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DESODORIZAÇÃO DE ÁGUA RESIDUÁRIA A PARTIR DO CULTIVO HETEROTRÓFICO MICROALGAL

Santa Maria, RS

2016

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

Orientador: Profª. Drª. Leila Queiroz Zepka

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Aprovado em 15 de agosto de 2016:

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RESUMO

DESODORIZAÇÃO DE ÁGUA RESIDUÁRIA A PARTIR DO CULTIVO HETEROTRÓFICO MICROALGAL

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Microalgas são seres de estrutura procarionte que reúnem interessantes características para a utilização biotecnológica em sistemas de tratamento de efluentes. A utilização destes micro-organismos baseada na bioconversão de material orgânico e nutrientes em compostos de interesse industrial tem sido amplamente explorada. No entanto, entre a diversidade de aplicações biotecnológicas destes micro-organismos, existe uma questão ainda pouco explorada que consiste no emprego de microalgas na desodorização de compostos provenientes das estações de tratamento de efluentes. Em face disto, o trabalho teve como objetivo avaliar a desodorização de águas residuárias a partir do cultivo heterotrófico microalgal de *Phormidium autumnale*. A estratégia utilizada baseou-se em três etapas, (i) Discutir a biogeração de compostos orgânicos voláteis em microalgas, (ii) avaliar a capacidade da microalga em desodorizar a água residuária (iii) identificar e quantificar os compostos orgânicos voláteis presentes no efluente agroindustrial. Os voláteis foram isolados no headspace por microextração em fase sólida em diferentes tempos de residência (0, 24, 48, 72, 96, 120 e 144 horas), separados pela coluna DB-WAX em um cromatógrafo gasoso e identificados por espectrometria de massas (GC/MS). A quantificação foi realizada a partir da área dos picos com o uso do padrão interno. Um total de 64 compostos foi detectado no cultivo. Sendo que 43 compostos desapareceram após 48 horas de processo. Concomitantemente, a partir de 24 horas de cultivo 21 novos compostos orgânicos voláteis foram formados. Adicionalmente, em paralelo as informações experimentais obtidas, foi publicado um capítulo de livro intitulado “Biogeneration of Volatile Organic Compounds by Microalgae: Occurrence, Behavior, Ecological Implications and Industrial Applications” que compõem o livro “Volatile Organic Compounds: Occurrence, Behavior and Ecological Implications”, publicado pela editora “Nova Science Publishers”.

ABSTRACT

DEODORIZING WASTEWATER FROM HETEROTROPHIC MICROALGAE CULTIVATION

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Microalgae are prokaryote structure of beings who gather interesting features for the biotechnological use in wastewater treatment systems. The use of such microorganisms based on bioconversion of organic matter and nutrients into compounds of industrial interest has been widely explored. However, among the variety of biotechnological applications of these micro-organisms, there is an issue little explored consisting in the microalgae employment in deodorizing compounds in the wastewater treatment plants. Given this, the study aimed to evaluate the deodorization of wastewaters the from heterotrophic microalgae bioreactors of *Phormidium autumnale*. The strategy is based on three stages, (i) discuss the bio-generation of volatile organic compounds in microalgae, (ii) evaluate the ability of microalgae to deodorize the wastewater, (iii) identify and quantify the volatile organic compounds present in the agro-industrial effluent. The volatiles were isolated by headspace solid-phase micro-extraction in different residence times (0, 24, 48, 72, 96, 120 e 144 hours), the compounds were separated on a column DB-Wax in the gas chromatograph and identified by mass spectrometry (SPME-GC/MS). The quantification of the volatiles was performed from the peak area, using one standard inside. Quantitation was performed from the peak area using the internal standard. A total of 64 compounds was detected in the culture. Being that 43 compounds disappeared after 48 hours of the process. Concomitantly, after 24 hours of cultivation 21 new volatile organic compounds were formed. Additionally, in parallel experimental information obtained, it was published a book chapter entitled Biogeneration of Volatile Organic Compounds by Microalgae: Occurrence, Behavior, Ecological Implications and Applications Industrial composing the book Volatile Organic Compounds: Occurrence, Behavior and Ecological Implications, published by publisher New Science Publishers.

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INTRODUÇÃO

As estações de tratamento de água residuária possuem uma alta carga de material orgânico e nutrientes. Devido à degradação destes compostos ocorre a liberação de compostos orgânicos voláteis (COV's), gerando um odor desagradável que provém de uma mistura complexa de sulfurados, nitrogenados, fenóis, aldeídos, álcoois e ácidos orgânicos (HWANG, 1994).

Estes compostos podem causar um incômodo para a população e contribuem significativamente para a poluição atmosférica (BURGESS, 2001). Nos últimos 30 anos os odores se tornaram um grave problema ambiental e a discussão sobre os efeitos negativos de odores na saúde humana ainda é uma questão amplamente estudada e debatida (CAPELLI, 2011). Os desconfortos causados pelos odores trazem efeitos negativos para a indústria e para a qualidade de vida da população vizinha.

Os COV's que ocorrem numa estação de tratamento de água residuária (ETAR) surgem principalmente a partir da biodegradação dos efluentes em diferentes etapas. Vários são os métodos de controle e tratamento de emissão de odores utilizados nas estações de tratamento de água residuária, que inclui basicamente a oxidação térmica, a oxidação química e biofiltros. A escolha de um único sistema ou de um conjunto de operações que possibilitem o efetivo abatimento das emissões requer estudos apurados, não apenas técnicos, mas também de custos de investimentos.

As características metabólicas das microalgas fazem com que estes micro-organismos apresentem potencial de aplicação no tratamento de efluentes. A utilização de microalgas baseada na bioconversão de material orgânico e nutrientes em compostos de interesse industrial tem sido amplamente explorada.

No entanto, entre a diversidade de aplicações biotecnológicas destes micro-organismos, existe uma questão ainda pouco explorada que consiste no emprego de microalgas na desodorização de compostos provenientes das estações de tratamento de efluentes. Vários compostos orgânicos podem ser metabolizados por microalgas.

A remoção substancial de nutrientes também é uma característica do metabolismo heterotrófico microalgal que converte simultaneamente estes poluentes no biorreator. Porém, a desodorização microalgal não pode ser atribuída exclusivamente à bioconversão. Há outros mecanismos físico-químicos que contribuem para a eliminação

destes compostos, em sistemas biológicos aerados (MARONEZE et al., 2014; QUEIROZ et al., 2007).

Em face disso, o desenvolvimento da tecnologia baseada em microalgas aplicáveis a conversão de resíduos agroindustriais, possibilitará avaliar o potencial de desodorização de efluentes agroindustriais.

OBJETIVOS

Objetivo Geral

- Avaliar a desodorização da água residuária a partir do cultivo heterotrófico microalgal de *Phormidium autumnale*.

Objetivos Específicos

- Discutir a biogeração de compostos orgânicos voláteis de microalgas;
- Avaliar a capacidade da microalga em desodorizar a água residuária;
- Identificar e quantificar os compostos orgânicos voláteis presentes no efluente agroindustrial;

CAPÍTULO 1
REVISÃO BIBLIOGRÁFICA

1. Microalgas

Microalgas são micro-organismos heterogêneos, microscópicos, unicelulares, coloniais ou filamentosos, fotoautotróficos, podendo ser procarióticos ou eucarióticos (OLAIZOLA, 2003). Tem hábito planctônico, embora haja também muitas espécies bentônicas e terrestres, não apresentando nenhum valor taxonômico (LOURENÇO, 2006). Segundo Reviers (2002), atualmente as microalgas estão classificadas em 11 divisões distintas: Cyanophyta, Glaucophyta, Rodophyta, Cryptophyta, Euglenozoa, Cercozoa, Haptophyta, Dinophyta, Ochroophyta, Streptophyta e Chlorophyta.

A diversidade deste grupo de micro-organismos é destacada por serem responsáveis pela estruturação da atmosfera terrestre, por sua importância ecológica e econômica. As algas são as maiores removedoras de carbono da biosfera. Os oceanos sequestram anualmente 2 gigatoneladas de carbono por meio de absorção de dióxido de carbono, enquanto a biosfera terrestre total, remove 1,4 gigatoneladas. As macroalgas (multicelulares) fornecem produtos como o alginato, carragena e ágar, que são valorizados por suas propriedades físicas como espessantes, emulsificantes e geleificantes. As microalgas (unicelulares) destacam-se pelo seu valor micronutricional, contendo diversos metabólitos que atuam diretamente no sistema imunológico humano (MOORE, 2001).

A aplicabilidade das cianobactérias é principalmente devido a seus perfis metabólicos únicos e diversos. Suas aplicações comerciais englobam nutrição, a produção de biocombustíveis, cosméticos, fertilizantes e controle da poluição (GAFFNEY et al., 2014). O número das espécies destes organismos não é conhecido exatamente, no entanto, são encontradas citações que relatam a existência de 2000 espécies e podem ser divididas em 150 gêneros. Sua diversidade é expressa por suas propriedades morfológicas, bioquímicas e fisiológicas, que lhes permite estabelecer e persistir em uma grande variedade de habitat (PALINSKA et al., 2011).

O gênero *Phormidium* sp. é uma cianobactéria filamentosa não ramificada que vivem aglomerados, com conteúdo celular geralmente azul esverdeado. Diversas espécies são conhecidas por viverem em ambientes extremos como fontes termais, solos desérticos e locais poluídos, e por isso eles têm um grande potencial para uso em

bioprocessos, devido à sua robustez e necessidades nutricionais simples (MARONEZE et al., 2014; GUIRY & GUIRY, 2013)

As cianobactérias são assim denominadas pela ausência de organização celular e estruturas definidas, fato que as assemelha às bactérias (REVIERS, 2002). Considerando a grande biodiversidade e os recentes desenvolvimentos na engenharia genética, as cianobactérias representam uma das fontes mais promissoras para novos bioprodutos, como proteínas, amido, celulose, lipídeos, incluindo metabólitos secundários como pigmentos e alguns fármacos, devido esses micro-organismos converterem substâncias inorgânicas como carbono, nitrogênio, fósforo, enxofre, ferro fazendo com que se obtenha uma biomassa rica em nutrientes (BATISTA et al., 2013).

A *Phormidium autumnale* é apontada como uma espécie em potencial no emprego de tecnologias de tratamento de efluentes a partir de sistemas que empregam cianobactérias devido a sua tolerância a determinadas condições como temperaturas extremas e concentrações elevadas de nutrientes (SU et al., 2012). Fatores ambientais, como temperatura, iluminação, pH, conteúdo mineral, densidade populacional, fase de crescimento e estado fisiológico podem modificar sua composição química. Assim, as condições de crescimento podem ser otimizadas e mapeadas para maximizar a produção de biomoléculas de interesse (HU, 2013; BATISTA et al., 2013).

Apesar de as microalgas serem principalmente fotoautotróficas, um número considerável destes microrganismos possui a habilidade de manutenção da estrutura na ausência de luz, assim sendo capaz de crescer também heterotroficamente, a partir da assimilação de substratos orgânicos para a manutenção de suas estruturas (WEN & CHEN, 2003). A microalga *Phormidium autumnale* faz parte deste grupo com potencial de exploração do metabolismo heterotrófico.

2. Cultivo heterotrófico

O metabolismo heterotrófico apresenta como características a ausência total de luminosidade e o emprego de uma fonte de carbono orgânica exógena utilizada na obtenção de energia. Este tipo de produção é suportado por carboidratos como glicose,

frutose, sacarose, acetato e glicerol, além da utilização direta de águas residuárias (SANTOS et al., 2016; PEREZ-GARCIA et al., 2011).

A glicose é a fonte de carbono exógena mais utilizada para as culturas heterotróficas microalgais, devido às elevadas taxas de crescimento e respiração obtidas com esse substrato. Em geral, quando se utilizam outros substratos, as microalgas requerem um período de adaptação, representadas pela extensa fase lag, necessária para a síntese das enzimas e dos sistemas de transporte específicos para a assimilação e o consumo das moléculas (PEREZ-GARCIA et al., 2011).

O glicogênio é o principal carboidrato de reserva, podendo, assim como a glicose exógena, ser convertido em glicose-6-fosfato e ser metabolizada pela via respiratória. Algumas enzimas do Ciclo de Krebs são detectadas com atividades extremamente baixas e o metabolismo no escuro está ligado à presença de oxigênio, sendo que a principal rota é a via da pentose-fosfato (BONINI et al., 2012; FAY, 1983). A glicose-6-fosfato é oxidada e descarboxilada em ribulose-5-fosfato. As reações são catalisadas pela glicose 6-fosfato-desidrogenase e 6-fosfo-gluconato-desidrogenase, respectivamente. Ambas as enzimas estão presentes em altas concentrações nas cianobactérias e duas moléculas de NADPH (nicotinamida adenina dinucleótido fosfato hidreto) são geradas, com subsequente oxidação na cadeia respiratória, rendendo 2 ATPs (adenosina trifosfato) (PEREZ-GARCIA et al., 2011; FAY, 1983). Análises dos extratos celulares revelaram que a ribulose-1,5-difosfato inibe a glicose-6-fosfato-desidrogenase, a primeira enzima da via oxidativa (PELROY; BASSHAM, 1973). A transferência das culturas para cultivo no escuro está ligada ao imediato desaparecimento deste metabólito, com ativação da via metabólica oxidativa.

O uso de substratos renováreis que incluem efluentes, resíduos e subprodutos de origem agrícola e industrial têm sido intensamente estudados para o cultivo de micro-organismos e produção de bioprodutos (FRANCISCO et al., 2014; PEREZ-GARCIA et al., 2011). A melhor maneira de reduzir o custo de substrato para a biotecnologia é a utilização de resíduos constituídos por nutrientes essenciais para o desenvolvimento do micro-organismo empregado, resultando ainda em benefícios ambientais (FRANCISCO et al., 2014).

3. Tratamento do odor em águas residuais

Estações de tratamento de águas residuais (ETAR) são consideradas importantes fontes de emissões gasosas, incluindo gases do efeito estufa e odorantes (ALFONSÍN et al., 2015). As emissões de odor desagradável associadas a processos de tratamento são considerados uma das principais preocupações das populações expostas que vive em áreas circundantes às ETAR.

As principais origens de odores numa ETAR convencional incluem as operações do tratamento preliminar e do tratamento dos lodos (VINCENT, 2001). Por outro lado, Metcalf & Eddy (2003), refere como principais origens de odores em ETAR locais como o tratamento preliminar, decantadores primários, tratamento secundário, armazenamento e tratamento de lodos, incluindo a digestão anaeróbia, relacionada nomeadamente com emissões de biogás. As lagoas anaeróbias e facultativas também podem constituir uma origem de odores sendo, no entanto, de ocorrência menos frequente (PICOT et al., 2001).

O tratamento de efluentes líquidos gera subprodutos que são responsáveis por emissões com maus odores em função da produção de constituintes pertencentes às famílias de compostos químicos tais como enxofre (H_2S, mercaptanas e outros polienxofres), nitrogênio (NH_3, aminas e aminas cíclicas), fenóis, aldeídos, cetonas, álcoois e ácidos graxos voláteis. Este efeito é resultado da decomposição das águas residuárias, ricas em lipídeos, proteínas e polissacarídeos (HWANG et al., 1995).

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 (ALFONSÍN et al., 2015).

No tratamento do odor por oxidação química os gases são captados pelo sistema de exaustão seguem para o lavador de gases do tipo torre de absorção com reação química. O controle é feito através da transferência dos poluentes da fase gasosa para a líquida (absorvente). Nos equipamentos com essa tecnologia, pode ocorrer a absorção física e a química. A eficiência deste sistema dependerá da solubilidade do poluente no líquido (absorção física) ou da velocidade da reação entre o poluente e o

líquido (absorção química). Os reagentes químicos usados são a soda cáustica (NaOH) + hipoclorito de sódio (NaOCl) e o peróxido de hidrogênio (H_2O_2).

As indústrias que utilizam a oxidação térmica possuem incineradores, onde é feita a oxidação de compostos odoríficos por combustão a temperaturas superiores a 800°C. O ar da combustão é misturado com gases de geradores de odores. Sua implantação apenas para o tratamento de odores não é viável devido ao alto custo do processo.

A desodorização por adsorção ocorre através da passagem de ar contaminado por um meio adsorvente com compostos químicos que fazem a oxidação ou inativação das substâncias odoríferas. Os gases captados pelo sistema de exaustão vão para um filtro de carvão ativado, principal meio adsorvente utilizado devido à sua alta eficiência e baixo custo, que fica saturado quando os poluentes são adsorvidos. A adsorção transfere uma molécula de uma fase gasosa para uma sólida, obedecendo às leis de equilíbrio entre a concentração na fase gasosa e na sólida. É importante a manutenção do ambiente livre de umidade e poeira e da troca regular do meio adsorvente. A adsorção é instantânea e com baixa energia e envolvendo as etapas de transferência do fluido em direção à camada limite gasosa e ao material poroso; difusão da molécula através da camada limite; difusão da molécula no interior dos poros do material adsorvente. A capacidade de adsorção de um filtro e sua eficiência dependem dos seguintes parâmetros: quantidade adsorvida no equilíbrio; velocidade e taxa de adsorção; percentagem volumétrica utilizada do filtro.

O ar contaminado dos exaustores vai para uma coluna de lavagem onde encontra o efluente adicionado em contracorrente. À medida que o gás sobe pelo meio úmido, é dissolvido e oxidado pelos produtos químicos adicionados ao efluente (ESTRADA et al., 2011).

Segundo Estrada et al., (2011) o tratamento de odor por aplicação de produtos químicos tem eficiência restrita, porque sua atuação em determinado gás pode não ser a mesma em outro. Os resultados obtidos são parciais ou ainda podem mascarar o odor. São utilizados oxigênio puro, nitrato, peróxido de hidrogênio, cloro, permanganato de potássio, sais metálicos, entre outros.

Nas últimas décadas, os sistemas biológicos, como os biofiltros, têm sido cada vez mais utilizados, devido à sua capacidade para tratar compostos orgânicos voláteis mal cheirosos porque eles trabalham em temperaturas e pressão ambiente, têm baixos custos de capital e tem um melhor desempenho ambiental do que os métodos químicos (RABBANI et al., 2016; ALFROSÍN et al., 2015).

O biofiltro é um sistema no qual os gases componentes das emissões são captados pelo sistema de exaustão, que remove compostos orgânicos voláteis e poluentes sulfurados. Os processos biológicos de tratamento de gases transferem compostos voláteis, mal odorantes, para uma fase líquida e, em seguida, degradam estes compostos por meio de micro-organismos (ação bacteriana) em compostos simples não agressivos. O bom funcionamento dos biofiltros exige um meio de contato (turfa) para o crescimento da biomassa bacteriana e suficiente suprimento de ar. De fácil manutenção, o biofiltro é indicado apenas para tratamento de baixas vazões de ar contaminado. Este processo é aplicado em produtos biodegradáveis e relativamente solúveis em solução aquosa. A biodegradabilidade de um composto depende das funções químicas que o constituem. O meio filtrante deve possuir: elevada capacidade de retenção líquida; grande área específica; capacidade de manter alta permeabilidade no decorrer do processo; pH neutro e poder tampão para eventuais produtos ácidos; alta porosidade; e integridade estrutural (RABBANI et al., 2016).

A partir do conhecimento que microalgas têm capacidade de remover matéria orgânica e nutrientes presentes nas águas residuais, podendo também produzir uma grande variedade de compostos orgânicos voláteis (SANTOS et al., 2016), torna-se de interesse avaliar o potencial destes micro-organismos em desodorizar compostos provenientes das estações de tratamento de efluentes.

4. Compostos orgânicos voláteis do efluente

A composição da água residual é um dos fatores impulsionadores ou limitantes da ocorrência de odores, dado que a presença de compostos odoríficos em solução resulta da composição original da água residual, das alterações químicas e bioquímicas

que tenham ocorrido durante o seu transporte e o processo de tratamento (VINCENT, 2001).

Segundo Vincent (2001), a liberação de compostos orgânicos voláteis fétidos na atmosfera por um líquido depende de três fatores: da concentração destes compostos no líquido; da área superficial do líquido exposta à atmosfera; e do grau de turbulência do fluxo deste líquido. Além disso, a liberação depende também do pH do meio, sendo que em condições ácidas, sulfetos e ácidos orgânicos são facilmente liberados, e em pH alcalino, amônia (NH_3) e aminas são favorecidas.

Os principais subprodutos que geram a emissão de odores são o sulfeto de hidrogênio, amônia, mercaptanos, aminas, aldeídos, cetonas, indol e escatol pode ser produzida por muitos dos processos de tratamento de águas residuais, que podem ser produzidos a partir da decomposição das águas residuais ricas em aminoácidos (HWANG, 1995).

De acordo com Joher et al., (1996) os compostos odoríficos de ocorrência em ETAR podem ser agrupados em quatro famílias: compostos nitrogenados, sulfurados, ácidos orgânicos, aldeídos e cetonas.

Referente a classificação os compostos nitrogenados possuem odor fecal irritante devido a decomposição orgânica. Estes compostos estão associados ao lançamento de efluentes industriais com alta concentração de proteínas. Amônia também é produzida a partir da quebra dos compostos orgânicos nitrogenados durante o tratamento anaeróbio de lodos (DINCER et al., 2008; HWANG, 1994).

Os compostos sulfurados são caracterizados por um odor a ovos podres ou a vegetais em decomposição (couve ou alho). O gás sulfídrico (H_2S) é formado a partir da ação de micro-organismos sobre sulfatos e outros compostos de enxofre em condições anaeróbias. Em ETAR o H_2S é produzido nos decantadores primários, adensadores por gravidade, tanques de estabilização e áreas de manejo de lodo. É facilmente liberado para a atmosfera, principalmente em locais de fluxo turbulento (DINCER, 2008; HWANG, 1995).

Os grupos dos ácidos orgânicos, aldeídos, cetonas e dos álcoois todos apresentam odor que potenciam odores irritantes. O cheiro característico dos ácidos alifáticos de peso molecular mais baixo passa progressivamente de forte e irritante nos

ácidos fórmico e acético a extremamente desagradável nos ácidos butírico, valérico e capróico (DINCER, 2008; JOHER et al.,1996). Os ácidos de peso molecular mais elevado não têm muito odor por serem pouco voláteis. Os aldeídos também apresentam odores penetrantes e altamente desagradáveis. Com o aumento da massa molecular, esses odores vão diminuindo até se tornarem agradáveis nos termos que contêm de 8 a 14 carbonos. (DINCER, 2008; HWANG, 1994). Os álcoois mais elementares (metanol, etanol e propanol) são altamente voláteis e de odor característico, uma vez que o grupo OH constitui importante porção da molécula (DINCER, 2008).

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 (HVITVED-JACOBSEN & VOLERTSEN, 2001). Quando o composto se mantém na fase líquida (água residual) são posteriormente oxidados, por via química ou biológica, a compostos potencialmente menos odoríficos (VINCENT, 2001). Em ETAR, os principais mecanismos associados à transferência dos compostos entre as fases líquida-gasosa incluem a volatilização e a lavagem (“gas stripping”) (METCALF & EDDY, 2003). A volatilização designa o processo de libertação de compostos dissolvidos a partir de uma superfície líquida para a atmosfera enquanto que a lavagem designa o processo de transferência devido à introdução de um gás num líquido (VINCENT, 2001).

5. Compostos orgânicos voláteis formados em bioprocessos microalgais

Os sistemas baseados em microalgas para a produção de produtos químicos são uma área emergente, o que representa uma grande possibilidade para aplicação industrial. No entanto, há pouca informação disponível sobre a biogeração de compostos orgânicos voláteis destes micro-organismos (SANTOS et al., 2016).

O interesse crescente em produtos naturais sugere o desenvolvimento de tecnologias que utilizam micro-organismos, incluindo microalgas, que são capazes de sintetizar compostos orgânicos voláteis (COV) específicos. Jacob-Lopes; Franco (2013) relataram que o COVs são os principais bioprodutos formados durante o cultivo de

microalgas. A análise do balanço de carbono indica que estes compostos representam até 90% do substrato total convertido no biorreator.

A ocorrência de compostos orgânicos voláteis em microalgas é uma consequência do seu metabolismo. Espécies de microalgas usam fotossíntese aeróbica para a fixação de CO_2 (KUMAR et al., 2011).

Adicionalmente, algumas espécies destes micro-organismos têm a versatilidade necessária para manter as suas estruturas, na ausência de luz, sendo capaz de crescer heterotroficamente através da assimilação de um ou mais substratos orgânicos como uma fonte de carbono na via da pentose fosfato oxidativa. Para usar esses compostos orgânicos, ocorre o transporte através da membrana. Este substrato será convertido em glicose 6-fosfato para que possa iniciar o percurso. Durante o metabolismo, há a formação de duas moléculas de ATP (adenosina trifosfato). E o produto final é também piruvato (FAY, 1983).

Independentemente do metabolismo, a biossíntese de compostos orgânicos voláteis ocorre através da formação de molécula de piruvato. As vias de formação destes compostos podem ser enzimaticamente ou por reação de degradação. Com base neste conhecimento, podem ser sugeridas rotas aplicáveis para a síntese de compostos orgânicos voláteis, tanto para a sua melhor compreensão ecológica como às suas potenciais aplicações comerciais.

Os compostos orgânicos voláteis a partir de microalgas podem pertencer a diferentes classes de compostos, tais como ésteres, álcoois, hidrocarbonetos, cetonas, terpenos, ácidos carboxílicos e os compostos de enxofre (PAPALEO et al., 2013). A biossíntese destes compostos orgânicos voláteis dependerá da disponibilidade dos blocos de construção, tais como carbono, nitrogênio, e o fornecimento de energia a partir do metabolismo primário. Por isso, a disponibilidade destes blocos de construção tem um grande impacto sobre a concentração dos metabolitos secundários, incluindo COVs, demonstrando o elevado nível de conectividade entre o metabolismo primário e secundário (SANTOS et al., 2016; DUDAREVA et al., 2013).

Microalgas contêm altas concentrações de carotenóides, com uma estrutura de ligações duplas conjugadas instáveis, sendo facilmente degradada, por exemplo, o β-caroteno, pode degradar formando β-ionona e β-ciclocitral. Nas fases posteriores de

degradação da β-ionona oxida, formando um produto de degradação chamado 5,6-epoxi-β-ionona (RODRIGUES et al., 2014).

Compostos como hexanol e hexanal são derivados de ácidos graxos saturados, como os ácidos linoleico ou linolênico. A biossíntese destes compostos baseia-se em acetil-CoA, que é gerado a partir do piruvato que é produto final da via da pentose fosfato. A via da lipoxigenase forma intermediários 9-hidroperoxido e 13-hidroperoxido. A ramificação do hidroperóxido liase converte ambos os hidroperóxidos em aldeídos C6 e C9, que são reduzidos a álcoois por desidrogenases (SANTOS et al., 2016; GIGOT et al., 2010).

Uma gama de compostos, incluindo classes, tais como aldeídos, cetonas e álcoois podem ser formados a partir da degradação de lipídeos (RZAMA, et al., 1995). Microalgas são relativamente ricos em ácidos graxos poliinsaturados (PUFAs). Microalgas marinhas contêm principalmente muito ácidos graxos poliinsaturados de cadeia longa tais como o ácido eicosapentaenóico e ácido docosa-hexaenóico, por exemplo, *Chlorella* contém principalmente cadeias curta de PUFA, tais como ácido α-linolênico. Espécies com baixas concentrações de PUFA possuem um número significativamente menor de aldeídos lineares, em comparação com as espécies tendo elevadas concentrações de PUFA (por exemplo, *Chlorella*, *Botryococcus*, *Rhodomonas*) (ZHANG et al., 2009). Os aldeídos lineares de cadeia curta, muitas vezes são quimicamente derivados da oxidação lipídica, aldeídos ramificados e aromáticos são tipicamente formados por causa da oxidação lipídica e enzimática da proteína (DURME et al., 2013).

Algumas reações químicas podem converter os compostos orgânicos voláteis em outros compostos. Por exemplo, os álcoois podem ser oxidados para aldeídos e, em seguida, para os ácidos carboxílicos, e cetonas podem reagir com os radicais hidroxila no ar para formar aldeídos (ATKINSON et al., 2000; KORPI et al., 2009). Aldeídos e cetonas podem ser reduzidos para os álcoois por redutases. Álcoois, aldeído ou cetona podem ser oxidados a aldeído por álcool-desidrogenase, e depois adicionalmente oxidado para o ácido por desidrogenase aldeído (KORPI et al., 2009). As cetonas podem ser formadas de muitas maneiras; cetonas alifáticas podem ser de produtos de

oxidação de lipídeos degradação ou metil e cetona (C3-C17) pode ser formada a partir da clivagem oxidativa de carotenoides (SANTOS et al., 2016; SUN et al., 2012).

Alguns compostos podem ser produzidos metabolicamente (por enzimas presentes em microalgas) e também por compostos de degradação primária, tais como lipídeos e proteínas. O estabelecimento de vias bioquímicas podem direcionar a produção de biomoléculas específicas do metabolismo microalgal para compostos de interesse comercial e também para um melhor conhecimento da sua função ecológica.

6. Compostos orgânicos voláteis de interesse comercial

O produto mais importante da biotecnologia de microalgas em relação a quantidade de produção e valor econômico é a sua biomassa. No entanto, verificou-se uma tendência crescente para o conhecimento da produção de compostos de baixo peso molecular a partir de fontes renováveis (CHOI et al., 2013; SCHIRMER et al., 2010).

As aplicações típicas de microalgas correspondem a uma variedade de metabolitos (enzimas, lípidos, pigmentos, biomassa) com aplicação potencial em produtos tais como cosméticos, ingredientes alimentares e bioenergia. Eles também podem ser utilizados como indicadores ambientais e para o tratamento de águas residuais (ABDEL-RAOUF et al., 2012; JACOB-LOPES et al., 2008). Ao lado de muitas propriedades benéficas, microalgas também produzem inúmeros compostos orgânicos voláteis, que poderiam ser utilizadas como uma importante fonte alternativa de fornecimento a granel e química fina.

Compostos orgânicos voláteis gerados por micro-organismos tem sido considerado como um avanço na pesquisa de laboratório. Os compostos com apelo comercial incluem o propanol, butanol, 3-metil-butanol, hexanol, hexanal, β-ciclocitral, β-ionona, e 5,6-epoxi-β-damascenona (SMITH et al., 2010; BERGER, 2009).

Berger (2009) relatou que aromas de micro-organismos podem competir com as fontes tradicionais. A triagem de espécies produtoras, a elucidação das vias metabólicas e aplicação de bioengenharia convencional resultaram em um conjunto de mais de 100 aromas químicos comerciais derivados através de biotecnologia.

Os aldeídos provaram serem os mais prevalentes, para cada espécie de microalga, em função do baixo limiar de percepção. Aldeídos saturados têm um odor semelhante a verde ou feno, enquanto aldeídos insaturados têm um odor óleo de fritura. Considerando que a cadeia mais curta dos aldeídos lineares são frequentemente derivados da oxidação química de lípidos, e os aldeídos ramificados e aromáticos são tipicamente formados devido à oxidação lipídica e enzimática das proteínas.

Muitas microalgas mostram a presença de cetonas e álcoois como compostos voláteis (DURME et al., 2013). A determinação de compostos voláteis mostra alcanos e alcenos que representam os principais componentes voláteis das estirpes investigadas de microalgas (MILOVANOVIC et al., 2015).

A utilização da fração volátil da biomassa de microalgas pode representar uma melhoria na oferta para diferentes tipos de indústria. Assim como usar a energia de biomoléculas de interesse, tais como hidrocarbonetos e álcoois de cadeia curta. Existe um interesse crescente na produção de biocombustíveis a partir de fontes renováveis, oferecendo soluções sustentáveis para o setor de energia como uma alternativa promissora para a indústria petroquímica tradicional (SI et al., 2014).

A produção de hidrocarbonetos é de particular interesse devido ao seu potencial para uso como biocombustíveis avançados. Compostos de cadeia longa podem substituir o diesel, e os de cadeia curta, a gasolina (CHOI&LEE, 2013).

Álcoois alifáticos com maior comprimento de cadeia de carbono ou igual a cinco, são alvos atraentes para os biocombustíveis, têm uma densidade de energia elevada e baixa solubilidade em água. A enzima responsável pela produção de tais compostos é a acetil-CoA-redutase que podem estar presentes nas reações do ciclo do ácido tricarboxílico, do mevalonato, e a biossíntese de leucina. Outro álcool de alta densidade energética é o 1-butanol, comparável ao valor energético da gasolina (ZHANG et al., 2008).

As microalgas podem produzir uma variedade de compostos voláteis industrialmente relevantes, e o conhecimento sobre a biossíntese destas estruturas a partir de microalgas pode ser útil para ajudar a elucidar a aplicação destas matérias-primas de base biológica para as indústrias alimentares e produtos não alimentares.

REFERÊNCIAS

ABDEL-RAOUF, N.; AL-HOMAIDAN, A. A.; IBRAHEEM, I. B. M. Microalgae and wastewater treatment. **Saudi Journal of Biological Science**, v.19, p.257-275, 2012.

ALFONSÍN, C.; LEBRERO, R.; ESTRADA, J. M. ; MUNÕZ, R.; KRAAKMAN, N. J. R.; FEIJOO, G.; MOREIRA, M. T. Selection of odour removal technologies in wastewater treatment plants: A guideline based on Life Cycle Assessment. **Journal of Environmental Management,** v.149, p.77 – 84, 2015.

ATKINSON, R.; TUAZON, E. C.; ASCHMANN, S. M. Atmospheric chemistry of 2-pentanone and 2-heptanone. **Environmental Science & Technology,** v.34, p.623-631, 2000.

BATISTA, A. P.; GOUVEIA, L.; BANDARRA, N. M.; FRANCO, J. M.; RAYMUNDO, A. Comparison of microalgal biomass profiles as novel functional ingredient for food products. **Algal Research,** v.2, p.164–173, 2013.

BERGER, R. G. Biotechnology of flavours- the next generation. **Biotechnology Letters,** v.31, p.1651-1659, 2009.

BONINI, M. A.; BASTOS, R. G. Produção de biomassa de *Aphanothece microscopica* e *Chlorella vulgaris* por cultivo heterotrófico a partir de Glicose. **Ciências Biológicas e da Saúde**, v.33, p.151-160, 2012.

BURGESS, J. E.; PARSONS, S. A.; STUETZ R. M. Developments in odour control and waste gas treatment biotechnology: a review. **Biotechnology Advances**, v.19, p.35-63, 2001.

CAPELLI, L.; SIRONI, S.; ROSSO, R. D.; CÉNTOLA, P.; ROSSI, A.; AUSTERI, C. Olfactometric approach for the evaluation of citizens' exposure to industrial emissions in the city of Terni, Italy. **Science of the Total Environment,** v.409, p.595–603, 2011.

CHOI, J. Y.; LEE, S. Y. Microbial production as short-chain alkane. **Nature**, p.1-6, 2013.

DINCER, F.; MUEZZINOGLU, A. Odor-causing volatile organic compounds in wastewater treatment plant units and sludge management areas. **Journal of Environmental Science and Health Part A,** v.43, p.1569–1574, 2008.

DUDAREVA, N.; KLEMPIEN, A.; MUHLEMANN, J. K.; KAPLAN, I. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. **New Phytologist**, v.198, p.16-32, 2013.

DURME, J. V.; GOIRIS, K., WINNE, A.; DE COOMAN, L.; MUYLAERT, K. Evaluation of the volatile composition and sensory properties of five species of microalgae. **Journal Agricultural and Food Chemistry**, v.61, p.10881-10890, 2013.

ESTRADA, J. M.; KRAAKMAN, N. J. R. B.; MUNOZ, R.; LEBRERO, R.; A Comparative Analysis of Odour Treatment Technologies in Wastewater. Treatment Plants. **Environmental Science & Technology**, v.45, p.1100–1106, 2011.

FAY, P. **The blue-greens(Cyanophytacyanobacteria)**. 5ª ed. London: ed. Edward Arnold, (Publishers), 88p. (Studies in Biology nº 160). 1983.

FRANCISCO, E. C.; FRANCO, T. T.; WAGNER; R.; JACOB-LOPES, E. Assessment of different carbohydrates as exogenous carbon source in cultivation of cyanobacteria. **Bioprocess and Biosystems Engineering**, v.1, p.2-11, 2014.

FUJISE, D.; TSUJI, K.; FUKUSHIMA, N.; KAWAI, K.; HARADA, K. Analytical aspects of cyanobacterial volatile organic compounds for investigation of their production behavior. **Journal of Cromatography A**, v.1217, p.6122-6125, 2010.

GAFFNEY, M.; O'ROURKE, R.; MURPHY, R. Manipulation of fatty acid and antioxidant profiles of the microalgae Schizochytrium sp. through flax seed oil supplementation, **Algal Research**,v.6, p.195-200, 2014.

GIGOT, C.; ONGENA, M.; FAUCONNIER, M. L.; WATHELET, J. P.; JARDIN, P. D.; THONART P. The lipoxygenase metabolic pathway in plants: potential for industrial production of natural green leaf volatiles. **Biotechnology Agronomy Society and Environment**, v.14, p.451–460, 2010.

GUIRY, M. D.; GUIRY, G.M. AlgaeBase. World-wide eletronic publication, National University of Ireland, Galway, 2013. http://www.algaebase.org; pequisa realizada em 23 de fevereiro de 2016.

HU, C. W.; CHUANG, L. T.; YU, P. C.; CHEN, C. N. N.; Pigment production by a new thermotolerant microalga Coelastrella sp. **Food Chemistry**,v.138, p.2071–2078, 2013.

HVITVED-JACOBSEN, T.; VOLERTSEN, J. Odour formation in sewers. Em R. Stuetz and F.-B. Frechen (Ed.), Odours in wastewater treatment: measurement, modelling and control (1st Ed.), 33-68. (2001) London, IWA Publishing.

HWANG, Y.; MATSUO, T.; HANAKI, K.; SUZUKI, N. Identification and quantification of sulfur and nitrogen containing odorous compounds in wastewater. **Water Research**, v.29, p.711-718, 1995.

HWANG, Y.; MATSUO, T.; HANAKI, K.; SUZUKI, N. Removal of odorous compounds in wastewater by using activated carbon, ozonation and aerated biofilter. **Water Research**, 28, 2309-2319, 1994.

IVERSON, T.M. Evolution and unique bioenergetic mechanisms in oxygenic photosynthesis. **Current Opinion in Chemical Biology,** v.10, p.91-100, 2006.

JACOB-LOPES, E.; FRANCO, T. T.; From oil refinery to microalgal biorefinery. **Journal of CO_2 Utilization,** v.2, p.1–7, 2013.

JACOB-LOPES, E.; SILVA, L.M.C.F.L.; FRANCO, T.T. Biomass production and carbono dioxide fixation by *Aphanothece microscopica Nagëli* in a bubble column photobioreactor. **Biochemical Engineering Journal**, v.40, p.27-34, 2008.

JOHER, J.B.; GARCIA, M.V.F.; CASTILLO, M.C.G.; AND ALEMANY, F.O. Control de olores em una EDAR. **Tecnologia del Água**, p.40-45, 1996.

KORPI, A.; JÄRNBERG, J.; PASANEN, A. L. Microbial volatile organic compounds. **Critical Reviews in Toxicology**, v.39, p.139-193, 2009.

KUMAR, K.; DASQUPTA, C. N.; NAYAK, B.; LINDBLAD, P.; DAS, D. Development of suitable photobioreactors for CO^2 sequestration addressing global warming using green algae and cyanobacteria. **Bioresource Technology,** v.102, p.4945-4953, 2011.

LOURENÇO, S. O. **Cultivo de Microalgas Marinhas: Princípios e Aplicações.** Editora RiMa, p.51-60, 2006.

MARONEZE, M. M. ; MENEZES, C. R.; BARIN, J. S.; QUEIROZ, M. I.; ZEPKA, L. Q.; JACOB-LOPES, E. Treatment of cattle-slaughterhouse wastewater and the reuse of sludge for biodiesel production by microalgal heterotrophic bioreactors. **Scientia Agricola** (USP. Impresso), v.71, p.521-524, 2014.

METCALF & EDDY. **Wastewater Engineering: Treatment, Disposal, Reuse.** 4ª ed. Singapura: ed. McGraw-Hill, 1851p. 2003.

MILOVANOVIC, I.; MISON, A.; SIMEUNOVIC, J.; KOVAC, D.; JAMBREC, D.; MANDIC, A. Determination of volatile organic compounds in selected strains of cyanobacteria. **Journal of Chemistry**, p.1-6, 2015.

MOORE, A. Blooming prospects. **EMBO reports**, v.21, p.462-464, 2001.

OLAIZOLA, M. Commercial development of microalgal biotechnology: from the test tube to the marketplace. **Biomolecular Engineering**, v.20, p.459-466, 2003.

PALINSKA, K. A.; DEVENTER, B.; HARIRI, K.; LOTOCKA, M. A taxonomic study on *Phormidium* group (cyanobacteria) based on morphology, pigments, RAPD molecular markers and RFLP analysis of the 16S rRNA gene fragmente. **Fottca**, v.11, p.41-55, 2011

PAPALEO, M. C; ROMOLI, R.; BARTOLUCCI, G.; MAIDA, I. Bioactive volatile organic compounds from Antartic (sponges) bacteria. **New Biotechnology**, v.30, p.824-838, 2013.

PELROY, R. A.; KIRK, M. R.; BASSHAM J. A. Photosystem II regulation of macromo[ecule synthesis in the blue-green alga Aphanocapsa 6714. **Journal Bacteriol**. v.128, p.623-632, 1976.

PEREZ-GARCIA, O.; ESCALANTE, F. M. E.; DE-BASHAN, L. E.; BASHAN, Y. Heterotrophic cultures of microalgae: Metabolism and potential products. **Water Research, v.**45, p.11-36, 2011.

PICOT, B.; PAING, J.; TOFFOLETTO, L.; SAMBUCO, J.P.; COSTA, R.H.R. Odor control of an anaerobic lagoon with a biological cover: floating peat beds. **Water Science and Technology**, v.44, p.309-316, 2001.

QUEIROZ, M. I.; HORNES, M. O.; MANETTI, A. G. S.; ZEPKA, L. Q.; JACOB-LOPES, E. Fish processing wastwater as a plataforma of the microalgal biorefineries. **Biosystems Engineering, v.**115, p.195-202, 2013.

QUEIROZ, M. I.; JACOB-LOPES, E.; ZEPKA, L. Q.; BASTOS, R. G.; GOLDBECK, R.; Kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor. **Bioresource Technology**, v.98, p.2163-2169, 2007.

RABBANI,K. A.; CHARLESA, W.; KAYAALPB, A.; , CORD-RUWISCHA, R.; HOA, G. Pilot-scale biofilter for the simultaneous removal of hydrogen sulphide and ammonia at a wastewater treatment plant. **Biochemical Engineering Journal,** v.107, p.1–10, 2016.

REVIERS, B. **Biologie et phylogénie des algues**. v.1. Paris: ed. Belin, 352p. 2002.

RODRIGUES, D. B.; FLORES, E. M. M.; BARIN, J. S.; MERCADANTE, A. Z.; JACOB-LOPES, E.; ZEPKA, L. Q. Production of carotenoids from microalgae cultivated using agroindustrial wastes. **Food Research International Journal**, v.65, p.144-148, 2014.

RZAMA, A.; BENHARREF, A.; ARRREGUY, B.; DUFOURC, E. J. Volatile compounds of green microalgae grown on reused wastewater. **Phytochemistry v.**38, p.1375-1379, 1995.

SANTOS, A. B.; FERNANDES, A. S.; WAGNER, R.; JACOB-LOPES, E.; ZEPKA, L. Q. Biogeneration of volatile organic compounds produced by *Phormidium autumnale* in hetrotrophic bioreactor. **Journal of Applied Phycology**, v.60, p.32-42, 2016.

SCHIRMER, A.; RUDE, M. A.; LI, X.; POPOVA, E.; CARDAYRE, S. B. Microbial Biosynthesis of Alkanes. **Science**, v.329, p.559-562, 2010.
SI, T.; LUO, Y.; XIAO, H.; ZHAO, H. Utilizing an endogenous pathway for 1-butanol production in *Saccharomyces cerevisiae*. **Metabolic engineering**, v.22, p. 60-68, 2014.

SMITH, K. M.; CHO, K. M.; LIAO, J. C. Engineering Corynebacterium glutamicum for isobutanol production. **Applied Microbiology and Biotechnology, v.**87, p.1045-1055, 2010.

SU, Y.; MENNERICH, A.; URBAN, B. Comparison of nutriente removal capacity and biomass settleability of four high-potential microalgal species. **Bioresource Technology**,v.124, p.157-162, 2012.

SUN, S. M.; CHUNG, G. H.; SHIN, T. S. Volatile compounds of the green alga, Capsosiphon fulvescens. **Journal of Applied Phycology**, v.24, p.1003-1013, 2012.

VINCENT, A. J. **Sources of odours in wastewater treatment**. Em R. Stuetz and F.-B. Frechen (Ed.), Odours in wastewater treatment: measurement, modelling and control (1st Ed.), 69-92. London, IWA Publishing.2001

WEN, Z. Y.; CHEN, F.; Heterotrophic production of eicosapentaenoic acid by microalgae. **Biotechnology Advances**, v.21, p.273–294, 2003.

ZEPKA, L. Q.; JACOB-LOPES, E.; GOLDBECK, R.; QUEIROZ, M. I. Production and biochemical profile of the microalgae *Aphanothece* microscopica *N¨ageli* submitted to different drying conditions. **Chemical Engineering and Processing,** v.47, p.1305–1310, 2008.

ZHANG, K.; SAWAYA, M. R.; EISENBERG, D. S.; LIAO, J. C. Expanding metabolism for biosynthesis of non-natural alcohols. **Proceedings of the National Academy of Sciences**, v.105, p.20653-20658, 2008.

ZHANG, Z.; LI, T.; WANG, D.; ZHANG, L.; CHEN, G. Study on the volatile profile characteristics of oyster Crassostrea gigas during storage by a combination sampling method coupled with GC/MS. **Food Chemistry**, v.115, p.1150–1157, 2009.

CAPÍTULO 2

DEODORIZING WASTEWATER FROM HETEROTROPHIC MICROALGAE CULTIVATION

O artigo será submetido para a revista Journal of Agricultural and Food Chemistry

DEODORIZING WASTEWATER FROM HETEROTROPHIC MICROALGAE CULTIVATION

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Abstract

The objective of this study was to investigate the capacity of microalgae *Phormidium autumnale* to deodorize volatile organic compounds from agroindustrial wastewater. The volatiles were isolated by headspace solid-phase micro-extraction in different residence times (0, 24, 48, 72, 96, 120 e 144 hours), separated by gas chromatography, and identified by mass spectrometry (SPME-GC/MS). A total of 64 compounds were detected in the cultive. A total of 43 compounds disappeared after 48 hours process, among these, compounds having a characteristic odor in fecal waste such as skatole and indole. Concomitantly, after 24 hours of cultivation 21 new volatile organic compounds were formed. The description of flavorings formed are among green, flower, fruit. In conclusion, the results have shown that the heterotrophic cultivation of the *Phormidiun autumnale* can be a potential biotechnological to reduce unpleasant VOCs and produce natural flavors.

1. Introduction

Wastewater treatment plants (WWTP) are known to be sources of unpleasant odors. And this is seen as a disturbance to the neighboring population, causing nausea and dizziness and also bring a bad image for the industry (Estrada, 2011; Capelli, 2011). The air pollutants originating from wastewater treatment are composed of a mixture of thousands of chemical compounds, including sulfur, nitrogen and other organic compounds (Rabbani, 2016).

Other odor that cause compounds in treatment plants are volatile organic compounds (Talaiekhozani et al., 2016). Volatile organic compounds (VOCs) are secondary metabolites derived from microalgae and cyanobacteria (Havel & Weuster-Botz, 2006). These low molecular compounds can enter into the air by evaporation, which can be inhaled by humans. The quantity of nutrients and organic matter still present in wastewater can increase the growth of microrganisms (Talaiekhozani et al., 2016; Fujise, 2010). Microalgae and cyanobacteria are able to release a variety of causing organic substances prevailing odor as geosmin, metilisoborneol and hydrogen sulfide in wastewater (Talaiekhozani et al., 2016) and also VOCs which could be used as an important alternative source of pharmaceuticals, flavors and fragrances at a low cost (Havel & Weuster-Botz, 2006).

The most wastewater treatment processes are designed to achieve acceptable removal of organic matter, nutrients and toxic substances, but they not always satisfactorily remove VOCs (Rabanni, 2016). Biotechnologies are now recognized as the best available technologies for treating odors due to their lower cost and operational environmental impact compared to their physico-chemical counterparts (Lebrero 2014; Estrada et al., 2012).

Among the conventional biotechnologies, biofiltration and biotrickling filtration technologies are by far the most commonly implemented to reduce odor probably due to its ease of operation and the extensive design and operation experience (Iranpour et al., 2005; Kraakman et al., 2011). However, these biotechnologies are required to support low removal efficiency of malodorous emissions, the elimination of which is required for efficient odor reduction (Lebrero 2014; Liu et al., 2009; Iranpour et al., 2005). Thus, new configurations of biological reactors should be developed to ensure cost-effective treatment.

Microalgal heterotrophic bioreactors have been shown to be the most cost effective way to remove organic matter in the waste water due to their tolerance to certain extreme conditions like high temperatures and nutrient concentrations (Maroneze et al., 2014; Su et al, 2012). The use of microalgae in biotechnological processes has grown in recent decades, due to its wide applicability because their metabolic profiles

are unique and diverse. Its commercial applications include nutrition, biofuel production, cosmetics, fertilizers, pollution control (Gaffney et al., 2014), and bio-generation of volatile organic compounds (Santos et al., 2016).

In this sense, the aim of this work was to evaluate the potential of the microalgae *Phormidium autumnale* in deodorizing of compounds from the wastewater treatment plants. The study focused on identify and quantify the volatile organic compounds, assess the acapacity of microalgae to deodorize the wastewater and the possibility in form new compounds.

2. Material and methods

2.1. Microorganism and culture conditions

A monoculture of *Phormidium autumnale* was originally isolated from the Cuatro Cienegas desert (26º59'N, 102º03'W-Mexico). Stock cultures were propagated and maintained in solidified agar-agar (20g.L^{-1}) containing synthetic BG11 (Rippka et al., 1979) medium with the following composition (mg L-1): K_2HPO_4 (30.0), $MgSO_4$ (75.0), $CaCl_2.2H_2O$ (36.0), ammonium citrate and iron (0.6), Na_2EDTA (1.0), NaCl (0.72), $NaNO_3$ (15.0), citric acid (0.6), Na_2CO_3 (1500.0), trace metals [H3BO3 (2.8), $MnCl_2.4H_2O$ (1.8), $ZnSO_4.7H_2O$ (0.22), $Na_2MoO_4.2H_2O$ (0.39), $CoSO_4.6H_2O$ (0.04). The incubation conditions used were 20ºC, photon flux density of 15 µmol$m^{-2}s^{-1}$ and photoperiod of 12h.

2.2. Wastewater

The poultry and swine slaughterhouse wastewater used in the experiments was obtained from an industry located in Santa Catarina, Brazil (27º14’02”S, 52º01’40”W). It was collected from 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-PO_4^{-3}), total solids (TS), suspended solids (SS) and volatile solids (VS), following the Standard Methods for the Examination of Water and Wastewater (APHA,

2005). The composition of the wastewater, in a one year of sampling, has the following composition (mg/L): pH of 5.9, COD of 1501,66, NTK-N of 69.54, P-PO_4^{-3} of 4.06, TS of 5.57, SS of 2.24, VS of 1.94. The carbon/nitrogen ratio (C/N) wea calculated through COD, N-TKN and adjusted glucose.

2.3. Bioreactor Configuration

Measurements were made in a bubble column bioreactor. The system was built of borosilicate glass and had an external diameter of 12.5 cm and a height of 16 cm, resulting in a height/diameter (h/D) ratio equal to 1.28 and a nominal working volume of 2.0 L. The dispersion system of the reactor consisted of a 2.5 cm diameter air diffuser located inside the bioreactor. The air flow was monitored by a flow meter (KI-Key Instruments®, Trevose-PA, USA) and the inlet of air and outlet of gases were filtered through filtering units made up of a polypropylene membrane with a pore diameter of 0.22 μm and total diameter of 50 mm (Millex FG®, Billerica-MA, USA). The bioreactor including filtering units was sterilized by autoclaving at 121 °C for 20 min.

2.4. Obtaining the Kinetic Data

Experiments were conducted in a batch bioreactor. The bioreactor was fed with 2.0 L of wastewater. The experimental conditions were as follows: initial cell concentration of 100 $mg.L^{-1}$, pH adjusted to 7.6, temperature of 25 ºC, absence of light and flow rate per unit volume of 1.0 VVM (volume of air per volume of wastewater per minute).

2.5. Biomass concentration and control pH

Cell biomass was determined gravimetrically by filtering a known volume of culture through a 0.45 μm membrane filter (Millex FG, Billerica-MA, USA) and drying at

60 °C for 24 h before weighing. Samples were collected over 7 days at 0, 24, 48, 72, 96, 120, and 144 hours. The experiments were performed twice and in duplicate for each substrate. Therefore, data refer to the mean value of four repetitions.

2.6. Isolation of the volatile organic compounds

The volatile compounds were isolated using solid-phase microextraction (SPME) with a 50/30-µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, USA). Sample preparation was performed using 10 mL of culture medium. Was analysed by SPME coupled with GC/MS for the quantitative determination of the volatile compounds. The aliquot was placed in a headspace septum vial containing 3 g of NaCl and 10 µL of an internal standard solution. The SPME fiber was inserted into the headspace of the vial containing the sample for 45 min at 40 °C, with agitation provided by a magnetic stir bar. After this period, the fiber was removed from the vial and immediately desorbed into the injector of the GC. The analytical procedure was performed in duplicate.

2.7. GC/MS analysis

The volatile compounds were separated on a DB-Wax fused silica capillary column, 60 m in length, 0.25 mm id, and 0.25 µm film thickness (Chrompack Wax 52-CB) in a Shimadzu QP 2010 Plus gas chromatograph mass spectrometer. The initial oven temperature for the DB-Wax column was set at 35 °C for 5 min, followed by a linear increase at 5 °C min−1 to 220 °C, and this temperature was held for 5 min. For identification, an electron-impact ionization voltage of 70 eV was applied, and helium was used as the carrier gas.

The volatile compounds were identified by a comparison of their MS spectra with those provided by the computerized library (NIST MS Search). In addition, to assist with identification, each volatile linear retention index (LRI) was calculated using the retention times of a standard mixture of paraffin homologues prepared in hexane and compared with the LRI values published in the literature for columns with the same polarity (Acree

and Arn 2016). Co-injection of the sample and the standard mixture provided experimental LRIs for the compounds, which were compared with those of standards analyzed under similar conditions.

The quantification of volatile organic compounds was from the peak area and the response factors internal standard 3-octanol added to the sample.

3. Results and discussion

The Table 1 and the Figure 1 shows the volatile organic compounds identified by GC-MS headspace of the bioreactor. The cultivations with wastewater 64 compounds were identified, including sulphurous, nitrogenous, aldehydes, alcohols, ketones, hydrocarbons, acids.

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

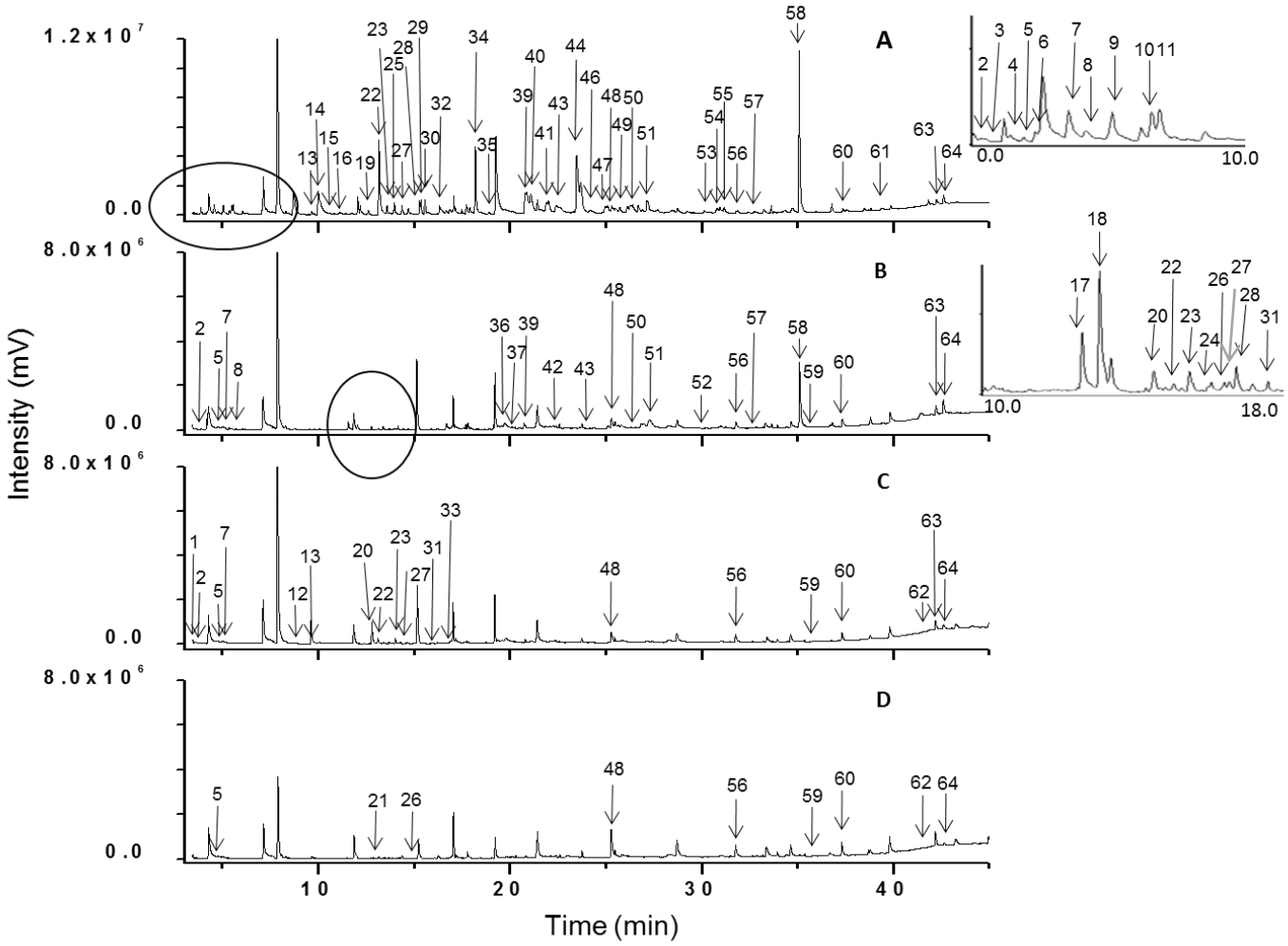
Peak	LRI DB-Wax[a]	Compounds	Odor descriptors[b]
1	699	Carbon disulfide	slightly sweet
2	700	Dimethyl sulfide	baked cauliflower
3	705	2-Methylpropanal	pungent, malt, green
4	710	2-Propenal	burnt cooking grease
5	711	2-Methylfuran	roasted meat, chocolate
6	713	Butyraldehyde	pungent, malt, green
7	714	1,1-Diethoxy-ethane	nd*
8	806	2-Butanone	camphor
9	807	2 Metylbutanal	cocoa, almond
10	808	3-Methylbutanal	malt
11	811	Benzene	nd*
12	904	2,4-Dimethylpentan-3-one	drink distilled from fruit
13	915	Dimethyl disulfide	stinky meat, eggy
14	916	Hexanal	grass, tallow, fat
15	1006	Ethyl Benzene	gasoline
16	1007	2-n-Butyl furan	nd*
17	1009	2-Methyl-3-Hexanone	fruity, onion
18	1010	2,3-Heptanedione	nd*
19	1012	1,4-Cineole	spice
20	1015	2-Heptanone	fruity

Peak	LRI DB-Wax[a]	Compounds	Odor descriptors[b]
21	1016	5-Methyl-2-Hexanone	nd*
22	1014	Limonene	lemon, orange
23	1017	1,8 Cineole	spice
24	1021	6-Ethyl-2-methyl-octane	nd*
25	1102	1-Pentanol	balsamic, fruit
26	1104	4,6-Dimethyl-dodecane	nd*
27	1103	2-Pentyl-furan	nd*
28	1110	Cyclohexanone	pepper, acetone
29	1201	2-Heptanol	herb
30	1202	Pyrrolidine-2,4-dione	nd*
31	1203	2-Phenylpropene	sweet
32	1204	2-Heptenal	cream
33	1205	6-Methyl-5-hepten-2-one	citrus, fruity
34	1207	Hexanol	flower, green
35	1210	Dimethyl trisulfide	stinky meat
36	1213	Dihexylsulfide	cooked chicken
37	1224	Cyclohexanol	camphor, menthol
38	1228	2,3,3-Trimethyl octane	nd*
39	1305	1,3-Dichloro-benzene	nd*
40	1307	1-Heptanol	chemical, green
41	1311	2-Ethyl-hexanol	heavy, earthy, slightly floral
42	1401	3-Hexyl-cyclopentene	nd*
43	1402	3-Propyl-cyclopentene	nd*
44	1403	Benzaldehyde	almond, burnt sugar
45	1407	Linalool	flower
46	1411	D-Fenchyl alcohol	camphor
47	1416	Pentadecane	alkane
48	1429	Menthol	peppermint
49	1503	Phenylacetaldehyde	nd*
50	1504	Acetophenone	must, flower, almond
51	1512	1,8-menthadien-4-ol	nd*
52	1703	2-Methoxy-phenol	vanilla, spice
53	1704	Benzyl alcohol	Sweet, flower
54	1705	Phenethyl alcohol	floral
55	1706	1-Dodecanol	nd*
56	1800	Benzothiazole	sulfury, rubbery, vegetable
57	1801	2-Methyl-phenol	fenol
58	1809	ρ-Cresol	fenol, smoke
59	1905	*m-t*-Butylphenol	nd*
60	2000	2,4-Di-*t*-butylphenol	nd*
61	2110	Indole	fecal, burned

Peak	LRI DB-Wax[a]	Compounds	Odor descriptors[b]
62	2398	Benzophenone	balsamic
63	2400	Skatole	fecal
64	2402	Diisobutyl phthalate	nd*

According to Acree and Arn (2016)

Figure 1. Chromatogram (total ion current) of the volatile organic compounds from the bioreactor. The letters correspond to the residence times with which the chromatograms were obtained: A=0h, B=24h, C=48h and D=144h



The Table 2 and the Figure 1 shows the volatile organic compounds identified in the experiments. From 24 hours, 27 compounds disappeared from cultivation, and that same period 11 new compounds are formed. Between 48 hous and 144 hours it is noted that compounds characteristic of the wastewater continue reduced their concentration up until total disappearance. With exception of 2-methylfuran, which has a concentration of 0.35 $\mu g.L^{-1}$

after 144 hours of process. This compound is not considered of odor unpleasant, has characteristic aroma of roast beef and chocolate.

The Table 3 shows 43 volatile organic compounds which disappeared from the headspace of the bioreactor, during 144 hours of experiment.

Table 2 Quantification of volatile compounds ($\mu g.L^{-1}$) deacrease from the headspace of microalgae bioreactor

Compostos	0 h	24 h	48 h	144 h
Dimethyl sulfide	0,43	0,38	0,18	nd*
2-Methylpropanal	0,5	nd*	nd*	nd*
2-Propenal	5,69	nd*	nd*	nd*
2-Methylfuran	2,56	2,38	2,27	0,35
Butyraldehyde	0,51	nd*	nd*	nd*
2-Butanone	1,78	0,52	nd*	nd*
2 Metylbutanal	3,78	nd*	nd*	nd*
3-Methylbutanal	4,94	nd*	nd*	nd*
Benzene	1,43	nd*	nd*	nd*
Hexanal	15,64	nd*	nd*	nd*
Ethyl benzene	1,64	nd*	nd*	nd*
2-n-Butyl furan	0,36	nd*	nd*	nd*
1,4- Cineole	1,92	nd*	nd*	nd*
Limonene	49,89	7,35	0,98	nd*
1,8 Cineole	4,92	0,87	0,39	nd*
1-Pentanol	6,3	nd*	nd*	nd*
2-Pentyl-furan	3,89	1,41	0,43	nd*
Cyclohexanone	2,73	2,3	nd*	nd*
2-Heptanol	1,11	nd*	nd*	nd*
Pyrrolidine-2,4-dione	2,19	nd*	nd*	nd*
2-Heptenal	6,15	nd*	nd*	nd*
Hexanol	30,34	nd*	nd*	nd*
Dimethyl trisulfide	0,93	0,43	nd*	nd*

Compostos	0 h	24 h	48 h	144 h
Dihexylsulfide	0,15	nd*	nd*	nd*
1,3-Dichloro-benzene	17,87	1,72	nd*	nd*
1-Heptanol	25,49	nd*	nd*	nd*
2-Ethyl-hexanol	9,58	nd*	nd*	nd*
3-Propyl-cyclopentene	3,88	nd*	nd*	nd*
Benzaldehyde	55,37	nd*	nd*	nd*
D-Fenchyl alcohol	4,31	nd*	nd*	nd*
Pentadecane	0,68	nd*	nd*	nd*
Menthol	6,06	1,81	1,57	nd*
Phenylacetaldehyde	7,87	nd*	nd*	nd*
Acetophenone	7,15	1,67	nd*	nd*
1,8-menthadien-4-ol	4,14	3,28	nd*	nd*
Benzyl alcohol	4,04	nd*	nd*	nd*
Phenethyl alcohol	1,55	nd*	nd*	nd*
1-Dodecanol	1,69	nd*	nd*	nd*
2-Methyl-phenol	0,24	0,2	nd*	nd*
ρ-Cresol	89,99	26,47	nd*	nd*
Indole	3,56	nd*	nd*	nd*
Skatole	7,09	5,93	nd*	nd*
Diisobutyl phthalate	0,37	1,23	2,47	1,36

nd* not detected

Were detected five sulfur compounds, dimethyl sulfide (baked cauliflower), dimethyl trisulfide (stinky meat), dihexylsulfide (cooked chicken), they disappeared in from 48 hours of cultivation as show in Table 1. Dimethyl disulfide emerged from 24 hours with a concentration of 8, 14 $\mu g.L^{-1}$ reducing its concentration to 0,54 $\mu g.L^{-1}$ in 144 hours of cultivation. Appeared in 48 hours the compound carbon disulfide (slightly sweet, irritating odor) and disappeared completely in 144 hours of process, as observed in table 2.

Sulfur compounds are primary sources of bad odors from wastewater due to decaying organic matter. Dimethyl disulfide, dimethyl trisulfide and 3-methyl butanal are especially potent VOSCs produced by bacterial activity, and largely responsible for the strong sulfurous and putrid odors produced during bacterial degradation of algae and plant material (Karlsson et al., 1995; Watson et al., 2016).

These compounds have multiple biosynthetic origins, sulfur and a major component of algal cells (Giordano, 2013). The importance of sulphur is it associated with its presence in numerous pivotal structural and functional compounds such as the

amino acids, sulpholipids, vitamins and cofactors, cell wall constituents (Takahashi et al., 2011). Algae, cyanobacteria and microalgae have the ability to reduce and assimilate sulfur amino acid and convert them into sulfate ester of formula (Takahashi et al., 2011; Borowitzka, 2016).

The nitrogenous compounds identified were indole and skatole, which are the cause of fecal odor typical of wastewater. The initial concentration at time zero was 3,56 $\mu g.L^{-1}$ and 7,09 $\mu g.L^{-1}$, respectively. Not was detected indole after 24 hour. Skatole reduced a 5,93 $\mu g.L^{-1}$ and disappeared in 48 hours. Nitrogen compounds are essential for the growth and metabolism of microalgae and cyanobacteria, nitrogen is one of the most abundant elements of microalgae intracellular (Lari et al., 2016; Fan et al., 2014).

An experiment conducted by Rabbani et al., in 2016 using biofilter to remove sulfur and nitrogen compounds in a wastewater treatment plant eliminated sulfur compounds into 96 hours of incubation. In heterotrophic cultivation, the microalgae *Phormidium autumnale* has the capacity to reduce and or remove sulfur compounds and nitrogen within 48 hours of the process. The fact of microalgae need of sulfur and nitrogen compounds for their metabolism, may have led to the disappearance of these VOCs from the headspace of heterotrophic bioreactor.

The compounds as ρ-cresol and 2-methylphenol has a concentration of 89.99 $\mu g.L^{-1}$ and 0.24 $\mu g.L^{-1}$, respectively, at the start of the process. The cresols are highly toxic compounds. ρ-Cresol is an isomeric phenol with a methyl substituent at the para position relative to hydroxy group ρ-cresol. It is a naturally occurring metabolic product that is formed from tyrosine by bacteria under anaerobic conditions. Even at very low concentration, In addition to being highly toxic have potential carcinogen (Singh, et al, 2007). In 24 hours of bioprocess these compounds reduced by 70% compared to the initial concentration. After 48 hours these compounds were not present in heterotrophic microalgal cultivation, thus demonstrating the capacity of using this technology to remove such VOCs.

The literature indicates that the biofilters and trickling filters removal of volatile organic compounds is not complete. Work performed by Deshusses et al., (2001) and Iranpour et al., (2005) reported that low pH may have been the cause of low VOC

removal in biofilters and biotrickling filters. However, the microorganisms that degrade VOCs prefer neutral pH (Rabbani, 2016).

The chromatograms (Fig. 1) show the reduction of volatile compounds of wastewaters from 24 hours of culture, this fact may have occurred because the microalgae *Phormidium autumnale* keep the medium at pH close to neutral (pH between 7.0 and 8.0) and also It may be linked to the use of these compounds for their metabolism.

During 144 hours of cultivation was not detected the presence of volatile organic compounds typical off-flavor in microalgae and cyanobacteria, such as geosmin, 2-methylborneol and hydrogen sulfide. The dibutyl phthalate compound is considered a toxic component (Sun et al., 2012), was detected in the wastewater in the time zero at a concentration of 0.37 $\mu g.L^{-1}$ and after 48 hours of residence time has arrived in its maximum of 2.47 $\mu g.L^{-1}$ and began its decline reducing the 1.36 $\mu g.L^{-1}$ at the end of 144 hours of experiment. More studies should be conducted to elucidate this case.

However, in parallel to the disappearance of volatile organic compounds from wastewater headspace of the bioreactor microalgal, we observed the appearance of new COV`s 21, which are shown in Table 3.

Table 3 Quantification of volatile compounds ($\mu g.L^{-1}$) that formed in the headspace of microalgae bioreactor

Compounds	0 h	24 h	48 h	144 h
Carbon disulfide	nd*	nd*	0,43	nd*
1,1-Diethoxy-ethane	nd*	1,03	1,46	nd*
2,4-Dimethylpentan-3-one	nd*	nd*	0,36	nd*
Dimethyl dissulfide	nd*	8,14	1,76	0,54
2-Methyl-3-hexanone	nd*	2,57	nd*	nd*
2,3-Heptanedione	nd*	1,42	nd*	nd*
2-Heptanone	nd*	0,43	1,81	nd*
5-Methyl-2-hexanone	nd*	nd*	nd*	0,74
6-ethyl-2-methyl-octane	nd*	nd*	0,14	nd*
4,6-Dimethyl-Dodecane	nd*	nd*	nd*	1,08
2-Phenylpropene	nd*	0,77	0,43	nd*
6-Methyl-5-hepten-2-one	nd*	nd*	nd*	0,56
Cyclohexanol	nd*	5,25	nd*	nd*
2,3,3-Trimethyloctane	nd*	nd*	0,42	nd*
3-Hexyl-cyclopentene	nd*	0,95	nd*	nd*

Compounds	24h	0 h	48 h	144 h
Linalool	nd*	0,5	nd*	nd*
2-Methoxy-phenol	nd*	0,99	nd*	nd*
Benzothiazole	3,05	3,26	3,38	6,36
m-t-Butylphenol	nd*	0,15	0,28	1,21
2,4-Di-t-butylphenol	1,27	1,35	2,39	0,64
Benzophenone	nd*	nd*	1,79	0,83

nd* not detected

The Table 3 shows the compounds formed from the 24 hours of residence time in heterotrophic microalgal cultivation. Was detected 3 alcohols, 7 ketones, 4 hydrocarbons and 8 micelaneous. The flavor descriptors are shown in Table 1.

One of the most abundant volatile groups produced in the experiment was ketones. Both 2-methyl-3-hexanone and benzophenone were found with 2.57 $\mu g.L^{-1}$ respectively, from 24 to 48 hours of cultivation. Aliphatic ketones can be products of lipid oxidation (Santos et al, 2016; Rodrigues et al., 2014.). Acree and Arn, (2016) reported the odor descriptors for ketones identified in *Phormidium autumnale* cultivation: 2-Butanone (camphor), 2,4-Dimethylpentan-3-one (distilled from fruit drink), 2-heptanone (fruit) and benzophenone (balsamic).

Hydrocarbons was the second group that appeared. Unlike bacteria that require genetic changes to synthesize alkanes, cyanobacteria have in their biosynthetic pathway (Schirmer et al., 2010). Hydrocarbons, alkanes and alkenes, are of particular interest because of its high potential for use in the production of bioenergy, to have 30% more energy than ethanol (Choi Lee, 2013). In experiment appeared a total of 9 volatile aliphatic hydrocarbons identified as alkanes (2,3,3-Trimethyl octane, 4,6-Dimethyl-Dodecane) and alkenes and cyclic chain (3-Hexyl-cyclopentene, 2-Phenylpropene). In contrast to higher organisms, microbial forms can be grown in bioreactors which allows the industrial production of hydrocarbons. In recent years, the microbial synthesis of volatile aliphatic hydrocarbons, non-methane extracellular has attracted considerable interest in the development of efficient and environmentally safe methods for producing biofuels (Ladygina, 2006).

All alcohols formed are short chain linear (C4–C10) compounds. These compounds are a chemical signature of cyanobacteria and have been confirmed in

studies involving the identification of organic volatiles in these microorganisms (Santos et al., 2016; Durme et al., 2013; Sun et al., 2012).

VOCs identified in the *P. autumnale* volatile profile have sweet fruity flavors (Table 1). These compounds are widely used in the food industry (Berger 2009; Sun et al., 2012).

4. Conclusion

Heterotrophic microalgal bioreactors are a technology high in potential to remove volatile organic compounds from cattle-slaughterhouse wastewater. Where the period between 24 and 48 hours was crucial to the disappearance of compounds characteristic of wastewater, in parallel the formation of new compounds that are commercial interest for fine chemicals.

5. References

Acree, T., Arn, H. Flavornet and human odor space, 2016. www.flavornet.org.

American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 20nd ed. Washington 2005.

Berger, R. G. Biotechnology of flavours- the next generation. Biotechnology Letters, v.31, p.1651-1659, 2009.

Borowitzka M. A.; Beardall J.; Raven J. A. The Physiology of Microalgae. Springer New York, Springer, 2016.

Capelli, L.; Sironi, S.; Rosso, R. D.; Céntola, P.; Rossi, A.; Austeri, C. Olfactometric approach for the evaluation of citizens' exposure to industrial emissions in the city of Terni, Italy. Science of the Total Environment, v.409, p.595–603, 2011.

Choi, J. Y.; Lee, S. Y. Microbial production as short-chain alkane. Nature, p.1-6, 2013.

Deshusses, M.A., Cox, H.H.J., Schroeder, E.D., & Converse, B.M. Controlling odor and VOCs with bioreactors, Final Report WERF Project 98-CTS-4, Alexandria, VA: Water Environment Research Foundation, 2001.

Durme, J.V., Goiris, K., Winne, A., De Cooman, L., Muylaert, K. Evaluation of the volatile composition and sensory properties of five species of microalgae. Journal of Agricultural and Food Chemistry.v.61, p.10881-10890, 2013.

Estrada, J. M.; Kraakman, N. J. R. B.; MUNOZ, R.; Lebrero, R.; A Comparative Analysis of Odour Treatment Technologies in Wastewater. Treatment Plants. Environmental Science & Technology, v.45, p.1100–1106, 2011.

Fan, J.; Cui, Y.; Wan, M.; Wang W.; Li, Y.; Lipid accumulation and biosynthesis genes response of the oleaginous Chlorella pyrenoidosa under three nutrition stressors. Biotechnol Biofuels, v.7, p.1–14, 2014.

Fujise, D.; Tsuji, K.; Fukushima, N.; Kawai, K.; Harada, K. Analytical aspects of cyanobacterial volatile organic compounds for investigation of their production behavior. Journal of Cromatography A. v.1217, p.6122-6125, 2010.

Gaffney, M.; O'Rourke, R.; Murphy, R. Manipulation of fatty acid and antioxidant profiles of the microalgae *Schizochytrium* sp. through flaxseed oil supplementation. Algal Research. v.6, p.195–200, 2014.

Giordano, M. Homeostasis: an underestimated focal point of ecology and evolution. Plant Science, v.211, p. 92–101, 2013.

Giordano, M.; Prioretti, L. Sulphur and algae: metabolism, ecology and evolution. In: Borowitzka M. A., Beardall, J., Raven JA (eds) Microalgal physiology. Springer, Dordrecht, pp 185–209, 2016.

Havel, J.; Weuster-Botz, D. Comparative Study of Cyanobacteria as Biocatalysts for the Asymmetric Synthesis of Chiral Building Blocks. Engineering in Life Sciences, v.6, p.176-179, 2006.

Iranpour, R., Cox, H.H.J., Deshusses, M.A., Schroder, E.D. Literature review of air pollution control biofilters and biotrickling filters for odor and volatile organic compound removal. Environmental Progress. V.24, p.254-267, 2005.

Jenkins, D.; Medsker, L.L.; Thomas, J.F. Odorous Compounds in Natural Waters. Some Sulfur Compounds Associated with Blue-Green Algae. Environmental Science and Technology. V. 9, p. 731-735, 1967.

Karlsson, S.; Grimvall, A., Boren, H. Drinking water off-flavour caused by the formation of short-chain fatty acids in slow sand filters. Water Science & Technology. v.31, p. 49–54, 1995.

Kunjapur, A. M.; Tarasova, Y.; Prather, K. L. J. Synthesis and Accumulation of Aromatic Aldehydes in an Engineered Strain of *Escherichia coli*. Journal of the American Chemical Society, v.136, p.11644−11654, 2014.

Ladygina, N.; Dedyukhina, E.G; Vainshtein, M. B. A review on microbial synthesis of hydrocarbons. Process Biochemistry. v.41, p.1001–1014, 2006.

Lari, Z.; Moradi-kheibari N.; Ahmadzadeh, H.; Abrishamchi, P.; Moheimani, N. R.; Murry, M. A. Bioprocess engineering of microalgae to optimize lipid production through nutrient management. Journal of Applied Phycology. 2016.

Lebrero R., Gondim A. C. Pérez R., Garcıa-Encina, P. A. Munõz R. Comparative assessment of a biofilter, a biotrickling filter and a hollow fiber membrane bioreactor for odor treatment in wastewater treatment plants. Water Research. V.49, p.339-350, 2014.

Maroneze, M. M. ; Menezes, C. R.; Barin, J. S.; Queiroz, M. I.; Zepka, L. Q.; Jacob-Lopes, E. Treatment of cattle-slaughterhouse wastewater and the reuse of sludge for biodiesel production by microalgal heterotrophic bioreactors. Scientia Agricola (USP. Impresso), v.71, p.521-524, 2014.

Rabbani, K.A.; Charles, W.; Kayaalp, A. R. Cord-Ruwisch, G. Ho Pilot-scale biofilter for the simultaneous removal of hydrogen sulphide and ammonia at a wastewater treatment plant. Biochemical Engineering Journal. V.107, p. 1–10, 2016.

Rodrigues, D.B., Flores, E.M.M., Barin, J.S., Mercadante, A.Z., Jacob-Lopes, E., Zepka, L.Q. Production of carotenoids from microalgae cultivated using agroindustrial wastes. Food Research International. v.65, p.144-148, 2014.

Santos, A.B.; Fernandes, A.S.; Wagner, R.; Jacob-Lopes, E.; Zepka, L.Q. Biogeneration of volatile organic compounds produced by Phormidium autumnale in hetrotrophic bioreactor. Journal of Applied Phycology, v.60, p.32-42, 2016.

Schirmer, A.; Rude, M. A.; Li, X.; Popova, E.; Cardayre, S. B. Microbial Biosynthesis of Alkanes. Science, v.329, p.559-562, 2010.

Singh, R. K.; Kumar, S.; Kumar, A. Biodegradation kinetic studies for the removal of p-cresol from wastewater using Gliomastix indicus MTCC 3869. Biochemical Engineering Journal, v.40, p.293–303, 2008.

Su, Y.; Mennerich, A.; Urban, B. Comparison of nutriente removal capacity and biomass settleability of four high-potential microalgal species. Bioresource Technology. v.124, p. 157-162, 2012.

Sun, S.M.; Chung, G.H.; Shin, T.S. (2012). Volatile compounds of the green alga, Capsosiphon fulvescens. Journal of Applied Phycology 24, 1003-1013.

Takahashi, H.; Kopriva, S.; Giordano, M.; Saito, K.; Hell, R. Sulfur assimilation in photosynthetic organisms: Molecular functions and regulations of transporters and assimilatory enzymes. Review of Plant Biology. v.62, p.157-84, 2011.

Talaiekhozani A.; Bagheri, M.; Goli A.; Khoozani, M. R. T. An overview of principles of odor production, emission, and control methods in wastewater collection and treatment systems. Journal of Environmental Management. v.170, p.186-206, 2016.

Vitko, T., Suffet, I.H., Zhou, Y., Abraham, S., 2014. OCSD odor control master plan. In: Proceedings of the Odors and Air Pollutants Meeting. Water Environment Federation, Miami, FL, USA, p. 2014.

Watson, S. B.; Monis, P., Baker, P.; Giglio, S. Biochemistry and genetics of taste- and odor-producing cyanobacteria. Harmful Algae. v.54, p.112–127, 2016.

Zhou Y.; Hallis S. A.; Vitko T.; Suffet I. H. Identification, quantification and treatment of fecal odors released into the air at two wastewater treatment plants. Journal of Environmental Management, v.180, p.257-263, 2016.

CONCLUSÃO GERAL

O perfil dos compostos orgânicos voláteis da água residuária no cultivo heterotrófico microalgal foi determinado por cromatografia gasosa acoplada ao detector de massas, apresentou 64 compostos classificados como álcoois, cetonas, aldeídos, ácidos, hidrocarbonetos, sulfurados e nitrogenados.

O período entre 24 e 48 horas percebeu-se uma mudança no perfil volátil no headspace do biorreator, no qual desapareceram 30 compostos em 24 horas de cultivo. A partir de 48 horas os compostos com odor característico dos efluentes como escatol e indol de odor fecal, desaparecu completamente.

Compostos sulforados, como o sulfeto de dimetil (couve-flor cozida), trisulfide dimetil (carne pútrida), dihexylsulfide (frango cozido) desapareceram em 48 horas de cultivo. Ao total, 43 compostos orgânicos voláteis sumiram do headspace do cultivo heterotrófico microalgal. Em paralelo, 21 novos compostos foram formados. O odor descritor dos compostos formados detectado nos experimentos foram principalmente classificados como frutados, especiarias e compostos florais. A microalga *Phormidium autumnale*, além de contribuir para a redução de compostos com descritores ruins podendo ser útil para as estações de tratamento de efluente agroindustrial, também produzem uma variedade de compostos voláteis com descritores de aroma de interesse comercial para a indústria de química fina.

CAPÍTULO 3

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

O capítulo foi publicado no livro "Volatile Organic Compounds: Occurrence, Behavior and Ecological Implications" pela editora "Nova Science Publishers".

Chapter

Biogeneration of Volatile Organic Compounds by Microalgae: Occurrence, Behavior, Ecological Implications and Industrial Applications

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Abstract

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

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

Introduction

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

Microalgae-based systems for chemicals production are an emergent area, representing a great promise for industrial application. However, there is little information available on the volatile organic compounds biogeneration of these microorganisms. The characterization of the volatile fraction of microalgal bioreactors can contribute to establishing routes for the bioconversion of substrates, and enable the identification of potential applications of the volatile bioproducts formed [2].

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

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

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

Thus, the aim of this chapter was to evaluate the biogeneration of volatile organic compounds produced by microalgae, with focus on the occurrence, behavior, ecological implications and industrial applications of these metabolites.

Occurrence and Behaviorof Volatile Organic Compounds from Microalgae

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

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

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

The volatile organic compounds from microalgae can belong to different classes of compounds such as esters, alcohols, hydrocarbons, ketones, terpenes, carboxylic acids and sulfur compounds [11]. In order to understand this diversity of compounds, Table 1 shows the main compounds and their cultivations previously found in studies. The biosynthesis of these volatile organic compounds will depend on the availability of building blocks, such as carbon, nitrogen, and energy supply from the primary

metabolism. Therefore, the availability of these building blocks has great impact on the concentration of secondary metabolites, including VOCs, demonstrating the high level of connectivity between the primary and secondary metabolism [12, 13].

The formation of compounds from pyruvate can follow the route of terpenoids or also via the keto acids via intermediate 2-ketoisovalerate. With the formation of Acetyl-CoA has the biosynthesis of fatty acids. On arriving at the tricarboxylic acid cycle, follows the route of keto acids by intermediates 2-ketobutyrate and 2-ketovalerate. Started by way 2-keto acids, amino acids, which are intermediates in the synthesis pathways. These 2-keto acids are formed by deamination followed by decarboxylation catalyzed by transaminase branched chain amino acids such as L-leucine, which has its synthesis from pyruvate, and L-isoleucine from the tricarboxylic acid or also by intermediate amino acid synthesis [14]. These 2-keto acids can be further subjected to decarboxylation, followed by reduction, oxidation and/or esterification, can be formed in addition to alcohols, aldehydes, acids and esters [12].

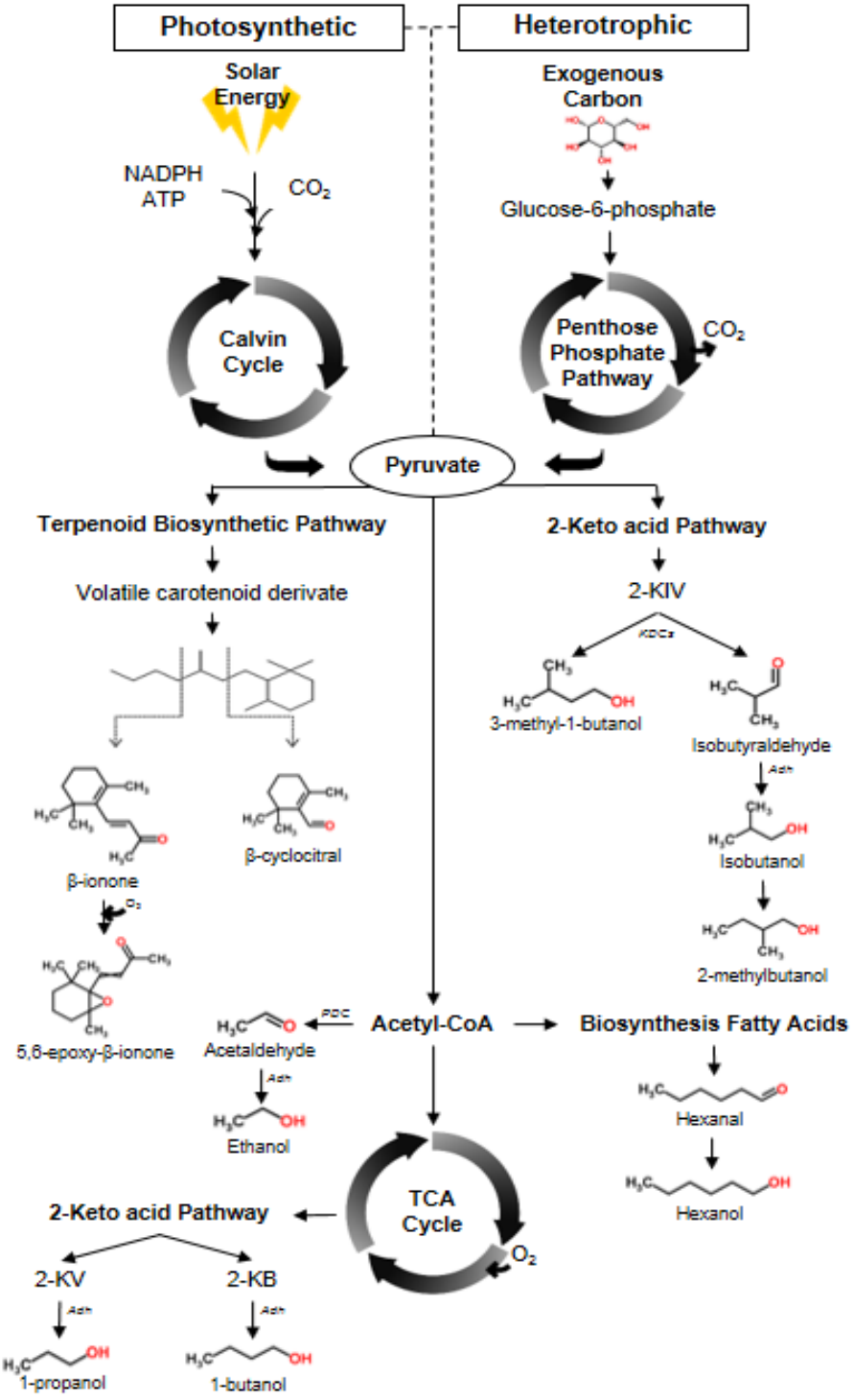

Figure 1. Overview of microalgae metabolism and their potential routes emission of volatile organic compounds.

Table 1. Major VOCs found in microalgae-based systems

Organic classes	Compounds	Microalgae	Cultivation	Reference
Acids	Acetic acid	*Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]
	Butanoic acid	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methylbutanoic acid	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Isovaleric acid	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
Sulfuric compounds	Dimethyl sulfide	*Tetraselmis* sp.	Photoautotrofic	Durme et al., 2013 [8]
	Dimethyl disulfide	*Tetraselmis* sp.*; Rhodomonas* sp.	Photoautotrofic	Durme et al., 2013 [8]
	Methional	*Rhodomonas* sp.	Photoautotrofic	Durme et al., 2013 [8]
	Dimethyl trisulfide	*Tetraselmis* sp.	Photoautotrofic	Durme et al., 2013 [8]
Furans	2-Ethylfuran	*Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	2-Pentylfuran	*Botryococcus braunii; Chlorella vulgaris; Spirulina platensis*	Photoautotrofic	Durme et al., 2013 [8]
Esters	Ethyl acetate	*Nannochloropsis oculata*	Photoautotrofic	Durme et al., 2013 [8]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]
	Methyl hexanoate	*Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	Methyl phenylacetate	*Nannochloropsis oculata*	Photoautotrofic	Durme et al., 2013 [8]
	Methyl octanoate	*Nannochloropsis oculata; Rhodomonas* sp.*; Chlorella vulgaris; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Isoamyl acetate	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Isobutyl acetate	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Methyl decanoate	*Nannochloropsis oculata*	Photoautotrofic	Durme et al., 2013 [8]
	2-Butyl acetate	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
Terpenes	β-Cyclocitral	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris; Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Durme et al., 2013[8]; Milovanovic et al., 2015 [38]
		Phormidium autumnale;	Heterotrophic	Santos et al., 2015 [13]
	β-Ionone	*Tetraselmis* sp.*: Rhodomonas* sp.; *Chlorella vulgaris;*	Photoautotrofic	Durme et al., 2013 [8]
		Phormidium autumnale	Heterotrophic	Santos et al., 2015 [13]

Table 1. (Continued)

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

Table 1. (Continued)

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

Table 1. (Continued)

Organic classes	Compounds	Microalgae	Cultivation	Reference
	Cis-4-heptenal	*Rhodomonas* sp.*; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Heptanal	*Rhodomonas* sp.*; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Tr,tr-2,4-heptadienal	*Rhodomonas* sp.*; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	2-Octenal	*Nannochloropsis oculata; Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Nonanal	*Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	2-Methylbutanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methylbutanal	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	Furfural	*Rhodomonas;*	Photoautotrofic	Durme et al., 2013 [8]
	Benzaldehyde	*Tetraselmis* sp.*; Rhodomonas* sp.*; Nannochloropsis oculata; Botryococcus braunii; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	Phenylacetaldehyde	*Nannochloropsis oculata; Chlorella vulgaris*	Photoautotrofic	Durme et al., 2013 [8]
	4-Ethylbenzaldehyde	*Botryococcus braunii*	Photoautotrofic	Durme et al., 2013 [8]
	Acetaldehyde	*Phormidium autumnale*	Heterotrophic	Santos et al, 2015 [13]
	Butanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	2-Methylbutanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	3-Methylbutanal	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Isobutyraldehyde	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
Hydrocarbons	Octane	*Phormidium autumnale*	Heterotrophic	Santos et al. 2015 [13]
	1-Heptene	*Phormidium autumnale*	Heterotrophic	Santos et al., 2015 [13]
	Hexadecane	*Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	Tetradecane	*Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	8-Methylheptadecane	*Spirulina platensis; Nostoc* sp.*; Anabaena* sp.	Photoautotrofic	Milovanovic et al., 2015 [38]
	3-Octadecene	*Nostoc* sp	Photoautotrofic	Milovanovic et al., 2015 [38]

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

As for the fatty acid synthesis, it occurs from the Acetyl-CoA molecule by Acetyl-CoA reductase enzyme. Using saturated fatty acids C_{18} as linoleic and linolenic acids, via the lipoxygenase 9-hydroperoxy form and intermediate 13-hydroperoxide. The branch hydroperoxide lyase converts both hydroperoxides C_6 and C_9 aldehydes such as 1-hexanal, hexanol and nonanal, which are reduced to alcohols by dehydrogenases [13]. In this synthesis may also be produced unbranched hydrocarbons by two families of enzymes: an acyl-acyl carrier protein reductase (AAR) and an aldehyde decarbonylase (AAD) that operate in the conversion of fatty acids [17].

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

Some chemical reactions may convert the volatile organic compounds into other compounds. For example, alcohols can be oxidized to aldehydes and then to carboxylic acids, and ketones may be reacted with the hydroxyl radicals in the air to form aldehydes [19, 20]. Aldehydes and ketones can be reduced to the alcohols by reductases aldehyde/ketone alcohols can be oxidized to aldehydes by alcohol dehydrogenase, and then further oxidized to the acid by aldehyde dehydrogenase [20]. Ketones can be formed in many ways; aliphatic ketones can be lipid oxidation products or ketone and methyl degradation (C_3-C_{17}) could be formed from the oxidative cleavage of carotenoids [13, 21].

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

Ecological Implications

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

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

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

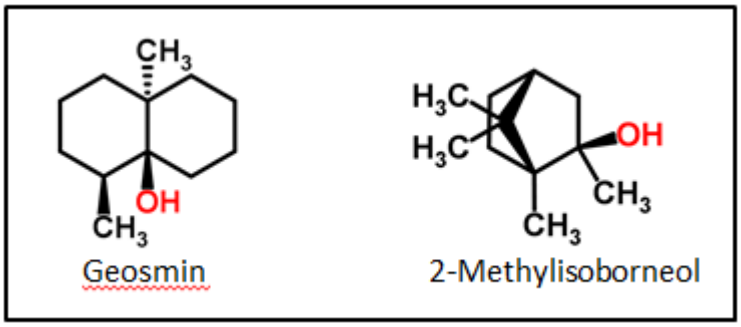

Figure 2. Chemical structure of geosmin and 2-methylisoborneol obtained from microalgae [50].

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

The formation of water blooms results from the redistribution, and often, rapid accumulation of buoyant planktonic populations. When such populations are subjected to optimal conditions, they respond by increasing their buoyancy and move

upward nearer to the water surface, causing change in color of the water and often also in taste and odor.

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

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

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

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

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

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

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

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

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

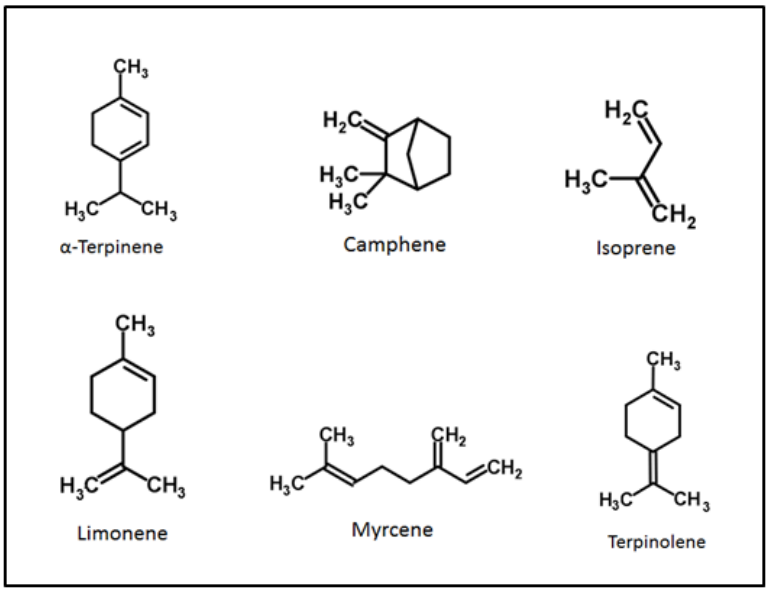

Figure 3. Dominant biogenic isoprenoids in microalgae [50].

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

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

Industrial Applications

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

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

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

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

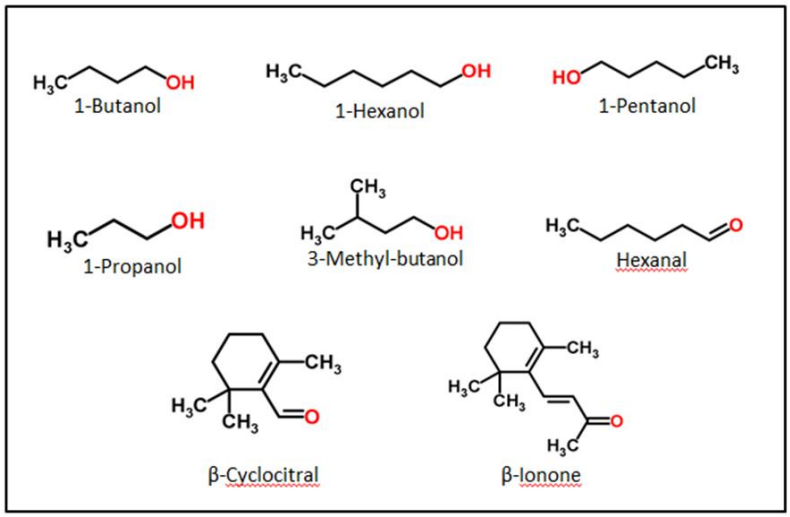

Figure 4. Volatile organic compounds with commercial application obtained from microalgae [50].

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

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

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

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

Aliphatic alcohols with higher carbon chain length or equal to five are attractive targets for biofuels have a high energy density and low water solubility (e.g., 1-pentanol 23 g/L; 1-hexanol 6.2 g/L; 1-heptanol 1.2 g/L). The enzyme responsible for the production of such compounds is the Acetyl-CoA-reductase that may be present in the reactions of the tricarboxylic acid cycle, mevalonate, and leucine biosynthesis. Other alcohol having substantial energy interest is the 1-butanol to have a

comparable gasoline energy (29.2 MJ/L and 32.5 MJ/L, respectively), this can be a substitute fuel or added in the place of ethanol It has a lower energy (21.2 MJ/L) [40].

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

References

[1] Buono, S.; Langellotti, A.L.; Martello, A.; Rinna, F.; Fogliano, V. (2014). Functional ingredients from microalgae. *Food & Function* 5, 1669-1685.

[2] Jacob-Lopes, E.; Scoparo, C.H.G.; Queiroz. M.I.; Franco, T.T. (2010). Biotransformations of carbon dioxide in photobiorreactors. *Energy Conversion and Management* 51, 894-900.

[3] Jacob-Lopes, E.; Franco, T.T. (2013). From oil refinery to microalgal biorefinery. *Journal of CO_2 Utilization* 2, 1-7.

[4] Nuccio, J.; Seaton, P.J.; Kieber, K.J. (1995). Biological production of formaldehyde in the marine environmental. *Limnology & Oceanography* 40, 521-527.

[5] Fay, P. (1983). *The blue-greens (Cyanophyta – Cyanobacteria)* (5th Ed.), Great Britain.

[6] Francisco, E.C.; Franco, T.T.; Wagner, R.; Jacob-Lopes, E. (2014). Assessment of different carbohydrates as exogenous carbon source in cultivation of cyanobacteria. *Bioprocess and Biosystems Engineering* 1, 2-11.

[7] Rzama, A.; Benharref, A.; Arrreguy, B.; Dufourc, E.J. (1995). Volatile compounds of green microalgae grown on reused wastewater. *Phytochemistry* 38, 1375-1379.

[8] Durme, J.V.; Goiris, K.; Winne, A.; De Cooman, L.; Muylaert, K. (2013) Evaluation of the volatile composition and sensory properties of five species of microalgae. *Journal of Agricultural and Food Chemistry* 61, 10881-10890.

[9] Kumar, K.; Dasqupta, C.N.; Nayak, B.; Lindblad, P.; Das, D. (2011). Development of suitable photobioreactors for CO2 sequestration addressing global warming using green algae and cyanobacteria. *Bioresource Technology* 102, 4945-4953.

[10] Iverson, T.M. (2006). Evolution and unique bioenergetic mechanisms in oxygenic photosynthesis. *Current Opinion in Chemical Biology* 10, 91-100.

[11] Papaleo, M.C; Romoli, R.; Bartolucci, G.; Maida, I. (2013). Bioactive volatile organic compounds from Antartic (sponges) bacteria. *New Biotechnology* 30, 824-838.

[12] Dudareva, N.; Klempien, A.; Muhlemann, J. K.; Kaplan, I. (2013) Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist* 198, 16-32.
[13] Santos, A.B.; Fernandes, A.F.; Wagner, R.; Jacob-Lopes, E.; Zepka, L.Q. (2015). Biogeneration of volatile organic compounds produced by Phormidium autumnale in heterotrophic bioreactor. *Journal of Applied Phycology* 1, 1-10.
[14] Fujise, D.; Tsuji, K.; Fukushima, N.; Kawai, K.; Harada, K. (2010). Analytical aspects of cyanobacterial volatile organic compounds for investigation of their production behavior. *Journal of Chromatography A* 1217, 6122-6125.
[15] Rodrigues, D.B.; Flores, E.M.M.; Barin, J.S.; Mercadante, A.Z.; Jacob-Lopes, E.; Zepka, L.Q. (2014). Production of carotenoids from microalgae cultivated using agroindustrial wastes. *Food Research International*, 65, 144-148.
[16] Mendes-Pinto, M.M. (2009). Carotenoid breakdown products – the norisoprenoids − in wine aroma. *Archives of Biochemistry and Biophysics* 483, 236−245.
[17] Ducat, D.C.; Way, J.C.; Silver, P.A. (2011). Engineering cyanobacteria to generate high-value products. *Trends in Biotechnology* 29, 95–103.
[18] Zhang, Z.; Li, T.; Wang, D.; Zhang, L.; Chen, G. (2009). Study on the volatile profile characteristics of oyster Crassostrea gigas during storage by a combination sampling method coupled with GC/MS. *Food Chemistry* 115, 1150–1157.
[19] Atkinson, R.; Tuazon, E.C.; Aschmann, S.M. (2000). Atmospheric chemistry of 2-pentanone and 2-heptanone. *Environmental Science & Technology* 34, 623-631.
[20] Korpi, A.; Järnberg, J.; Pasanen, A.L. (2009). Microbial volatile organic compounds. *Critical Reviews in Toxicology* 39, 139-193.
[21] Sun, S.M.; Chung, G.H.; Shin, T.S. (2012). Volatile compounds of the green alga, Capsosiphon fulvescens. *Journal of Applied Phycology* 24, 1003-1013.
[22] Guttman, L.; Rijn, J.V. (2008). Identification of conditions underlying production of geosmin and 2-methylisoborneol in a recirculating system. *Aquaculture* 279, 85–91.
[23] Wanke, M.; Skorupinska-Tudek, K.; Swiezewska, E. (2001). Isoprenoid biosynthesis via 1-deoxy-D-xylulose 5-phosphate/2-C-methyl- D-erytritol 4-phosphate (DOXP/MEP) pathway. *Acta Biochimica Polonica Warsaw* 48, 663-672.
[24] Jüttner, F.; Watson, S.B. (2007). Biochemical and Ecological Control of Geosmin and 2-Methylisoborneol in Source Waters. Minireview. *Applied and Environmental Microbiology* 73, 4395–4406.
[25] Smith, J.L.; Boyer, G.L.; Zimba, P.V. (2008). A review of cyanobacterial odorous and bioactive metabolites: impacts and management alternatives in aquaculture. *Aquaculture* 280, 5–20.
[26] Watson, S.B.; Jüttner, F.; Köster, O. (2007). Daphnia behavioural responses to taste and odour compounds: ecological significance and application as an inline treatment plant monitoring tool. *Water Science & Technology* 55, 23–31.

[27] Watson, S.B. (2003). Cyanobacterial and eukaryotic algal odour compounds: signals or by-products? A review of their biological activity. *Phycologia* 42, 332–350.
[28] Kesselmeier, J.; Staudt, M. (1999). Biogenic Volatile Organic Compounds (VOC): An Overview on Emission, Physiology and Ecology. *Journal of Atmospheric Chemistry* 33, 23–88.
[29] Graedel, T.E. (1979). Terpenoids in the atmosphere. *Reviews of Geophysics and Space Physics* 17, 937–947.
[30] Jenkins, D.; Medsker, L.L.; Thomas, J.F. (1967). Odorous Compounds in Natural Waters. Some Sulfur Compounds Associated with Blue-Green Algae. *Environmental Science and Technology* 9, 731-735.
[31] Cheremisinoff, P.N. (1993). *Air Pollution Control and Design for industry*. Marcel Dekker Inc.
[32] Schirmer, A.; Rude, M.A.; Li, X.; Popova, E.; Cardayre, S.B. (2010). Microbial Biosynthesis of Alkanes. *Science* 329, 559-562.
[33] Choi, J.Y.; Lee, S.Y. (2013). Microbial production as short-chain alkane. *Nature*, 2013, 1-6.
[34] Jacob-Lopes, E.; Lacerda, L.M.C.F.; Franco, T.T. (2008). Biomass production and carbono dioxide fixation by Aphanothece microscopica Nagëli in a bubble column photobioreactor. *Biochemical Engineering Journal* 40, 27-34.
[35] Abdel-Raouf, N.; Al-Homaidan, A.A.; Ibraheem, I.B.M. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Science* 19, 257-275.
[36] Berger, R.G. (2009). Biotechnology of flavours - the next generation. *Biotechnology Letters* 31, 1651-1659.
[37] Smith, K.M.; Cho, K.M.; Liao, J.C. (2010). Engineering Corynebacterium glutamicum for isobutanol production. *Applied Microbiology and Biotechnology* 87, 1045-1055.
[38] Milovanovic, I.; Mison, A.; Simeunovic, J.; Kovac, D.; Jambrec, D.; Mandic, A. (2015). Determination of volatile organic compounds in selected strains of cyanobacteria. *Journal of Chemistry*, 2015, 1-6.
[39] Si, T.; Luo, Y.; Xiao, H.; Zhao, H. (2014). Utilizing an endogenous pathway for 1-butanol production in Saccharomyces cerevisiae. *Metabolic engineering* 22, 60-68.
[40] Zhang, K.; Sawaya, M. R.; Eisenberg, D. S.; Liao, J. C. (2008). Expanding metabolism for biosynthesis of non-natural alcohols, *Proceedings of the National Academy of Sciences*, 105, 20653-20658.
[41] Jüttner, F.; Hoflacher, B.; Wurster, K. (1986). Seasonal analysis of volatile organic biogenic substances (VOBS) in freshwater phytoplankton populations dominated by Dinobryon, Microcystis and Aphanizomenon. *Journal of Phycology* 22, 169–175.
[42] Izaguirre, G.; Taylor, W.D. (1995). Geosmin and 2-methylisoborneol production in a major aqueduct system. *Water Science & Technology* 31, 41–48.
[43] Schrader, K.K.; Blevins, W.T. (1993). Geosmin-producing species of Streptomyces and Lyngbya from aquaculture ponds. *Canadian Journal of Microbiology* 39, 834–840.

[44] Izaguirre, G.; Hwang, C.J.; Krasner, S.W.; McGuire, M.J. (1982). Geosmin and 2- methylisoborneol from cyanobacteria in three water supply systems. *Applied and Environmental Microbiology* 43, 708–714.
[45] Tsuchiya, Y.; Matsumoto, A.; Okamoto, T. (1981). Identification of volatile metabolites produced by blue-green algae Oscillatoria splendida, Oscillatoria amoena, Oscillatoria geminata and Aphanizomenon sp. *Yakugaku Zasshi Journal of the Pharmaceutical Society of Japan* 101, 852–856.
[46] Naes, H.; Utkilen, H.C.; Post, A.F. (1988). Factors influencing geosmin production by the cyanobacterium Oscillatoria brevis. *Water Science & Technoogy* 20, 125–131.
[47] Persson, P.E. (1988) Odorous algal cultures in culture collections. *Water Science & Technology* 20, 211–213.
[48] Zimmerman, W.J.; Soliman, C.M.; Rosen, B.H. (1995) Growth and 2-methylisoborneol production by the cyanobacterium Phormidium LM689. *Water Science & Technology* 31, 181–186.
[49] Höckelmann, C.; Jüttner, F. (2005). Off-flavours in water: hydroxyketones and β-ionine derivatives as new odour compounds of freshwater cyanobacteria. *Flavour and Fragrance Journal* 20, 387–394, 2005.
[50] Royal Society of Chemistry (2015). *ChemSpider: search and share chemistry* (http://www.chemspider.com/). Access on 20 February 2015.

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