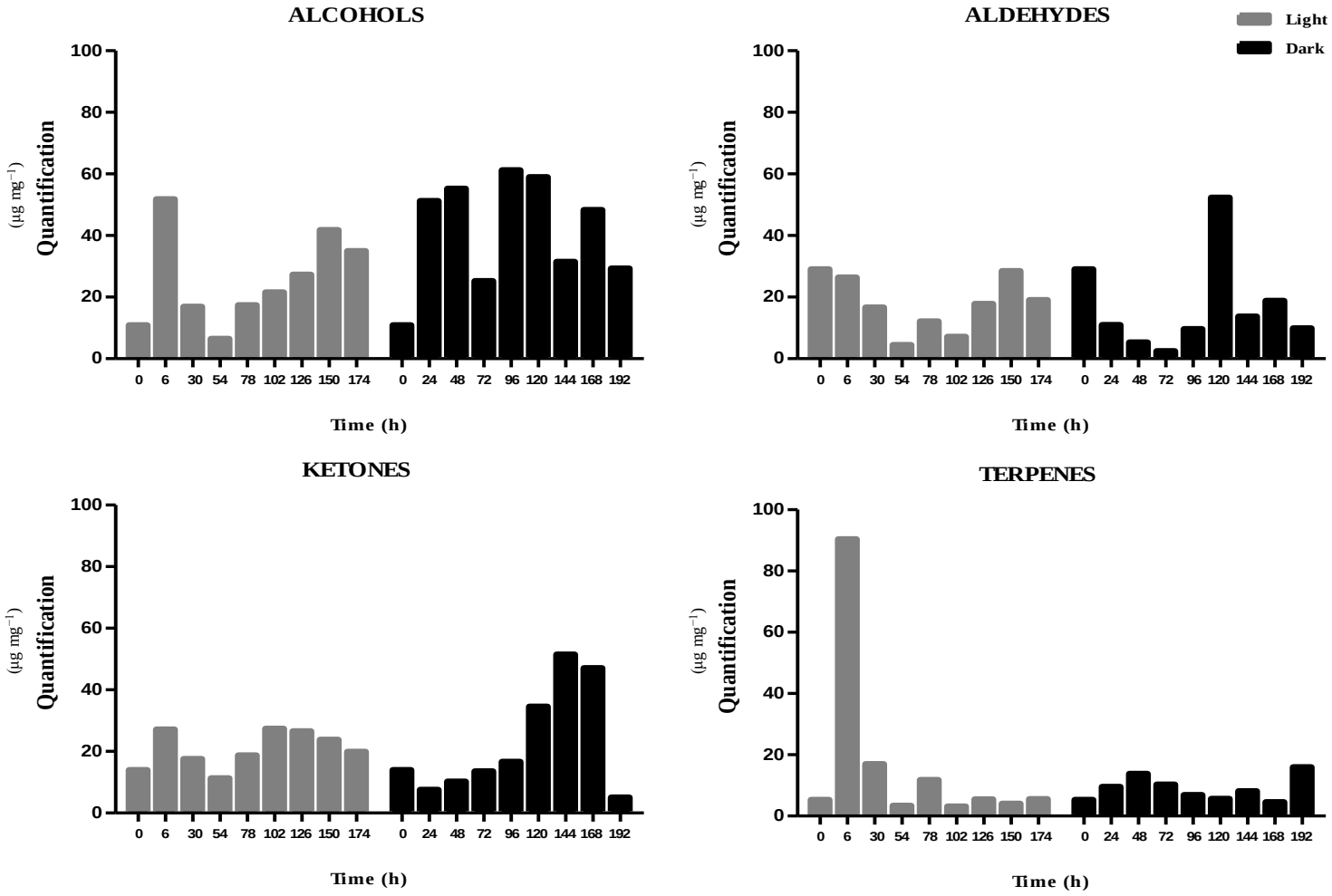


Figure 1. Quantification of each volatile groups identified over time in the light/dark periods.

Table 1 shows the volatile organic compounds identified in all experiments. A total of 37 compounds was identified, of which 10 compounds were only produced in the light cycle (2-Pentenol, 1-Undecanol, 1-Tetradecanol, Heptanal, Nonanal, Decanal, 3-Hydroxy-2-butanone, Limonene, β-Citronellol, and (E) Geranyol), while 5 compounds were identified only in the dark cycle (2-Methyl propanol, 1-Pentanol, 2-Ethyl hexanol, 3,5-Octadien-2-one, Linalool).

The results show that the main volatile compounds found in *Scenedesmus obliquus* for the light cycle were 3-Methyl butanol compound (120.52 µg $mL^{-1}$), followed by 6-Methyl-5-hepten-2-one (75.18 µg $mL^{-1}$). Also known as isoamyl alcohol, 3-Methyl butanol is one of the main volatile compounds found in fermented foods and beverages, formed primarily by the amino acid leucine. Some phototrophic studies also recognized this compound as the major volatile product of *Phormidium autumnale* (SANTOS et al., 2016) and red algae *Palmaria*

*palmata* (LE PAPE et al., 2004), reaching concentrations of 142 μg mg $^{-1}$ and 0.96 μg kg $^{-1}$ (HOSOGLU, 2018). Already, for the dark cycle, the most relevant compounds were the alcohols 2-Ethyl hexanol, formed during the oxidative cleavage of carotenoids, and 1-Pentanol, with maximum yields of up to 126.63 and 95.97 μg mL$^{-1}$, respectively.

One of the most abundant volatile groups produced in the experiment was alcohols. Relatively high amounts of these compounds were found for cycles light and dark (218.07 and 360.19 μg mg$^{-1}$, respectively). Most of the alcohols formed are short chain linear compounds and have been confirmed in several microalgae studies in the identification of volatile organic compounds (OZAKI et al., 2008; VAN DURME et al., 2013). Most alcohols have a strong impact and pungent odor, which can make them substantial contributors to the microalga odor (HOSOGLU, 2018).

Ketones also showed relatively high yields (172.33 μg mg$^{-1}$ for light cycle, and 164.31 μg mg$^{-1}$ for dark cycle), followed by the aldehydes and terpenes groups with the productivity of 132.05 and 140.67 μg mg$^{-1}$ for the light and 123.65 and 74.65 μg mg$^{-1}$ for the dark, respectively. Ketones can be formed starting in be products of lipid oxidation or degradation and from the oxidative cleavage of carotenoids, such formation of a compound as 6-Methyl- 5-hepten-2-one. As expected, some aldehydes formed can provide several notes to food matrices depending on the number of carbon atoms and the degree of saturation. According to Kunjapur et al. (2014), the main barrier to overproduction of aldehydes in microorganisms is a rapid conversion of aldehydes desired in alcohols by numerous endogenous enzymes. Finally, terpenes are characterized by low odor threshold values which make them important aromatic compounds (VAN DURME et al., 2013, SANTOS et al., 2016).

Changes in light/dark cycles through generation time induce alterations in the metabolism and consequently on biochemical composition of microalgae. Figure 2 show the impact of the metabolic transformation as a function of time on the composition of volatile compounds obtained in the experiments.

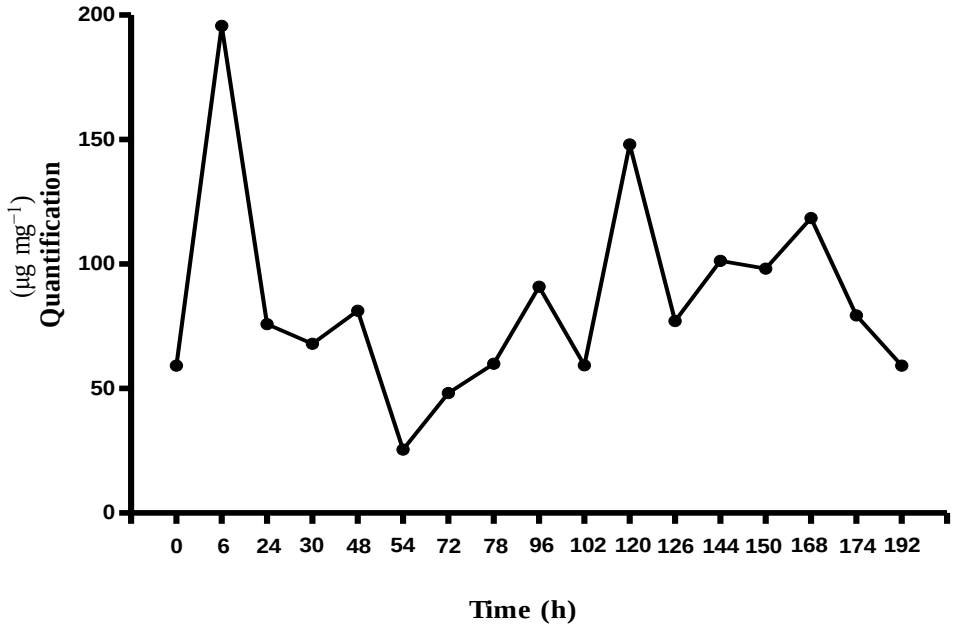


Figure 2. Quantification of all periods analyzed over time in light/dark cycles.

According to Figure 2 between 0 and 24 h of culture, there was a clear change in the volatile profile of *Scenedesmus obliquus*. Of the 37 compounds formed throughout the culture, 30 were formed only in this period. The terpenics compounds (Citronellol, Geranylacetone, and β-Citronellol) appear as the most relevant compounds in this period formed mainly in the first hours of cultivation when the first maximum luminous intensity is achieved. In fact, terpenes play a crucial role in the defense of microorganisms to biotic and abiotic challenges, such as protection of photosynthetic products and performance in response to thermal stress and/or oxidative stress often associated with high light intensities, probably due to stimulation of activity of the enzymes involved in the synthesis of VOCs (SPINELLI et al., 2011). These data are also confirmed by Achyuthan et al. (2017), which correlates the isoprenes and monoterpenes production when obtained at higher intensities of light under photoperiods of 14:10 (light:dark) by three diatomaceous strains (*Thalassiosira pseudonana*, *Thalassiosira weisflogii*, and *Chaetoceros neogracile*).

In particular, observed changes in the various levels of terpene compounds in microalgae are a demonstration of GPP (Geranyl pyrophosphate) pathways changes, often associated with pathways of defense of photosynthetic processes. Therefore, they not only demonstrate that these microorganisms exhibit a high tolerance to high light intensities, but also demonstrate that this strategy can be widely used to modulate in a controlled way the profile and important levels of VOCs from the industrial perspective (MACHADO et al., 2017).

Between 24 and 48 h of culture, it can be observed that the productivity remained constant until reaching minimum values of concentration in the residence time of 54 h under the light cycle. At this time, the productivity of VOCs has reduced dramatically and probably this behavior may be associated with the phenomena of photoinhibition under high illumination condition. This occurs when photosynthetic organisms are exposed to strong light and results in inhibition of the activity of photosystem II (MURATA et al., 2007). After this period the system presented a gradual increase in the productivity of COVs, which may be related to adaptation of the microalga to the cultivation process used.

On reaching the residence time of 120 h the yield under cycles in the dark reached 148.05 μg $mg^{-1}$, and the production of compounds such as alcohols, aldehydes, and ketones were higher at this stage. These VOCs increased with inhibition of light and then decreased to values close to those of the control (0h).

The high production of VOCs in the dark period may be related to stress due to lack of light (SILVA, 2017), and according to data by Maroneze et al. (2016) when the cultures of *Scenedesmus obliquus* were submitted to 12 hours of dark the low productivity and growth, when compared to periods with constant lighting, ended up affecting the photosynthetic metabolism of the microalga. These results suggest that algae are not able to store enough energy to use during the long, dark period. A consequence of the long dark period is that the basic carbon compounds, which have been synthesized by light, will be breathed for maintenance purposes and, consequently will produce larger amounts of volatile organic compounds.

### 3.2 Exploratory multivariate analysis

Principal component analysis (PCA) was used to better visualize the light/dark cycles against the production of VOCs separated by groups. Figures 3a and 3b show the scores (samples over time) and loadings (volatile groups), respectively, of the two main components. Together, main component 1 (PC1) and main component 2 (PC2) explained 80.58% of the global variance. This analysis differentiated the samples light/dark cycles based on the four volatile groups identified in the experiment.

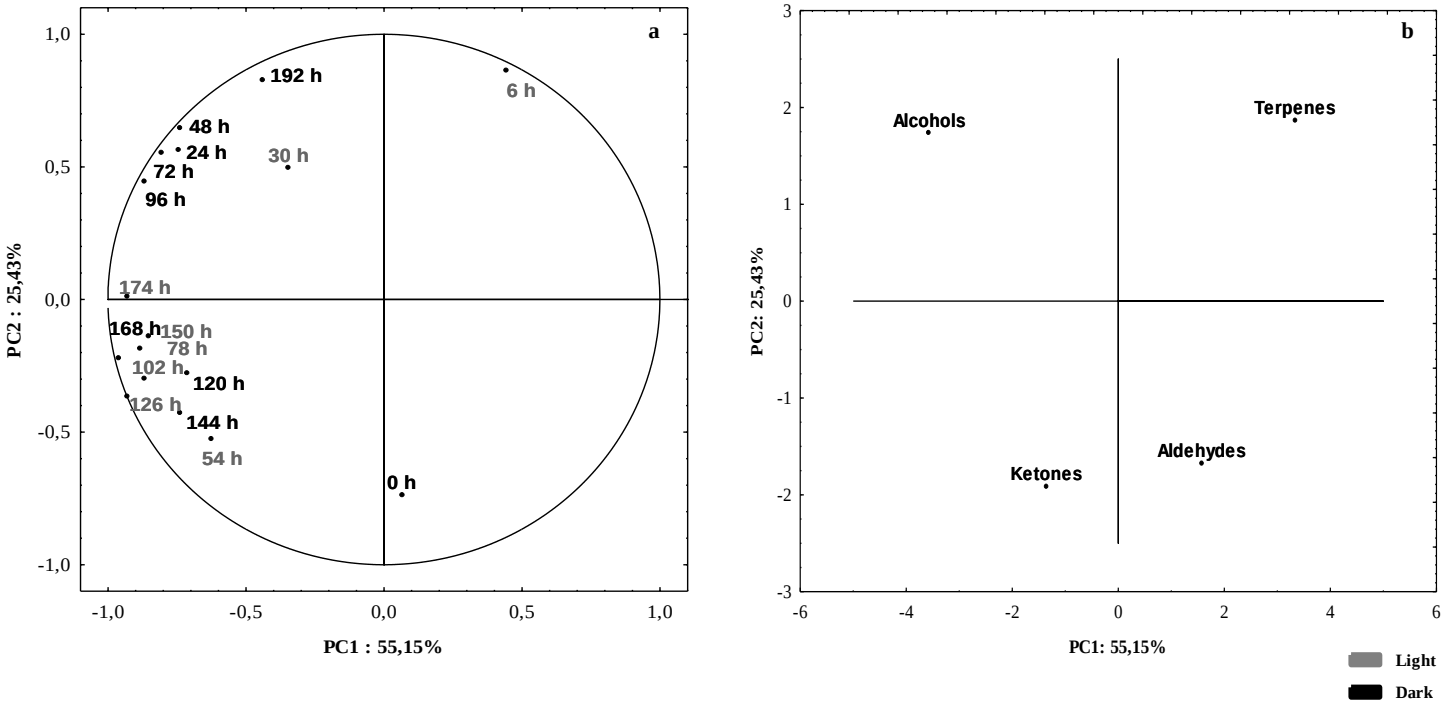


Figure 3. Principal component analysis of the volatile groups found for *Scenedesmus obliquus* in the different cycles of light/dark.

As shown in PC 1, the right quadrant of the volatile groups terpenes and aldehydes could be separated into a group due to their high concentration in the first hours of analysis (between 0 and 6h). It is also verified that the group of terpenes were higher correlated to the time of 6 h of the experiment to the light cycle mainly because it is close to the reference circle. It is referenced that the closer to the circle and further from the origin, the greater the influence of the point in the analysis.

On the left side, alcohols and ketones presented the highest concentrations of volatiles throughout the experiment time. It is also observed that ketones were correlated mainly to light cycles (except in 120, 144 and 168 h), while alcohols were correlated mainly through the formation of compounds in the dark at time 24, 48, 72, 96, and 192 h.

Based on the data already presented, the PCA confirms that the formation of terpenes had its peak in the first hours of the experiment when it reached its first point of high luminosity. After this period, the formation of terpenes declined, as the formation of the other volatile groups increased over time, especially in the dark cycles, until reaching their final decline of production. With this, it is possible to verify the formed compounds against different culture conditions through the light/dark cycles and select those conditions that are most favorable for the production of VOCs.

## 4. Conclusion

The cultivation analysis of *Scenedesmus obliquus* through the proposed conditions of simulation in the summer the tropical climate of Brazil allowed to produce a significant variety of interesting volatile organic compounds and helped to elucidate the influence of the light/dark cycles in the production of these compounds. It appears that for each light/dark cycle over the time of the experiment, these metabolites are at least metabolically, quite distinct. The intensification of research in this area can add to understanding the different ways of applying light/dark cycles and determining the most suitable process for the production of precursors or bioproducts of interest.

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# CONCLUSÃO GERAL

O perfil de compostos orgânico voláteis por *Scenedesmus obliquus* em diferentes climas e estações do ano de regiões extremas do Brasil, apresentou um total combinado de 47 compostos identificado em todas as amostras estudadas. O clima subtropical foi o que apresentou a maior abundância de área total no verão, seguido pelos climas tropical, tropical Atlântico e equatorial, enquanto no inverno o tropical Atlântico apresentou maior área, seguido do clima tropical, subtropical e equatorial. Os hidrocarbonetos foram identificados como os COVs mais abundantes em termos de composição percentual. Esta classe química apresentou um crescimento relativo em seu conteúdo total ao longo dos tratamentos e foi o principal biomarcador do clima subtropical, atingindo um rendimento de 80,46% e 89,25% no verão e inverno, respectivamente. Em seguida, o grupo cetona atingiu um percentual maior de COVs no clima equatorial no verão, com um percentual de 51,29%, mas diminuiu drasticamente no inverno, obtendo cerca de apenas 18,95%.

De fato, o impacto das condições de estresse tem sido documentado no nível genético e proteico e sua reflexão é relatada na alteração do pool de metabólitos secundários em microrganismos afetados. Consequentemente, as diferenças observadas entre as amostras de verão e inverno podem ser atribuídas às condições ambientais, que variam consideravelmente com a estação durante o crescimento, o que logicamente dá origem à produção alterada de COVs.

Ao analisar a influência dos ciclos claro e escuro na produção de COVs no clima tropical do verão, os compostos terpênicos aparecem como os compostos mais relevantes neste período formados principalmente nas primeiras horas de cultivo, quando a primeira intensidade luminosa máxima é alcançada. De fato, os terpenos desempenham um papel crucial na defesa de microrganismos, como proteção de produtos fotossintéticos e desempenho em resposta a estresse térmico e/ou estresse oxidativo frequentemente associado a intensidades de luz elevadas.

Ao atingir o tempo de permanência de 120 h, o rendimento sob ciclos no escuro atingiu 148,05 $\mu g\ mg^{-1}$, e a produção de compostos como álcoois, aldeídos e cetonas foi maior neste estágio. A alta produção de COVs no período escuro pode estar relacionada ao estresse devido à falta de luz, o que logicamente pode estar relacionada a produção de COVs nessas condições.

Os resultados indicaram que a fração de compostos orgânicos voláteis de cultivos fotoautotróficos de *Scenedesmus obliquus* apresentaram um expressivo potencial de aplicação em diferentes condições de cultivos e colaboraram tanto para elucidação metabólica destes

compostos, como a identificação de uma fração de biocompostos microalgais com grande potencial de exploração comercial.