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DINÂMICA DE COMPOSTOS ORGÂNICOS VOLÁTEIS A PARTIR DE BIOPROCESSOS MICROALGAIS

Santa Maria, RS
2021

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

Orientador (a): Profª. Drª. Leila Queiroz Zepka

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Karem Rodrigues Vieira

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Aprovado em 16 de novembro de 2021

Leila Queiroz Zepka, Drª.(UFSM)
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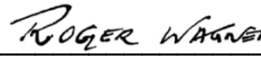

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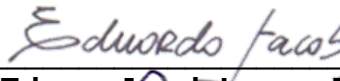

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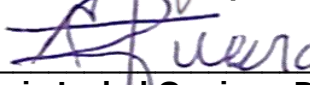

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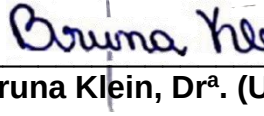

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"*O vento sopra onde quer, e ouves a sua voz, mas não sabes de onde vem, nem para onde vai. Assim é todo aquele que é nascido do Espírito".*

(*Jo. 3:8 – Bíblia Sagrada*)

RESUMO

DINÂMICA DE COMPOSTOS ORGÂNICOS VOLÁTEIS A PARTIR DE BIOPROCESSOS MICROALGAIS

AUTORA: Karem Rodrigues Vieira
ORIENTADORA: Leila Queiroz Zepka

A versatilidade metabólica das microalgas oportuniza soluções para desafios tecnológicos da indústria de alimentos. Questões que parecem controversas, como a remoção do odor desagradável de estações de tratamento de águas residuárias, e a produção de compostos de aroma desejáveis é uma hipótese possível em processos utilizando microalgas, porém ainda pouco explorada. Em face disso, o objetivo desta pesquisa foi avaliar a dinâmica dos compostos orgânicos voláteis (COVs) durante experimentos realizados com efluentes agroindustriais, bem como potencial de produção de compostos voláteis de interesse comercial. O trabalho foi dividido em duas etapas: Na primeira, foi avaliada a eficiência dos sistemas baseados em microalgas na desodorização de água residuária do processamento de carnes e a bioconversão destes compostos em voláteis de interesse industrial. Esta etapa deu origem ao artigo intitulado "*The role of microalgae-based systems in the dynamics of the odors compounds in the meat processing industry*". Esta pesquisa apresentou remoção de 99,6% dos COVs desagradáveis da água residuária em 72 horas de tempo de residência, e concomitantemente 15 compostos voláteis foram formados no decorrer do processo. A segunda etapa do trabalho foi realizado com o intuito de comprovar a eficiência de remoção dos compostos desagradáveis da água residuária através de análise cromatográfica olfatométrica. Os resultados originou um segundo artigo intitulado "*The role of microalgae-based systems in the dynamics of odorous compounds in the meat processing industry. Part II - Olfactometry and sensory relevance*". Os resultados mostraram que os compostos indol e escatol, sendo estes os principais marcadores de odor da água residuária, não apresentaram percepção sensorial após 72 horas de tempo de residência, sugerindo que foram completamente removidos. Ao mesmo tempo, um total de 11 compostos formados foram perceptíveis e classificados como frutados, cítricos, verdes e resinosos pelos jurados. Além disso, foi realizado uma revisão a respeito dos COVs das microalgas, abordando tópicos sobre, biossíntese dos voláteis microalgais, fatores que afetam na formação de compostos voláteis, aplicação dos compostos voláteis e técnicas de recuperação dos COVs e publicado em forma de capítulo de livro, initulado "*Volatile organic compounds from microalgae*". Assim, foi concluído que sistemas baseados em microalgas são capazes de mitigar os odores mais desagradáveis da água residuária do processamento de carnes, além de gerar uma variedade de compostos voláteis de interesse comercial, sugerindo a possibilidade de explorá-los para aplicação na indústria de química fina ou alimentícia.

Palavras-chave: Microalgas/cianobactérias, água residuária, análises cromatográficas, olfatometria, desodorização, bioprodutos

ABSTRACT

DYNAMICS OF VOLATILE ORGANIC COMPOUNDS FROM MICROALGAL BIOPROCESSES

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SUPERVISOR: Leila Queiroz Zepka

The metabolic versatility of microalgae provides solutions to technological challenges in the food industry. Issues that sound controversial, such as the removal of unpleasant odor from wastewater treatment plants, and the production of desirable aroma compounds is a possible hypothesis in processes using microalgae, but still little explored. In view of this, the objective of this research was to evaluate the dynamics of volatile organic compounds (VOCs) during experiments carried out with agro-industrial effluents, as well as the potential production of volatile compounds of commercial interest. The work was divided into two stages: In the first, the efficiency of microalgae-based systems in the deodorization of wastewater from meat processing and the bioconversion of these compounds into volatiles of industrial interest was evaluated. This step gave source to the article entitled: "*The role of microalgae-based systems in the dynamics of the odors compounds in the meat processing industry*". This research showed the removal of 99.6% of unpleasant VOCs from wastewater in 72 hours of residence time, and concomitantly 15 volatile compounds were formed during the process. The second stage of the work was carried out in order to prove the efficiency of removing unpleasant compounds from wastewater through chromatographic olfactory analysis. The results generated a second article entitled: "*The role of microalgae-based systems in the dynamics of odorous compounds in the meat processing industry. Part II - Olfactometry and sensory relevance*". The results showed that the compounds indole and skatol, which are the main odor markers in wastewater, did not present sensory perception after 72 hours of residence time, suggesting that they were completely removed. At the same time, a total of 11 compounds formed were perceptible and classified as fruity, citrus, green, and resinous by the judges. In addition, a review of microalgal VOCs was carried out, covering topics on microalgal volatile biosynthesis, factors that affect the formation of volatile compounds, application of volatile compounds, and VOC recovery techniques and published as a book chapter, entitled "*Volatile organic compounds from microalgae*". Thus, it was concluded that microalgae-based systems are capable to mitigate the most unpleasant odors of wastewater from meat processing, in addition to generating a variety of volatile compounds of commercial interest, suggesting the possibility of exploring them for application in fine chemical or food industry.

Keywords: Microalgae/cyanobacteria, wastewater, chromatographic analysis, olfactometry, deodorization, bioproducts.

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INTRODUÇÃO

1. INTRODUÇÃO

A indústria frigorífica e os matadouros representam um impacto ambiental significativo devido à descarga de efluente com elevada concentração de matéria orgânica nos corpos d'água, além disso são responsáveis por incômodos olfativos, gerados por diferentes processos químicos ou biológicos durante o tratamento da água residuária. Os odores são formados principalmente por compostos orgânicos voláteis (COVs) como aldeídos, cetonas, ácidos orgânicos, além de aminas e sulfurados (SADDOUD, A. & SAYADI, 2007; LEBRERO et al., 2013; LEWKOWSKA et al., 2016).

Compostos orgânicos voláteis são definidos como qualquer composto orgânico cujo ponto de ebulição está na faixa de (50-260 °C), correspondendo a pressões de vapor de saturação maiores que 102 kPa a 25 °C (ISO16000-6, 1989). Uma variedade de COVs, como acroleína, acetofenona e fenóis, são tóxicos, sendo a maioria proveniente de atividades antropogênicas oriundos de emissões veiculares, indústrias petroquímicas e indústrias de manufaturas (BERENJIAN et al., 2012; EPA, 2012). A exposição humana a COVs de origem antropogênica pode resultar em um espectro de doenças que variam de leves, como irritação, a efeitos muito graves, incluindo câncer (POVEDA, 2021).

Além disso, COVs são conhecidos por seus odores variados que vai do agradável ao mais intenso e fétido. O odor é certamente o mais complexo de todos os problemas de poluição do ar. A poluição por odor contribui para a formação fotoquímica de fumaça, assim como emissões secundárias de contaminantes. Portanto, é uma ameaça à saúde e bem-estar humano e à qualidade do ar (CAPELLI et al., 2009; BAJPAI, 2014; LEWKOWSKA et al., 2016).

Diferentes métodos para o tratamento de COVs são utilizados nas estações de tratamento de água residuária. Dentre eles estão os processos físico, químico e o tratamento biológico (DOMENO et al., 2010). Métodos biológicos para a remoção de odores e COVs são tecnologias econômicas quando se trata de baixas concentrações, no entanto, têm sido relatadas por suas desvantagens em relação a mitigação dos poluentes hidrofóbicos, além do crescimento excessivo do biofilme e desempenho instável devido às mudanças da comunidade microbiana (BURGESS et al., 2001; MUÑOZ et al., 2015; CHENG et al., 2021).

A biotecnologia de microalgas é uma área emergente da tecnologia industrial, que vem se consolidando em função da sua potencialidade de exploração. As microalgas são consideradas uma fonte potencialmente nova e valiosa de compostos biologicamente ativos (LAURITANO et al., 2018). Além disso, o uso de microalgas desempenha um papel vital na conversão de resíduos em uma infinidade de produtos, por exemplo, biocombustíveis, nutracêuticos, polímeros, pigmentos, uma variedade de produtos químicos e COVs. Microalgas também apresentam potencial em transformar gases de efeito estufa industriais, bem como águas residuais em produtos úteis, servindo assim como uma plataforma eficaz de captura e utilização de carbono (WANG et al., 2017; JACOB-LOPES et al., 2020).

Embora existe uma variedade de usos comerciais para microalgas, não se sabe muito sobre a aplicação de sistemas baseados em microalgas para remoção de poluição de odores e a bioconversão potencial de produtos de valor agregado usando substratos de resíduos de odores. Em face disso, o objetivo desta tese foi avaliar a dinâmica de compostos odoríferos na indústria de processamento de carnes a partir de processos à base de microalgas. Num primeiro momento, o estudo se concentrou na caracterização do perfil odorante de águas residuais brutas, desodorização de compostos voláteis e formação de COVs de interesse industrial. A segunda parte do trabalho foi realizado a caracterização do perfil olfatométrico odorante de águas residuais brutas, avaliação sensorial do processo de desodorização e avaliação sensorial de compostos orgânicos voláteis de alto valor gerados pela microalga.

1.1. OBJETIVOS

1.1.1 Objetivo geral

Avaliar a dinâmica dos compostos voláteis em efluentes agroindustriais, bem como o potencial de produção de COVs de interesse comercial, a partir de bioprocessos microalgais.

1.1.2 Objetivos específicos

Primeira etapa do estudo:

- Identificar e quantificar os compostos orgânicos voláteis na água residuária bruta;
- Identificar e quantificar os compostos orgânicos voláteis do cultivo heterotrófico suplementado com água residuária;
- Avaliar a capacidade de remoção dos compostos de odor;
- Estabelecer o potencial de exploração comercial dos bioprodutos formados.

Segunda etapa do estudo:

- Avaliar olfatometricamente o perfil volátil da água residuária bruta;
- Avaliar olfatometricamente o perfil volátil do processo de desodorização microalgal;
- Realizar um estudo da relevância sensorial dos compostos orgânicos voláteis formados durante o bioprocesso microalgal.

CAPÍTULO 3

REVISÃO BIBLIOGRÁFICA

2. REVISÃO BIBLIOGRÁFICA

2.1 Microalgas

Microalgas são um grupo de microrganismos fotossintéticos tipicamente unicelulares e eucarióticos. Embora as cianobactérias pertençam ao domínio das bactérias e sejam procariontes fotossintéticos, são frequentemente consideradas microalgas. Desenvolvem-se principalmente em ambientes aquáticos (água doce e salgada), estão presentes em solos, rochas, e em ambientes extremos, como geleiras e fossas termais, bem como, associados simbioticamente a outros organismos (líquens, pteridófitas, protozoários) auxiliando-os na fixação de nitrogênio (HERRERO et al., 2001; LOURENÇO, 2006; SANTOS et al., 2017).

A presença desses organismos em ambientes tão diversos deve-se ao seu metabolismo que podem explicar sua capacidade em responder rapidamente a alterações no meio onde vivem (ACHYUTHAN et al., 2017). A diversidade deste grupo de microrganismos é destacada por serem responsáveis pela estruturação da atmosfera terrestre, por sua importância ecológica e econômica, sendo que as algas são as maiores removedoras de carbono da biosfera (MOORE, 2001). Algumas cianobactérias podem ser fixadoras de nitrogênio atmosférico. Estas cianobactérias são caracterizadas por formarem células especializadas, denominadas heterócitos que se diferenciam quando há baixa concentração de compostos nitrogenados do meio (HERRERO et al., 2001).

Considerando a grande biodiversidade e com o avanço tecnológico na engenharia genética, as cianobactérias representam uma das fontes mais promissoras para novos bioprodutos, como proteínas, amido, celulose, lipídeos, aminoácidos, antioxidantes, carotenoides, glicerol, ácidos graxos poli-insaturados, esteróis, vitaminas B, C e E e compostos orgânicos voláteis (RODRIGUES et al., 2014; SATHASIVAM et al., 2017; DURME et al., 2013; SANTOS et al., 2016a; HOSOGLU, 2018).

Phormidium é um género de algas de uma única célula azul-verde, pertencentes às cianobactérias. É filamentoso, não ramificado e possui cerca de 3 a 4 µm de diâmetro. *Phormidium autumnale*, é uma cianobactéria robusta que pode crescer em diferentes temperaturas ambientais, tolera altas concentrações de nutrientes e tem altas taxas de crescimento celular, tornando-se facilmente adaptável

a culturas. Além disso, tem a capacidade de operar com dois meios metabólicos, heterotrófico e fotoautotrófico, variações na cultura podem levar a diversos metabólitos (FRANCISCO et al., 2014; MARONEZE et al., 2014; FAGUNDES et al., 2019).

Metabolicamente, a fotossíntese é a rota energética preferida de microalgas. O processo fotossintético em microalgas e cianobactérias ocorre nos cloroplastos e tilacóides (localizados no citoplasma), respectivamente. Este mecanismo envolve um metabolismo complexo e pode ser subdividido em dois estágios: (i) a fotoquímica (ou reações de luz) e (ii) a bioconversão de carbono (reações no escuro). Normalmente, as microalgas usam energia luminosa para gerar equivalentes redutores e incorporar CO_2 em moléculas orgânicas (CALVIN & BENSON 1948; SUGANYA et al., 2016; SEVERO et al., 2020).

As microalgas podem se adaptar a diferentes concentrações de carborno. Portanto, existem mecanismos de bioconversão do carbono inorgânico que envolvem muitas reações bioquímicas nesses processos biológicos que darão origem aos COVs.

2.2 Metabolismo microalgal

Metabolicamente, as espécies de microalgas têm três vias de fixação de carbono: (i) fotoautotrófica, (ii) heterotrófica ou (iii) mixotrófica (PEREZ-GARCIA & BASHAN, 2015; SANTOS et al., 2016a). Destas, a via fotoautotrófica é a principal rota energética dos microrganismos relacionados (SUGANYA et al., 2016; SEVERO et al., 2019a). Este mecanismo envolve o uso de carbono inorgânico (CO_2) ou íons bicarbonato HCO_3^- dissolvido em meio aquoso (de acordo com pH: CO_2 (pH <5); HCO_3^- (7 <pH <9)) como fonte de carbono na presença de luz, principalmente regulado por carbono do metabolismo fotossintético e mecanismos de concentração (KONG et al., 2021).

Em geral, o metabolismo fotossintético do carbono microalgal ocorre através do ciclo Calvin-Benson-Bassham. Portanto, as microalgas usam energia luminosa para gerar equivalentes redutores e fixar CO_2 em moléculas orgânicas (por meio das reações dependentes e independentes de luz) (CALVIN & BENSON 1948; SEVERO et al., 2019a; SU, 2021). O ciclo de Calvin é composto por 13 etapas catalisadas por cerca de 11 enzimas diferentes e subdivididas em 3 reações: (i) carboxilação, (ii)

redução e (iii) regeneração (NOREÑA-CARO & BENTON, 2018; SEVERO et al., 2020).

Como alternativa à condição de baixa concentração de CO_2 viabilizando a fotossíntese, a maioria das microalgas tem diferentes mecanismos de concentração de CO_2: como assimilação de íons HCO_3^- por meio de transportadores ativos na membrana plasmática; e usando a enzima anidrase carbônica extracelular para conversão aumentada de HCO_3^- em CO_2 intracelular (KONG et al., 2021).

Por outro lado, algumas espécies de microalgas também podem crescer heterotroficamente na ausência de luz, suportadas por uma fonte de carbono exógena. No metabolismo heterotrófico, o substrato é convertido em glicose 6-fosfato para que possa iniciar a via oxidativa da pentose fosfato. Durante o metabolismo, ocorre a formação de duas moléculas de ATP (trifosfato de adenosina). O produto final, assim como no cultivo fotossintético, também é o piruvato (SANTOS et al., 2016b; PINHEIRO et al., 2019).

Além disso, algumas espécies de microalgas são mixotróficas e podem causar fototrofia e heterotrofia simultaneamente. Isso porque, o CO_2 é fixado pela fotossíntese, enquanto os substratos orgânicos são assimilados pela respiração aeróbia (PEREZ-GARCIA & BASHAN 2015; PINHEIRO et al., 2019).

2.3 Mecanismo de biossíntese de compostos orgânicos voláteis em microalgas

A bioconversão de diferentes formas de carbono metaboliza o piruvato ou acetil-CoA. Além disso, as vias biossintéticas e metabólicas das microalgas podem converter esses substratos em COVs como terpenos, álcoois, cetonas, aldeídos, ésteres, hidrocarbonetos, ácidos carboxílicos e compostos sulfurados. Em geral, a produção desses compostos é alcançada por meio das vias de 2-cetoácidos, isoprenóides e derivados de ácidos graxos (ZARGAR et al., 2017; SEVERO et al., 2019b; VIEIRA, PINHEIRO & ZEPKA, 2020; JACOB-LOPES et al., 2020).

A via dos 2-cetoácidos é uma rota importante para a obtenção de compostos voláteis de diferentes classes químicas, como aldeídos, álcoois, ésteres e ácidos carboxílicos. Esta via envolve reações bioquímicas sequenciais, como extensão, descarboxilação, isomerização, redução, desidratação e esterificação de alguns aminoácidos de cadeia ramificada (como, leucina e valina). Os compostos 1-butanol,

3-metil-butanal e 2-metil-butanal são reduzidos a 3-metil-butanol e 2-metil-butanol, além disso a reação pode ser estendida para formar 1-hexanol e outros álcoois (LIAO et al., 2016; VIEIRA, PINHEIRO & ZEPKA, 2020).

Até o momento, três vias diferentes foram relatadas para sintetizar os isoprenóides: o ácido mevalônico (MVA); fosfato de metileritritol (MEP); e MVA modificado. No entanto, para espécies de microalgas, apenas as vias MVA / MEP foram descritas, ou ambas as vias em combinação (PINHEIRO et al., 2019; VIEIRA, PINHEIRO & ZEPKA, 2020).

O isopentenil difosfato e o dimetilalil difosfato são os intermediários centrais do mecanismo dos isoprenóides. Em sequência, essas estruturas iniciais são transformadas em geranil difosfato seguido de farnesil difosfato (MEENA et al., 2017; PINHEIRO et al., 2019). Posteriormente, esses precursores de carbono são convertidos em terpenóides diversificados, por meio de uma série de reações catalisadas por três enzimas distintas: geranil difosfato sintase, farnesil difosfato sintase e geranilgeranil difosfato sintase, respectivamente. Finalmente, os carotenóides e seus produtos de clivagem oxidativa e enzimática são formados, como β-ionona e 6-metil-5-hepten-2-ona (DUDAREVA et al., 2013; SANTOS et al., 2016b).

Além disso, através do geranil difosfato como substrato de partida, são produzidos a geosmina e o 2-metilisoborneol (2-MIB), no qual foram amplamente estudados devido a indesejáveis manifestações de sabor e odor. A síntese de 2-metilisoborneol, inicia com a metilação do precursor geranil difosfato em 2-metilgeranil difosfato que é ciclizado em 2-MIB (LEE et al., 2017). Em microalgas, a ciclização de farnesil difosfato pode formar geosmina, catalisada pela geosmina sintase por meio de três etapas (farnesil difosfato para germacradienol, germacradienol para 8,10-dimetil-1-octalina e 8,10-dimetil-1-octalina para geosmina) (DURME et al., 2013; LIATO & AÏDER, 2017; MEENA et al., 2017).

A via dos ácidos graxos começa com acetil-CoA usando malonil-CoA como bloco de construção, com base em uma série de reações cíclicas catalisadas pelo sistema multienzimático, denominado ácido graxo sintase. Compostos orgânicos voláteis como cetonas, aldeídos, hidrocarbonetos e álcoois podem ser produzidos a partir da degradação de ácidos graxos (SANTOS et al., 2016a; KERKHOVEN & NIELSEN, 2018).

Cetonas estruturalmente diversificadas são produtos metabólicos de ácidos graxos precursores (PINHEIRO et al., 2019; VIEIRA, PINHEIRO E ZEPKA, 2020).

Exemplos recentes incluem a produção de 2-heptanona a partir da oxidação do ácido linoléico (HAN et al., 2019).

Os aldeídos produzidos pela via dos ácidos graxos são aldeídos C6 e C9 que podem ser rapidamente metabolizados em álcoois por meio da enzima desidrogenase. Por exemplo, os ácidos graxos linoléico e ácido linolênico são conhecidos por serem precursores biossintéticos para 2,4-decadienal, 2-heptanol, 2-octenal e 1-hexanal, que podem ser subsequentemente reduzidos a álcoois como 1-hexanol (BRAVO-LAMAS et al., 2018; JERKOVIĆ et al., 2018; PINHEIRO et al., 2019; VIEIRA, PINHEIRO & ZEPKA, 2020).

A conversão do ácido graxo em hidrocarboneto ocorre usando aldeídos como substratos. Pelo menos duas enzimas, redutase de proteína transportadora acil-acil e oxigenase deformiladora de aldeído, são responsáveis por catalisar a reação (PINHEIRO et al., 2019; VIEIRA, PINHEIRO & ZEPKA, 2020; BASRI et al., 2020).

As microalgas também liberam compostos de enxofre, como dimetilsulfeto, dimetildissulfeto e dimetiltrissulfeto (ACHYUTHAN et al., 2017; WATSON & JÜTTNER, 2017), sendo que o sulfeto volátil mais importante produzido é dimetilsulfeto (WATSON & JÜTTNER, 2017). Esses compostos podem ser derivados de aminoácidos, como a metionina, formando dimetilsulfoniopropionato (GIORDANO & PRIORETTI, 2016). A partir da desmetiolação do dimetilsulfoniopropionato, ele forma metanotiol que pode ser convertido em dimetilsulfeto por metilação (ACHYUTHAN et al., 2017; CURSON et al., 2017).

2.4 Fatores ambientais que afetam a produção de COVs a partir de microalgas

Os compostos de odor desejáveis e indesejáveis estão presente no meio ambiente, decorrentes de processos naturais e artificiais, pela geração de COVs. Os compostos orgânicos voláteis são os principais bioprodutos formados durante o cultivo de microalgas (JACOB-LOPES & FRANCO, 2013). Considerando a biossíntese de COVs pelas microalgas, embora dependente da espécie, sua produção pode ser modificada por vários fatores bióticos e abióticos, como fase de crescimento, estresses (temperatura, intensidade de luz, pH, salinidade), nutrientes, gases (H_2O, CO_2, O_3), aeração (mistura / turbulência) ou cultura estática (MILOVANOVIC et al., 2015; DURME et al., 2013; AYCHUCHAN et al. 2017).

Diferentes sistemas de cultivo microalgal também interferem na produção de COVs. No cultivo fotoautotrófico as microalgas absorvem energia luminosa e bioconvertem CO_2 biotranformando-os em COVs (PEREZ-GARCIA et al., 2011; PEREZ-GARCIA & BASHAN, 2015; CLAASSENS et al., 2016; GONG et al., 2018). No cultivo heterotrófico, os substratos orgânicos são assimilados por meio da respiração aeróbia. Com fontes de carbono exógeno como glicose, frutose e sacarose as microalgas demonstraram um perfil variável de compostos voláteis (FRANCISCO et al., 2014; PEREZ-GARCIA & BASHAN, 2015; SANTOS et al., 2016b; SANTOS et al., 2018). O cultivo mixotrófico pode aumentar a produtividade da biomassa e consequentemente a formação de compostos voláteis (SANTOS et al., 2018; VIEIRA, PINHEIRO & ZEPKA, 2020).

A variedade na concentração de nutrientes, como fósforo e nitrogênio, podem afetar o metabolismo secundário das microalgas, influenciando na emissão de COVs desses microrganismos (ZUO et al., 2019; VIEIRA, PINHEIRO & ZEPKA, 2020). Nitrato de sódio ($NaNO_3$), nitrito de sódio ($NaNO_2$), cloreto de amônio (NH_4Cl) e alguns aminoácidos (serina, lisina e arginina), influenciam na produção de diferentes classes químicas de compostos voláteis gerando compostos de enxofre, terpenóides, hidrocarbonetos, aldeídos e ésteres (ZUO et al., 2018; XU et al., 2017).

A incidência de luz influencia na emissão de terpenóides, como isopreno e monoterpenos (LIAO et al., 2016). Altas temperaturas causam a degradação oxidativa dos ácidos graxos, que promovem a emissão de álcoois, aldeídos, hidrocarbonetos. Além disso, compostos derivados de carotenóides podem ser formados como β-ciclocitral, α-ionona, β-ionona e geranilacetona (JÜTTNER, 1984; GARCÍA-PLAZAOLA et al., 2017). Temperaturas entre 20 °C e 30 °C são as mais favoráveis para a emissão de COVs em sistemas baseados em microalgas como já demonstrado em pesquisas publicadas (SANTOS et al., 2016b; SANTOS et al., 2018; HOSOGLU, 2018).

A fase de crescimento das microalgas também podem afetar a formação de compostos voláteis desses microrganismos. Compostos voláteis como aldeídos e álcoois podem surgir, em diferentes espécies de microalgas cultivadas em fotoautotrofia. Alcanos apresentam maior concentração durante a fase exponencial, porém reduzem a partir da fase estacionária. Cetonas tendem a surgir da fase exponencial para a estacionária (ZHOU et al., 2017).

Nos cultivos heterotróficos, utilizando glicose como substrato, podem influenciar na maior concentração de compostos de enxofre durante a fase exponencial, reduzindo nas fases seguintes. Compostos como álcoois, aldeídos e ésteres podem apresentar maior concentração na fase estacionária (HOSOGLU, KARAGUL-YUCEER & GUNESER., 2020).

Independente do tipo de metabolismo, fotossintético ou heterotrófico, a biossíntese de compostos orgânicos voláteis ocorre por meio da formação da molécula de piruvato e com as condições de crescimento de microalgas controladas e adequadas, esses microrganismos têm a capacidade de produzir compostos voláteis desejáveis com um limiar de percepção agradável (SANTOS et al. 2016a; HOSOGLU, 2018; HOSOGLU et al., 2020.) Portanto, compreender as condições do cultivo de microalgas pode fornecer uma melhor estrutura para a produção de COVs com potencial industrial.

2.5 Aplicação Industrial de compostos orgânicos voláteis de microalgas

Produtos à base de microalgas têm ganhado atenção nos setores acadêmico e industrial. O valor de mercado global de produtos à base de microalgas é estimado em cerca de US$ 6,5 bilhões, dos quais cerca de US$ 2,5 bilhões são gerados pelo setor de alimentos saudáveis, US $ 1,5 bilhão para a produção de ácido docosahexaenóico e US$ 700 milhões para a aquicultura (MOBIN & ALAM, 2017; DEPRÁ et al., 2019). As microalgas apresentam potencial aplicação industrial nos setores químico, petroquímico, alimentício e farmacêutico (JACOB-LOPES & FRANCO 2013; CLAASSENS et al., 2016; GONG et al., 2018).

As microalgas produzem uma variedade de COVs que podem ser aplicados como uma importante fonte alternativa de produtos químicos a granel e finos (SANTOS et al., 2016b). Os compostos propanol, butanol, 3-metil-butanol, hexanol, hexanal, β-ciclocitral e β-ionona produzidos por microalgas possuem um apelo comercial (SANTOS et al. 2016b). Berger (2009), relatou que compostos de aromas de microrganismos podem competir com fontes tradicionais. A triagem de superprodutores, a elucidação das vias metabólicas e precursores e a aplicação da bioengenharia convencional resultou em um conjunto de mais de 100 aromas químicos derivados da biotecnologia.

O mercado global de compostos aromáticos foi avaliado em US$ 5,5 bilhões em 2019 e deve crescer a uma taxa composta de crescimento anual de 5,8% atingindo US$ 8,2 bilhões em 2027, sendo os terpenos a classe de compostos predominante neste mercado (GLOBAL AROMA CHEMICALS MARKET, 2021). Outras classes também de grande interesse são os álcoois e os aldeídos, sendo importantes componentes do aroma amplamente aplicados em cosméticos, perfumaria e indústrias alimentícias (SANTOS et al. 2016b; VIEIRA, PINHEIRO & ZEPKA, 2020).

A identificação detalhada de tais compostos é muito importante devido aos seus impactos diretos nas propriedades aromáticas do produto final enriquecido com biomassa de microalgas (ROBERTSON et al., 2016; SANTOS et al., 2016a; ZHOU et al., 2017). Segundo Hosoglu (2018), compostos identificados como sendo responsáveis por tais características aromáticas são diferenciados em categorias tais como hidrocarbonetos, aldeídos, álcoois, ésteres, cetonas, lactonas, ácidos graxos livres de cadeia curta a média, compostos fenólicos e enxofre.

A utilização da fração volátil da biomassa de microalgas pode representar uma melhoria na oferta para diferentes tipos de indústria. As microalgas são bem conhecidas pela capacidade de produção futura de biodiesel por apresentarem alta produtividade, biossíntese lipídica eficiente e praticamente não competem com as terras agricultáveis para a produção de alimentos (JACOB-LOPES, ZEPKA & QUEIROZ, 2018). Também possuem robustez para capturar gases de efeito estufa, principalmente o CO_2 atmosférico, e bioconverter em múltiplos bioprodutos. Esses excedentes provavelmente estarão disponíveis a um custo mínimo ou nenhum custo, o que favorece a abordagem da biorrefinaria de microalgas (DEPRÁ et al., 2018).

Combustíveis gasosos, como biohidrogênio e biometano, podem ser produzidos por meio de um sistema baseado em microalgas, com o uso de fermentação e digestão anaeróbia, respectivamente. Esses biocombustíveis são os mais eficientes em termos de ganho líquido de energia entre todas as tecnologias de conversão de biocombustíveis (DEPRÁ et al., 2018; LIN et al., 2019). Biohidrogênio é o biocombustível com maior teor de energia em comparação com outros combustíveis (142 MJ kg^{-1}) e pode ser usado em células de combustão para produzir eletricidade com alta eficiência (BUX & CHISTI, 2016; LIN et al., 2019).

Os compostos orgânicos voláteis são considerados bioprodutos gasosos do metabolismo microalgal, podendo ser recuperados na forma de gases de exaustão. Esses sistemas podem produzir COVs com potencial energético. O potencial de

energia dos COVs produzidos no biorreator heterotrófico pode variar de 3,48 × 10^9 a 8,67 × 10^9 MJ kg^{-1}, totalizando conteúdo de energia de 1,22 × 10^{13} MJ kg^{-1}. Além disso, a taxa de geração de energia pode chegar a 1,01 × 10^{12} MJ. m^{-3}. d^{-1} sob essas condições de cultivo (SEVERO et al., 2019a; JACOB-LOPES et al., 2020).

Considerando as estruturas químicas deste compostos, alguns álcoois apresentaram potencial energético comparável ao da gasolina (SANTOS et al., 2016b; PINHEIRO et al., 2019). Além disso, Halfmann, Gu e Zhou (2014), demonstraram que os compostos terpênicos possuem características atrativas como biodiesel e querosene de aviação. Da mesma forma, os hidrocarbonetos voláteis fornecem características de combustão desejáveis (JAHANDIDEH et al., 2017; BASRI et al., 2020). Os aldeídos e cetonas podem ser considerados compostos intermediários de álcoois e hidrocarbonetos (SANTOS et al., 2016a; BASRI et al., 2020).

2.6 Compostos orgânicos voláteis na água residuária

Estações de tratamento de águas residuais são uma rede complexa de sistemas tecnológicos interligados com diferentes condições de processo em todas as etapas do tratamento. As águas residuais, que percorrem até a estação de tratamento, podem diferir consideravelmente em termos de suas propriedades físico-química. Como resultado, vários grupos de poluentes atmosféricos podem ser gerados em cada etapa do tratamento da água residual. Água de processamento, água de escoamento e águas pluviais, bem como precipitações que chegam às estações de tratamento, juntamente com águas residuais, podem causar a formação de precursores de compostos odoríferos (LEBRERO et al., 2013; LEWKOWSKA et al., 2016).

Odores são caracterizados por propriedades sensoriais específicas, e odorantes coexistentes podem estar sujeitos a efeitos interativos. Por essas razões, a pesquisa sobre a relação entre a intensidade do odor e a concentração química ainda se concentra principalmente em odorantes individuais ou grupos de odores, ao invés de misturas de odor (YAN et al., 2015; RAVINA et al., 2020).

Estações de tratamentos de água residuária geralmente estão localizadas próximas a assentamentos urbanos. Por esse motivo, podem ocorrer situações críticas do ponto de vista do impacto do odor. Os odores no tratamento de águas residuais surgem da biodegradação do efluente. A maioria dos odores ambientais

desagradáveis estão relacionadas a compostos derivados de enxofre, em particular o sulfeto de hidrogênio (CAPELLI et al., 2009; CAPELLI et al., 2012).

Recentemente, compostos orgânicos voláteis (COVs) também foram estudados devido a sua contribuição para a formação de odores (CARRERA-CHAPELA et al., 2014). Um grande número de COVs é emitido pelas estações de tratamento de água residuária (ETARs) e a identificação completa dos principais odorantes é desafiador. Em geral, compostos aromáticos, terpenos, aminas, aldeídos e ácidos graxos também podem ser encontrados além de compostos de enxofre reduzidos (KOTOWSKA et al., 2012 ; DINCER & MUEZZINOGLU, 2008; RAVINA et al., 2020).

Muitos fatores podem influenciar na emissão de COVs odoríferos como: a concentração destes compostos; a área superficial do líquido exposta à atmosfera; o grau de turbulência do fluxo deste líquido. Além disso, a liberação depende também do pH do meio, sendo que em condições ácidas, compostos sulfurados e ácidos orgânicos são facilmente liberados, e em pH alcalino, compostos aminas são favorecidas (HWANG, 1994; HWANG, 1995).

Compostos como indol e escatol, são aminas que possuem odor fecal irritante devido a decomposição orgânica. Estes compostos estão associados ao lançamento de efluentes industriais com alta concentração de proteínas e gorduras, como os provenientes da indústria de abate e processamento de bovinos, aves e suínos (DINCER & MUEZZINOGLU, 2008; HWANG, 1994; FANG et al., 2012; LEWKOWSKA et al., 2016).

Os compostos que contém enxofre são caracterizados por odor pútrido ou a vegetais em decomposição. Estes compostos são formados a partir da ação de microrganismos sobre sulfatos e durante a decomposição de proteínas que contém enxofre (DINCER & MUEZZINOGLU, 2008; HWANG, 1995).

Alguns ácidos orgânicos, aldeídos, cetonas e álcoois apresentam odores irritantes. O cheiro característico dos ácidos alifáticos de peso molecular mais baixo passa progressivamente de forte e irritante a extremamente desagradável em água residuária. Os aldeídos apresentam odores penetrantes. Os álcoois são altamente voláteis e de odor característico, uma vez que o grupo hidroxila (OH) constitui importante porção da molécula (DINCER & MUEZZINOGLU, 2008).

Águas residuais apresentam uma mistura de todos os compostos mencionados acima. Como resultado, a identificação e a determinação quantitativa de compostos

químicos, que causam sensações desagradáveis, muitas vezes provam ser muito complexas, especialmente se a composição da mistura com odor é condicionada pela presença de vários grupos de compostos com odor (LEBRERO et al., 2013; LEWKOWSKA et al., 2016).

A identificação de COVs odoríferos, mesmo em baixas concentrações, é importante, pois eles influenciam o caráter geral de uma emissão (RYAN et al., 2008). A caracterização de COVs sensorialmente relevantes é, portanto, necessária para uma melhor estimativa do impacto do odor. A ocorrência de odores está diretamente relacionada com a presença de compostos odoríficos na fase líquida e com a transferência desses compostos da fase líquida para a fase gasosa (FISHER et al., 2017).

Estudos demonstram que, em geral, os tratamentos preliminares, decantadores primários e o lodo são as principais causas de problemas de odor em ETARs. Além disso, pode ocorrer a formação de odores em reatores biológicos aeróbicos resultante da sobrecarga do sistema e, consequentemente, liberar compostos odoríferos (LEWKOWSKA et al., 2016; RAVINA et al., 2020).

A ocorrência da emissão de COVs odorantes para a atmosfera é inevitável. No entanto, minimizar o efeito destes odores constitui em um dos principais aspectos que deverá ser avaliado. Embora várias tecnologias tenham sido amplamente divulgadas no passado (ESTRADA et al., 2011; LEBRERO et al., 2013), uma redução de odores econômica e ecologicamente correta ainda é urgentemente necessária, uma vez que as preocupações cada vez maiores do público e a legislação rigorosa também são urgentes (REN et al., 2019).

2.7 Biotecnologia de tratamento de odor na água residuária

O tratamento de água residuária gera subprodutos que são responsáveis por emissões de maus odores. Este efeito é resultado da decomposição das águas residuárias, ricas em lipídeos, proteínas e polissacarídeos (HWANG et al., 1995). Os princípios de geração e requisitos de desempenho para o controle de odores e ventilação em ETARs encontram-se especificados na Norma Europeia EN12255-9:2002. Tecnologias de redução de odor têm sido amplamente investigadas como alternativas economicamente eficientes e confiáveis para a mitigação dos odores.

Estas tecnologias são comumente classificadas em técnicas físicas, químicas e biológicas (METCALF & EDDY, 2003; ALFONSÍN et al., 2015).

Tecnologias físicas, a remoção dos compostos odoríficos do ar ocorre por transferência de massa, da fase gasosa para a fase líquida (absorção) ou da fase gasosa para a fase sólida (adsorção). Os processos químicos, induzem a oxidação, a redução e, ou a precipitação dos compostos odoríficos. A degradação destes compostos também pode ser realizada através de processos térmicos de combustão ou oxidação. Nos processos biológicos, microrganismos são responsáveis pela remoção dos compostos odoríficos através da sua decomposição e incorporação na biomassa (METCALF & EDDY, 2003; ESTRADA et al., 2011; LEBRERO et al., 2013).

Entre as diferentes tecnologias disponíveis para o tratamento de odores, a Norma Europeia EN12255-9:2002 recomenda a oxidação biológica, a oxidação química, a adsorção e oxidação térmica. No entanto, os métodos físicos e químicos de purificação de compostos de odor, apesar de comprovarem sua eficiência e confiabilidade e continuarem a ocupar seu nicho, ainda existem diversas desvantagens. Entre eles o alto custo de investimento e operação, além da possível geração de fluxos secundários de resíduos (BAJPAI, 2014; ALFONSÍN et al., 2015).

Em contrapartida, os sistemas de tratamento biológico de odores utilizam processos bioquímicos para decompor compostos odoríferos. Estes métodos possuem a vantagem de converter os poluentes em produtos de oxidação como por exemplo, dióxido de carbono, água etc. São métodos de baixo custo, com simplicidade operacional e são considerados "tecnologias limpas", pois reduzem ou eliminam a necessidade de tratamento adicional dos produtos finais (BURGES et al., 2011; BAJPAI 2014; CHENG et al., 2021).

Os métodos biológicos têm um amplo espectro de aplicações. São considerados os sistemas mais competitivos para a desodorização de poluentes do ar caracterizados por altas taxas de fluxo e baixas concentrações de contaminantes (SADDOUD & SAYADI, 2007; VIKRANTE et al., 2017). Além disso, o tratamento biológico é ambientalmente seguro, pois não produz compostos tóxicos prejudiciais a saúde ou ao meio ambiente. Geralmente é operado em condições naturais (temperatura e pressão atmosféricas normais). Estes métodos para o tratamento de COVs incluem biofiltros, *bioscrubbers* (bio-lavador), biorreatores de membrana e filtros de *biotrickling* (filtro biológico percolador). Nestes métodos, os poluentes são

degradados biologicamente por microrganismos aeróbios (KIM & DESHUSSES, 2005; VIKRANTE et al., 2017).

A biofiltração é um processo que utiliza o crescimento de microrganismos imobilizados em um meio de suporte orgânico ou inorgânico, que podem ser partículas sólidas, como turfa, aparas de madeira ou espuma de poliuretano embalada em uma coluna. Os microrganismos imobilizados são responsáveis pela depuração dos gases e vapores, removendo odor e toxicidade dos mesmos. À medida que o fluxo de ar poluído passa através do filtro, os COVs são particionados no biofilme (KUMAR et al. , 2011; VIKRANTE et al., 2017).

A biofiltração possui como a principal vantagem a eficiência em termos de energia e econômica, ao mesmo tempo que convertem os poluentes em produtos finais inofensivos. Custos relativamente baixos e excelente estabilidade operacional (RENE et al., 2012). As desvantagens desta tecnologia são quedas excessivas de pressão, acúmulo gradual de subprodutos ácidos, dificuldade em controlar os parâmetros biológicos de operação, entupimento devido ao acúmulo de grande quantidade de biofilme e redução da eficiência do tratamento em altas concentrações de poluentes (LEWKOWSKA et al.,2016; VIKRANTE et al., 2017).

Bioscrubber (bio-lavador) fundamenta-se em unidade de duas etapas em que a absorção ocorre em uma etapa e a biodegradação por microrganismos em suspensão ocorre no outra. Compostos químicos são geralmente usados para o tratamento de COVs solúveis na corrente de ar residual (KELLENER & FLAUGER, 1998). *Bioscrubbers* são estáveis permitindo um melhor controle dos parâmetros operacionais. A principal desvantagem é a geração de excesso de lodo e resíduos líquidos que, com o tempo, reduzem consideravelmente a eficiência do processo de desodorização (VIKRANTE et al., 2017).

Nos biorreatores de membrana ocorre a transferência de massa dos COVs da fase gasosa para uma fase líquida contendo microrganismos ativos. As vantagens deste método de tratamento de odor são a facilidade de aumento de escala e a capacidade de variar o fluxo de gás e líquido, sem problemas de inundação ou formação de espuma. As desvantagens são o alto custo de investimento e o entupimento dos canais de líquido devido à formação de excesso de biomassa (VIKRANTE et al., 2017).

Filtro biológico percolador, também conhecido como filtro *biotrickling*, os compostos voláteis percolam através de um leito compactado, que é continuamente

irrigado com uma solução aquosa contendo os nutrientes essenciais necessários aos miorganismos (COX & DESHUSSES, 1999). O filtro biológico percolador apresentam vantagens sobre os métodos anteriores, como economia do processo, apresentam baixa queda de pressão. O pH e umidade, podem ser controlados por gotejamento contínuo. Além disso, estes filtros são capazes de tratar produtos de degradação ácida de COVs (LU et al., 2001). No entanto, filtros biológico percolador, apresentam desvantagens como a necessidade do fornecimento contínuo de nutrientes e entupimento do sistema devido ao acúmulo do biofilme. O entupimento aumenta a queda de pressão no reator, o que leva a redução da remoção de poluentes (OKKERSE et al., 1999 ; COX & DESHUSSES, 1999).

Processos baseados em microalgas pode ser uma tecnologia inovadora para desodorização de água residuária em estações de tratamento por ser uma alternativa econômica e ecologicamente correta, cuja flexibilidade metabólica é uma vantagem, pois converte moléculas polares e apolares de efluentes. Além disso, microalgas convertem resíduos orgânicos em uma infinidade de produtos, como, biocombustíveis, nutracêuticos, polímeros, pigmentos e variedades de produtos químicos, sendo considerada uma fonte potencialmente nova e valiosa de compostos biologicamente ativos para aplicações em diversos setores da biotecnologia (SANTOS et al., 2016a; XANG et al., 2017; LAURITANO et al., 2018).

CAPÍTULO 4

MANUSCRITO 1

3. MANUSCRITO 1

The role of microalgae-based systems in the dynamics of odors compounds in the meat processing industry

Karem Rodrigues Vieiraa, Pricila Nass Pinheiroa, Andriéli Borges Santosa, Alexandre José Cichoski, Cristiano Ragagnin de Menezes, Roger Wagnera, Leila Queiroz Zepka, Eduardo Jacob-Lopes

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The role of microalgae-based systems in the dynamics of odors compounds in the meat processing industry

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ABSTRACT

The aim of this work was to evaluate the dynamics of odors compounds in the meat processing industry through microalgae-based processes. The study focused on the characterization of odorant profile from raw wastewater, on the deodorization of the compounds and the formation of the volatile organic compounds as co-products of the process. The results showed that emissions from the wastewater treatment plant are composed of 4 sulfur, 7 aldehydes, 1 furan, 2 hydrocarbon, 10 terpenes, 7 alcohols, 2 ketones, 3 amines, and 4 phenolic compounds. The levels of these volatile organic compounds from wastewater, regardless of polarity range, decreased with concomitant formation of other compounds, usually with desirable odor description, as residence time increased. A total of 15 compounds of various chemical structures (such as aldehydes, alcohols, ketones, esters, terpenes, acids, and nitrogen compounds) ware formed. Regardless of these organic classes, three main odor categories (fruity, spicy, and resinous) emerged. Based on these results, we found the potential of the microalgae-based processes for odor abatement of the meat industry in parallel to production of desirable compounds.

Keywords: Algae/cyanobacteria; Agro-industrial wastes; Volatile organic compounds; Deodorization; Bioproducts

1. Introduction

Two typical human behaviors are meat eating and food processing. Evidence indicates that people began to increase meat consumption at least 2.6 million years ago, contributing for the growth of the meat product industry to the point of making it become one of the largest in the food sector [1,2]. Thus, the meat supply chain is a complex operation, with global sourcing strategies to secure supply. However, managing this segment can be difficult and can expose vulnerabilities which include environmental issues [2,3].

Thus, some facts have affected the food industry; for example, complaints from people living near meat processing facilities have prompted regulatory agencies to address public concerns [4] officially. The production of animal protein, in particular, is a substantial and growing driver of odor pollution, accounting for approximately half of all food production-related emissions [5].

The volatile profile from meat processing plants includes specific groups of odorants such as alcohols, volatile fatty acids, aldehydes, and ketones, which are products derived from decomposition of carbohydrates, proteins, and lipids. Meat spoilage can contribute to emissions of amine compounds, indole, and skatole, which poses challenges to the deodorization process. The technological challenge

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of removing indole and skatole is posed by the simultaneous presence of hydrophobic and hydrophilic structural components; benzene, and pyrrole rings, together with CH bonds are hydrophobic surfaces while the center is hydrophilic because of the N heteroatom [6,7]. Also, these compounds are malodorous and have very low odor thresholds, potentially resulting in an impact of odors on nearby populations [8,9].

The simultaneous presence of hydrophobic and hydrophilic chemical moieties in odor pollution and the significant contribution of these compounds to unpleasant odors from industrial facilities pose technological challenges for odor removal in waste treatment. In this sense, the use of microalgae-based processes can be an innovative technology for deodorization in the meat processing industry because it is a cost-effective, environmental friendly alternative, whose metabolic plasticity is an advantage, as it converts polar and nonpolar molecules of wastewater.

Microalgae are considered to be a potentially new and valuable source of biologically active compounds for applications in several biotechnology sectors [10]. Moreover, the use of microalgae plays a vital role in conversion of waste to a multitude of products, e.g., biofuels, nutraceuticals, polymers, pigments, and varieties of chemicals. Algae inherently have the potential to transform industrial greenhouse gases as well as wastewater into useful products, thus serving as an effective carbon capture and utilization platform [11].

Although, a number of commercial uses have been found for microalgae, not much is known on the application of microalgae-based systems for odor pollution removal, and the potential bioconversion of value-added products using odor waste substrates. At times when people perceive waste as wealth, this hypothesis should be investigated. Thus, the objective of this study was to evaluate the dynamics of odorous compounds in the meat processing industry in microalgae-based processes. The study focused on the (i) characterization of the odorant profile of raw wastewater, (ii) deodorization of compounds, and (iii) formation of volatile organic compounds as co-products of the process. In addition, to the best of our knowledge, it is the first time that a heterotrophic microalgal bioreactor, using *Phormidium autumnale*, was simultaneously applied for deodorization in meat industry facilities and production of desirable industrial compounds.

2. Material and methods

2.1. Standards

The standards benzyl alcohol, 2-heptanone, butanal, toluene, α-terpineol, hexanol, linalool, limonene, a-terpinene, ρ-cresol, and 6-methyl-5-hepten-2-one, as well as 3-octanol (which were used as an internal standard) were purchased from Sigma-Aldrich (Bellefonte-PA, USA). The paraffin homologues were obtained from Polyscience (Chicago-IL, USA). The identities of volatile compounds were confirmed with retention indices and comparison with the MS spectral database.

2.2. Microalgae and culture media

Axenic cultures of *Phormidium autumnale* were used in the experiments. Stock cultures were propagated and maintained in solidified agar-agar (20 $g.L^{-1}$) containing synthetic BG11 medium [12]. The incubation conditions were 25°C, photon flux density was 15 $\mu mol.m^{-2}.s^{-1}$ and the photoperiod was 12 h. To obtain the inoculums in liquid form, 1 mL of sterile synthetic medium was transferred to slants; the colonies were scraped and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

2.3. Food processing wastewater

Slaughterhouse wastewater used in the experiments was obtained from an industry located in Santa Catarina, Brazil (27°14′02″ S, 52°01′40″ W). It was collected at the discharge point of an equalization tank over a period of one year, and analyzed for pH, chemical oxygen demand (COD), total nitrogen (N-TKN), total phosphorus ($P\text{-}PO_4^{-3}$), total solids (TS), suspended solids (SS), volatile solids (VS), and fixed solids (FS) following the Standard Methods for the Examination of Water and Wastewater [13]. This is the average composition of the wastewater: pH of 5.9 ± 0.05, COD of 4.100 ± 874 ($mg.L^{-1}$), NTK-N of 128.5 ± 12.1 ($mg.L^{-1}$), $P\text{-}PO_4^{-3}$ of 2.84 ± 0.2 ($mg.L^{-1}$), TS of 3.8 ± 2.7 ($mg.L^{-1}$), SS of 1.9 ± 0.8 ($mg.L^{-1}$), VS of 2.9 ± 0.4 ($mg.L^{-1}$), and FS of 0.9 ± 0.3 ($mg.L^{-1}$).

2.4. Heterotrophic microalgal bioreactor

Measurements were made in a batch bubble column bioreactor [14], fed on 2.0 L of wastewater. The bioreactor, which included filtering units, was previously autoclaved at 121°C for 30 min. The experimental conditions were determined as follows: initial concentration of inoculum 100 $mg.L^{-1}$, temperature 25°C, pH adjusted to 7.6, and aeration of 1.0 VVM (volume of air per volume of culture per minute), absence of light and residence time of 144 h. To confirm the dynamics of formation and degradation of volatile organic compounds by microalgae, an experiment control (without inoculum addition) was used. The wastewater was pneumatically aerated in the bubble column bioreactor at a rate of 1.0 VVM. The experiments were performed twice and in duplicate. Therefore, data refer to the mean value of four repetitions.

2.5. Analytical methods

2.5.1. Isolation of the volatile organic compounds

The volatile compounds were isolated from the matrix by 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). A wastewater sample of 20 mL was collected and equally separated into two portions. Each portion was placed in a vial containing 3 g of NaCl and 10 μL of a 3-octanol internal standard solution. The SPME fiber was exposed into the headspace of the vial containing the sample for 45 min at 40°C, under constant stirring (400 rpm) with a magnetic stir bar. After this period, the fiber was removed from the vial and submitted to chromatographic analysis. The analytical procedure was performed twice and in duplicate. Therefore,

data refer to the mean value of four repetitions. HS-SPME was coupled with GC/MS for the quantitative determination of the volatile compounds [15].

2.5.2. GC/MS analysis

The volatile compounds were analyzed in a Shimadzu QP 2010 Plus gas chromatograph coupled to a mass spectrometer (Shimadzu, Kyoto, Japan). The fiber was thermally desorbed for 10 min in a split/splitless injector, operating on the splitless mode (1.0 min splitter off) at 250°C. Helium was used as a carrier gas at a constant flow rate of 1.6 mL.min^{-1}. 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 on 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). Also, the linear retention index (LRI) was calculated for each volatile compound using the retention times of a standard mixture of homologous series of paraffins (C_6–C_{24}) to aid identification [16]. The sample and the standard mixture were injected both separately and together to obtain the experimental LRI and mass spectra values for the purpose of compound identification by directed comparison. Analytes were quantified by internal standard calibration. The relative concentration of the investigated compounds was determined by relating the standard internal area with a known concentration (0.082 μg.mL^{-1}) to the area of the compound of interest. The response factor between internal standard and analytes was assumed as one.

3. Results and discussion

A characterization of odorant composition and profile of raw wastewater is the first step to improve the understanding of the mechanism of odor formation and degradation as well as to optimize treatment technology with high deodorization performance. The initial data analysis (Table 1) shows the volatile profile from agro-industrial wastewater. A total of 40 different compounds were separated in the raw wastewater, ρ-cresol, peak 52, was the major volatile compound (19.1%), followed by benzaldehyde, peak 35, (11.9%), limonene, peak 17, (10.8%), linalool, peak 36, (7.5%) and hexanol, peak 27, (6.2%).

The odors and air pollutants from wastewater treatment plants are a complex mixture of chemical compounds, including a range of volatile organic compounds that contribute to malodor. As shown in Table 1, the emissions from the wastewater treatment plant are composed of about 4 sulfur compounds (peaks 1, 2, 10, and 28), 7 aldehydes (peaks 3, 6, 7, 8, 11, 35, and 43), 1 furan (peak 4), 2 hydrocarbons (peaks 9 and 34), 10 terpenes (peaks 15, 17, 18, 21, 22, 36, 37, 38, 45, and 46), 7 alcohols (peaks 19, 24, 27, 33, 39, 42, and 47), 2 ketones (peaks 23 and 44), 3 amines (peaks 25, 54, and 55) and 4 phenolic compounds (peaks 48, 50, 51, and 52). A large number of the compounds detected in this study show low concentrations and have very low odor thresholds, and agree with data available in the literature [4,8,28–30].

Odors compounds have a threshold value (odor unit), in which an odor is not detectable below a given concentration. Most volatile malodors present trace level concentration and potent odor. Weber's law (1834) and Steven's Law (1970) mathematically confirm that odor perception relates psychological interpretation to physiological reception. Thus, the minor compounds found in the wastewater, mainly peaks 1, 2, 10, 28, 50, 51, 54, and 55, are indispensable for the complex evaluation of odor released from wastewater facilities.

Table 1 also includes each volatile odor intensity (ranging from 2.0×10^8 μg.m^{-3} for benzyl alcohol to 5.6×10^{-4} μg.m^{-3} for skatole) and odor description. As shown Table 1, important minor malodors compounds such as dimethyl disulfide (1.1%), indole (1.3%) and skatole (0.3%), and compounds with higher contents, ρ-cresol (92.0 μg.m^{-3}) and benzaldehyde (11.9 μg.m^{-3}) show the lowest threshold, which makes deodorization of this wastewater a challenging task. In this context, odor removal utilizing microalgal heterotrophic bioreactor is an interesting biotechnology that should be taken into consideration.

Fig. 1 and Table 2 show the impact of residence time on the performance of the bioreactor in the treatment of the volatile organic compounds of wastewater. As expected, similar qualitative and quantitative volatile organic compounds profiles were found in the raw wastewater (40 peaks) and in the microalgal heterotrophic bioreactor at time 0 h (44 peaks), although, 4 compounds (peaks 26, 40, 49, and 53), not previously detected in the raw wastewater, were detected at time 0 h. In fact, detection of 6-methyl-5-hepten-2-one, menthol, benzothiazole and 1-penten-3-ol at the initial residence time is not surprising, considering that these volatile components were present in the microalgae biomass utilized in the experiment. The natural biosynthesis of volatile from microalgae is derived from the carotenoid cleavage (6-methyl-5-hepten-2-one), carbon-rearranged monoterpenes (menthol), amino acid (benzothiazole), and fatty acid (1-penten-3-ol) pathways [25,31]. As previously reported [15], off-flavors were not identified in *Phormidium autumnale* biomass; this is a technological advantage when compared with other microalgae that are capable of releasing a range of malodorous compounds into surface waters. The levels of volatile organic compounds from wastewater decreased with concomitant formation of the others compounds (in general with desirable odor description) as time of cultivation increased. A combined total of 55 compounds were identified (Fig. 1 and Table 2). At the initial residence time (0 h), 97.5% of volatile organic compounds from raw wastewater along with 4 compounds in small amounts (2.5%) were found (Table 2). As a consequence of residence time in the microalgal bioreactor, the ratio values were found for volatile organic compounds from wastewater in comparison to the volatile organic compounds formed (VOCw/VOCf), changing from 98:2 to 10:90 after 72 h of residence time.

There was a clear change in the volatile profile of the heterotrophic microalgal bioreactor at residence time between 0–24 h; 25 compounds disappeared, and all of the 15 compounds were formed in this period. However, following 24 h of treatment, removal of the volatile organic

Table 1
Quantification of volatile compounds ($\mu g.m^{-3} \pm \sigma$) of wastewater and their corresponding threshold values and odor descriptors

Peak	Compound	Chemical formula	Molecular weight ($g.mol^{-1}$)	Concentration[a] ($\mu g.m^{-3}$)	Odor threshold[b] ($\mu g.m^{-3}$)	Odor description[b]
1	Carbon disulfide	CS_2	76.1	1.1 ± 0.1	3×10^2	Disagreeable, sweet
2	Dimethyl sulfide	C_2H_6S	62.1	0.6 ± 0.2	2×10^4	Decayed cabbage, sulfurous
3	2-propenal	C_3H_4O	56.1	6.0 ± 0.4	7×10^2	Burnt, sweet
4	2-methylfuran	C_5H_6O	82.1	7.1 ± 1.9	3.5×10^3	Roasted meat, chocolate
6	Butanal	C_4H_8O	72.1	4.9 ± 0.1	1.5×10^4	Sweet
7	2-methylbutanal	$C_5H_{10}O$	86.1	4.0 ± 0.3	1×10^3	Cocoa, almond
8	3-methylbutanal	$C_5H_{10}O$	86.1	5.2 ± 0.3	2×10^2	Malt, smell of oil
9	Toluene	C_7H_8	92.1	23.8 ± 1.4	5.95×10^5	Rubbery, tarry, mothballs
10	Dimethyl disulfide	$C_2H_6S_2$	94.2	5.2 ± 1.9	3.5×10^3	Rotten cabbage, putrefaction
11	Hexanal	$C_6H_{12}O$	100.1	18.1 ± 3.4	2×10^2	Grass, tallow, fat
15	1,4-cineole	$C_{10}H_{18}O$	154.3	2.0 ± 0.1	na[c]	Spice
17	Limonene	$C_{10}H_{16}$	136.2	51.9 ± 2.9	1.7×10^3	Lemon
18	1,8-cineole	$C_{10}H_{18}O$	154.3	4.5 ± 0.5	1.3×10^3	Spice
19	1-pentanol	$C_5H_{12}O$	88.1	6.2 ± 0.1	5×10^2	Balsamic, fruity
21	α-terpinene	$C_{10}H_{16}$	136.2	3.9 ± 0.3	na	Lemon
22	ρ-cymene	$C_{10}H_{14}$	134.2	6.7 ± 0.1	7.1×10^3	Lemon, fruity, fuel like
23	Cyclohexanone	$C_6H_{10}O$	98.1	4.3 ± 1.6	3×10^2	Pepper, acetone
24	2-heptanol	$C_7H_{16}O$	116.2	1.6 ± 0.1	1×10^5	Herb
25	Pyrrolidine-2,4-dione	$C_4H_5NO_2$	99.1	2.1 ± 0.1	na	na
27	Hexanol	$C_6H_{14}O$	102.2	29.7 ± 1.1	1×10^1	Flower, green
28	Dimethyl trisulfide	$C_2H_6S_3$	126.3	1.0 ± 0.1	1×10^2	Rotten, vegetables
33	1-heptanol	$C_7H_{16}O$	116.2	24.7 ± 1.1	2.5×10^6	Chemical, green
34	3-propylcyclopentene	C_8H_{14}	110.2	4.5 ± 0.9	na	na
35	Benzaldehyde	C_7H_6O	106.1	57.5 ± 3.9	1×10^1	Burnt, sweet
36	Linalool	$C_{10}H_{18}O$	154.2	36.0 ± 0.1	1.4×10^2	Flower, lavender
37	Fenchol	$C_{10}H_{18}O$	154.2	4.8 ± 0.7	5×10^4	Camphor
38	4-terpineol	$C_{10}H_{18}O$	154.2	4.1 ± 0.9	3.4×10^{-1}	Turpentine, nutmeg, must
39	2-octen-1-ol	$C_8H_{16}O$	128.2	7.8 ± 0.9	5×10^4	Soap, plastic
42	1-nonanol	$C_9H_{20}O$	144.3	6.5 ± 0.6	5×10^1	Fat, green
43	Phenylacetaldehyde	C_8H_8O	120.1	9.4 ± 2.2	4×10^3	Honey, sweet
44	Acetophenone	C_8H_8O	120.1	6.4 ± 1.1	6.5×10^{-1}	Must, flower, almond
45	Limonen-4-ol	$C_{10}H_{16}O$	152.2	4.7 ± 1.6	na	Fresh, mint
46	α-terpineol	$C_{10}H_{18}O$	154.2	15.6 ± 1.4	2.5×10^5	Oil, anise, mint
47	Benzyl alcohol	C_7H_8O	108.1	4.3 ± 0.4	2×10^8	Sweet, flower
48	2-phenylethanol	$C_8H_{10}O$	122.1	1.9 ± 0.2	8.6×10^4	Rosy
50	o-cresol	C_7H_8O	108.1	0.4 ± 0.1	2×10^1	Medicinal, phenolic
51	Phenol	C_6H_6O	94.1	2.9 ± 0.1	2×10^4	Medicinal, phenolic plastic rubber
52	ρ-cresol	C_7H_8O	108.1	92.0 ± 2.9	2×10^1	Fecal, horse stable-like
54	Indole	C_8H_7N	117.1	6.5 ± 0.5	3×10^{-1}	Manure, fecal, nauseating
55	Skatole	C_9H_9N	131.2	1.6 ± 0.7	5.6×10^{-4}	Fecal, nauseating

[a]Mean and standard deviation often independent experiments.
[b]According to: [4, 7, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27].
[c]na: not available in the literature.

Table 2
Dynamics of conversion and production of volatile compounds (μg.m^{-3} ± σ) and removal efficiency in the heterotrophic microalgal bioreactor

Peak	Compound	Chemical formula	LRI DB-Wax[a]	Residence time[b]				Removal efficiency (%)
				0 h	24 h	48 h	72 h	
1	Carbon disulfide	CS_2	762	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	nd[c]	100
2	Dimethyl sulfide	C_2H_6S	771	0.4 ± 0.1	nd	nd	nd	100
3	2-propenal	C_3H_4O	856	5.7 ± 0.1	nd	nd	nd	100
4	2-methylfuran	C_5H_6O	872	7.9 ± 1.1	5.5 ± 1.5	5.0 ± 1.9	nd	100
5	Acetaldehyde	$C_6H_{14}O_2$	890	nd	2.6 ± 0.3	nd	nd	na[d]
6	Butanal	C_4H_8O	883	5.5 ± 1.2	nd	nd	nd	100
7	2-methylbutanal	$C_5H_{10}O$	917	3.8 ± 0.8	nd	nd	nd	100
8	3-methylbutanal	$C_5H_{10}O$	921	4.5 ± 0.1	nd	nd	nd	100
9	Toluene	C_7H_8	1049	22.8 ± 0.1	nd	nd	nd	100
10	Dimethyl disulfide	$C_2H_6S_2$	1080	5.8 ± 0.1	2.3 ± 0.4	1.9 ± 0.2	1.8 ± 0.4	69.0
11	Hexanal	$C_6H_{12}O$	1092	15.7 ± 0.1	nd	nd	nd	100
12	2-methylpentanol	$C_6H_{14}O$	1099	nd	0.5 ± 0.1	nd	nd	na
13	2-methyl-3-hexanone	$C_7H_{14}O$	1140	nd	4.2 ± 0.5	nd	nd	na
14	Acetyl valeryl	$C_7H_{12}O_2$	1153	nd	2.5 ± 0.6	nd	nd	na
15	1,4-cineole	$C_{10}H_{18}O$	1168	3.6 ± 0.1	nd	nd	nd	100
16	2-heptanone	$C_7H_{14}O$	1181	nd	1.2 ± 0.4	5.0 ± 0.5	5.0 ± 0.7	na
17	Limonene	$C_{10}H_{16}$	1182	49.9 ± 0.1	20.4 ± 0.4	13.4 ± 0.5	nd	100
18	1,8-cineole	$C_{10}H_{18}O$	1193	4.9 ± 0.7	1.1 ± 0.1	nd	nd	100
19	1-pentanol	$C_5H_{12}O$	1203	6.3 ± 0.7	nd	nd	nd	100
20	3-methylbutanol	$C_5H_{12}O$	1221	nd	0.4 ± 0.1	nd	nd	na
21	α-terpinene	$C_{10}H_{16}$	1226	3.7 ± 0.5	nd	nd	nd	100
22	ρ-cymene	$C_{10}H_{14}$	1253	6.8 ± 1.4	nd	nd	nd	100
23	Cyclohexanone	$C_6H_{10}O$	1285	5.4 ± 0.4	nd	nd	nd	100
24	2-heptanol	$C_7H_{16}O$	1301	1.1 ± 0.8	nd	nd	nd	100
25	Pyrrolidine-2,4-dione	$C_4H_5NO_2$	1311	2.2 ± 0.9	nd	nd	nd	100
26	6-methyl-5-hepten-2-one	$C_8H_{14}O$	1327	3.8 ± 0.4	3.8 ± 0.4	2.0 ± 0.6	nd	na
27	Hexanol	$C_6H_{14}O$	1338	30.3 ± 0.7	nd	nd	nd	100
28	Dimethyl trisulfide	$C_2H_6S_3$	1363	1.2 ± 0.2	0.9 ± 0.2	nd	nd	100
29	2-nonanone	$C_9H_{18}O$	1382	nd	1.1 ± 1.6	1.4 ± 0.8	2.3 ± 0.8	na
30	Methyl 3-methyl 2-hydroxybutanoate	$C_6H_{12}O_3$	1390	nd	2.4 ± 0.4	nd	nd	na
31	Cyclohexanol	$C_6H_{12}O$	1395	nd	6.5 ± 1.0	nd	nd	na
32	5-ethyl-2-nonanol	$C_{11}H_{24}O$	1399	nd	2.3 ± 0.2	nd	nd	na
33	1-heptanol	$C_7H_{16}O$	1447	25.5 ± 0.1	nd	nd	nd	100
34	3-propylcyclopentene	C_8H_{14}	1510	3.9 ± 1.0	3.0 ± 1.2	nd	nd	100
35	Benzaldehyde	C_7H_6O	1545	55.4±0.1	nd	nd	nd	100
36	Linalool	$C_{10}H_{18}O$	1552	36.0 ± 2.3	nd	nd	nd	100
37	Fenchol	$C_{10}H_{18}O$	1574	4.3 ±1.2	nd	nd	nd	100
38	4-terpineol	$C_{10}H_{18}O$	1605	4.8 ± 0.8	3.7±0.5	nd	nd	100
39	2-octen-1-ol	$C_8H_{16}O$	1611	8.5 ± 0.9	nd	nd	nd	100
40	Menthol	$C_{10}H_{20}O$	1642	4.4 ± 0.2	5.7 ± 0.4	7.3 ± 0.8	7.6 ± 0.6	na
41	3-methylpentanoic acid	$C_6H_{12}O_2$	1655	nd	0.7 ± 0.2	nd	nd	na
42	1-nonanol	$C_9H_{20}O$	1655	6.0 ± 0.1	nd	nd	nd	100
43	Phenylacetaldehyde	C_8H_8O	1662	7.9 ± 0.1	nd	nd	nd	100
44	Acetophenone	C_8H_8O	1679	7.2 ± 0.4	3.2 ± 0.4	nd	nd	100
45	Linomen-4-ol	$C_{10}H_{16}O$	1687	5.3 ± 0.9	4.1 ± 0.7	nd	nd	100
46	α-terpineol	$C_{10}H_{18}O$	1697	17.3 ± 1.9	14.6 ± 1.4	nd	nd	100

(continued)

Table 2 *(continued)*

Peak	Compound	Chemical formula	LRI DB-Wax[a]	Residence time[b]				Removal efficiency (%)
				0 h	24 h	48 h	72 h	
47	Benzyl alcohol	C_7H_8O	1848	4.0 ± 0.7	nd	nd	nd	100
48	2-phenylethanol	$C_8H_{10}O$	1865	1.6 ± 0.6	nd	nd	nd	100
49	Benzothiazole	C_7H_5NS	1896	3.3 ± 0.7	2.2 ± 0.7	3.8 ± 1.0	5.0 ± 0.1	na
50	o-cresol	C_7H_8O	1909	0.8 ± 0.1	0.3 ± 0.1	0.2 ± 0.4	nd	100
51	Phenol	C_6H_6O	1915	3.0 ± 0.3	0.6 ± 0.9	0.6 ± 0.7	nd	100
52	ρ-cresol	C_7H_8O	1991	90.0 ± 0.7	47.3 ± 1.0	nd	nd	100
53	1-penten-3-ol	$C_5H_{10}O$	2041	0.6 ± 0.3	5.0 ± 0.9	1.0 ± 0.7	0.3 ± 0.5	na
54	Indole	C_8H_7N	2390	7.3 ± 1.2	3.0 ± 0.9	1.0 ± 0.7	0.3 ± 0.5	95.9
55	Skatole	C_9H_9N	2437	1.2 ± 0.6	nd	nd	nd	100

[a]Linear retention indices in the DB-Wax column.
[b]Mean and standard deviation of the independent experiments.
[c]nd: not detected.
[d]na: not applicable.

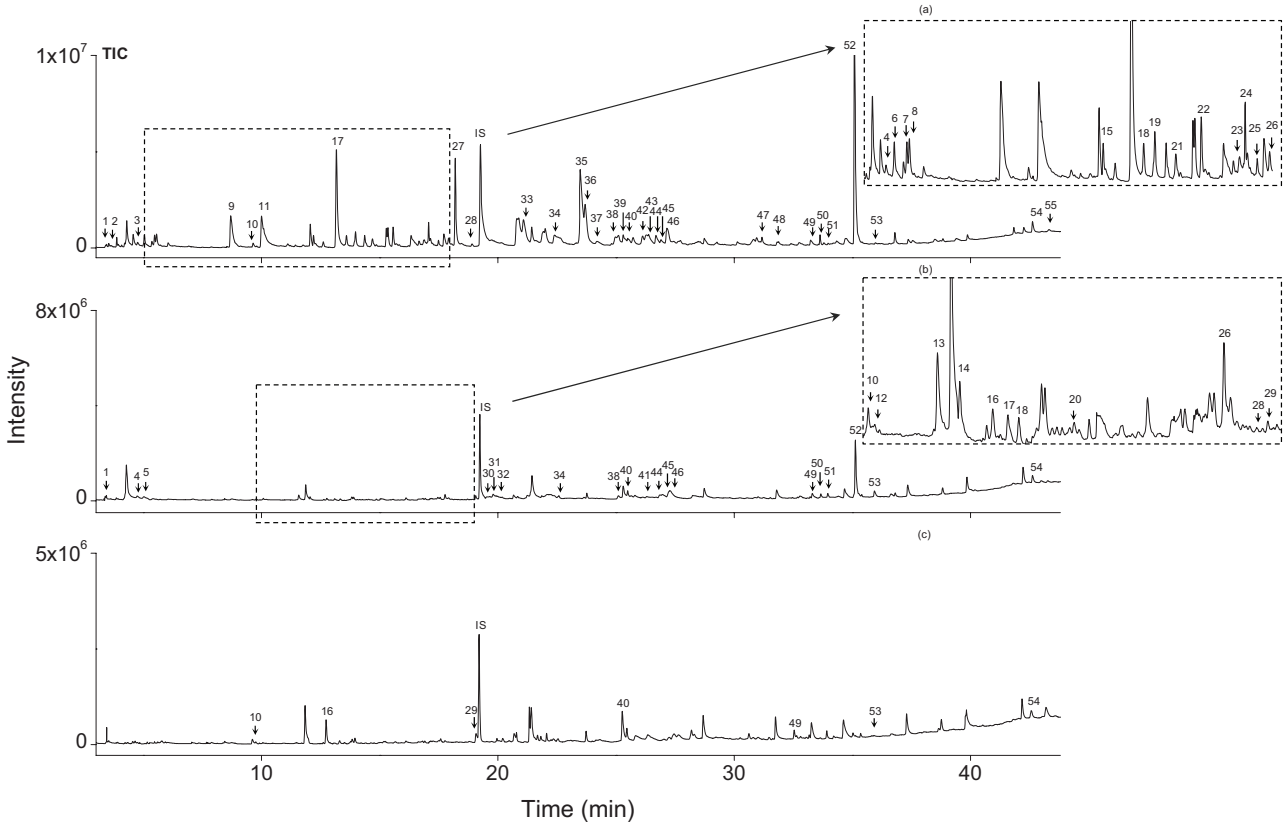

Fig. 1. Chromatogram (total ion current) of the volatile organic compounds from the heterotrophic microalgal bioreactor. The letters correspond to the residence times with which the chromatograms were obtained: (a) 0 h, (b) 24 h, (c) 72 h.

compounds did not exceed 76.8%. Between 24 and 72 h of residence time, just 8 compounds disappeared with odor abatement efficiencies of 95.1%, and in the complete cycle of treatment (48 h), more 5 compounds from wastewater disappeared, and total odorant concentration was reduced by 99.6%.

Studies about 7 usual odor treatment technologies in wastewater treatment plants, e.g., those carried out by Estrada [32], reported that odor removal ranged from 70% to 95%. According to the Logan [33], total residence time of 260 h was necessary to reduce 99.7% of odor emission from swine wastewater by using microbial fuel cells.

Considering that compounds with low odor threshold values play an important role in the negative effects on odor release from wastewater treatment plants, the indolic, phenolic, and sulfur compounds are a key group

in malodors of agro-industrial wastewaters. In this work, these categories contain carbon disulfide (peak 1), dimethylsulfide(peak2),dimethyldisulfide(peak10),dimethyl trisulfide (peak 28), o-cresol (peak 50), phenol (peak 51), ρ-cresol (peak 52), indole (peak 54), and skatole (peak 55). All these compounds were totally degraded as a function of residence time, with exception of dimethyl disulfide and indole. However, these compounds showed 69.0% and 95.9% removal efficiency respectively.

The major compounds ρ-cresol and benzaldehyde were totally removed at 48 and 24 h of residence time (Fig. 2) while other important compounds for nuisance odor from raw wastewater had the following results: skatole and dimethyl sulfide were totally removed at 24 h; dimethyl trisulfide at 48 h; and carbon disulfide, o-cresol, and phenol at 72 h.

In the present study, there was removal of apolar compounds. A total of 10 terpenes were completely removed: limonene (peak 17) and its derivatives (peaks 21, 22, 45), α-terpineol (peak 46) and its derivatives (peaks 15, 18, 38), linalool (peak 36) and fenchol (peak 37). Limonene (49.9 μg.m^{-3}), was totally removed at 72 h; the isomers of limonene, α-terpinene, and ρ-cymene disappeared at 24 h of residence time and the same occurred with 1,4-cineole, linalool, and fenchol. Moreover, α-terpineol, 4-terpineol, and 1,8-cineole were degraded at 48 h of residence time.

This fact is interesting, considering that the best available techniques for odor abatement show severe mass transfer limitations when treating hydrophobic odorants [34].

The results reported by previous studies in literature for removal limonene from wastewater did not exceed 90% [35]. In this work, terpenes were completely removed. Volatile organic compounds are usually resistant to biodegradation, thereby limiting the performance of traditional biotechnology dealing with waste gas containing such pollutants, especially in the mixture [36]. Therefore, a unique process of odor abatement that shows good performance for removal of a functional group of hydrophobic and hydrophilic character, sometimes in the same molecule (mainly peaks 48, 50, 51, 52, 54, and 55), is one of the main challenges of bioprocess engineering for degradation of malodors gases.

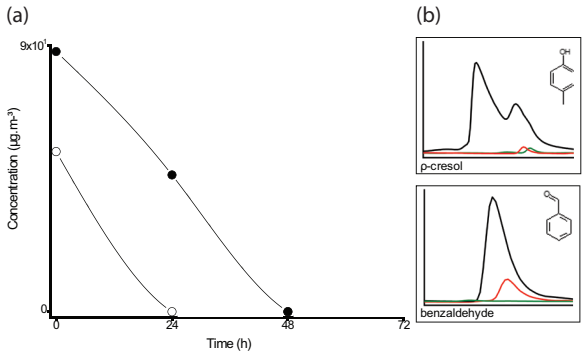

Fig. 2. Changes in the volatile organic compounds observed during residence time of the bioreactor, (a) dynamics of degradation of ρ-cresol (○) and benzaldehyde (●), (b) Chromatogram detail with degradation of the peak during residence time of the heterotrophic microalgal bioreactor 0 h (black line), 24 h (red line), 48 h (green line).

In this context, in the present study, there was a high removal performance of volatile organic compounds from meat process wastewater, hence one of the main technological advantages of the microalgal heterotrophic bioreactor was the polarity range of odor compounds removed from raw wastewater. This fact was not a surprise, and it can be explained by the metabolic diversity of microalgae. The dominant growth physiology of the diverse cyanobacteria is phototrophic. However, these organisms also display other metabolic capabilities. One of them is of particular importance to cyanobacteria: the maintenance of the structure in the dark [37]. Under heterotrophic conditions, the growth of microalgae is dependent on exogenous organic compounds; in this case, organic compounds provide the organism with a source of carbon and energy. In this particular culture condition, the microalgae show a very different ability from those commonly found in the phototrophic environment, for example, the removal of odorous compounds from water, despite the information reported in the literature that microalgae produced unpleasant odors mainly in the form of geosmin and 2-methylisoborneol in drinking water [38–40]. Based on the results of our previous works [41,42], which show carotenoids and volatile profile from different microalgae cultivated under heterotrophic and phototrophic conditions, it can be suggested that the hypothesis for total degradation of terpenes found in this study is related to carotenoid production in the dark.

Cyanobacteria produce a wide variety of carotenoids, and for many years it was believed that carotenoid production depends on high light irradiance under photosynthetic conditions [43,44]. However, more recent studies have focused on carotenoid production in the heterotrophic microalgal bioreactor and identified pigments with very different structural characteristics, such as a greater number of carbon atoms, conjugated double bonds, and hydroxyl groups, all of which contribute to their great antioxidant capacity [42,45].

Taking into account the structures of terpenes identified in this work and of the tetraterpenes detected in previous works [41,42], the mechanism for degradation of terpenes and production of microalgal carotenoids in the dark was proposed (Fig. 3). Limonene and other terpenes (Table 2 and Fig. 1) were metabolized in the heterotrophic growth via an oxidative pentose-phosphate cycle. These catabolic routes are yield precursors in the methylerythritol phosphate pathway (MEP). Synthesized by this pathway, geranyl pyrophosphate (GPP) is produced, and a head to head condensation of the two GPP C_{20} compounds formed the first carotene, the phytoene (C_{40}) precursor of keto and acetylated microalgae carotenoid.

Also, the volatile organic compounds formed by *Phormidium autumnale* cultivated in the heterotrophic bioreactor were found in this work (Fig. 1, Table 2). A total of 15 compounds were formed, 14 of which had odor description of various chemical structures such as aldehyde (peak 5), alcohols (peaks 12, 20, 31, and 53), ketones (peaks 13, 14, 16, 26, and 29), ester (peak 30), terpene (peak 40), acid (peak 41), and nitrogen compound (peak 49).

Regardless of the organic class of the compounds formed, three odor categories (fruity, spicy, and resinous) emerged. The literature [16] reported that peaks 12, 13, 30, and 53 show an odor descriptor that may be classified as fruity.

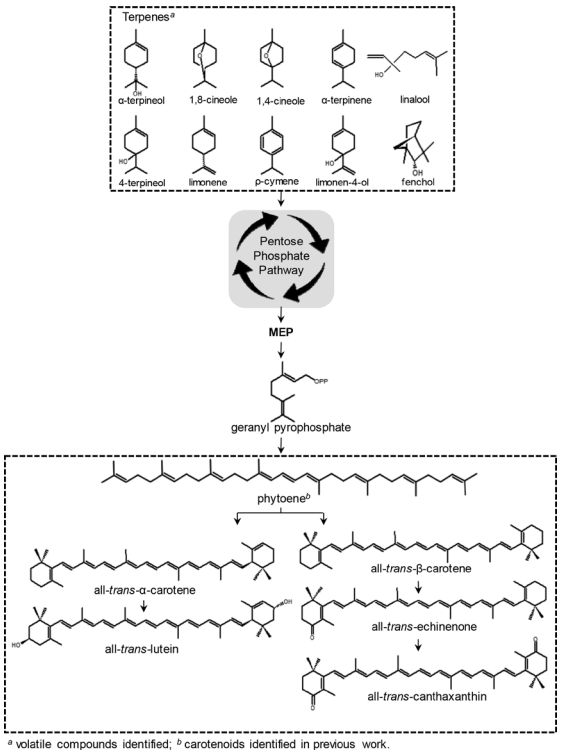

Fig. 3. Overview of proposed the mechanism for degradation of terpenes and production of microalgal carotenoids in the dark.

The compounds (peaks 5, 14, 16, 29, 41, and 49) were classified with a resinous odor, peaks 20, 40 were classified with a burnt odor, and peaks 26 and 31 showed a spicy odor (Fig. 4). Among the chemical compounds identified, menthol (peak 40) showed 7.6 μg.m^{-3} at 72 h, followed by cyclohexanol (peak 31), with 6.5 μg.m^{-3} at 24 h of residence time.

The predominant volatile compound was formed as time of cultivation increased: menthol (peak 40), an isomer of limonene (Fig. 4). Altogether, this result supports the hypothesis of the present research that the terpene compounds was the main volatile organic compound to be removal from meat processing wastewater and metabolized for production of microalgae-based products.

These compounds could, therefore, be a source of useful chemicals products, based on a nonconventional technological route. Thirteen compounds produced by *Phormidium autumnale* in the heterotrophic microalgal bioreactor are commercially available from other biotechnological routes. The flavor biotechnology will be the next generation of the industrial biotechnology. The chemicals obtained from biobased technologies are sold at prices up to 1,000 times higher than synthetic chemicals, hence there is great potential for exploitation of such processes [15,30].

Finally, to confirm whether the volatile organic compounds had been removed from raw wastewater by biological mechanisms, a parallel experiment containing only wastewater and pneumatic aeration was conducted (Table 1, Supplementary data). In this experiment with the absence of microalgae, only 26 compounds were totally removed – in general, with

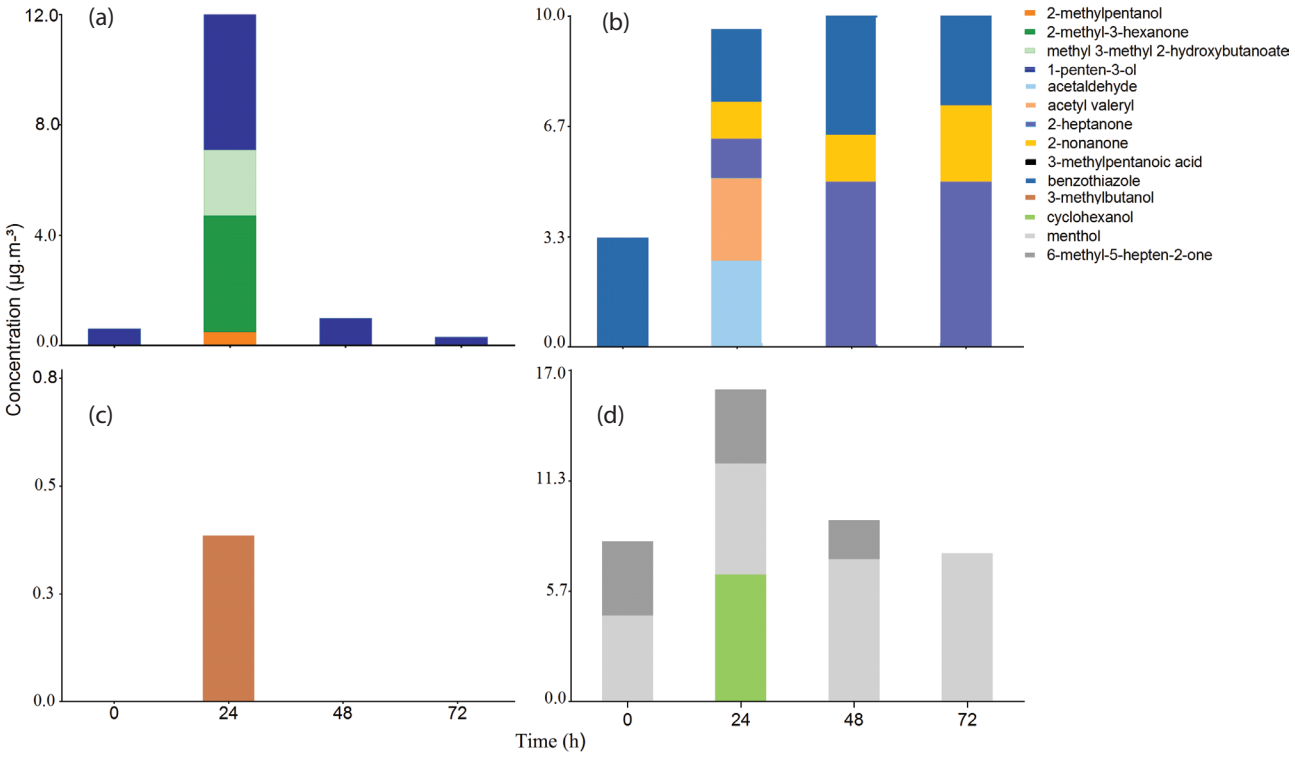

Fig. 4. Dynamics of production of the volatile organic compounds in the bioreactor: (a) fruity, (b) resinous, (c) burnt and (d) spice.

substantially increased residence time of the bioreactor. Between the more recalcitrant compounds, terpenes (limonene, 1,8-cineole, and linalool) practically were not removed. This result shows the potential of the microalgal heterotrophic bioreactor in odor emission abatement in meat processing wastewater, particularly in the terpene family.

4. Conclusions

The meat processing wastewater presents a total of 40 odor compounds, with a wide range of odor thresholds. The microalgal heterotrophic bioreactor was able to totally remove 38 volatile organic compounds. Dimethyl disulfide and indole were the most recalcitrant compounds, with removal efficiencies in the order of 69.0% and 95.9%, respectively.

In parallel to this odor abatement, 13 industrially interesting volatile compounds were produced (menthol, 25.0 $\mu g.m^{-3}$; benzothiazole, 14.3 $\mu g.m^{-3}$; 2-heptanone, 11.2 $\mu g.m^{-3}$; 6-methyl-5-hepten-2-one, 9.6 $\mu g.m^{-3}$; 1-penten-3-ol, 6.9 $\mu g.m^{-3}$; cyclohexanol, 6.5 $\mu g.m^{-3}$; 2-nonanone, 4.8 $\mu g.m^{-3}$; 2-methyl-3-hexanone, 4.2 $\mu g.m^{-3}$; acetaldehyde, 2.6 $\mu g.m^{-3}$; acetyl valeryl, 2.5 $\mu g.m^{-3}$; 3-methylpentanoic acid, 0.7 $\mu g.m^{-3}$; 2-methylpentanol, 0.5 $\mu g.m^{-3}$ and 3-methylbutanol, 0.4 $\mu g.m^{-3}$), thus potentializing the application of these biobased feedstocks for both food and non-food industries.

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Symbols

COD	–	Chemical oxygen demand, $mg.L^{-1}$
N-TKN	–	Total nitrogen, $mg.L^{-1}$
$P\text{-}PO_4^{-3}$	–	Total phosphorus, $mg.L^{-1}$
TS	–	Total solids, $mg.L^{-1}$
SS	–	Suspended solids, $mg.L^{-1}$
VS	–	Volatile solids, $mg.L^{-1}$
FS	–	Fixed solids, $mg.L^{-1}$
VVM	–	Volume of air per volume of wastewater per minute
HS-SPME	–	Headspace solid-phase microextraction
DVB/Car/PDMS	–	Divinylbenzene/carboxen/ polydimethylsiloxane
GC/MS	–	Gas chromatography-mass spectrometry
LRI	–	Linear retention index
VOC_w	–	Volatile organic compounds from wastewater
VOC_f	–	Volatile organic compounds formed
MEP	–	Methylerythritol phosphate pathway
GPP	–	Geranyl pyrophosphate pathway
C_{20}	–	Eicosapentaenoic acid
C_{40}	–	Phytoene

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Supporting information

Table S1
Odor concentration (μg.m-^{3}±σ) in the wastewater using aeration (1.0 volume of air per volume of wastewater per minute) in the heterotrophic bioreactor.

Peak	Compound	Residence time[a]			
		0 h	24 h	48 h	72 h
1	Carbon disulfide	1.1±0.1	0.1±0.7	nd[b]	nd
2	Dimethyl sulfide	0.6± 0.2	0.4±0.2	0.4±0.2	0.5±0.2
3	2-propenal	6.0±0.4	nd	nd	nd
4	2-methylfuran	7.1±1.9	4.9±0.4	4.8±0.1	4.3±0.4
6	Butanal	4.9±0.1	nd	nd	nd
7	2-methylbutanal	4.0±0.3	nd	nd	nd
8	3-methylbutanal	5.2±0.3	1.0±0.2	nd	nd
9	Toluene	23.8±1.4	16.4±0.5	12.8±1.1	7.1±2.1
10	Dimethyl disulfide	5.2±1.9	5.4±2.2	5.5±2.5	5.4±2.5
11	Hexanal	18.1±3.4	nd	nd	nd
15	1,4-cineole	2.0±0.1	nd	nd	nd
17	Limonene	51.9±2.9	50.0±3.1	51.0±1.8	51.0±1.4
18	1,8-cineole	4.5±0.5	2.7±1.1	2.3±1.6	2.9±2.1
19	1-pentanol	6.2±0.1	6.4±0.1	6.0±0.1	1.0±0.1
21	α-terpinene	3.9±0.3	nd	nd	nd
22	ρ-cymene	6.7±0.1	nd	nd	nd
23	Cyclohexanone	4.3±1.6	3.2±2.3	1.0±0.3	nd
24	2-heptanol	1.6±0.1	nd	nd	nd
25	Pyrrolidine-2,4-dione	2.1±0.1	nd	nd	nd
27	Hexanol	29.7±1.1	8.7±0.6	3.5±0.5	3.3±0.3
28	Dimethyl trisulfide	1.0±0.1	1.1±0.1	1.2±0.8	nd
33	1-heptanol	24.7±1.1	1.1±0.2	nd	nd
34	3-propylcyclopentene	4.5±0.9	4.0±0.7	nd	nd
35	Benzaldehyde	57.5±3.9	nd	nd	nd
36	Linalool	36.0±0.1	32.8±1.7	37.4±3.4	33.4±2.2
37	Fenchol	4.8±0.7	0.9±0.2	0.6±0.4	nd
38	4-terpineol	4.1±0.9	4.4±0.5	nd	nd
39	2-octen-1-ol	7.8±0.9	nd	nd	nd
42	1-nonanol	6.5±0.6	nd	nd	nd
43	Phenylacetaldehyde	9.4±2.2	nd	nd	nd
44	Acetophenone	6.4±1.1	5.8±1.8	3.2±0.9	nd
45	Limonen-4-ol	4.7±1.6	5.8±1.2	3.4±0.9	nd
46	α-terpineol	15.6±1.4	14.0±0.4	nd	nd
47	Benzyl alcohol	4.3±0.4	5.7±0.4	2.4±0.8	nd
48	2-phenylethanol	1.9±0.2	nd	nd	nd
50	o-cresol	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.1
51	Phenol	2.9±0.1	2.8±0.6	1.7±0.8	0.4±0.3
52	ρ-cresol	92.0±2.9	71.0±2.6	74.9±2.7	63.8±2.0
54	Indole	6.5±0.5	6.1±2.2	6.5±1.7	6.3±1.3
55	Skatole	1.6±0.7	1.6±2.4	1.6±1.1	1.5±1.1

[a] Mean and standard deviation often independent experiments.
[b] nd: not detected.

CAPÍTULO 5

MANUSCRITO 2

4. MANUSCRITO 2

Volatile organic compounds from microalgae

Karem Rodrigues Vieira, Pricila Nass Pinheiro, Leila Queiroz Zepka

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CHAPTER 24

Volatile organic compounds from microalgae

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Chapter outline

24.1 Introduction

Microalgae comprise a diverse group of photosynthetic microorganisms; however, the term "microalgae" is not of a taxonomic term, but it is a common collective term a commercial terminology (Borowitzka et al., 2016). Regarding biotechnological exploitation, the most widely used species of microalgae belong to these classes: *Cyanophyceae*, *Chlorophyceae*, *Bacillariophyceae*, and *Chrysophyceae* (Borowitzka, 2018).

There is global interest in the exploitation of microalgae-based processes and products, fundamentally supported in the diversity chemical composition of the biomass, in addition to the broad spectrum of its secondary metabolites. Volatile organic compounds are secondary metabolites naturally emitted by microalgae (Santos et al., 2016a; Amavizca et al., 2017; Jacob-Lopes et al., 2019).

The profile of volatile compounds released by microalgae and cyanobacteria has been reported as a thriving source for the production of mixtures of VOCs from different chemical classes such as alcohol, aldehydes, ketones, hydrocarbons, esters, terpenes, and sulfur compounds (Santos et al., 2016b; Hosoglu, 2018).

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The emission of VOCs in the microalgae-based system depends on abiotic and biotic factors. Thus, the biosynthesis knowledge and environmental factors affecting the production can help identify them and target the most appropriate industrial application sector (Santos et al., 2016a; Achyuthan et al., 2017).

The use of the volatile compounds of microalgal culture may represent an improvement in the supply of inputs to a distinct sector of the industry, and once there is a growing interest in natural products guiding the development of the technologies that employ microorganisms, including microalgae, which can synthesize specific volatile organic compounds (Lukin et al., 2018).

Exploring the volatile compounds of microalgae is a possibility; however, it is scientifically challenging to apply these metabolites. Recent research shows that the VOCs produced by microalgae have a high energy potential. Substantial concentrations of VOCs are released and simultaneously reused as fuels in biocombustion processes (Santos et al., 2016a; Deprá et al., 2018; Severo et al., 2018).

In addition, microalgae have emerged as a promising technology for environmental applications because they balance sustainable vectors by reuse of pollutants, which are present in wastewater generated by industries (Santos et al., 2019). Wastewater, in addition to water pollution, also contributes to air pollution. These facts have affected industries, such as complaints from residents living near industrial facilities (Filipy et al., 2006; Lebrero et al., 2014). However, current research demonstrates that microalgae-based processes are an innovative technology for wastewater deodorization (Vieira et al., 2019).

Therefore, the objective of this chapter is to provide a comprehensive view of the volatile organic compounds formed in microalgae-based systems, focusing on the biosynthesis, culture conditions, and environmental factors that affect VOCs production, VOCs application, and the recovery techniques.

24.2 Biosynthesis mechanism of volatile organic compounds in microalgae

Microalgae-based systems release a wide spectrum of volatile organic compounds (VOCs). Metabolically, these VOCs are secondary metabolites, and biosynthesis depends mainly on the availability of carbon and nutrients as well as energy provided by primary metabolism (Dudareva et al., 2013; Santos et al., 2016a; Zuo, 2019).

Fundamentally, VOCs are produced from simple molecules through enzymatic pathways or degradation. Among the pathways for the production of these compounds are ketoacids, fatty acid derivatives, and the isoprenoid pathway. The VOCs belong to innumerable organic classes such as terpenes, alcohol, ketones, aldehydes, esters, hydrocarbons, carboxylic acids, and sulfurized compounds (Liao et al., 2016; Santos et al., 2016a,b).

From the 2-keto acid pathway, a diversity of volatile compounds is obtained, such as aldehydes, alcohols, esters and carboxylic acids, which can be synthesized through the 2-keto acid pathway (Fig. 24.1). The 2-ketoacid pathway covers sequential biochemical reactions such as extension, decarboxylation, isomerization, reduction, dehydration, and esterification of some branched-chain amino acids (e.g., leucine and valine). For example, for 1-butanol, 3-methyl-butanal, and 2-methyl-butanal subsequently reduced to 3-methyl-butanol and 2-methyl-butanol, the reaction can be extended to form 1-hexanol and other alcohols (Hasegawa et al., 2012; Lan and Liao, 2012; Liao et al., 2016).

Most microalgae groups possess two isoprenoid biosynthesis pathways: the mevalonic acid (MVA) pathway and the methylerythritol phosphate (MEP) pathway (Table 24.1), responsible for the synthesis of isopentenyl diphosphate (IPP) and its molecular isomer dimethylallyl diphosphate (DMAPP) (Chappell, 2003; Lichtenthaler et al., 1997).

For both MEP and MVA routes (Fig. 24.2), DMAPP serves as the primer for the sequential and linear chain elongation, catalyzed by the respective enzymes. Consecutive additions of IPP in a head-to-tail fashion yield in sequence C10 geranyl diphosphate (GPP), C15 farnesyl diphosphate (FPP), and C20 GGPP. The series of reactions are catalyzed by enzymes geranyl diphosphate synthase (GPPS), farnesyl diphosphate synthase (FPPS), and geranylgeranyl diphosphate synthase (GGPPS), respectively (Liao et al., 2016).

These carbon precursors are transformed rapidly into different terpenoids, as carotenoids and their oxidative and enzymatic cleavage products—for example, VOCs as α-ionone, β-ionone, and β-cyclocitral (Hosoglu, 2018; Lee et al., 2017; Van Durme et al., 2013).

Among numerous cyanobacteria volatile compounds, geosmin and 2-methylisoborneol (2-MIB) have been extensively studied due to undesirable outbreaks of taste and odor. Synthesis of 2-methylisoborneol (2-MIB) (Fig. 24.3) starts with the methylation of the precursor geranyl diphosphate (GPP) in 2-methylgeranyl diphosphate, which is cyclized in

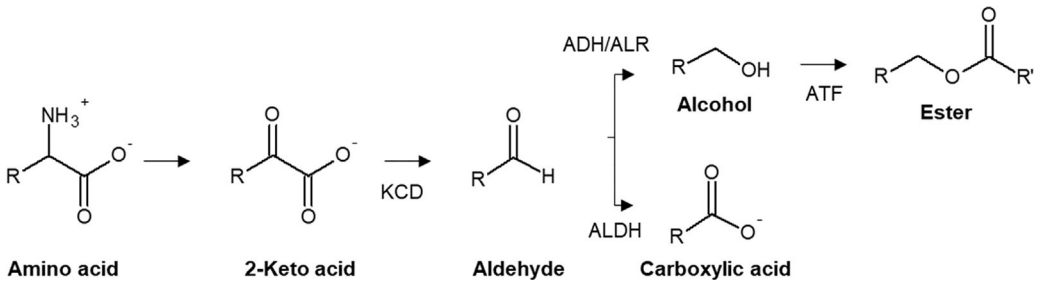

Fig. 24.1

Scheme of metabolic 2-ketoacid pathway for production of VOCs of different organic classes. *KDC*, 2-keto acid decarboxylase; *ADH*, alcohol dehydrogenase; *ALR*, aldehyde reductase; *ALDH*, aldehyde dehydrogenase; *ATF*, alcohol O-acyltransferase.

Table 24.1: Distribution MVA and the MEP pathways in different species.

Phylum	Class	Representative species	Pathway		Reference
			MVA	MEP	
Cyanophyta	*Cyanophyceae*	*Synechocystis* sp.	–	+	Disch et al. (1998)
Glaucophyta	*Glaucophyceae*	*Cyanophora paradoxa*	–	+	Grauvogel and Petersen (2007)
Rhodophyta	*Cyanidiophyceae*	*Galdieria sulphuraria*	+	+	Schwender and Seemann (1996)
		Cyanidium caldarium	+	+	Disch et al. (1998)
Chlorophyta	*Chlorophyceae*	*Scenedesmus obliquus*	–	+	Disch et al. (1998), Schwender and Seemann (1996)
	Trebouxiophyceae	*Chlorella fusca*	–	+	Disch et al. (1998)
	Prasinophyceae	*Tetraselmis striata*	–	+	Schwender and Gemu (2001)
Euglenophyta	*Euglenophyceae*	*Euglena gracilis*	+	+	Disch et al. (1998), Kim et al. (2004)
Heterokontophyta	*Chrysophyceae*	*Ochromonas danica*	+	+	Disch et al. (1998)
	Bacillariophyceae	*Phaeodactylum tricornutum*	+	+	Cvejic and Rohmer (2000)
		Nitzschia ovalis	+	+	Cvejic and Rohmer (2000)
Cryptophyta	*Cryptophyceae*	*Guillardia theta*	?	+	Frommolt et al. (2008)

This table is not comprehensive. "+" indicates proof of existence of pathway, "–" indicates absence of the pathway, and "?" indicates unknown if present.

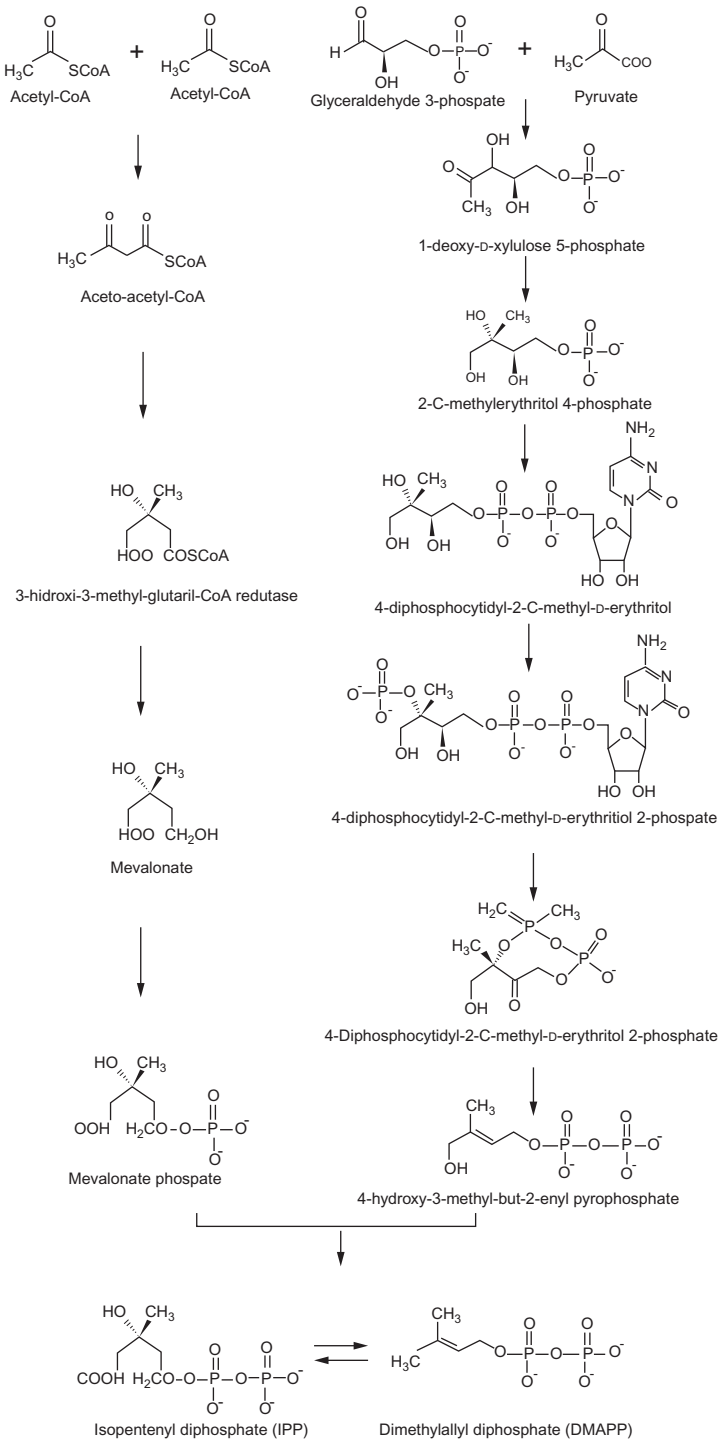

Fig. 24.2

Two pathways for the formation of isoprenoid. (A) Mevalonic acid (MVA) pathway. (B) Methylerythritol phosphate (MEP) pathway.

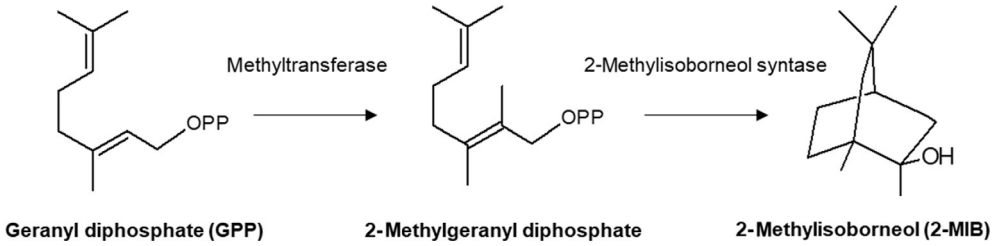

Fig. 24.3
Scheme of 2-methylisoborneol (2-MIB) biosynthetic pathway.

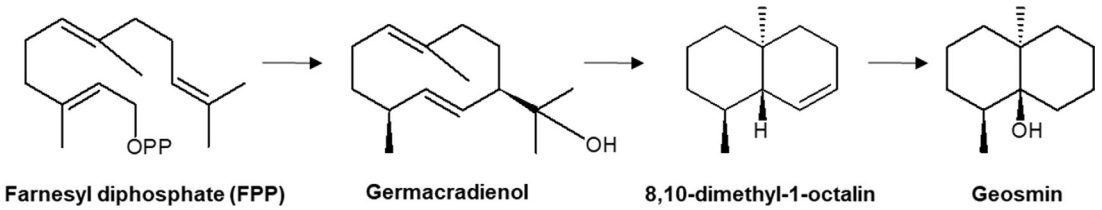

Fig. 24.4
Overview of geosmin synthesis route.

2-MIB (Lee et al., 2017). The cyclization of farnesyl diphosphate (FPP) to geosmin occurs in four stages (Fig. 24.4); farnesyl diphosphate to germacradienol is converted to 8,10-dimethyl-1-octalin, forming to geosmin, and is catalyzed by geosmin synthase (Liato and Aïder, 2017; Meena et al., 2017; Van Durme et al., 2013; Watson et al., 2016).

A range of VOCs, including classes such as ketones, aldehydes, hydrocarbons, and alcohols, can be produced from fatty acid degradation (Santos et al., 2016a). The fatty acid pathway starts with acetyl-CoA using malonyl-CoA as a building block, based on a series of cyclic reactions catalyzed by the multienzymatic system, denominated fatty-acid synthase (Fig. 24.5) (Peralta-Yahya et al., 2012; Zhou et al., 2018).

Aliphatic ketones can be formed from lipid degradation (Santos et al., 2016a). The aldehydes2,4-decadienal and 2,4,7-decatrienal are derivative products of arachidonic or eicosapentaenoic acid, catalyzed by lipoxygenase/hydroperoxid lyase. The fatty acids linoleic or linolenic acid are the precursors of aldehydes compounds such as nonanal, hexanal, and2-pentanal, which can subsequently be reduced to alcohols by dehydrogenases (Adolph et al., 2003; Jerković et al., 2018; Santos et al., 2016a,b; Yu et al., 2014).

Unbranched hydrocarbon production is achieved mainly by two families of enzymes: acyl-acyl carrier protein reductase (AAR) and an aldehyde decarbonylase (AAD), which catalyzes a

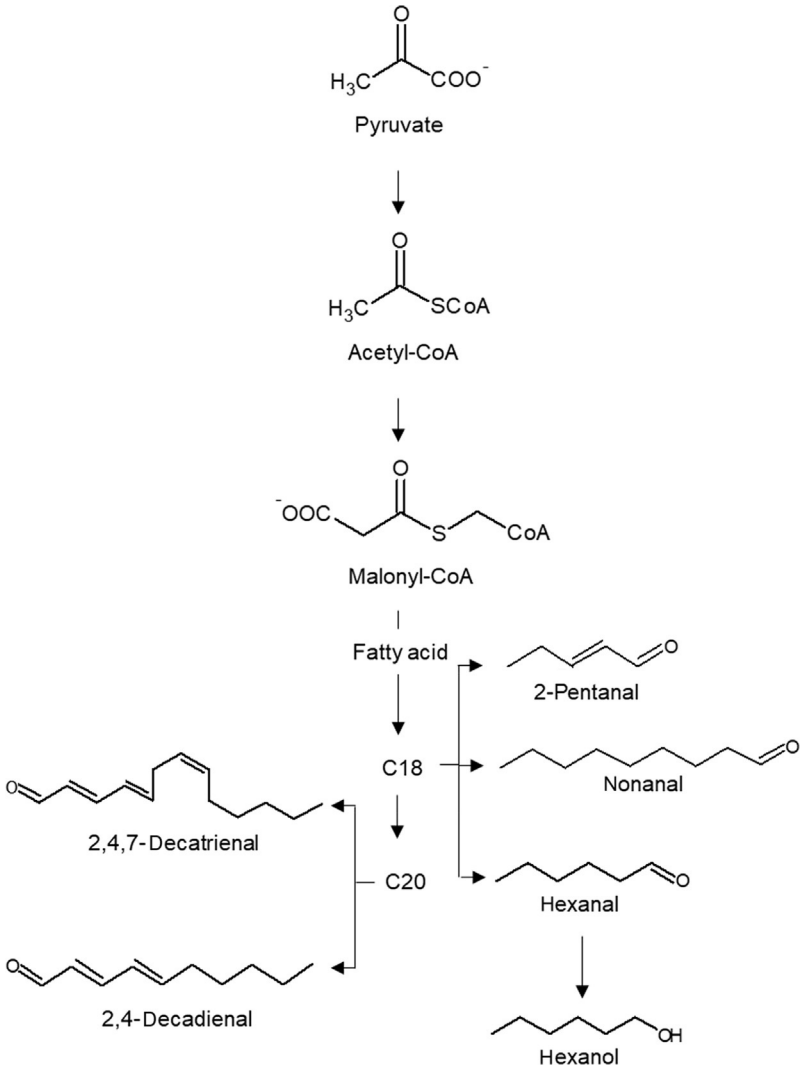

Fig. 24.5
Schematic representation of biosynthetic pathways of the fatty acids and its volatile derivatives.

number of mechanisms converting fatty acid intermediates into alkanes and alkenes (Milovanović et al., 2015; Santos et al., 2016a).

Sulfur compounds, such as dimethylsulfide (DMS), dimethyldisulfide (DMDS), and dimethyltrisulfide (DMTS), are potent volatile compounds due to their low odor threshold values, liberated by many microalgae (Achyuthan et al., 2017; Watson and Jüttner, 2017). The most important volatile sulfide produced is dimethylsulfide (DMS) (Watson and Jüttner, 2017).

The DMSP arises from the amino acid methionine, which is the forerunner of the 2-keto acid 4-methylthio-2-oxobutyrate, through transamination (see Giordano et al. (2005) and their references), followed by a reduction reaction catalyzed by 4-methylthio-2-oxobutyrate reductase, transforming in 4-methylthio-2-hydroxybutyrate, using a nicotinamide adenine dinucleotide phosphate molecule (Giordano and Prioretti, 2016).

The next stage in the mechanism is the *S*-methylation of 4-methylthio-2-hydroxybutyrate to 4-dimethylsufonio-2-hydroxybutyrate, which is finally transformed at the DMSP compound through oxidative decarboxylation (Giordano et al., 2005; Giordano and Prioretti, 2016). The demethiolation of dimethylsulfoniopropionate produces methanethiol, which can be converted into dimethylsulfide (DMS) by methylation (Fig. 24.6) (Achyuthan et al., 2017; Curson et al., 2017).

In order to exploit VOCs in microalgae-based systems successfully, a good understanding of physiology, biosynthesis, and mode of cultivation is essential. This will enable selection of controlled and appropriate growth conditions and optimization of yield biomass as the productivity of desirable volatile compounds (Santos et al., 2016a).

24.3 Environmental factors affecting VOCs production from microalgae

When we consider the biosynthesis of VOCs by microalgae, though dependent on the species, their production can be modified by various factors, such as culture system, nutritional conditions, light intensity, temperature, and growth phase (Milovanović et al., 2015; Van Durme et al., 2013; Achyuthan et al., 2017).

In general, the most widely used system for microalgae cultivation is the photoautotrophic system, where species are cultivated by inorganic carbon (CO_2) bioconversion and light energy absorption. In photosynthetic cultures, these microorganisms can biosynthesize CO_2 very efficiently and biotransform it into VOCs (Perez-Garcia et al., 2011; Perez-Garcia and Bashan, 2015; Claassens et al., 2016; Gong et al., 2018).

In addition, some microalgae species have the versatility to grow in the absence of light, where organic substrates are assimilated through aerobic respiration. In heterotrophic cultures, exogenous carbon sources such as glucose, fructose, and sucrose showed a variable profile of volatile compounds (Francisco et al., 2014; Perez-Garcia and Bashan, 2015; Santos et al., 2016b, 2018).

In mixotrophic cultivation, microalgae employ the phototrophy and heterotrophy systems simultaneously, using different energy sources, such as organic carbon and inorganic carbon in the presence of light. This cultivation system has an additive effect that increases biomass productivity and consequently the formation of volatile compounds (Bhatnagar et al., 2011; Perez-Garcia and Bashan, 2015; Santos et al., 2018).

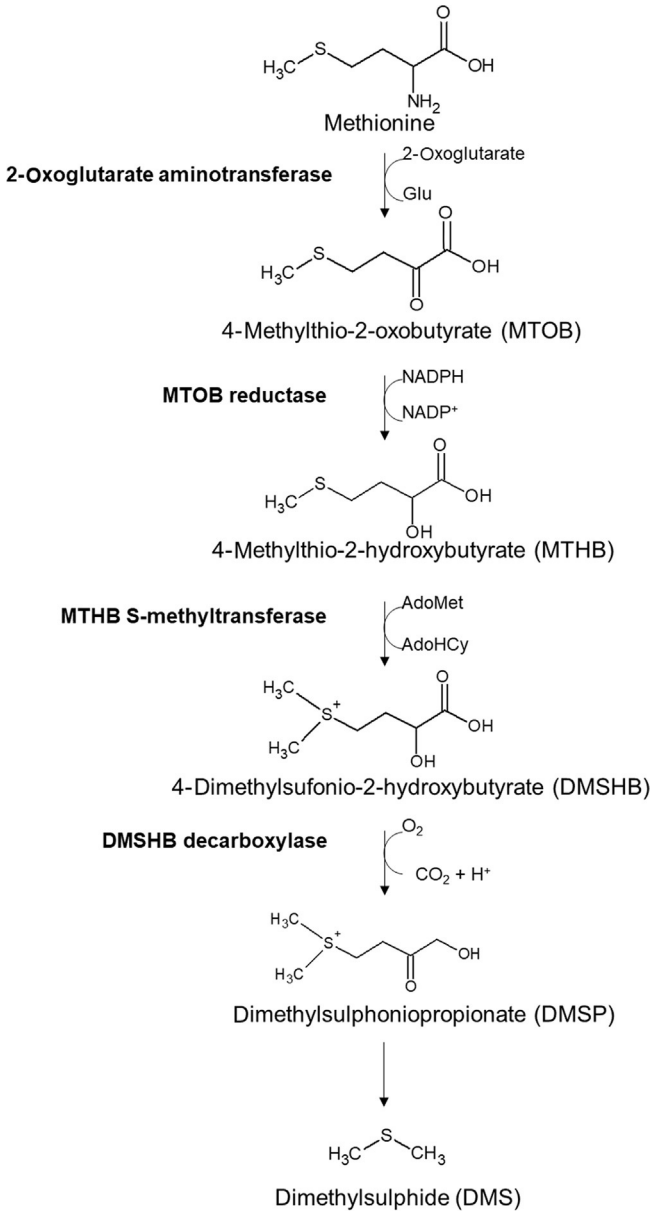

Fig. 24.6

Dimethylsulfide biosynthetic pathway in microalgae. *MTOB*, 4-methylthio-2-oxobutyrate; *MTHB*, 4-methylthio-2-hydroxybutyrate; *DMSHB*, 4-dimethylsufonio-2-hydroxybutyrate.

In microalgae-based systems, the nutrition conditions can influence the emission of the VOCs from algae. In addition to phosphorus and nitrogen sources, their concentrations may also affect the secondary metabolism of this microorganism (Zuo et al., 2018a; Zuo, 2019).

The cyanobacteria *Microcystis flos-aquae* released different VOCs, (sulfur compounds, terpenoids, hydrocarbons, aldehydes, and esters) when they were supplied with different nitrogen sources such as $NaNO_3$, $NaNO_2$, NH_4Cl, urea, serine, lysine, and arginine (Zuo et al., 2018a; Xu et al., 2017).

In previous studies, Hasegawa et al. (2012) demonstrated that *Microcystis aeruginosa* cyanobacteria cultures increased the emission of β-cyclocitral, 2-methyl-1-butanol, 2-phenylethanol, and 3-methyl-1-butanol under non-N condition (Hasegawa et al., 2012). Similar results have also been reported for cyanobacteria *Microcystis flos-aquae* and *Microcystis aeruginosa* under distinct sources and phosphorus concentration (Zuo et al., 2018b; Ye et al., 2018).

Light promotes terpenoid emission, which due to the availability of energetic cofactors and carbon intermediates increases the availability of DMAPP, the immediate precursor of the MEP pathway. Thus, isoprene and monoterpenes are synthesized via MEP and are released from microalgae after direct synthesis, due to no storage structures (Shaw et al., 2003; Niinemets and Sun, 2015; Liao et al., 2016; Englund et al., 2018).

Elevated temperatures promote the emission of alcohols, aldehydes, and hydrocarbons, which are formed via oxidative degradation of fatty acids and carotenoid derivatives as β-cyclocitral, α-ionone, β-ionone, and geranylacetone (Jüttner, 1984; García-Plazaola et al., 2017).

Another factor that affects the emission of volatile compounds in microalgae-based systems is the growth phases. Zhou et al. (2017) reported that the chemical classes of VOCs produced showed differences between the three growth phases. Aldehydes and alcohols of different microalgae species did not show the same tendency and concentration in the growth phases. Alkanes presented the highest concentration in the exponential phase, but decreased from the stationary phase, while ketones in the species studied showed similar increasing trends from the exponential to the stationary phase (Zhou et al., 2017).

The occurrence of VOCs in microalgae is a consequence of their versatile metabolism; thus, understanding the microalgae culture conditions can provide a better knowledge basis for the production of VOCs with industrial potential (Santos et al., 2016a, 2018).

24.4 Application of VOCs from microalgae

Chemicals obtained from microalgae-based systems are sold at prices 1000 times higher than those of synthetic chemicals (Santos et al., 2016b). The most important product of microalgae biotechnology in relation to the amount of production and economic value is its biomass.

However, an emerging trend toward knowledge production of low molecular weight compounds from renewable sources has been noted (Schirmer et al., 2010; Choi and Lee, 2013).

Volatile organic compounds generated by microalgae with commercial appeal include propanol, butanol, 3-methyl-butanol, hexanol, hexanal, β-cyclocitral, and β-ionone (Smith et al., 2010; Santos et al., 2016b). Berger (2009) reported that flavors from microorganisms can compete with traditional sources. The screening for overproducers, elucidation of metabolic pathways and precursors, and application of conventional bioengineering has resulted in a set of more than 100 commercial aroma chemicals derived via biotechnology. Table 24.2 shows a diversity of volatile compounds that were detected in different strains of microalgae from controlled cultures, as well as a comparison of chemically synthesized compounds and those found naturally in plants.

The global market of VOCs was worth US$3.85 billion in 2015, and has a predicted compound annual growth rate of 6.2% until 2024. Terpenes are the predominant class of compounds in this market (Sales et al., 2018). Other classes also of great interest are the alcohols and the aldehydes, which are important aroma components widely applied to the cosmetic, perfumery, and food industries (Longo and Sanromán, 2006).

The VOCs from microalgae have specific advantages regarding extraction from natural sources and chemical synthesis, mainly in terms of not having seasonal and environmental issues, due to the ability of microalgae to be cultivated on non-arable land, making their use commercially attractive for the source of fine chemicals and the food sector, despite the higher production costs (Borowitzka, 2018).

However, the full use of the volatile fraction of microalgal biomass may represent an improvement in the supply of a large volume of inputs to many different types of industry. Concerning the petrochemical industry, hydrocarbons and short-chain alcohols are interesting to generate bioenergy (Severo et al., 2018).

VOCs from microalgae have demonstrated an energy potential of 86.32 $MJ\,kg^{-1}$ (Table 24.3), representing nearly twice as much energy content when compared to traditional fuels, such as gasoline (47.30 $MJ\,kg^{-1}$) and diesel (44.80 $MJ\,kg^{-1}$). However, to meet the energy demands that a combustion system requires, a biotechnological process becomes necessary that produces these compounds in high volumes, which does not currently exist (Deprá et al., 2018).

Microalgae-based systems can also be used for wastewater deodorization (Vieira et al., 2019). The treatment techniques commonly employed in WWTPs for odor removal are chemical scrubbing and bio-filter (Lebrero et al., 2011; Alinezhad et al., 2019). However, these technologies present disadvantages such as produce secondary pollutants, a long period of adaptation required for the microbial population (weeks or even months), and high water consumption, which can make the long-term process onerous and costly (Lebrero et al., 2011).

Table 24.2: VOCs detected in different strains of microalgae, and a comparative with chemical production and naturally extracted.

Chemical name	Microalgae	Chemical	Natural	References
		Terpenes		
α-ionone	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochlopsis*	+	+	Van Durme et al. (2013)
β-cyclocitral	*Botryococcus braunii*, *Chlorella vulgaris*, *Nannochloropsis oculata*, *Nostoc* sp., *Phormidium autumnale*, *Rhodomonas* sp., *Spirulina platensis*, *Tetraselmis chuii*	+	–	Van Durme et al. (2013), Milovanović et al. (2015), Santos et al. (2016b), Lee et al. (2017)
β-ionone	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochlopsis*, *Spirulina platensis*, *Nostoc* sp.,	+	+	Van Durme et al. (2013), Milovanović et al. (2015)
Geosmin	*Anabaena lemmermannii*, *Anabaena circinalis*, *Anabaena solitaria*, *Anabaena viguieri*, *Aphanizomenon gracile*, *Geitlerinema splendidum*, *Leibleinia subtilis*, *Microcoleus* sp., *Phormidium allorgei*, *Phormidium amoenum*, *Phormidium breve*, *Phormidium cortianum*, *Phormidium formosum*, *Phormidium simplicissimum* *Phormidium* sp.,	+	–	Watson (2003), Liato and Aïder (2017), Lee et al. (2017)
2-methylisoborneol	*Oscillatoria curviceps*, *Oscillatoria limosa*, *Oscillatoria tenuis*, *Oscillatoria variabilis*, *Phormidium autumnale*, *Phormidium breve*, *Phormidium calcícola*, *Phormidium favosum*, *Phormidium tenue*, *Phormidium* sp.	+	–	Watson et al. (2016), Lee et al. (2017)
Geraniol	*Synechococcus*			Jüttner and Hans (1986)
Menthol	*Phormidium autumnale*	+	+	Vieira et al. (2019)

Table 24.2: VOCs detected in different strains of microalgae, and a comparative with chemical production and naturally extracted—cont'd

Chemical name	Microalgae	Chemical	Natural	References
Citronellol	*Oocystis pusilla*	+	+	Ghasemi et al. (2009)
Linalool	*Chlorella* sp., *Chlamydomonas* sp., *Oocystis pusilla*	+	+	Ghasemi et al. (2009), Rasoul-Amini et al. (2010)
		Aldehyde		
Benzaldehyde	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	+	Van Durme et al. (2013), Zhou et al. (2017)
Heptanal	*Botryococcus braunii*, *Rhodomonas* sp., *Thalassiosira weissflogii*, *Dicrateria inornata*	+	+	Van Durme et al. (2013), Zhou et al. (2017)
Hexanal	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Phormidium autumnale*, *Schizochytrium limacinum*	+	+	Van Durme et al. (2013), Santos et al. (2016b), Hosoglu (2018)
2-methylpropanal	*Phormidium autumnale*, *Nannochloropsis oculata*, *Chaetoceros calcitrans*, *Thassiosira weissflogii*, *Platymonas helgolandica*, *Nitzschia closterium*	+	+	Santos et al. (2016b), Zhou et al. (2017)
3-methylbutanal	*Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Phormidium autumnale*	+	+	Van Durme et al. (2013), Santos et al. (2016b)
Nonanal	*Botryococcus braunii*, *Rhodomonas* sp., *Nannochloropsis oculata*, *Chlorella vulgaris*, *Thalassiosira weissflogii*, *Nitzschia closterium*, *Chaetoceros calcitrans*, *Platymonas helgolandica*, *Crypthecodinium cohnii*, *Schizochytrium limacinum*, *Chlorella prothecoides*	+	+	Van Durme et al. (2013), Zhou et al. (2017), Xu et al. (2017), Hosoglu (2018)

Continued

Table 24.2: VOCs detected in different strains of microalgae, and a comparative with chemical production and naturally extracted—cont'd

Chemical name	Microalgae	Chemical	Natural	References
2,6-nonadienal	*Nitzschia closterium*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, *Platymonas helgolandica*, *Nannochloropsis* sp., *Dicrateria inornata*, *Chlorella vulgaris*	+	–	Zhou et al. (2017), Hosoglu (2018)
2-octenal	*Botryococcus braunii*, *Nannochloropsis oculata*, *Thalassiosira weissflogii*, *Nitzschia closterium*, *Chaetoceros calcitrans*, *Dicrateria inornata*	+	+	Van Durme et al. (2013), Zhou et al. (2017)
2-pentenal	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	–	Durme et al. (2013), Zhou et al. (2017)
Acetaldehyde	*Phormidium autumnale*	+	+	Vieira et al. (2019)
	Sulfurs			
Benzothiazole	*Phormidium autumnale*, *Nitzschia closterium*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, *Platymonas helgolandica*, *Nannochloropsis* sp., *Dicrateria inornata*	+	–	Santos et al. (2016b), Zhou et al. (2017), Vieira et al. (2019)
Dimethyl disulfide	*Rhodomonas* sp., *Tetraselmis chuii*	+	–	Van Durme et al. (2013), Lee et al. (2017)
Dimethyl sulfide	*Chaetoceros calcitrans*, *Chlorella protothecoides*, *Chlorella vulgaris*, *Crypthecodinium cohnii*, *Nannochloropsis* sp., *Oscillatoria chalybea*, *Oscillatoria tenuis*, *Phormidium autumnale*, *Plectonema boryanum*, *Synechococcus cedrorum*, *Tetraselmis chuii*, *Thalassiosira weissflogii*	+	+	Watson (2003), Van Durme et al. (2013), Zhou et al. (2017), Hosoglu (2018), Lee et al. (2017)
Dimethyl trisulfide	*Rhodomonas* sp., *Tetraselmis chuii*	+	–	Van Durme et al. (2013), Lee et al. (2017)

Table 24.2: VOCs detected in different strains of microalgae, and a comparative with chemical production and naturally extracted—cont'd

Chemical name	Microalgae	Chemical	Natural	References
		Alcohol		
Benzyl alcohol	*Phormidium autumnale*, *Crypthecodinium cohnii*, *Schizochytrium limacinum*, *Chlorella prothecoides*, *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	+	Santos et al. (2016b), Zhou et al. (2017), Hosoglu (2018)
cis-2-penten-1-ol	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	–	Van Durme et al. (2013), Zhou et al. (2017)
Ethanol	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	+	Van Durme et al. (2013), Zhou et al. (2017)
1-hexanol	*Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Phormidium autumnale*	+	+	Van Durme et al. (2013), Santos et al. (2016b)
3-hexen-1-ol	*Chlorella vulgaris*	+	–	Van Durme et al. (2013)
2-ethyl-1-hexanol	*Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*, *Spirulina platensis*, *Nostoc* sp.	+	–	Milovanović et al. (2015), Zhou et al. (2017)
Cyclohexanol	*Phormidium autumnale*	+	–	Vieira et al. (2019)
Isobutanol	*Phormidium autumnale*	+	+	Santos et al. (2016b)
2-methylbutanol	*Tetraselmis* sp., *Nannochloropsis*, *Chlorella vulgaris*	+	+	Van Durme et al. (2013)
3-methylbutanol	*Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Phormidium autumnale*	+	+	Hasegawa et al. (2012), Van Durme et al. (2013), Santos et al. (2016b), Vieira et al. (2019)

Continued

Table 24.2: VOCs detected in different strains of microalgae, and a comparative with chemical production and naturally extracted—cont'd

Chemical name	Microalgae	Chemical	Natural	References
1-octen-3-ol	*Rhodomonas* sp., *Nannochloropsis oculata*, *Chlorella vulgaris*, *Crypthecodinium cohnii*, *Chlorella prothecoides*, *Tetraselmis chuii*, *Schizochytrium limacinum*	+	+	Van Durme et al. (2013), Hosoglu (2018)
2-phenylethyl alcohol	*Crypthecodinium cohnii*	+	+	Hosoglu (2018)
1-pentanol	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	+	Van Durme et al. (2013), Zhou et al. (2017)
1-penten-3-ol	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia Closterium*, *Phormidium autumnale*	+	+	Van Durme et al. (2013), Zhou et al. (2017), Vieira et al. (2019)
2-methyl-1-pentanol	*Phormidium autumnale*	+	–	Vieira et al. (2019)
	Hydrocarbons			
2,4-dimethylheptane	*Scenedesmus obliquus*	+	+	Severo et al. (2018)
Dodecane	*Microcystis flos-aquae*, *Microcystis aeruginosa*	+	–	Xu et al. (2017), Zuo et al. (2018a,b)
Heptadecane	*Spirulina platensis*, *Nostoc* sp., *Nitzschia closterium*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, *Platymonas helgolandica*, *Nannochloropsis* sp., *Dicrateria inornata*, *Microcystis flos-aquae*, *Microcystis aeruginosa*	+	–	Milovanović et al. (2015), Zhou et al. (2017), Xu et al. (2017), Zuo et al. (2018a,b)
Hexadecane	*Spirulina platensis*, *Nostoc* sp., *Nitzschia closterium*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, *Platymonas helgolandica*, *Nannochloropsis* sp., *Dicrateria inornata*, *Microcystis flos-aquae*, *Microcystis aeruginosa*	+	–	Milovanović et al. (2015), Zhou et al. (2017), Xu et al. (2017), Zuo et al. (2018a,b)

Table 24.2: VOCs detected in different strains of microalgae, and a comparative with chemical production and naturally extracted—cont'd

Chemical name	Microalgae	Chemical	Natural	References
Pentadecane	*Spirulina platensis*, *Nostoc* sp., *Nitzschia closterium*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, *Platymonas helgolandica*, *Nannochloropsis* sp., *Dicrateria inornata*	+	–	Milovanović et al. (2015), Zhou et al. (2017)
Tetradecane	*Spirulina platensis*, *Nostoc* sp., *Nitzschia closterium*, *Chaetoceros calcitrans*, *Thalassiosira weissflogii*, *Platymonas helgolandica*, *Nannochloropsis* sp., *Dicrateria inornata*, *Microcystis flos-aquae*, *Microcystis aeruginosa*	+	–	Milovanović et al. (2015), Zhou et al. (2017), Xu et al. (2017), Zuo et al. (2018a,b)
Tridecane	*Microcystis flos-aquae*, *Microcystis aeruginosa*	+	–	Xu et al. (2017), Zuo et al. (2018a,b)
	Furan			
2-ethylfuran	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	–	Van Durme et al. (2013), Zhou et al. (2017)
2-penthylfuran	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	+	Van Durme et al. (2013), Zhou et al. (2017)
	Ketones			
3-hydroxy-2-butanone	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	+	Van Durme et al. (2013), Zhou et al. (2017)
Acetyl valeryl	*Phormidium autumnale*	+	+	Vieira et al. (2019)
2,3-butanedione	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	+	Van Durme et al. (2013), Zhou et al. (2017)

Continued

Table 24.2: VOCs detected in different strains of microalgae, and a comparative with chemical production and naturally extracted—cont'd

Chemical name	Microalgae	Chemical	Natural	References
2-heptanone	*Phormidium autumnale*	+	+	Vieira et al. (2019)
6-methyl-5-hepten-2-one	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochlopsis*, *Phormidium autumnale*	+	+	Van Durme et al. (2013), Santos et al. (2016b), Vieira et al. (2019)
2-octanedione	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	–	Van Durme et al. (2013), Zhou et al. (2017)
2-nonanone	*Phormidium autumnale*	+	+	Vieira et al. (2019)
3,5-octadien-2-one	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*, *Chaetoceros calcitrans*, *Dicrateria inornata*, *Platymonas helgolandica*	+	–	Van Durme et al. (2013), Zhou et al. (2017)
2-propanone	*Scenedesmus obliquus*	+	+	Severo et al. (2018)
2,3-pentenedione	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochloropsis oculata*, *Chlorella vulgaris*, *Nitzschia closterium*	+	+	Van Durme et al. (2013), Zhou et al. (2017).
1-penten-3-one	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Chlorella vulgaris*, *Nitzschia closterium*, *Chaetoceros calcitrans*, *Dicrateria inornata*, *Platymonas helgolandica*	+	+	Van Durme et al. (2013), Zhou et al. (2017)
Ester				
Methyl octanoate	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis* sp., *Nannochlopsis*, *Crypthecodinium cohnii*, *Chlorella prothecoides*, *Tetraselmis chuii*, *Schizochytrium limacinum*	+	+	Van Durme et al. (2013), Hosoglu (2018)

Table 24.2: VOCs detected in different strains of microalgae, and a comparative with chemical production and naturally extracted—cont'd

Chemical name	Microalgae	Chemical	Natural	References
Methyl 3-methyl 2-hydroxybutanoate	*Phormidium autumnale*	+	–	Vieira et al. (2019)
Methyl phenylacetate	*Botryococcus braunii*, *Rhodomonas* sp., *Tetraselmis chuii*, *Nannochlopsis*, *Crypthecodinium cohnii*, *Chlorella prothecoides*, *Schizochytrium limacinum*	+	–	Van Durme et al. (2013), Hosoglu (2018)
2-methoxy-2-methylpropane	*Scenedesmus obliquus*	+	–	Severo et al. (2018)

(+) indicates production or (−) no VOCs production chemically produced and naturally extracted.

Table 24.3: Volatile organic compounds generated by microalgae and their energy potential.

Volatile organic compounds	Energy potential ($MJ\,kg^{-1}$)
2-ethyl-1-hexanol	5.42
2-propil-1-heptanol	6.72
2-methylbutanal	3.24
Hexanal	3.88
2,4-heptadienal	2.97
2,4-decadienal	6.0
2-methoxy-2-methyl-propane	3.48
3,3-dimethyl-hexane	5.42
2,4-dimethyl-heptane	6.07
4,7-dimethyl-undecane	8.39
2-propanone	1.94
2,4-dimethyl-3-pentanone	4.53
4-octen-3-one	5.18
6-methyl-5-hepten-2-one	4.94
Acetophenone	4.22
β-ionone	7.70
2-phenylpropene	4.87
Total	86.32

Adapted from Deprá et al. (2018).

Limited research is available on the economic implications of investment and operational costs of microalgae-based systems (Banerjee and Ramaswamy, 2019). However, microalgae have the advantage of performing wastewater treatment, removing inorganic nutrients and fetid compounds, and in parallel, providing high-value biomass production with the potential to exploit multiple products (Leite et al., 2019; Jacob-Lopes et al., 2019).

These facts make it clear that microalgae-based systems are seen as an alternative that allows for marked improvements in wastewater treatment plants, and may result in the extinction of some traditional unit operations, like odor removal technologies. In addition, this process results in the production of VOCs with odor descriptors of interest to different industries (Vieira et al., 2019).

Finally, although hundreds of VOCs have been identified in cultures of microalgae, the induction of synthesis is in most cases unknown, and the separation and recovery of the compounds need to be optimized. Thus, their insertion into commercial products is subject to further research and development (Pinheiro et al., 2019).

24.5 Techniques for VOCs recovery

Commercial production of volatile organic compounds obtained biotechnologically requires economic profitability. The biosynthesis of microalgae-based products is generally limited by low productivity or low concentrations of main compounds in the bioreactor. In order to achieve high yields and productivity, it is important to choose a reactor design carefully and select a convenient system for the recovery of volatile compounds (Akachaa and Gargouri, 2015).

Currently, some techniques can be exploited for the separation and recovery of VOCs in bioreactors, such as adsorption, condensation, absorption, and membrane-based techniques, which may assist microalgae-based processes when a compound or a group thereof need to be obtained separately (Wylock et al., 2015; Try et al., 2018; Saffarionpour and Ottens, 2018).

In the condensation-based recovery system, the gas stream of the headspace of the bioreactor passes through the vertical trap column placed in a cryogenic bath containing liquid nitrogen, which allows VOC vapor to condense (Saffarionpour and Ottens, 2018). Condensation is used to separate VOCs from a plant matrix, or microalgae biomass, that consists mainly of carbohydrates and nonvolatile lipids (Lukin et al., 2018).

Another technique is adsorption, widely used in the recovery of VOCs from the bioreactors, being a process based on the ability of a solid (e.g., adsorbent) to connect a gaseous component (e.g., adsorbate) to its surface (Saffarionpour and Ottens, 2018). The adsorption of volatile compounds in solid materials, as in microalgae biomass, is more widely used for the quantitative analysis of these compounds on a laboratory scale (Lukin et al., 2018). This type of adsorbent is used to remove VOCs from industrial gases, as well as in wastewater treatment plants (Lebrero et al., 2011).

In the absorption technique, a gas stream is put into contact with a liquid in order to transfer one or several gaseous components into the liquid phase. The absorption devices can be used as a single operation with a reactant dissolved in the liquid phase, or can be used with a

non-reacting liquid. This device is coupled with an adsorbent, in order to regenerate the absorbing liquid (Wylock et al., 2015). This is the principle of bioscrubbers, which are applied in wastewater treatment plants as a biological treatment in the odor removal (Lebrero et al., 2011).

Overall, adsorption is in principle highly comparable with absorption and can be useful for both wastewater odor abatement and industrial recovery of VOCs, of the liquid and gaseous phase in microalgae-based systems (Lebrero et al., 2011; Lebrero et al., 2014; Lukin et al., 2018).

Membrane-based techniques, known as pervaporation, have as the principle of separating liquid mixtures through a dense membrane with the gas flow (Try et al., 2018). Pervaporation demonstrates significant advantages for the recovery of aroma compounds and hydrophobic molecules (Lukin et al., 2018). Pervaporation is an emerging technology with significant potential to recover alcohols and other biofuels efficiently from a microalgae bioreactor (Vane, 2005). Heymes et al. (2007) investigated the possibility of removing VOCs from industrial gases by a combination of absorption and pervaporation. Table 24.4 shows the technologies and VOC recovery efficiency.

The techniques proposed for the recovery of VOCs aim to minimize their losses and recover the major components, which are valuable in producing a high-quality final product for industrial application. These technologies can be applied in different industry for VOCs recovery such as the chemical, petrochemical, and pharmaceutical industries, and the food processing industry. In addition, they can be used in the treatment of gaseous wastewater released by these industries, contributing to reduction of olfactive and environmental pollution (Wylock et al., 2015; Saffarionpour and Ottens, 2018).

The recovery of volatile compounds from microalgae is challenging because the compounds are present at low concentrations; the biomass is present as solid content; within the solid phase the volatile may be intracellular or membrane-bound; and the compound may be located and distributed in different phases, such as solid, liquid, and gaseous (López-pérez et al., 2017; Achyuthan et al., 2017).

Thus, industrial recovery may be particularly limiting; as such, VOCs are expected to partition between different phases, sometimes requiring different recovery techniques for each phase, and in some cases additional steps. Fig. 24.7 shows the application possibilities of VOCs recovery techniques in microalgae-based systems.

Aeration and production of CO_2 can lead to loss of VOCs in the headspace of microalgae bioreactors as a result of the volatility of the molecules. According to Mackay and Yuen (1980), chemical substances can be found in the following volatility classes, based on Henry's law constant H: highly volatile, $H > 1 \times 10^{-3}$ atm m^3 mol^{-1}; volatile, $1 \times 10^{-5} < H < 1 \times 10^{-3}$ atm m^3 mol^{-1}; with slow volatilization for $3 \times 10^{-7} < H < 1 \times 10^{-5}$ atm m^3 mol^{-1}; and with negligible

Table 24.4: Comparison of the characteristics of the potential technologies for recovery of VOCs.

Technologies	Membranes separation	Condensation	Adsorption	Absorption
Industrial applications	Environmental depollution; solvent recovery	Environmental depollution; food volatile compounds recovery	Environmental depollution; capture of VOCs	Environmental depollution; odor reduction
Type of VOCs	Alcohols, alkanes, aromatic hydrocarbons	Hydrocarbons, ketones, aldehydes, alcohols, furan	Esters, aldehydes, alcohols, hydrocarbons	Hydrocarbons
Mediators	Polymer membranes	Liquid nitrogen	Activated carbon; porous resin	Water; high-boiling hydrocarbons
Recoveries efficiency	>90%	>95%	>99%	95%–99%
Advantages	Cyclic operating; easy recycling of membranes; no additive required; no further treatment of recovered VOCs; operates under mild conditions	Ideal for high concentrated gas stream	Good recovery efficiency	Easy to set up; reuse of absorbent liquid; used in a wide range of concentration
Disadvantages	Costly and rarely available membranes; susceptibility of membranes to fouling and bacterial growth (inducing clogging and possibly VOCs alteration)	High energy consumption; cooling fluid use; not suitable for compounds with boiling points above 37°C	Less selectivity; poor regeneration of adsorbent; use of solvent for desorbing; susceptible to clog; not suitable for cyclic operation; require humidity control	Use of a large amount of absorbing liquid; the need for posttreatment for the regeneration of absorbing liquid

Adapted from Wylock, C., Eloundou Mballa, P.P., Heilporn, C., Debaste, F., Fauconnier, M.-L., 2015. Review on the potential technologies for aromas recovery from food industry flue gas. Trends Food Sci. Technol. 46(1), 68–74. https://doi.org/10.1016/j.tifs.2015.08.002.

volatilization for $H < 1 \times 10^{-7}\,\mathrm{atm\,m^3\,mol^{-1}}$. With vast diversity across VOCs from microalgae, the volatility of individual molecules varies greatly, requiring different recovery approaches (Lukin et al., 2018).

From the techniques presented, condensation seems less suitable for the recovery of hydrophobic VOCs from microalgae-based processes, because of the large volumes of water evaporated and the presence of compounds with low concentration. However, pervaporation shows higher potential for hydrophobic VOCs recovery from a bioreactor. The wide use of absorption for the removal of odorous volatile organic compounds from industrial gases, like

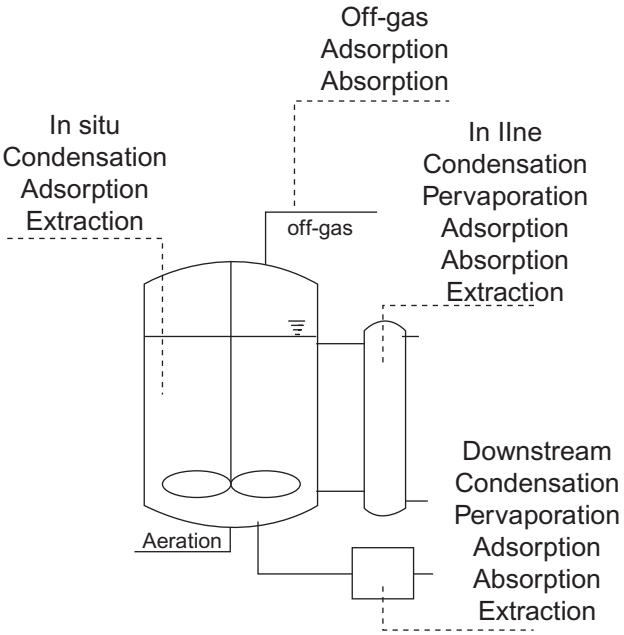

Fig. 24.7

Application possibilities of VOCs recovery techniques at different points in the microalgae-based system. Definitions: *in situ*, product recovery in the bioreactor during production; *off-gas*, product recovery from the reactor off-gas during production; *in line*, product recovery in the external loop during production; *downstream*, external product recovery after production.

wastewater odor abatement, makes its application for VOCs recovery from a microalgae-based system imaginable (Heymes et al., 2007; Lukin et al., 2018).

24.6 Conclusions and future perspectives

Microalgae can produce a variety of volatile compounds, and knowledge about the characterization and morphology of the microalgae, metabolic pathways, VOCs biosynthesis, and optimization of culture systems enables exploitation for many relevant commercial applications. However, some hurdles must be overcome for these bioprocesses to be included in the market, such as improving biochemical and genetic engineering strategies to boost VOCs production, because until now the yields of the products have been too low to make the biotechnological process competitive. Moreover, microalgae VOCs are a blend of compounds, and in order to select the most advantageous volatile compound recovery technique, it is necessary to investigate the location of the target compound within the biochemical system as well as the volatility of the target molecule and its partitioning between the phases under real production conditions.

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CAPÍTULO 6

MANUSCRITO 3

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The role of microalgae-based systems in the dynamics of odorous compounds in the meat processing industry. Part II - Olfactometry and sensory relevance

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The role of microalgae-based systems in the dynamics of odorous compounds in the meat processing industry. Part II – olfactometry and sensory relevance

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ABSTRACT

This research evaluated the role of microalgae-based systems in deodorizing the meat processing industry by analyzing gas chromatography-olfactometry (GC-O). The olfactometric odorant profile of raw wastewater, the deodorization process along the residence time, and the high-value volatile organic compounds generated by heterotrophic cultures of *Phormidium autumnale* were assessed. The results presented thirty-seven compounds identified by GC-O in the raw wastewater. Indole and skatole were considered the main odor markers with the modified frequency of 91% and 75%, respectively. These compounds did not present sensory perception after 72 h of residence time, suggesting that were completely removed. At the same time, a total of 11 compounds were formed in the microalgae-based process. These compounds were classified as fruity, citrus, green, and resinous by the judges and can be used as a flavoring agent. Finally, the microalgal heterotrophic bioreactor was able to mitigate the most unpleasant odors of the meat processing wastewater, and, in addition, compounds of commercial interest were generated, suggesting the possibility of exploring them for application in the fine chemical or food industry.

Keywords: Microalgae/cyanobacteria; Agro-industrial wastes; Olfactometric analysis; Deodorization; Bioproducts

1. Introduction

Unpleasant odors emissions from wastewater treatment plants (WWTPs) represent a prominent threat to society by causing degradation of environmental quality, interference with business activities. In addition, the odor can cause effects on human health, ranging from mild discomfort (skin and eye irritation, headaches, dizziness, and nausea) to more severe symptoms (coughing, wheezing, and even breathing problems), depending on its intensity and time of exposure. If the odor lasts for a long time, it can affect a human's mood, anxiety, and stress level [1–3]. With the global trend of urbanization, the increasing population, and the shortage of land resources, the distance between residential areas and WWTPs has decreased, leading to a rise in public grievances against the occurrence of odorous compounds in areas adjacent to these facilities [5,6].

Odor can be defined as a sensation resulting from the interaction of volatile chemical species with relatively low molecular weight (30–200 g mol^{-1}) and pungent smell inhaled through the nose [7]. Among these molecules are volatile organic compounds (VOCs), which are the main pollutants in the atmospheric environment [8]. Some of these compounds have very low odor threshold values in

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terms of ppbv or pptv, where even at low concentrations, they can cause negative psychosomatic symptoms [9].

One of the main sources of environmental odors of anthropogenic origin is the food industry, especially meat processing plants. Although emissions of bad odors have always been associated with the animal protein production chain, only in recent decades has this attracted greater attention. This is related to the intensification of animal production in many countries since the global population growth has increased the demand for animal food sources. Representative VOCs emitted from meat processing facilities are mainly terpenes, alcohols, aldehydes, sulfuric compounds, amines, phenolic compounds, esters, and ketones [9].

To alleviate the issues related to odor emissions, strict environmental regulations are continually being developed and strengthened by the administrative authorities worldwide [10]. In this regard, a variety of odor treatment technologies have been proposed, which can be classified into physical/chemical (e.g., adsorption and chemical scrubbers) and biological (e.g., biofilters, biotrickling filters, bioscrubbers) techniques. Each available technology has advantages and disadvantages, cost, and specific application ranges since the wastewater from WWTPs is a complex mixture of compounds with different molecular weights, volatilities, and chemical functionalities [6,10]. Still, biological technologies are preferable in practical applications based on their efficiency and sustainability [11]. An innovative technology that has emerged is the application of microalgae-based systems for odor removal and the potential bioconversion of value-added products [12].

Microalgae-based systems applied to wastewater treatment have been used for almost 60 y [11,12]. However, the application of these microorganisms for deodorization of the volatile organic compounds of the wastewater treatment plant was first proposed by Vieira et al. [12], in Part I of this sequential research. In this study, the microalgae *Phormidium autumnale* was used to deodorize volatile organic compounds from wastewater, which regardless of polarity range and molecular weight, were removed with 99.6% of efficiency. In addition, was possible to observe the concomitant formation of compounds industrially interesting.

To characterize the olfactory impact of odorants, techniques that combine analytical and sensory measurements, such as olfactometry, have been key tools in odor control processes. Gas chromatography coupled with olfactometry (GC-O) allows to characterize compounds using odor descriptors, evaluate the potential sensorially relevant VOCs, thought the odor intensity and, so allow better estimation of odor impact [13,14]. As far as we know, there have been no reports on the olfactometric evaluation of wastewater deodorization processes.

Thus, the objective of this study was to evaluate the sensorial relevance of volatile organic compounds emitted by a deodorization process based on microalgae of meat processing wastewater. The study focused on the (i) characterization of the olfactometric odorant profile of raw wastewater, (ii) sensory evaluation of the deodorization process, and (iii) evaluation of high-value volatile organic compounds generated by *Phormidium autumnale*.

2. Material and methods

2.1. Microalgae and culture media

Axenic cultures of *Phormidium autumnale* were used in the experiments. Stock cultures were propagated and maintained in solidified agar-agar (20 g L^{-1}) containing synthetic BG11 medium [15]. The incubation conditions were 25°C, the photon flux density was 15 μmol m^{-2} s^{-1} and the photoperiod was 12 h. To obtain the inoculums in liquid form, 1 mL of sterile synthetic medium was transferred to slants; the colonies were scraped and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

2.2. Meat processing wastewater

Meat processing wastewater (MPWW) samples were collected from industry in Santa Catarina, Brazil (27°14'02″S, 52°01'40″W). Samples were collected from the discharge point of an equalization tank over a period of 1 y. The collected MPWW samples were transferred to the analytical laboratory and stored at 4°C according to the standard methods for the examination of water and wastewater [16]. The characteristics of MPWW included chemical oxygen demand (COD), total Kjeldahl nitrogen (N-TKN), total phosphorus ($P\text{-}PO_4^{-3}$), total solids (TS), volatile solids (VS), fixed solids (FS), suspended solids (SS), and pH was determined according to APHA. The average composition of the wastewater was COD 4,100 ± 874 mg L^{-1}, N-TKN 128.5 ± 12.1 mg L^{-1}, $P\text{-}PO_4^{3-}$ 2.84 ± 0.2 mg L^{-1}, TS 3.8 ± 2.7 mg L^{-1}, VS 2.9 ± 1.4 mg L^{-1}, FS 0.9 ± 0.3 mg L^{-1}, SS 1.9 ± 0.8 mg L^{-1}, and pH 5.9 ± 0.05.

2.3. Experimental condition

Cultivations were performed in a bubble column bioreactor, operating under a batch regime and fed on 2.0 L of wastewater [17]. The experimental conditions were determined as follows: initial concentration of inoculum 100 mg L^{-1}, temperature 25°C, pH adjusted to 7.6, and aeration of 1.0 VVM (volume of air per volume of culture per minute), absence of light, and residence time of 72 h. The experiments were performed twice and in duplicate. Therefore, data refer to the mean value of four repetitions.

2.4. Analytical methods

2.4.1. Isolation of the volatile organic compounds

The volatile compounds were isolated from the sample using a headspace solid-phase microextraction (HS-SPME) technique, employing a divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (50/30 μm film thickness × 20 mm; Supelco®, Bellefonte, PA). Sample aliquots of 20 mL were collected each 24 h (0, 24, 48, 72) and equally separated into two portions. The same procedure was repeated for the wastewater and microalgae. Each portion was placed in a 20 mL amber glass vial containing 3 g of NaCl and 10 μL of a 3-octanol internal standard solution with a known concentration (0.082 μg mL^{-1}). The SPME fiber was exposed in the sample headspace for 45 min at 40°C, under constant stirring (400 rpm) with a magnetic

stir bar. After this period, the fiber was removed from the vial and submitted to chromatographic analysis. The analytical procedure was performed twice and in duplicate. Therefore, data refer to the mean value of four repetitions.

2.4.2. GC-O and GC–FID analyses

The volatile compounds were quantified and sniffed by a Varian Star 3400 CX (CA, USA) gas chromatograph equipped with a flame ionization detector (GC–FID) and a sniffing port both interconnected by a flow splitter to the column exit. Eluting compounds were split at the end of the column at a 1:1 ratio between the FID detector and the olfactometric port. The fiber was thermally desorbed into the injection port at a temperature of 250°C for 10 min, in a splitless mode for 1.0 min. Hydrogen was used as carrier gas at constant pressure (15 psi) and flow rate (1.2 mL min^{-1}). The compounds were separated in a polar fused silica capillary column DB-WAX (CHROMPACK, USA; 30 m × 0.25 mm × 0.25 μm of film thickness). 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 temperature in the detector was kept at 250°C. Purified compressed air (flow rate 3.5 L min^{-1}) was used to carry the analytes from the heated GC transfer line until the sniffing port. The air was pre-heated and reach the judge's nose at 28°C.

The protocol of the study was approved by the Ethics Committee of the Federal University of Santa Maria (CAAE 98758718.8.0000.5346). A modified frequency technique was used for the evaluation of odors and their relative influence on the aroma of the sample. Sniffings were carried out by a panel composed of six experienced judges belonging to the laboratory staff. Sniffing time was approximately 47 min, and each judge evaluated a half part in one chromatographic run, and they participated one time per day. The panelists were asked to score the intensity of each volatile stimulus using a categorical 4-point scale: 0 = no odor; 1 = weakly recognizable odor; 2 = clear but not intense odor, and 3 = very intense odor. The olfactometric strategy carried out in this study combined measurements of intensity and frequency of detection, as has been reported in previous papers [18,19]. The signal obtained was the modified frequency (MF, %), a parameter which was calculated by Eq. (1) proposed by Dravnieks [20]:

$$\mathrm{MF}(\%) = \sqrt{F(\%)} \times I(\%) \quad (1)$$

where F (%) is the detection frequency of an aromatic attribute expressed as a percentage of the total number of judges and I (%) is the average intensity expressed as a percentage of the maximum intensity.

The linear retention index (LRI) was calculated for each volatile compound using the retention times of a standard mixture of homologous series of n-alkanes (C6-C24) to aid identification [21]. This parameter was used to calculate the LRI of odoriferous stimuli.

2.4.3. GC/MS analysis

The volatile compounds were separated and identified in a Shimadzu QP2010 Plus gas chromatography coupled to a mass spectrometer (Shimadzu, Kyoto, Japan). The fiber was thermally desorbed for 10 min in a split/splitless injector, operating on the splitless mode (1.0 min splitter off) at 250°C. Helium was used as a carrier gas at a constant flow rate of 1.6 mL min^{-1}. Analytes were separated as described for a GC-O-FID. The MS detector was operated on electron impact ionization mode +70 eV and mass spectra were obtained by scan range from m/z 35 to 350.

The volatile compounds were identified by a comparison of experimental, mass spectra, and LRI with those provided by the computerized library (NIST MS Search) considering over 80% of similarity. Additionally, volatile olfactory descriptions were taken into account to identification when compounds possess odoriferous stimuli. The sample and the standard mixture were injected both separately and together to obtain the experimental LRI and mass spectra values for the purpose of compound identification by directed comparison.

3. Results and discussion

3.1. Compounds identified

Towards control odor at WWTPs, the first step is identifying the sensorially relevant VOC emitted, which should be monitored and managed [2]. Table 1 provides a complete list of VOCs identified in this study, along with their corresponding identifications, where the components are listed in order of their LRI on the DB-WAX column.

The compounds presented molecular weights ranged from 44.0 to 156.2 g mol^{-1} and included four sulfur compounds (compounds 1, 2, 10, and 28), eight aldehydes (compounds 3, 5, 6, 7, 8, 11, 35 and 43), one furan (compounds 4), two hydrocarbons (compounds 9 and 34), twelve alcohols (compounds 12, 19, 20, 24, 27, 31, 32, 33, 39, 42, 47, and 53), seven ketones (compounds 13, 14, 16, 23, 26, 29, and 44), eleven terpenes (compounds 15, 17, 18, 21, 22, 36, 37, 38, 40, 45, and 46), three amines (compounds 25, 54, and 55), 1 ester (compound 30), 1 carboxylic acid (compound 41), 4 phenolic compounds (compounds 48, 50, 51, and 52), and 1 nitrogen heterocyclic compounds (compound 49). Among them, sulfides, indoles, and phenols are generally listed as the most impacting odor classes in meat processing wastewater [22,23].

Among all the fifty-five odor compounds detected in this study, following the criteria of other authors [18,24], we considered odor-active compounds that were detected in at least half of the total sniffing analyses and reached a modified frequency value (MF) higher than 30%. Therefore, a total of 48 odor-active compounds were considered in this study.

3.2. Evaluation of odor characteristics along deodorization process with microalgae

Table 2 shows the volatile composition of the raw wastewater and the impact of the metabolic transformation as a function of time on the composition of volatile compounds in the microalgal heterotrophic bioreactor.

Thirty-seven compounds were identified by CG-O in the raw wastewater, and among them, indole had the highest MF value (91%). This compound is considered one of the main odor markers from animal production facilities

Table 1
List of VOCs identified by GC-O in this study

Compound number	LRI DB-WAX[a]	Identity	Chemical formula	Molecular weight (g mol^{-1})	Odor description[b]
1	<1000	Carbon disulfide	CS_2	76.1	Disagreeable, sweet
2	<1000	Dimethyl sulfide	C_2H_6S	62.1	Decayed cabbage, sulfurous
3	<1000	2-Propenal	C_3H_4O	56.1	Burnt, sweet
4	<1000	2-Methylfuran	C_5H_6O	82.1	Roasted meat, chocolate
5	<1000	Acetaldehyde	$C_6H_{14}O_2$	44.0	Pungent, fresh, green
6	<1000	Butanal	C_4H_8O	72.1	Sweet
7	<1000	2-Methylbutanal	$C_5H_{10}O$	86.1	Cocoa, almond
8	<1000	3-Methylbutanal	$C_5H_{10}O$	86.1	Malt, smell of oil
9	1053	Toluene	C_7H_8	92.1	Rubbery, tarry, mothballs
10	1089	Dimethyl disulfide	$C_2H_6S_2$	94.2	Rotten cabbage, putrefaction
11	1102	Hexanal	$C_6H_{12}O$	100.1	Grass, tallow, fat
12	1103	2-Methylpentanol	$C_6H_{14}O$	102.1	Pungent
13	1120	2-Methyl-3-hexanone	$C_7H_{14}O$	114.1	Fruity
14	1128	Acetyl valeryl	$C_7H_{12}O_2$	128.1	Butter, cheese, oily
15	1129	1,4-Cineole	$C_{10}H_{18}O$	154.3	Spice
16	1145	2-Heptanone	$C_7H_{14}O$	114.1	Fruity, spicy, sweet, herbal
17	1156	Limonene	$C_{10}H_{16}$	136.2	Lemon
18	1159	1,8-Cineole	$C_{10}H_{18}O$	154.3	Spice
19	1162	1-Pentanol	$C_5H_{12}O$	88.1	Balsamic, fruity
20	1166	3-Methylbutanol	$C_5H_{12}O$	88.1	Oil, alcoholic, fruity, banana
21	1169	α-Terpinene	$C_{10}H_{16}$	136.2	Lemon
22	1182	ρ-Cymene	$C_{10}H_{14}$	134.2	Lemon, fruity, fuel like
23	1184	Cyclohexanone	$C_6H_{10}O$	98.1	Pepper, acetone
24	1185	2-Heptanol	$C_7H_{16}O$	116.2	Herb
25	1184	Pyrrolidine-2,4-dione	$C_4H_5NO_2$	99.1	na[c]
26	1210	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	126.1	Citrus, green, musty
27	1251	Hexanol	$C_6H_{14}O$	102.2	Flower, green
28	1247	Dimethyl trisulfide	$C_2H_6S_3$	126.3	Rotten, vegetables
29	1230	2-Nonanone	$C_9H_{18}O$	142.2	Fruity, sweet, cheese, green
30	1322	Methyl 3-methyl 2-hydroxy-butanoate	$C_6H_{12}O_3$	132.1	Apple
31	1341	Cyclohexanol	$C_6H_{12}O$	100.1	Camphor
32	1356	5-Ethyl-2-nonanol	$C_{11}H_{24}O$	172.3	na
33	1415	1-Heptanol	$C_7H_{16}O$	116.2	Chemical, green
34	1427	3-Propylcyclopentene	C_8H_{14}	110.2	na
35	1502	Benzaldehyde	C_7H_6O	106.1	Burnt, sweet
36	1511	Linalool	$C_{10}H_{18}O$	154.2	Flower, lavender
37	1522	Fenchol	$C_{10}H_{18}O$	154.2	Camphor
38	1528	4-Terpineol	$C_{10}H_{18}O$	154.2	Turpentine, nutmeg, must
39	1526	2-Octen-1-ol	$C_8H_{16}O$	128.2	Soap, plastic
40	1534	Menthol	$C_{10}H_{20}O$	156.2	Peppermint
41	1586	3-Methylpentanoic acid	$C_6H_{12}O_2$	116.1	Acidic, cheese, green
42	1591	1-Nonanol	$C_9H_{20}O$	144.3	Fat, green
43	1669	Phenylacetaldehyde	C_8H_8O	120.1	Honey, sweet
44	1685	Acetophenone	C_8H_8O	120.1	Must, flower, almond
45	1699	Linomen-4-ol	$C_{10}H_{16}O$	152.2	Fresh, mint
46	1741	*α*-Terpineol	$C_{10}H_{18}O$	154.2	Oil, anise, mint
47	1780	Benzyl alcohol	C_7H_8O	108.1	Sweet, flower
48	1819	2-Phenylethanol	$C_8H_{10}O$	122.1	Rosy
49	1832	Benzothiazole	C_7H_5NS	135.1	Gasoline, rubber
50	1829	o-Cresol	C_7H_8O	108.1	Medicinal, phenolic
51	1877	Phenol	C_6H_6O	94.1	Medicinal, phenolic plastic rubber
52	1876	ρ-Cresol	C_7H_8O	108.1	Fecal, horse stable-like
53	2015	1-Penten-3-ol	$C_5H_{10}O$	86.3	Pungent, green, vegetable
54	2264	Indole	C_8H_7N	117.1	Manure, fecal, nauseating
55	2500	Skatole	C_9H_9N	131.2	Fecal, nauseating

[a]Linear retention índices in the DB-WAX column;
[b]According to: Vieira et al. [12]; Acree and Arn [21];
[c]na: not available in the literature.

Table 2
Odorants found in the microalgal heterotrophic bioreactor: gas chromatographic retention data, identify, and modified frequency percentage (MF, %)

			Modified frequency (%)				
Group	Peak	Identify	Waste	0 h	24 h	48 h	72 h
Sulfur compounds	1	Carbon disulfide	47	41	–	–	–
	2	Dimethyl sulfide	–	–	–	–	–
	10	Dimethyl disulfide	51	51	33	33	–
	28	Dimethyl trisulfide	71	58	41	–	–
Aldehydes	3	Acrolein	58	30	–	–	–
	5	Acetaldehyde	–	–	–	–	–
	6	Butanal	30	–	–	–	–
	7	2-Methylbutanal	58	51	–	–	–
	8	3-Methylbutanal	51	–	–	–	–
	11	Hexanal	–	–	–	–	–
	35	Benzaldehyde	68	68	–	–	–
	43	Phenylacetaldehyde	–	–	–	–	–
Furans	4	2-Methylfuran	47	37	–	–	–
Hydrocarbons	9	Toluene	31	30	–	–	–
	34	3-Propylcyclopentene	61	54	30	–	–
Alcohols	12	2-Methylpentanol	–	–	–	–	–
	19	1-Pentanol	88	78	–	–	–
	20	3-Methylbutanol	–	–	37	–	–
	24	2-Heptanol	71	74	–	–	–
	27	Hexanol	54	44	–	–	–
	31	Cyclohexanol	–	–	–	–	–
	32	5-Ethyl-2-nonanol	–	–	37	–	–
	33	1-Heptanol	51	58	–	–	–
	39	2-Octen-1-ol	51	30	–	–	–
	42	1-Nonanol	54	30	–	–	–
	47	Benzyl alcohol	68	–	–	–	–
	53	1-Penten-3-ol	–	51	43	51	41
Ketones	13	2-Methyl-3-hexanone	–	–	58	–	–
	14	Acetyl valeryl	–	–	–	–	–
	16	2-Heptanone	–	–	44	37	30
	23	Cyclohexanone	54	40	–	–	–
	26	6-Methyl-5-hepten-2-one	–	54	41	68	–
	29	2-Nonanone	–	–	41	–	–
	44	Acetophenone	30	30	–	–	–
Terpenes	15	1,4-Cineole	41	–	–	–	–
	17	Limonene	85	54	44	–	–
	18	1,8-Cineole	58	44	30	–	–
	21	α-Terpinene	41	51	–	–	–
	22	ρ-Cymene	71	97	–	–	–
	36	Linalool	41	47	–	–	–
	37	Fenchol	85	54	–	–	–
	38	4-Terpineol	58	54	41	–	–
	40	Menthol	–	–	44	53	58
	45	Linomen-4-ol	61	68	61	–	–
	46	α-terpineol	41	85	51	–	–
Amines	25	Pyrrolidine-2,4-dione	54	68	–	–	–
	54	Indole	91	82	71	50	–
	55	Skatole	75	44	–	–	–
Ester	30	Methyl 3-methyl 2-hydroxybutanoate	–	–	68	–	–
Carboxylic acid	41	3-Methylpentanoic acid	–	–	41	–	–
Phenolic compounds	48	2-Phenylethanol	58	–	–	–	–
	50	o-Cresol	44	33	30	–	–
	51	Phenol	61	44	41	–	–
	52	ρ-Cresol	65	54	54	–	–
Nitrogen heterocyclic compounds	49	Benzothiazole	–	41	54	47	33

by several authors [25–27]. Indole, as well as skatole, which had an MF of 75% in wastewater, are produced in the large intestine of animals and in manure by microbial deamination and decarboxylation of tryptophan. Both are detected low threshold concentration and contribute to the unpleasant and nauseating feces odors [28,29]. The other major compounds in the raw wastewater included 1-pentanol (88%), limonene (85%), skatole (75%), p-cymene (71%), 2-heptanol (71%), and dimethyl trisulfide (71%), whose main descriptors were balsamic/fruit, lemon, fecal/nauseating, lemon/fruit/fuel like, herb, and rotten, respectively.

Unsurprisingly, between the raw wastewater and the initial residence time (0 h), that is, shortly after inoculation, little change in the volatile profile was perceived. However, 3 compounds not identified in the wastewater, were detected at 0 h, 6-methyl-5-hepten-2-one, benzothiazole, and 1-penten-3-ol. These compounds are naturally found in the volatile fraction of microalgal cultures since they are derived from the carotenoids cleavage (6-methyl-5-hepten-2-one), fatty acids (1-penten-3-ol), and amino acids (benzothiazole) pathways [12,30,31].

A day after inoculation, important reductions in VOCs were noticed, as shown in Table 2. In this period 19 compounds were removed, mainly alcohols, terpenes, and aldehydes. Aldehydes are a group of great concern as air pollutants due to their reactivity and toxicity [32], so it is important to note that in 24 h all compounds in this class were removed. The term "removed" used in this article refers to changes in which it is unclear whether the compounds are biotransformed, metabolized, or removed from wastewater by any other mechanism. In addition, as a result of the microalgal heterotrophic metabolism, 8 new compounds were generated in the first 24 h of residence time, which are 3 ketones, 2 alcohols, 1 carboxylic acid, 1 terpene, and 1 ester, that will be discussed later.

Between 24 and 48 h, 9 compounds from the raw wastewater were removed, including compounds associated with malodors, such as dimethyl trisulfide, o-cresol, phenol, and ϱ-cresol. Moreover, 6 compounds formed by the microalgae disappeared. During this period no new compound was noticed.

Part I of this sequential research [12] showed that dimethyl sulfide and indole were the most recalcitrant compounds, which were not completely removed, with efficiencies of 69% and 96%, respectively. In terms of sensory perception, these compounds were also the most persistent, being the last odors from wastewater to disappear. Both compounds play an important role in the negative effects on odor release from wastewater treatment plants. The odor impact of these compounds was assessed by the judges, and after 72 h of residence time, presented a modified frequency below 30%, concluding, therefore, that these compounds were completely removed (Fig. 1).

The VOCs identified by the panelists in the treated sample (72 h) were menthol (58%), 2-nonanone (57%), 1-penten-3-ol (41%), and benzothiazole (33%). Note that all of these compounds are the result of microalgae biotransformations since most of these structures were present in the inoculum and others, such as 2-nonanone and menthol were perceived during the process. Except for menthol, the compounds showed a reduction in their

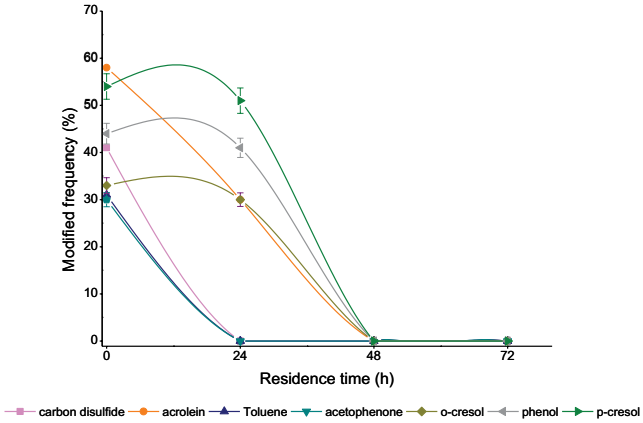


Fig. 1. Hazardous air pollutants biodegradation by *P. autumnale*.

modified frequency in 72 h, characterizing the beginning of the senescence phase. According to the literature [33–35], the production rates of microalgal VOCs follow the same pattern as cell growth, which increases by several orders of magnitude during the exponential phase and decreases during senescence.

Although some VOCs are considered pollutants due to their toxicity to many organisms, they have the potential to serve as sources of carbon for microalgae cultures, and consequently, as substrates for bioconversion into high-value products [36]. Six compounds found in the meat processing wastewater are listed as hazardous air pollutants (HAPs) by the United States Environmental Protection Agency [37]. The adverse effects on health from exposure to these toxic compounds can be as diverse as the substances themselves and therefore, their monitoring and controlling is imperative. The compounds classified as HAPs were carbon disulfide (1), acrolein (3), toluene (9), acetophenone (44), o-cresol (50), phenol (51), and ρ-cresol (52). Fig. 1 shows the HAPs biodegradation as a function of residence time.

In the raw wastewater, acrolein was found to be the most abundant species, followed by ρ-cresol, phenol, carbon disulfide, o-cresol, toluene, and acetophenone. The results obtained indicate that in one day of operation the heterotrophic bioreactor was able to reduce 43% of the compounds (carbon disulfide, acetophenone, and toluene) to levels undetectable by humans panelists in olfactometry. The phenolic compounds (ρ-cresol, phenol, and o-cresol) and acrolein were only eliminated in 48 h.

To help the study of the odor profile of each sample, the panel of six experienced judges generated a consensual list with twelve sensory descriptors: resinous, putrid, wood, hospital, fruity, sweet, mold, green, spice, floral, burnt, and fat, which are shown in Fig. 2.

The results presented in Fig. 2 corroborate what has already been discussed, where showed a clear change in the volatile profile of the wastewater along the residence time, were no longer detected. In 24 h of residence time, it can be observed that putrid odors were no longer detected. The changes were even more evident between 24 h and 48 h of process, where the descriptors wood, hospital, fruity, mold, spice, floral, and burnt disappeared. On the other hand, in all the samples analyzed, resinous was the descriptive term with the greatest impact.

The odors can be classified into pleasant, neutral, or unpleasant and the relative pleasantness of an odor can be measured by the hedonic tone. A comparative spider chart of the data from the raw wastewater and the end of the cultivation (72 h), considered the treated wastewater is shown in Fig. 3. Dravinieks et al. [38] developed a robust list of 150 odor descriptors and their respective hedonic tone. In this way, Fig. 3 also shows the hedonic tone values determined by these authors regarding the descriptors assigned in this study.

A total of eleven descriptors were generated in the raw wastewater, with resinous, putrid, and hospital being the most impact descriptors. The highest modified frequency occurred among the compounds, putrid (indole, 91%), resinous (1-pentanol, 88%), and fruity (limonene, 85%). At the end of the process, four compounds were perceived by the judges; a terpene, a ketone, alcohol, and a heterocyclic nitrogen compound, which have been described as green, spice, resinous and hospital.

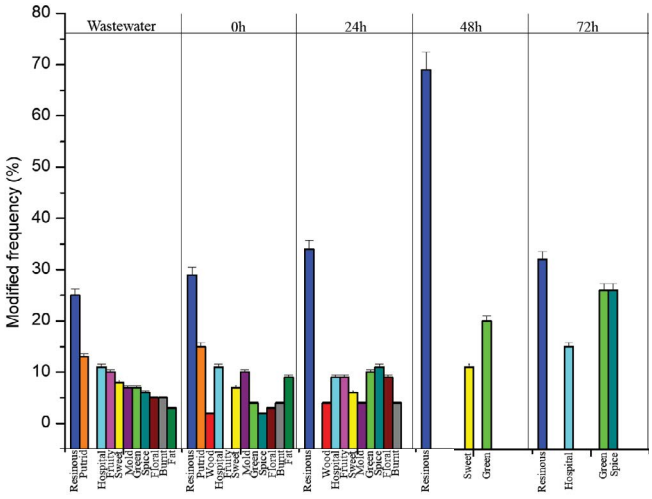


Fig. 2. Consensual list with twelve sensory descriptors the panel of six experienced judges generates.

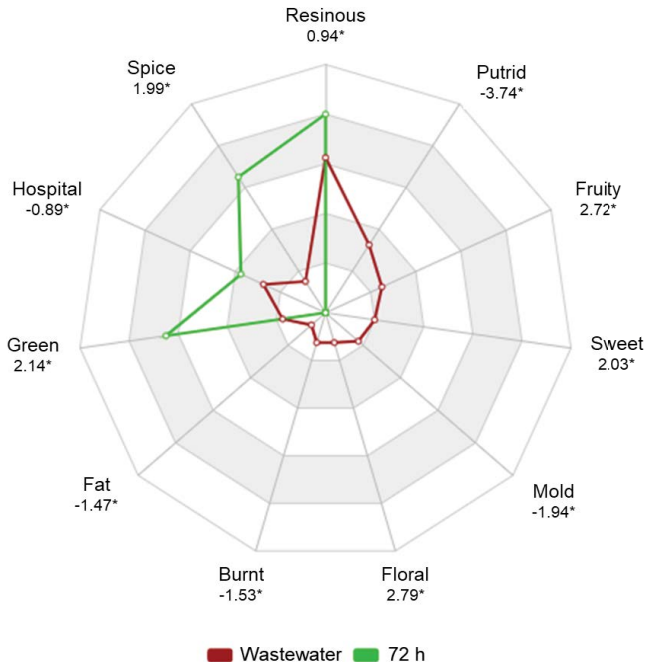


Fig. 3. Spider chart of the sensory profile of mean attribute values for the raw wastewater and the treated wastewater. *Hedonic tone determined by Dravnieks et al. [38].

Regarding the hedonic tone, the 11 descriptors associated with the raw wastewater presented values from –3.74 to 2.79, as showed in Fig. 3. The odor annoyance is subjective, and the perception of pleasantness or dislike depends on the individual's level of tolerance, the exposure time, the emotions of the moment, in addition to being influenced by intercultural differences. Typically, the hedonic tone, that is the level of odor pleasantness or unpleasantness, is measured in a numeric scale ranging from –4 to 4, where –4 is the most unpleasant odor, 0 is neutral, and 4 is the least unpleasant odor. Among the eleven descriptors, five were classified as unpleasant due to their negative value, namely putrid (–3.74), mold (–1.94), burnt (–1.53), fat (–1.47), and hospital (–0.89).

The diagram of comparison (Fig. 3) shows that seven odor characteristics disappeared after microalgae treatment, including the four with the lowest hedonic tone value (putrid, mold, burnt, and fat). At the same time, the descriptors resinous, green, spice, and hospital, with a hedonic tone of 0.94, 2.14, 1.99, and –0.89, increased the impact with the microalgae treatment. By analyzing the results presented above, it can be seen that the microalgal heterotrophic bioreactor was capable of mitigating the most unpleasant odors of the meat processing wastewater.

3.3. Biogeneration of volatile organic compounds

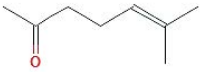
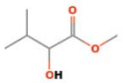
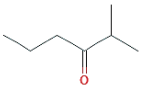
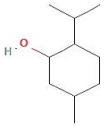
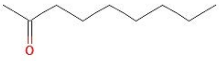
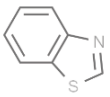
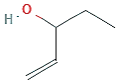
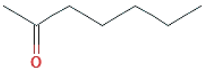
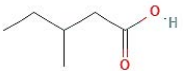
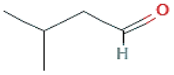
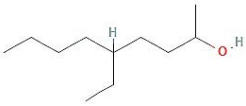
Volatile organic compounds represent an important part of the microalgae metabolome, with expressive possibilities for industrial applications. These structures could be used as a significant alternative source of aromas, fragrances, food additives, pharmaceutical products, and energy [35]. Still, VOCs have been neglected for a long time. However, scientific advances in recent years and the increasing consumers' preference for natural compounds have driven researchers and companies to explore the volatile fraction of microalgae-based processes [39,40].

In this sense, the volatile organic compounds produced by *Phormidium autumnale* cultivated in meat processing wastewater under heterotrophic conditions are presented in Table 3, as well as its potential industrial applications and chemical structure.

A total of 11 compounds produced in the microalgae-based process were identified, with 4 ketones, 3 alcohols, 1 terpene, 1 ester, 1 carboxylic acid, and 1 heterocyclic nitrogen compound. Among the chemical classes identified, 6-methyl-5-hepten-2-one (68%), methyl-3-methyl-2-hydroxybutanoate (68%), 2-methyl-3-hexanone (58%), menthol (58%), and 2-nonanone (57%) were the most impactful in terms of modified frequency.

Ketones, such as 6-methyl-5-hepten-2-one, 2-nonanone, and 2-heptanone are used mainly as flavors, and fragrance agents, due to their description as fruity, citrus, and green [35]. 2-Methyl-3-hexanone, another ketone produced by

Table 3
Ranking of the volatile profile by average modified frequency percentage of the compounds formed

Compound	MF %	Main applications	Structure
6-Methyl-5-hepten-2-one	68	Analytical standard, flavor, fragrance agents	
Methyl-3-methyl-2-hydroxybutanoate	68	Research chemical	
2-Methyl-3-hexanone	58	Analytical standard, research chemical	
Menthol	58	Medicines, ointments, flavor, fragrance agents	
2-Nonanone	57	Flavor, fragrance agents	
Benzothiazole	54	Analytical standard, fragrance agents, cosmetic, chemical industry	
1-Penten-3-ol	51	Analytical standard, flavor, fragrance agents	
2-Heptanone	44	Flavoring agent, adjuvant	
3-Methylpentanoic acid	41	Flavoring agent, adjuvant	
3-Methylbutanol	37	Flavoring agent, adjuvant	
5-Ethyl-2-nonanol	37	Research chemical, building blocks	

P. autumnale is applied as a research chemical and analytical standard and is not recommended for flavor use [41]. Except for 6-methyl-5-hepten-2-one, which was already present in the microalgal inoculum, the other ketones were noticed only in 24 h of culture.

In the alcohol class, 1-penten-3-ol was identified in all samples after inoculation, which might exert an important effect on the flavor of microalgae, which was defined as resinous by the judges and generally is used as a flavoring agent. This compound is typically found in microalgae since it is a product of the lipid oxidation of n-3 fatty acids [42,43]. 3-Methylbutanol and 5-ethyl-2-nonanol were only perceived in 24 h of cultivation, being described as wood and green, respectively. 3-Methylbutanol is allowed to be used in foods, as a flavoring and adjuvant agent, while 5-ethyl-2-nonanol is used for other purposes, such as research chemicals and building blocks [42].

Terpenes are particularly important in the flavor market, especially menthol-flavored compounds, that are used extensively as additives in oral hygiene products and flavors in food and beverages. Menthol isomers are derived from limonene, and it is possible to see (Table 2) that the modified frequency of menthol increases as that of limonene decreases, which may give evidence of the biotransformation of these compounds [44]. Benzothiazole is another natural component of the VOCs of *P. autumnale*, where its biggest modified frequency was in 24 h (54%). Nitrogen heterocycles compounds, especially benzothiazole and its derivatives

are of great interest to the fine chemical and pharmaceutical industries due to their wide range of biological activities, like anticancer, antifungal, antiviral, anticonvulsant, anti-inflammatory, and antidiabetic activities [45].

Finally, among the VOCs originating from *P. autumnale*, typical microalgal compounds that cause an unpleasant odor, such as 2-methylisoborneol and geosmin, were not detected, as already reported by Santos et al. [35]. Despite the possibility of broad industrial application of microalgal VOCs, the unit operations of isolation, fractionation, and purification operations are still substantial bottlenecks in the process that need to be solved.

4. Conclusions

GC-O analysis has demonstrated been a key tool in odor control processes and contributed to proving that there was a transformation in the volatile profile of compounds released in wastewater treatment plants by the microalgae-based system proposed. This research shows the potential of the microalgal heterotrophic bioreactor in odor emission abatement in meat processing wastewater and production concomitant of new compounds. Thus, the microalgae-based systems can become essential support in the consolidation of new technologies in the wastewater treatment industry, with simultaneous odor and wastewater treatment by microalgal heterotrophic bioreactor.

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Symbols

COD	—	Chemical oxygen demand (mg L^{-1})
N-TKN	—	Total nitrogen (mg L^{-1})
P-PO_4^{-3}	—	Total phosphorus (mg L^{-1})
TS	—	Total solids (mg L^{-1})
SS	—	Suspended solids (mg L^{-1})
VS	—	Volatile solids (mg L^{-1})
FS	—	Fixed solids (mg L^{-1})
VVM	—	Volume of air per volume of wastewater per minute
HS-SPME	—	Headspace solid-phase microextraction
DVB/Car/PDMS	—	Divinylbenzene/carboxen/polydimethylsiloxane
GC/MS	—	Gas chromatography-mass spectrometry
LRI	—	Linear retention index
GC-FID	—	Gas chromatography equipped with a flame ionization detector
GC-O	—	Gas chromatography coupled with olfactometry
MF	—	Modified frequency
F (%)	—	Detection frequency of an aromatic attribute
I (%)	—	Intensity

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6. DISCUSSÃO

Com o aumento da população global e níveis de industrialização, a demanda por tecnologias de controle de odor de COVs sustentáveis, tornou-se importante para garantir melhoria da qualidade do ar dentro e ao redor das fontes de emissão. Como consequência, o desenvolvimento de técnicas biológicas mais ecológicas e com maior eficiência de remoção de poluição de ar, vem ganhando grande atenção, pois pode superar várias limitações das técnicas convencionais (CHEN et al., 2021).

As tecnologias atuais de controle de odor, ainda apresentam problemas para remover o indol e o escatol, ambos COVs são considerados um dos principais marcadores de odor de instalações de tratamento de água residuária do abate e processamento animal. Além disso, estes compostos estão presentes simultaneamente dos componentes estruturais hidrofóbicos e hidrofílicos (MATIAS et al., 2015; LEWKOWSKA, et al. 2016). Esta pesquisa forneceu os resultados do uso de bioprocessos microalgais empregado para o controle de COVs desagradáveis encontrados em água residuária de processamento de aves e suínos. O composto com maior FM identificados por GC-O na água residuária bruta foi o indol com valor de 91%, seguido deste, o escatol apresentou 75% de FM, e ambos não apresentaram percepção olfativa após o período de 72 horas de tempo de residência, obtendo valores de FM abaixo de 30%, sendo interpretados como ruídos (BRAVOS-LAMAS et al., 2018).

Além disso, foi observado a remoção de compostos hidrofóbicos. Um total de 10 terpenos foram completamente removidos, o limoneno e seus derivados, α-terpineol e seus derivados, linalol e fenchol. Os compostos dissulfeto de carbono, acroleína, tolueno, acetofenona, o-cresol, fenol e ρ-cresol são classificados como poluentes atmosféricos perigosos e podem ter efeitos adversos à saúde decorrentes da exposição a esses compostos tóxicos (EPA, 2008). Nesta pesquisa foi observado a biodegradação destes poluentes.

O sistema baseado em microalga, após o tempo de residência de 72 horas apresentou uma eficiência de remoção de 100% dos compostos de odor desagradáveis, pois não houve mais percepção sensorial detectada pelos juízes. Outras tecnologias de tratamento de odores em estações de tratamento de água residuária existentes foram relatados que a remoção de odores variou de 70 a 95% (LEBRERO et al., 2011). Pesquisas também mostraram uma eficiência de remoção

de 99,7% da emissão de odores de águas residuárias de suínos com o uso de células microbianas, no entanto o tempo de residência total foi de 260 horas (LOGAN et al., 2008). Assim, é possível perceber a capacidade do biorreator heterotrófico microalgal em mitigar os odores mais desagradáveis do efluente do processamento de carnes em um curto período de tempo.

Muitas desvantagens são encontradas nas tecnologias existentes de tratamento de odor biológico, entre eles formação de biofilmes, entupimento dos biorreatores, necessidade de suplementação de nutrientes, alto custo de processo, entre outros (KELLENER & FLAUGER, 1998; LEWKOWSKA et al.,2016; VIKRANTE et al., 2017). A tecnologia que utiliza microalgas, apesar de apresentar um alto custo inicial de implantação (DEPRA et al. 2019), não apresentou problemas de processo significativos. Além do mais, biorreatores microalgais podem ser utilizados em conjuntos com outras etapas de processo, como conversão de matéria orgânica e nutrientes da água residuária em uma infinidade de bioprodutos, reduzindo assim o custo de processo (SANTOS et al., 2016a; XANG et al., 2017; LAURITANO et al., 2018; DEPRA et al. 2019).

Muitos estudos relataram a produção de compostos de mau cheiro em microalgas, como a presença de 2-MIB e geosmina (LIATO & AÏDER, 2017; MEENA et al., 2017; LEE et al., 2017). No entanto, estes compostos não foram detectados por GC/MS e nem pelos juízes por GC-O na microalga *Phormidium autumnale* utilizada neste estudo. Sabe-se que estes compostos podem ser facilmente liberados por muitas microalgas devido a fatores biótico e abióticos. Neste estudo, foi utilizados biorreatores heterotróficos, onde foi facilitado o controle de temperatura e pH, além do tempo de residencia celular, evitando assim a liberação de 2-MIB e geosmina.

Este estudo mostrou que a microalga *Phormidium autumnale* além de biodegradar compostos de mau cheiro da água residuária, também conseguiu biotransformar e produzir novos compostos voláteis. Pesquisas com relação a COVs gerados por microalgas tem sido realizados atualmente, e estas apresentam um amplo espectro de classes de voláteis, podendo ser utilizadas como aromas, aditivos alimentares ou na geração de energia (SANTOS et al. 2016b; VIEIRA, PINHEIRO & ZEPKA, 2020).

7. CONCLUSÃO GERAL

Sistemas baseados em microalgas podem ser amplamente considerados como uma solução para resolver os diversos desafios da humanidade em relação aos problemas ambientais. As microalgas já são conhecidas por apresentarem potencial no processo de recuperação de efluentes, reduzindo o uso de energia de estratégias de gerenciamento de resíduos e regenerar nutrientes como carbono, fósforo e nitrogênio. Além disso, este estudo demontrou a viabilidade do uso de microalgas na desodorização de água residuária, atingindo uma eficiência de remoção de compostos orgânicos voláteis de 100% após 72 horas de tempo de residência, não apresentando percepção sensorial.

Além da remoção dos compostos de mal cheiro, paralelamente, as microalgas geraram 11 novos compostos (4 cetonas, 3 álcoois, 1 terpeno, 1 éster, 1 ácido carboxílico e 1 composto de nitrogênio heterocíclico) perceptíveis e classificados como frutados, cítricos, verdes e resinosos pelos jurados. Os compostos mais impactantes foram 6-metil-5-hepten-2-ona, metil-3-metil-2-hidroxibutanoato, 2-metil-3-hexanona, mentol e 2-nonanona. Essas estruturas apresentam possibilidades expressivas para aplicações industriais, em diferentes setores.

A partir destes estudos, foi concluído que sistemas baseados em microalgas são capazes de mitigar os odores mais desagradáveis da água residuária do processamento de carnes, além de gerar uma variedade de compostos voláteis de interesse comercial, maximizando o aproveitamento da biomassa microalgal, ao mesmo tempo que se reduz o impacto ambiental gerado por estes resíduos. Essa integração de processo pode abordar as questões de sustentabilidade energética e reciclagem de resíduos no âmbito da bioeconomia circular, reduzindo os custos de produção de microalgas, aumentando a eficiência e a rentabilidade do processo.

Apesar de o futuro para aplicações de sistemas baseados em microalgas pareça promissor, um longo caminho ainda precisa ser percorriddo para se tornar uma parte importante da indústria moderna. São necessários mais pesquisa e investimentos biotecnológicos. Espera-se que a colaboração proativa e o envolvimento de diferentes setores, como tecnólogos, economistas, engenheiros, empresários e políticos, sejam cruciais para impulsionar os processos e produtos baseados em microalgas em direção a uma sociedade cada vez mais verde.

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