JACKELINE DE SIQUEIRA CASTRO

VALORIZAÇÃO AGRÍCOLA E ENERGÉTICA DA BIOMASSA ALGAL CULTIVADA EM ÁGUAS RESIDUÁRIAS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Engenharia Civil, para obtenção do título de *Doctor Scientiae*.

Orientadora: Maria Lúcia Calijuri

Coorientadora: Paula Peixoto Assemany

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Assentimento:

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Maria Caly

Maria Lúcia Calijuri
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Aos meus pais Clério e Teresinha;
Às minhas irmãs Francielle e Danielle;
Ao meu amado esposo Vinícius;
E ao meu filho Samuel;
Dedico.

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BIOGRAFIA

Jackeline de Siqueira Castro, filha de Clério Vicente de Castro e Teresinha Maria de Siqueira Castro, nasceu na cidade Patos de Minas-MG, em 08 de abril de 1990. Cursou ensino médio na Escola Estadual “Coronel Cristiano”, em Lagoa Formosa-MG, concluindo-o em 2007. Ingressou em 2009, no Centro Universitário de Patos de Minas – UNIPAM para ser formar Engenheira Ambiental e Sanitarista em 2013. Em março de 2014 iniciou mestrado no Programa de Pós-graduação em Engenharia Civil, área de concentração em Engenharia Sanitária e Ambiental, na Universidade Federal de Viçosa. Em fevereiro de 2016 defendeu sua dissertação intitulada Biofilme de microalgas no solo: emissão de gases de efeito estufa, volatilização de amônia e crescimento de *Pennisetum glaucum*. Na mesma instituição de ensino e Programa de pós-graduação iniciou, em março de 2016, as atividades do doutorado. Submeteu sua pesquisa à banca de qualificação em outubro de 2018 e em 04 de março de 2020 defende sua tese intitulada Valorização agrícola e energética da biomassa algal cultivada em águas residuárias. No mestrado e doutorado foi orientada pela Professora D. Sc. Maria Lúcia Calijuri.

“ Forte é quem, depois de tanto perder, reergue-se e segue lutando. ”

Ayrton Senna

RESUMO

CASTRO, Jackeline de Siqueira, D.Sc, Universidade Federal de Viçosa, março de 2020. **Valorização agrícola e energética da biomassa algal cultivada em águas residuárias.** Orientadora: Maria Lúcia Calijuri. Coorientadora: Paula Peixoto Assemany.

Essa pesquisa abordou a valorização agrícola e energética da biomassa de microalgas cultivada em efluente agroindustrial. As rotas de valorização estudadas foram definidas com base em uma revisão sistemática de literatura realizada entre o ano de 1999 a 2017, em revistas classificadas no quartil 1 pelo Scimago Journal & Country Rank. Os periódicos selecionados pertecem a área de ciências ambientais e sub-área de manejo e disposição de resíduos. A palavra "microalgas" foi usada como termo de busca na página da *web* de cada revista. Assim, os principais temas pesquisados e as lacunas dentro de cada um foram elencados. Um tema no âmbito da valorização agrícola e um no âmbito da valorização energética foram selecionados para serem alvo de investigação técnica e ambiental. No âmbito da valorização agrícola foram testadas diferentes proporções de biomassa de microalgas adicionadas ao fertilizante superfosfato triplo. Após a definição de uma proporção ideal (12 % m/m) de biomassa de microalgas na massa do biofertilizante, vários testes foram realizados com relação à dinâmica do fertilizante após aplicação no solo e sua absorção pelas plantas. Do ponto de vista técnico trata-se de uma potencial fonte de nutrientes visto que contribuiu para o aumento na absorção de P pelas plantas aproximadamente 2 % mais que o fertilizante convencional. No entanto, quando tal rota foi analisada na perspectiva do ciclo de vida, o fertilizante químico foi mais viável que o biofertilizante. Acredita-se que numa análise a longo prazo e levando em consideração os benefícios que o biofertilizante trás, em termos de aumento de matéria orgânica e nutrientes para o solo, contribuindo para a construção da fertilidade, o mesmo poderia se tornar competitivo. Quanto a rota de valorização energética, a carbonização hidrotérmica, que atua na presença de água, mostrou-se interessante do ponto de vista técnico, visto que foi possível obter um material mais concentrado em carbono e outros nutrientes, quando comparado à matéria prima de origem, sob temperaturas e tempos de reação relativamente baixos. O maior rendimento de sólidos (77,72 %) e energético (78,21 %) foi obtido à temperatura de 170 °C e tempo de reação de 10 minutos. Na perspectiva do ciclo de vida, a carbonização hidrotérmica se torna mais interessante se a fase aquosa for reutilizada no processo termoquímico. Entre as possibilidades de utilização da fase aquosa cita-se o cultivo de microalgas e a recirculação no próprio reator de carbonização. Conclui-se que, do ponto de vista técnico ambas as rotas são viáveis. Por outro lado, melhorias nos processos de cultivo e concentração da biomassa de microalgas devem ser realizados para tornar as rotas ambientalmente sustentáveis.

Palavras-chave: Biofertilizantes. Carbonização hidrotérmica. Plantas - Ciclo de Vida.

ABSTRACT

CASTRO, Jackeline de Siqueira, D.Sc, Universidade Federal de Viçosa, March, 2020. **Agricultural and energetic valorization of algal biomass cultivated in wastewater.** Adviser: Maria Lúcia Calijuri. Co-adviser: Paula Peixoto Assemany.

This research addressed the agricultural and energetic valorization of algal biomass grown in agroindustrial wastewater. The valorization routes studied were defined based on a systematic literature review carried out between 1999 and 2017 in journals classified in quartile 1 by the Scimago Journal & Country Rank. The selected journals belong to the environmental sciences area and the sub-area of waste management and disposal. The word "microalgae" was used as a search term on each journal's web page. Thus, the main themes researched and the gaps within each one were listed. One theme in the scope of agricultural valorization and one in the scope of energetic valorization were selected to be the target of research. Within the scope of agricultural valorization, different proportions of microalgae biomass added to the triple superphosphate fertilizer were tested. After defining an ideal proportion (12% w / w) of microalgae biomass in the biofertilizer mass, several tests were carried out regarding the dynamics of the fertilizer after application to the soil and its absorption by plants. From a technical point of view it is a potential source of nutrients since it contributed to the increase in the absorption of P by plants approximately 2% more than conventional fertilizer. However, when the route was analyzed from the perspective of the life cycle, chemical fertilizer was more viable than biofertilizer. It is believed that in a long-term analysis and taking into account the benefits of the biofertilizer, both for the plant and for the soil, it could become competitive. As for the energetic recovery route, the hydrothermal carbonization, which acts in the presence of water, proved to be interesting from a technical point of view, since it was possible to obtain a material more concentrated in carbon and other nutrients, when compared to the raw material source, under relatively low temperatures and reaction times. The highest yield of solids (77.72%) and energy (78.21%) was obtained at a temperature of 170 ° C and a reaction time of 10 minutes. From the perspective of the life cycle, hydrothermal carbonization becomes interesting, especially if the aqueous phase is reused in the thermochemical process. Among the possibilities of using the aqueous phase are the cultivation of microalgae and recirculation in the carbonization reactor. It is concluded that from a technical point of view both routes are viable. On the other hand, improvements in the cultivation and concentration processes of microalgae biomass must be made to make the routes environmentally desirable.

Keywords: Biofertilizers. Hydrothermal carbonization. Plants – Life Cycle.

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ABREVIATURAS, SIGLAS E SÍMBOLOS

ACV	Avaliação do ciclo de vida
AQI	Análise química imediata
BB	Biomassa bruta
CHT	Carbonização hidrotérmica
CF	Carbono fixo
COT	Carbono orgânico total
DBC	Randomised blocks
DTG	Termogravimetria derivada
EEFs	Enhanced efficiency fertilizers
Effective CEC	Effective cation exchange capacity
EFFs	Environmentally friendly fertilizers
EOPs	Emerging organic pollutants
ETAR	Estação de tratamento de águas residuárias
FA	Fase aquosa
FT-IR	Espectroscopia de infravermelho por Transformada de Fourier
GHT	Gaseificação hidrtotérmica
H + Al	Hydrogen plus aluminum
HRAPs	High-rate algal ponds
HRT	Hydraulic retention time
HTC	Hydrothermal carbonization
HTG	Hydrothermal gaseification
HTL	Hydrothermal liquefaction
kg CO_2 eq	Quilograma de CO_2 equivalente
kg SO_2 eq	Quilograma de SO_2 equivalente
kg P eq	Quilograma de P equivalente
kg PM10 eq	Quilograma de material particulado inferior a 10 micrômetros equivalente
kg 1,4-DB eq	Quilograma de 1,4 diclorobenzeno equivalente
kg óleo eq	Quilograma de óleo equivalente
LAT	Lagoa de alta taxa
LCA	Life cycle analysis
LHT	Liquefação hidrotérmica
MB	Microalgae biomass
Mbiomassa	Massa de biomassa
mchar	Massa de biochar
m/m	Massa/massa
mMS	Dry matter mass
Mn	Manganese ou Manganês
MV	Material volátil
m/v	Massa/volume

NC	Nutrient content
NPK	Nitrogênio-fósforo-potássio
NT	Nutrient concentration
NTK	Nitrogênio total Kjehdal
OM	Organic Matter
PCS	Poder calorífico superior
Potential CEC	Potential cation exchange capacity
P_{rem}	Remaining phosphorus
Re	Rendimento energético
Rs	Rendimento de sólidos
SDMM	Shoot dry mass matter
SEM	Scanning electron microscope
SFT	Superfosfato triplo
SJR	Scimago Journal & Country Rank
Sr	Estrôncio
SSV	Sólidos suspensos voláteis
TG	Termogravimetria
TSP	Triple superphosphate
v/v	Volume/volume
WT	Wet torrefaction

SUMÁRIO

1. APRESENTAÇÃO

Esse documento foi estruturado em 5 capítulos. O primeiro refere-se a uma revisão sistemática de literatura, dos principais temas pesquisados dentro do universo das microalgas. A partir desse estudo foi possível observar quais são as principais pesquisas já realizadas com relação à biomassa de microalgas produzida em efluentes, bem como as lacunas e desafios, desde a produção até a utilização em diversas rotas de aproveitamento. Dentro das rotas que ainda apresentavam lacunas foram selecionadas duas rotas de aproveitamento para serem alvo dos estudos dessa tese. Tratar-se-ão de uma rota de valorização agrícola e uma de valorização energética da biomassa de microalgas. Além dos experimentos desenvolvidos dentro de cada uma dessas rotas, buscou-se realizar uma avaliação dos impactos ambientais das mesmas, com o objetivo de identificar os processos e insumos mais impactantes e realizar proposições de melhorias.

Destaca-se a aderência dos temas selecionados aos projetos em desenvolvimento no grupo de pesquisa do qual a estudante de doutorado fez parte desde o ingresso na pós-graduação.

Na abordagem da valorização energética, destaca-se o projeto “From wastewater to bioenergy: a microalgae biorefinery approach in Indian and Brazilian scenarios” MCTI/CNPq/DBT Nº 17/2015 aprovado em 2016 e finalizado em 2020. Esse projeto teve como objetivo geral, propor um sistema de biorrefinaria otimizado que integra o tratamento de águas residuárias com a produção de biomassa e a produção de bioenergia. Mais especificamente, um dos objetivos deste trabalho foi otimizar um tratamento termoquímico da biomassa visando a obtenção de um combustível. Em decorrência da inovação do assunto e dos poucos estudos envolvendo a carbonização hidrotérmica da biomassa algal, decidiu-se propor nesta tese um estudo específico sobre esse assunto como rota alternativa de aproveitamento de biomassa.

No que se refere a valorização agrícola, destaca-se o projeto “Biofilme de microalgas: tecnologia inovadora para mitigação das mudanças climáticas e desenvolvimento sustentável no setor agropecuário”, Processo nº 37/2013 – Mudanças Climáticas, aprovado em 2013 e encerrado em 2018. O projeto foi financiado pelo CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico e teve como objetivo, desenvolver e avaliar tecnologia inovadora para a redução de emissões e aumento da produtividade agrícola a partir da ação de microalgas. Na abrangência desse projeto, alguns trabalhos foram realizados, dentre eles uma dissertação defendida em fevereiro de 2016, que investigou os efeitos da aplicação de biomassa de microalgas no solo, com o objetivo de formar um biofilme na superfície do solo e o efeito dessa prática na emissão de gases de efeito estufa (GEE), perdas de nitrogênio por volatilização e crescimento de plantas (Castro, 2016).

Nessa tese, as duas rotas de valorização foram estudadas, experimentadas e os resultados foram dispostos nos capítulos 2 e 4. Além disso, foi realizada avaliação do ciclo de vida de ambas as rotas de valorização, com o auxílio do software SimaPro e as mesmas constam dos capítulos 3 e 5.

2. INTRODUÇÃO GERAL

O uso das algas como matéria prima para a produção de alimento, ração e combustível remonta da década de 1930 (Wang et al., 2015). Porém, foi a partir dos anos 1970s, diante da forte dependência por combustíveis fósseis e da crise energética, que as pesquisas em geral tomaram um novo rumo. A produção de energia a partir de gases como o hidrogênio e o metano (Algae Biomass Organization, 2015) e mais propriamente dito, a utilização de matérias primas alternativas para a obtenção dessa energia receberam mais enfoque. Entre as matérias primas alternativas destaca-se a biomassa de microalgas.

A principal característica das microalgas é a versatilidade para a produção de diversos tipos de biocombustíveis, como o biodiesel, bioetanol, biogás e biohidrogênio (Zhu, 2015). As vantagens de se produzir biocombustíveis a partir de microalgas são a alta eficiência fotossintética, o alto teor de lipídios (que pode ser acumulado na biomassa), a não-competição com terras agricultáveis e a tolerância a águas residuárias como meio de cultivo (Zhu e Ketola, 2012).

Em relação ao meio de cultivo utilizado para a produção da biomassa de microalgas, pode-se citar, basicamente, dois tipos. Um deles é o meio de cultivo sintético, em que fertilizantes comerciais são adicionados à água, e nesse meio são inoculadas as espécies de microalgas desejadas na biomassa final. O outro meio de cultivo é constituído pelas águas residuárias que contém além de outros elementos, água, nutrientes e microrganismos como algas, bactérias, fungos e protozoários. Em condições ambientais propícias, o crescimento dos microganismos ali presentes se dará, levando à obtenção de uma biomassa mista, contendo microalgas e outros organismos. Uma vez que o meio e as condições de cultivo e as espécies de organismos no meio são diversos e variáveis, a composição da biomassa final pode ser alterada.

Devido a essa composição variável, estudos realizados com biomassa algal produzidas em efluentes mostraram, por exemplo, que nem toda rota de aproveitamento da biomassa produzida nesse meio de cultivo é viável do ponto de vista econômico e ambiental. Como exemplo, cita-se as rotas de produção de biogás e extração lipídica. Jankowska et al. (2017) mencionam o biogás produzido a partir de efluente tratado com microalgas como uma rota potencial, mas somente quando analisada sob o contexto da biorrefinaria.

Assemany et al. (2016) realizaram estudo cujo objetivo foi avaliar o potencial energético, em termos de lipídios e biogás da biomassa cultivada em um fotobiorretor de coluna de bolhas utilizando efluentes da indústria de processamento de carne como meio de cultivo. Para isso, consideraram as rotas de produção de energia: lipídios, biogás antes da extração de lipídios, biogás após a extração de lipídio e biogás + lipídios. Os autores concluíram que nenhuma rota apresentou taxa de energia líquida maior que 1, sendo que o pouco acúmulo de energia causado pela extração de lipídio não tornou o processo viável energeticamente. No caso da extração lipídica, Couto et al. (2018) citam que a biomassa produzida em lagoas de alta taxa (LATs) utilizando esgoto como meio de cultivo possui baixo teor lipídico. O cultivo em efluentes sob condições pouco apropriadas e com competição com outros microrganismos podem ser apontados como causa do reduzido acumulo lipídico. De acordo com Mehrabadi et al. (2015), a principal razão para o baixo conteúdo de lipídios da biomassa cultivada em LAT com águas residuárias é a mistura de algas e bactérias. Como o conteúdo lipídico das bactérias é tipicamente inferior a 10% (Brown et al., 1996), o conteúdo global lipídico é reduzido e consequentemente o conteúdo energético da biomassa.

Os processos de conversão termoquímica da biomassa algal em biocombustíveis, têm ganhado destaque nos últimos anos. Esses processos de conversão incluem a carbonização hidrotérmica (CHT), liquefação hidrotérmica (LHT) e gaseificação hidrotérmica (GHT) (ocorrem na presença de água); torrefação, pirólise e gaseificação (ocorrem na ausência de água) (Barreiro et al., 2013).

O principal gargalo das rotas que atuam na ausência de água é a necessidade da secagem da biomassa, que aumenta consideravelmente o consumo de energia necessária para a retirada da umidade. Por outro lado, as rotas que atuam na presença de água, tem se mostrado mais favoráveis, como o estudo realizado por Couto et al. (2018), que obteve elevados rendimentos de bio-óleo, utilizando a biomassa produzida a partir do tratamento de efluentes domésticos em LATs. Os autores mencionam que a utilização da biomassa diretamente após a produção nas LATs pode conduzir a um consumo excessivo de energia devido ao elevado conteúdo de água.

No entanto, a necessidade de secagem é reduzida quando comparada a outros processos de obtenção de energia, o que garante balanço energético positivo na LHT.

Nesse contexto, um assunto em crescente na literatura é a CHT da biomassa de microalgas. Esse novo conceito é promissor, visto que não há necessidade de secagem da biomassa, o que, por conseguinte reduz a demanda por energia, além de necessitar de condições operacionais mais brandas (temperatura e pressão) do que comparativamente à LHT, sendo mais vantajoso economicamente e operacionalmente. A CHT refere-se a um tratamento termoquímico que ocorre em meio aquoso, cujo objetivo é converter componentes carboidratados de determinada biomassa em material rico em carbono. O tratamento ocorre em meio hidrotérmico ou água quente comprimida a temperaturas entre 180-270ºC (Chen et al., 2012; Bach et al., 2013; Bach et al., 2016; Lee et al., 2018; Zhao et al., 2019). Porém, no que se refere à CHT da biomassa de microalgas, estudos têm sido realizados com diferentes faixas de temperatura, tempos de espera e diferentes proporções de biomassa/água inicial no reator (Bach e Skreiberg, 2016; Bach et al., 2017; Wilk e Magdziarz, 2017), não havendo um consenso sobre os parâmetros operacionais a serem adotados, denotando ainda mais a necessidade de aprimoramento da rota. Adicionalmente, são poucos os trabalhos que reportam a CHT de biomassa de microalgas cultivada em efluentes (Lee et al., 2019; Liu et al., 2019; Marin-Batista et al., 2019).

No conceito da biorrefinaria de microalgas, uma outra possibilidade de aproveitamento da biomassa algal é a sua aplicação no solo como biofertilizante. Existem poucas publicações fazendo referência a esse tema. Castro et al. (2017) obtiveram resultados favoráveis quanto ao crescimento de plantas e melhoria da qualidade do solo, para a aplicação de biomassa algal no solo quando comparada com um fertilizante químico comercial. Porém, com a elevada exigência da indústria com relação ao padrão dos grânulos de fertilizantes, a aplicação da biomassa tal qual é retirada do reator de cultivo e tratamento de efluentes não é um processo viável, tanto no que diz respeito ao padrão da aplicação, quanto à logística dessa operação. Por isso, faz-se necessário que ainda sejam pesquisadas formas de obtenção de um fertilizante comercial a partir da biomassa. Além disso, mais estudos devem ser realizados, para averiguar a influência desse biofertilizante no sistema solo-planta e principalmente sua atuação no sistema fisiológico das plantas. Marks et al. (2017) também obtiveram resultados favoráveis quando da aplicação da biomassa de microalgas no solo, mostrando que a aplicação da biomassa teve efeito direto na disponibilidade de carbono, nitrogênio e fósforo no solo.

Diante do exposto, propõe-se nesse trabalho a avaliação de duas rotas de aproveitamento potenciais da biomassa mista, sendo elas CHT e biofertilizante, escolhidas com base em estudo prévio e a avaliação do ciclo de vida (ACV) dessas rotas com relação aos potenciais impactos ambientais das mesmas. A ACV é um importante aliada, uma vez que a metodologia avalia aspectos ambientais e potenciais impactos negativos de um bem, com base na coleta e quantificação de materiais e energia requeridos em seus processos produtivos, assim como os resíduos e emissões liberados ao meio ambiente (ISO 14040, 2001; Souza et al., 2019).

3. HIPÓTESES DE PESQUISA

- O biofertilizante, produzido a partir da biomassa de microalgas produzida durante o tratamento de efluentes com fertilizante químico comercial, promove maior absorção de P pelas plantas;
- O biofertilizante é mais viável ambientalmente quando comparado com o fertilizante químico convencional;
- A CHT representa rota viável, técnica e ambientalmente, de obtenção de energia a partir da biomassa de microalgas produzida em lagoas de alta taxa durante o tratamento de efluentes;
- A CHT de biomassa de microalgas pode ser realizada em condições mais amenas de temperatura e tempo de reação do que aquelas reportadas na literatura para biomassa lignocelulósica.

4. OBJETIVO GERAL

Avaliar e comparar técnica e ambientalmente, a recuperação de nutrientes de efluente agroindustrial via valorização agrícola e energética de biomassa produzida em lagoas de alta taxa.

Objetivos Específicos

- Realizar uma revisão sistemática de literatura visando a identificação de rotas potenciais de aproveitamento da biomassa de microalgas produzida em efluentes;

- Utilizar os nutrientes recuperados nas LATs por meio do cultivo de biomassa durante o tratamento de efluentes na produção de fertilizantes organominerais e avaliar sua eficiência em plantas;
- Comparar os fertilizantes organominerais produzidos a partir da biomassa com um fertilizante químico comercial na ACV;
- Utilizar os nutrientes recuperados nas LATs, por meio do cultivo de biomassa durante o tratamento de efluentes, na produção de biocombustível via CHT, sob diferentes condições de temperatura e tempo de reação;
- Realizar análise de ciclo de vida da CHT de biomassa cultivada em efluentes.

5. ARTIGO I. Valorization of wastewater grown microalgal biomass: gaps and challenges

Abstract: Microalgae are referenced to as of the most viable alternatives to solve future energy and food supply issues of the world population. This is due to the very superior productivity of terrestrial plants and the viability of their cultivation independently of land and freshwater availability. The cultivation of microalgae in wastewater has been known for about half a century and has therefore been investigated. However, there are still many questions to be answered about this technology before the implementation of large-scale projects as not every route of cultivation, harvesting and utilization of the biomass produced in wastewater is feasible from the economic and environmental points of view. The objective of this study was to carry out a systematic literature review on the biotechnology of microalgae cultivated in wastewater and to determine the main gaps and future challenges in this area. Therefore, the database provided by SCImago was used to select only journals from the area of Environmental Science with a sub-category Management and Waste Disposal that were available between 1999 and 2017. Journals classified in quartile 1 which consequently had the highest SJR were selected. The term "microalgae" was used as the search term on each journal's *web* page. In algae cultivation, the main gaps are the impacts of the pollutants on the growth reactors affecting the final biomass characteristics. The study of the wastewater beforehand is highly recommended in order to get an algae biomass with the desired characteristics at the end of the production stage. Biomass harvesting needs improvement, and drying must be avoided, leading to a choice of wet biomass valorization routes. Biofuel and biofertilizer are products with the potential to exploit the biomass of algae grown in wastewater. LCA is an important tool to support decision-making and needs to be further explored in the microalgae universe.

Keywords: systematic literature review; algae cultivation; wastewater treatment; nutrient recovery; valorization of algal biomass.

5.1. Introduction

Microalgae are opportunistic organisms found in diverse environments and are important primary producers in several communities (Qiao et al., 2015). They are useful in the production of various types of biofuels, such as biodiesel, bioethanol, biogas and biohydrogen (Zhu, 2015). In addition, the advantages of producing biofuels from microalgae biomass include the high photosynthetic efficiency of microalgae, its high lipid content that can, depending on the cultivation conditions, be accumulated in the biomass, the lack of competition for arable land for production and its tolerance to wastewater during cultivation (Zhu and Ketola, 2012).

There are many research topics related to microalgae including the following: wastewater treatment such as polishing secondary effluent (Cheng et al., 2017) in concomitance with microalgae biomass production; different growth reactors, such as high rate algal ponds (HRAPs) (Drira et al., 2016; Gutiérrez et al., 2016); photobioreactors (Assemany et al., 2016; Michels et al., 2014) and attached growth reactors (Assis et al., 2017; Christenson and Sims, 2012); as well as studies that investigate biomass harvesting techniques (Coppens et al., 2016;

Sutherland et al.,2014).

In the context of biomass valorization, a relevant research focus is energy use, with the generation of different types of biofuels, such as biogas (Bohutskui et al., 2016; Wieczorek et al., 2015), biodiesel (Komolafe et al., 2014; Tran et al., 2013) and biohydrogen (Batista et al., 2015; Nagarajan et al., 2017). Another research area is related to biomass pretreatment in order to optimize the energy conversion process, such as hydrothermal pretreatment (Passos and Ferrer, 2015; Wang et al., 2015). In addition to energy use, another valorization route that has been highlighted is the application of wastewater-grown biomass on soil as a biofertilizer (Castro et al., 2017) or to stimulate heterotrophic activity and promote bacterial growth in soil (Marks et al., 2017).

Despite being a recent issue, numerous studies have been conducted on microalgae biomass and include topics from biomass production to its application in various valorization routes. However, few studies use wastewater-grown biomass. There are several advantages of using effluent for microalgae cultivation, for example (i) microalgae removes nutrients that are present in effluents, so treating effluent with microalgae prevents the eutrophication of water bodies; (ii) microalgae capture CO_2 and thus use atmospheric or greenhouse gas emissions; and (iii) it saves financial resources by maintaining the cultivation medium at zero cost. Despite these benefits, some studies have shown that not every route of biomass valorization using wastewater-grown biomass is economic and environmentally feasible (Assemany et al., 2016; Jankowska et al., 2017). Therefore, a systematic literature reviews to identify the trends related to this subject and the current gaps in the literature are necessary. This will provide guidance for future researchers, mainly regarding the cultivation and use of wastewater-grown biomass.

The objective of this study was to carry out a survey of publication sources related to the area of “waste management and disposal”, more specifically in wastewater-grown microalgae biomass, from cultivation to valorization. Moreover, journals were selected based on quality indicators. Finally, the geographical distribution of publications and the main research topics regarding wastewater-grown biomass, indicating subjects that require more attention, new trends and future challenges, were identified.

5.2. Materials and Methods

5.2.1. Selection of journals

In the database provided by Scimago Journal & Country Rank (http://www.scimagojr.com) journals from the Environmental Science area, the category "waste management and disposal" and that were available between 1999 and 2017 were selected. In addition to the title of each journal were obtained SJR and Quartile indicators. The definition of each of these indicators is described below (SJR, 2017):

- SJR: The average number of citations of articles published three years prior to the analysis;
- Quartile: The Scimago Journal & Country Rank database has its own methodology for classifying the database into quartiles, which is divided into four groups taking into account the SJR index. The journals with the highest SJRs are classified as quartile 1 and as the SJR decreases in value, the quartile increases until reaching quartile 4.

Journals classified in quartile 1 were selected, which consequently had the highest SJR. The criteria used to select the analyzed articles are summarized in Figure 5.1.

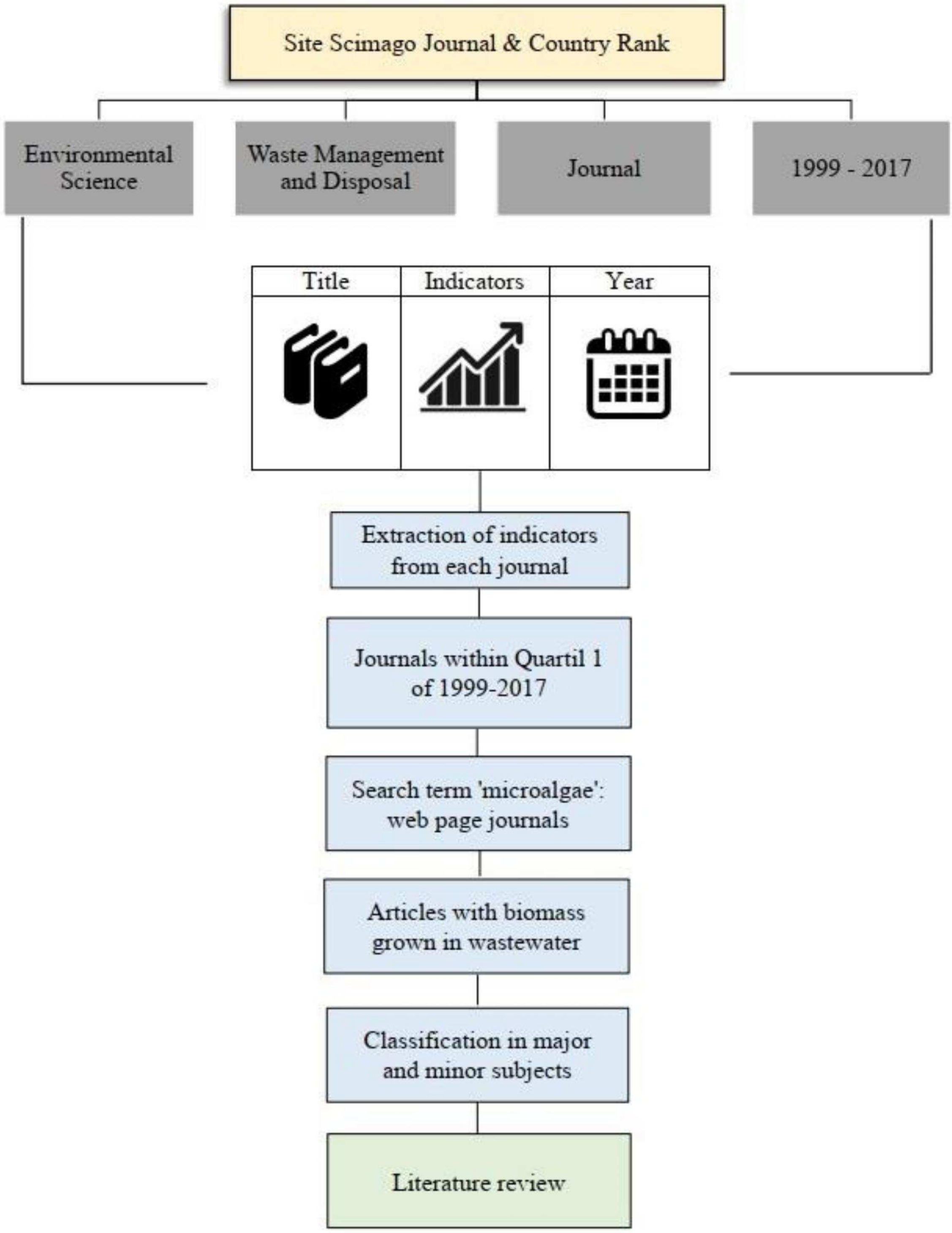

Figure 5.1. Fluxogram with the criteria used to select articles for literature review.

5.2.2. Researches related to microalgae biomass

The journals that were selected according to the criteria described in section 2.1 were analyzed between the years 1999 and 2017 for publications regarding microalgae. The term "microalgae" was used as the search term on each journal's *web* page. This search term was chosen because it allowed to find articles that have the words microalgae, micro-algae or algae if any of them appeared at least once in the article.

Subsequently, the selected articles were analyzed one by one with the objective of identifying the publications related to the cultivation of microalgae in wastewater. The classification scheme of articles according to the subject was adapted from Patel et al. (2016), as shown in Figure 5.2.

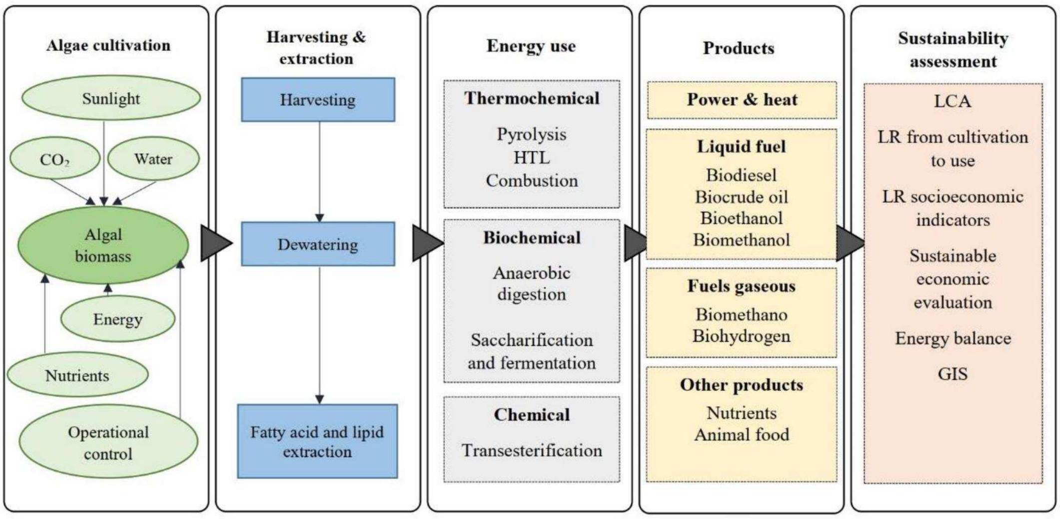

Figure 5.2. Themes for article classification**.**

The five categories illustrated themes related to the subjects described below:

Algae cultivation: Gas dynamics in growth reactors; mixotrophic, heterotrophic or autotrophic culture conditions; operational control of reactors; interaction between algae and bacteria in the wastewater treatment; microalgae biomass production; wastewater treatment; comparison of algal species; impacts of environmental pollutants on microalgae growth;

Harvesting and extraction: Harvesting/concentration of biomass, drying and extraction of fatty acids and lipids;

Energetic use: Use through thermochemical processes, biochemical and chemical process of algal biomass;

Final products: Solid biofuels; liquid biofuels; biofuels; value-added bioproducts; electricity; source of plant nutrients; food and animal feed;

Sustainability assessment: Life cycle analysis; economic and sustainable assessment; energetic balance; geographical information system used to define the best sites to install growth reactors; literature review from the cultivation to the use of biomass; literature review on socioeconomic indicators.

It is important to highlight that an article may have been classified in one or more categorie, since it may cover more than one subject.

With this data, it was possible to determine the main topic explored regarding the production/optimization of microalgae produced in effluents and biomass valorization, as well as guiding new research to advance further studies on the theme.

5.3. Results and Discussion

Studies related to microalgae that were published between 1999 and 2017 in the journals classified by SJR Rank as being in quartile 1 (i.e., those with higher SJR and H-Indexes) were also investigated. A total of 2283 articles related to microalgae were published, 19.23% (439 articles) of them were related to microalgae cultivation in effluents (Figure 5.3). The great majority of them used synthetic culture medium for their tests.

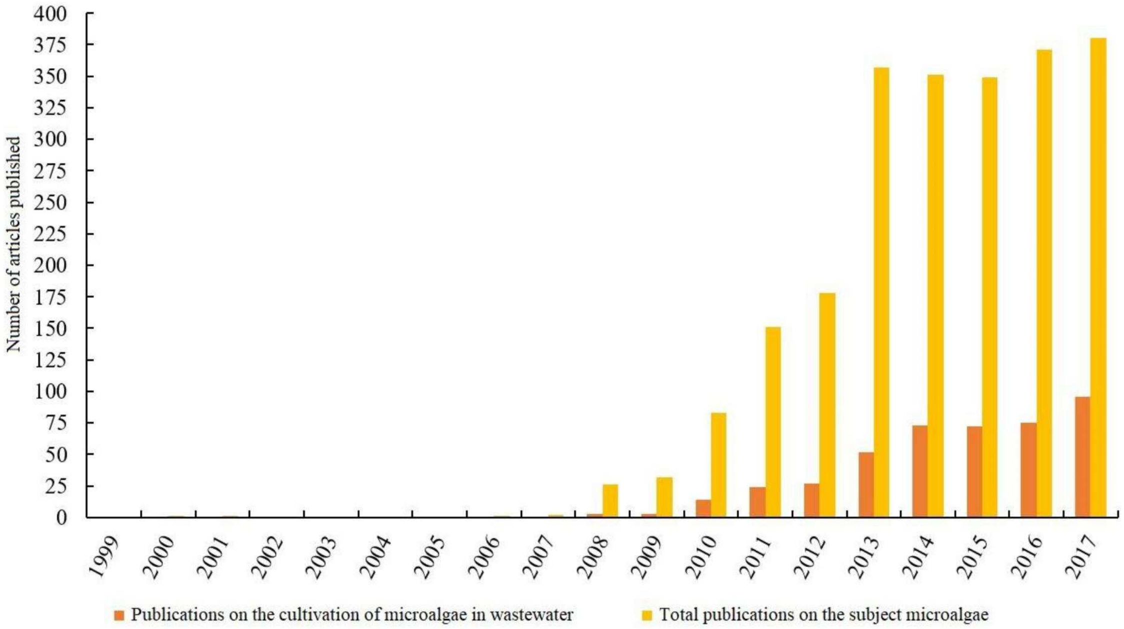

Figure 5.3. Number of publications on the theme "Microalgae" over time.

It is noteworthy that the theme 'microalgae' is a recent approach. Most of the studies appeared in the year 2008 and from the year 2013 onwards there was a significant increase in the number of publications in this theme, but most of them are related with biomass grown in synthetic medium.

The theme "cultivation of microalgae" was the one that presented the largest number of publications (60.63%), followed by "harvest and extraction of lipids and fatty acids", "final products", "energy utilization" and finally "sustainability assessment" (Figure 5.4).

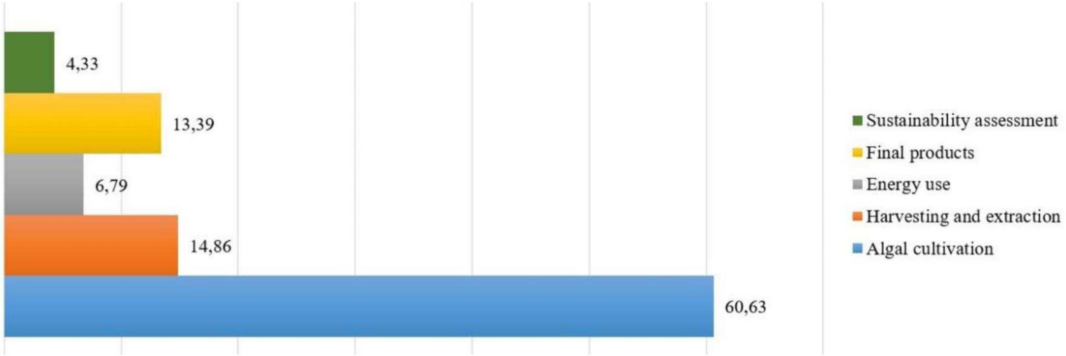

Figure 5.4. Percentage of articles published by subject classification.

5.3.1. Cultivation of microalgae

“Wastewater treatment" and “biomass production" were the most researched subjects within this theme (69.64%). The biomass production associated with effluent treatment may make algal biomass more cost-competitive compared to other biomasses, since the effluent contains the necessary nutrients to promote algal growth, avoiding the addition of fertilizers and potable water. On the other hand, regarding the quality of the biomass, microalgae produced in effluent has worse quality than microalgae cultivated in pure culture media, and is therefore used for less noble industries.

Also, if a lot of input or energy is used to produce a raw material whose final characteristic does not reach the desired quality, for example in terms of lipids, proteins, carbohydrates, etc., the chosen valorization route can become unfeasible. Therefore, the effluent should be studied, before biomass treatment and production, to know if its characteristics will provide an appropriate material for the desired use.

Other subjects in this theme corresponded to 29.06% of the total and explore topics related to operational control of growth reactors (Arbib et al., 2017; Arcila and Buitrón, 2016;

Cheng et al., 2017; Cho et al., 2015; Hom-Diaz et al., 2017; Maza-Márquez et al., 2017; Sutherland et al., 2014; Úbeda et al., 2017), emission, capture and gas addition in high rate ponds (Fang et al., 2017; Mehrabadi et al., 2016; Yadav and Sen, 2017), the study of mixotrophic, heterotrophic and autotrophic culture conditions (Chiranjeevi and Mohan, 2017; Cho et al., 2017; Santos et al., 2016; Sarkar et al., 2015; Wang et al., 2015), algae in wastewater treatment (Anbalagan et al., 2017; Solimeno et al., 2017; Toledo-Cervantes et al., 2017) and comparisons of algal species during wastewater treatment and the use of algal biomass (Arbib et al., 2014; Calixto et al., 2016).

One subject that has received less research under the theme "algal culture" was the "impact of environmental pollutants on microalgae cultivation" (1.30%). One of the main problems associated with the use of effluents in microalgae cultivation is the production of a mixed biomass, which consists of viruses, bacteria and other organisms in addition to algae, which can affect biomass productivity (Rawat et al., 2013) and its quality.

Farooq et al. (2013) in an evaluation of different types of microalgae culture in brewery effluent aiming to increase lipid productivity observed that two types of algae metabolism (photoautotrophic metabolism followed by photoheterotrophic metabolism) proved to be an attractive and economically viable option. This system promoted an increase of lipid productivity in microalgae biomass obtained after wastewater treatment and contributed to a reduction in the incidence of bacteria.

Zhang et al. (2012) examined the impacts of bacteria on algae biomass production, lipid content and treatment efficiency of soybean processing effluent during the heterotrophic cultivation of *Chlorella pyrenoidosa*. The authors observed that the bacteria that coexisted with the algae improved the degradation of total nitrogen, total phosphorus, glucose and chemical oxygen demand. Although algal biomass productivity was not significantly affected, total lipid content and the lipid production rate were slightly reduced when bacteria coexisted with the algae. Therefore, it should be emphasized that the formation of mixed biomass can be beneficial from the point of view of effluent treatment, but from the energy use purpuse, medicine production and animal feed, among other uses, mixed biomass still needs to be resolved. The lipid productivity of wastewater-grown biomass deserves to be highlighted in this discussion and will be separately treated in the topic "Harvest and extraction of fatty acids and lipids".

In addition to microorganisms, emerging organic pollutants (EOPs) have caught the attention of several researchers (Deng and Tam, 2015; Matamoros et al., 2015; Ramakrishnan et al., 2013). They include a wide range of compounds belonging to different chemical categories such as pharmaceuticals, personal care products, plasticizers, flame retardants, surfactants and some pesticides, among others, whose ecotoxicological effects are relatively unknown (Murray et al., 2010). These products are worrisome due to their presence in natural water bodies (Ternes et al., 2004) where they may exert ecotoxicological effects in relatively low concentrations (Henry and Black, 2008; Muñoz et al., 2009), and typical wastewater treatment plants are not designed to remove them.

Matamoros et al. (2015) evaluated the effect of hydraulic retention time (HRT) and seasonality on the removal efficiency of 26 micro-organic contaminants from domestic sewage in two pilot scale HRAPs. This study showed that microalgae-based wastewater treatment systems (such as HRAPs) allow for the removal of a wide range of EOPs from domestic sewage. The most frequently occurring compounds (caffeine, acetaminophen and ibuprofen) had removal efficiencies of up to 90% and they were minimally affected by seasonality and HRT.

Emerging pollutants are mostly present in domestic sewage. Although most of the studies published in the theme "algae culture" are on the topic of effluent treatment (234 articles), mostly with domestic sewage (125), only nine of these publications cited EOPs in the studies.

In addition to EOPs, heavy metals are noteworthy pollutants, causing serious toxicity and long-term problems to living beings due to their cumulative and non-degradable properties. Subramaniyam et al. (2016) highlighted the great potential of microalgae biomass for remediation of contaminants, including heavy metals. Out of 234 articles listed in the theme "algae cultivation", 22 performed metal analysis and described the influence of these elements in biomass production.

Heavy metals can severely inhibit photosynthesis by blocking or replacing prosthetic metal atoms in enzyme active sites (Kumar et al. 2010). On the other hand, microalgae can act in heavy metal removal from the wastewater. Palma et al. (2017) carried out a study that has the objective to investigate the growth and the metal removal capacity of fungal/microalgae consortium treating the wastewater of tailings dams, polluted with metal and ammonia. The dominant genera in the consortium was Chlorella. The consortium showed significant potential for the removal of heavy metals (Ni, Co, Mn, Sr) from the wastewater with 24.8%, 10.5%, 24.8% and 26.4% reduction of Ni, Co, Mn and Sr, respectively.

The main obstacles within the theme "microalgae cultivation" are i) mixed biomass when the objective is to obtain raw material for lipid production and ii) a focus on better understanding and removal of EOPs in domestic sewage treatment. Knowledge of the effluent to be used as a culture medium is also extremely important, as its characteristics will directly influence the composition of the biomass, making it more or less suitable for the desired valorization route.

5.3.2. Harvest and fatty acid and lipid extraction

Within the topic "harvest and fatty acid and lipid extraction" the most majority of the articles was on the subject of lipid and fatty acid extraction (72.85 %), in contrast 21.85 % wew on harvest/dewatering and 5.30 % on drying.

Based on the temporal evaluation of the articles published on the subject “lipid and fatty acids extraction” it could be observed from Figure 5.5 that researches began in 2010 reaching a higher number of published articles (26) in the year 2014. Since then, there has been a decrease of approximately 10 articles in the years 2016 and 2017. This reduction may be related to the low lipid productivity experienced when lipid extraction was done from a mixed biomass as mentioned in item 5.3.1 or the exhaustion of the theme.

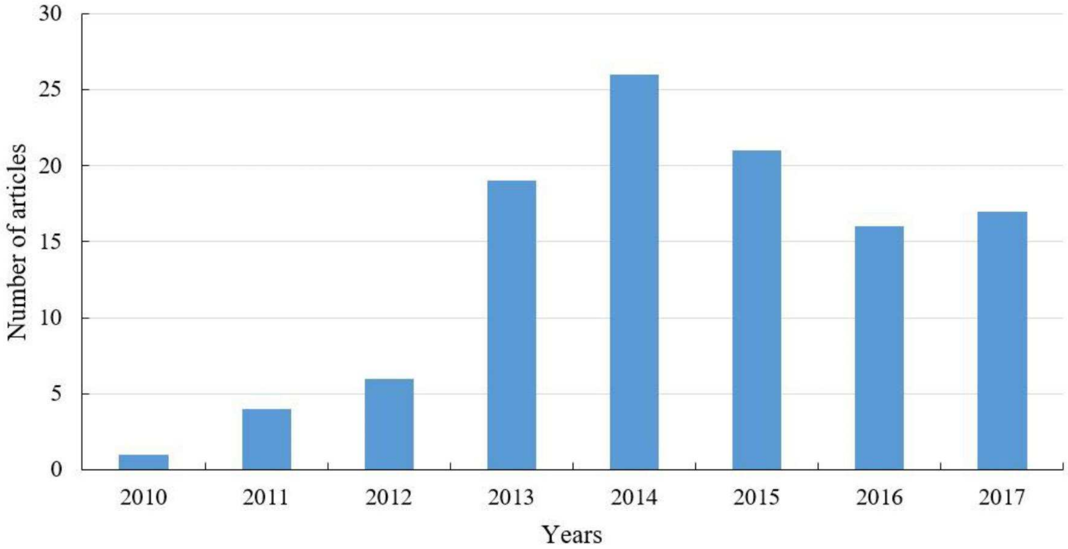

Figure 5.5. Temporal evolution of articles on the subject “lipid and fatty acid extraction”.

Table 5.1 presents different studies that aimed to obtain lipids from algal biomass grown in wastewater.

Table 5.1. Lipid content of algal biomass grown in different effluents.

Culture medium	Reactor type	Lipid content (%)	Reference
Filtered municipal secondary effluent -5% CO_2	Bottles (0.5 L)	19.70	Ji et al., 2015
Municipal secondary effluent / Food industry effluent (99.5:0.5) filtered - 5% CO_2		18.40	
Municipal secondary effluent / Food industry effluent (99:1) filtered - 5% CO_2		18.70	
Municipal secondary effluent / Food industry effluent (98:2) filtered - 5% CO_2		22.90	
Effluent from the alcohol / wastewater industry of anaerobically digested starch (0.176:1, v/v) filtered	Conical flasks (2 L)	23.35	Yang et al., 2015
Effluent from the alcohol / wastewater industry of anaerobically digested starch (0.053:1, v/v) filtered		25.44	
Effluent from the alcohol / wastewater industry of anaerobically digested starch (0.26:1, v/v) filtered		24.27	
Anaerobically filtered residual starch		18.68	
Municipal effluent after preliminary screening, primary sedimentation, activated sludge and secondary sedimentation - continuous treatment in HRAP with 0.3 m depth - CO_2 addition	HRAP (533 L)	23.00	Arbib et al., 2017
Municipal effluent after preliminary screening, primary sedimentation, activated sludge and secondary sedimentation - continuous treatment in HRAP with 0.15 m depth - CO_2 addition		19.00	
Dairy effluent (without any pre-treatment)	Erlenmeyer flasks (0.5 L)	25.00	Chokshi et al., 2016

Mixed biomass is often expected to have a lower lipid content compared to pure algal biomass. This can be explained by the presence of other organisms, such as bacteria, which have a lower lipid content than algae, helping to decrease the total lipid content of the biomass. Moreover, it is questionable whether all of the small operations that are used to obtain high productivities will be economically and environmentally costly when scaled up.

Assemany et al. (2016) evaluated the energy potential of the biomass cultivated in a photobioreactor using effluents from the meat processing industry. Three different energy production routes were considered: lipids, biogas before lipid extraction, biogas after lipid extraction and biogas + lipids. The authors concluded that none of the tested routes had a net energy ratio greater than 1, and that the low lipid accumulation did not make the process energetically viable. In the case of lipid extraction, Mehrabadi et al. (2015) mentioned that the biomass produced in HRAPs using domestic sewage as a culture medium might have a low lipid content.

Maranduba et al. (2015) conducted a biodiesel life cycle analysis (LCA) on the microalgae *Chlorella vulgaris* that was cultivated in HRAP using greenhouse gas emissions. One of the evaluated scenarios was the cultivation of microalgae for biodiesel production only (Cultivation Area: 100 ha y^{-1}. Biodiesel production: 2081 t y^{-1}, without considering the integration with other production routes. The results showed that even considering 46% final oil production, this route alone was not feasible.

Again, it is important to evaluate the effluent prior to cultivation, in addition to the need for feasibility studies of lipid extraction in the biomass grown in effluent. Another important issue is the drying of the biomass for lipid extraction, which may contribute to the unfeasibility of the production chain. The biorefinery approach can be a way to increase the viability of lipid extraction in wastewater-grown biomass. Biorefinery refers to the integration of processes, where by-products are used as inputs to subsequent processes and the flow of energy, heat and materials is directed towards the cost reduction of isolated processes and the minimization or even elimination of waste and related negative environmental impacts.

The biomass produced in HRAPs has a high water content, which requires a high-energy consumption for the harvest/dewatering and drying processes. The use of chemicals and energy increases the negative impacts on the environment and the economy of these steps. Despite their importance, these themes are still poorly researched today when compared to lipid production, which is a more consolidated subject.

Several methods are mentioned in the literature regarding biomass harvesting/dewatering. Among them, centrifugation (Mennaa et al., 2015), filtration (Milledge and Heaven, 2013), ozoflotation (Velasquez-Orta et al., 2014), coagulation (Udom et al., 2013) and coagulation/flocculation (Udom et al., 2013) are quite usual. Such methods include the addition of chemicals or the use of mechanical equipment that could increase costs and impacts related to the process. Udom et al. (2013) evaluated the costs and benefits and the environmental impact of some harvesting techniques, such as centrifugation, solar drying beds and belt presses. The authors concluded that centrifugation had the highest energy consumption in the life cycle (700 MJ/ton of dry algae) and GHG emissions (50 kgCO_2eq/ton of dry algae), while solar drying beds had the lowest impacts (2.8 MJ/ton of dry algae and 0.2 kgCO_2eq/t of algae). However, solar drying beds only achieved a solid content of approximately 40%.

As an alternative method, some research have mentioned bioflocculation or spontaneous flocculation as a form of harvesting (Gutierrez et al., 2016, Úbeda et al., 2017, Yunos et al., 2017). Furthermore, the possibility of using supports for attached biomass growth, instead of suspended growth in cultivation reactors has also been studied (Assis et al., 2017; Eliseus et al., 2017). This technology has been shown to be effective in microalgae harvesting, since it guarantees the complete retention of biomass and offers a more economic option for separation (Billad et al., 2012 a, b). Therefore, studies on the position of support materials regarding solar exposition and their composition, reactor design and operation are of great importance for the advancement of the subject.

Many processes can be used for drying such as solar drying, roller drying, atomization, spray and freeze-drying. Solar drying does not require energy from fossil sources and is therefore a less expensive option. On the other hand, it depends on the climate, demands a very large area and can cause considerable denaturation of organic compounds (Milledge and Heaven, 2013). Among the cited processes, freeze-drying tends to cause less damage to organic materials than spraying, but is a more expensive option (Brennan et al., 1969).

The best option is to invest in a more efficient dewatering process during harvesting. Milledge and Heaven (2013) suggested minimizing the water content of the harvested microalgae prior to drying and later selecting energy routes that do require wet biomass.

5.3.3. Energy use

The energy valorization route that had the most publications was biochemical valorization through anaerobic digestion, corresponding to 79.71% of the total publications (Figure 5.6). Anaerobic digestion is a competitive route, since it is widely known for other types of biomass and there is no need to dry the biomass, making its application more feasible.

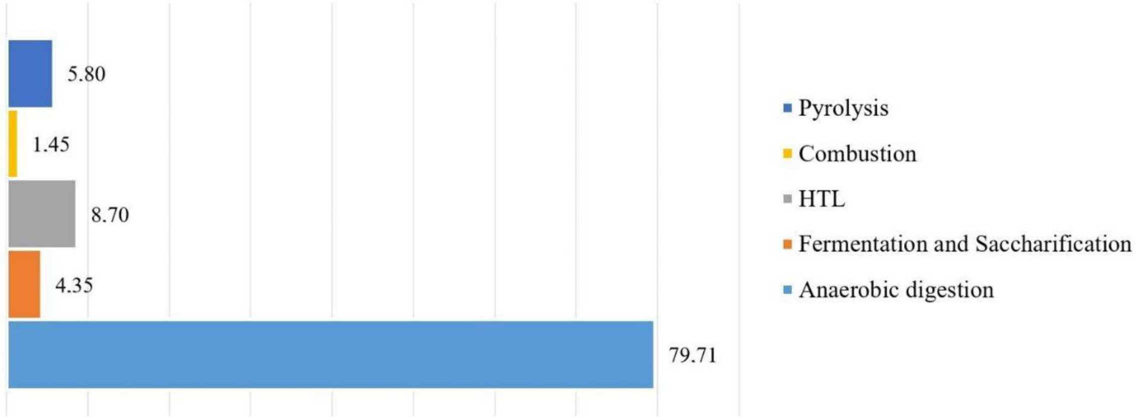

Figure 5.6. Percentage of subjects researched within the theme "energy use".

Of the 55 published articles regarding anaerobic digestion, the largest volume of an anaerobic digestion reactor was the one operated by Hidaka et al. (2014) that worked with a 100 L semi-continuous complete mixing reactor under mesophilic conditions (35°C) and a HRT of 50 days. Other volumes varied from 8 L (Bohutskyi et al., 2016) or less. Since it is a well-known process, it is highly recommended that future studies scale up the reactors (i.e. a commercial scale). This would allow a breakthrough in this subject, which has already been extensively investigated on a pilot scale. The development of specific reactors for algal biomass substrates, in which the hydrolysis phase is critical, is also suggested. Currently, the most used reactor is the continuous stirred tank reactor, which is not necessarily the best and the most suitable type of reactor. Others important issues relating to algal biomass anaerobic digestion are: i) more studies of the microbial anaerobic community present in the digestion process of this type of substrate and ii) the development of economically and energetically feasible pre-treatments to overcome low biodegradability, which is characterized by the presence of the microalgae cell wall (Composed of hard degradation substances such as lignin, cellulose and hemicellulose in some species).

In addition to the biochemical process, the thermochemical conversion process of algal biomass into biofuels has caught the attention of researchers. Among them, hydrothermal carbonization (HTC), hydrothermal liquefaction (HTL) and hydrothermal gasification (HTG) occur in the presence of water, and torrefaction, pyrolysis and gasification occur in absence of water (Barreiro et al., 2013). The main bottleneck of the routes that operate in the absence of water is the need to dry the biomass, which considerably increases the energy consumption required for the removal of moisture, as already mentioned in section 3.3.2. Biomass drying influences a demand for electric energy that makes these methods impractical, as in the cases of pyrolysis (Borges et al., 2014; Castine et al., 2013, Sarkar et al., 2015, Sharara et al., 2014) and combustion (Tang et al., 2011). Dry torrefaction is another possible thermochemical route, but no publications were found using this process in the used database.

Hydrothermal process was shown to be more favorable, as in the study carried out by Couto et al. (2018), which obtained high yields of bio-oil (approximately 40 %), using biomass produced during domestic effluent treatment in HRAPs. The authors mentioned that the use of biomass directly after production in HRAPs could lead to excessive energy consumption due to the high water content. However, the need for drying is reduced when compared to other conversion processes, which ensures a positive energy balance in HTL. The hydrothermal processes differ in terms of the main product and operational conditions. HTC generates hydrochar (a kind of biochar, but in aqueous media), HTL generates bio-oil, while HTG produces synthetic gas. A common feature of the three processes is the generation of water-soluble products in addition to the main fuel, since the reaction occurs in an aqueous media (Tian et al., 2014).

Among these three processes, only HTL was the subject of research in the consulted database (Arun et al., 2017; Chen et al., 2014 ab; Lemoine et al., 2013; Mehrabadi et al., 2017; Patel et al., 2016). When it comes to HTG, the main difficulty is affording the high prices of the reactors, as they should support very high operating temperatures.

Regarding HTC, Kubo (2013) cited the following points as the main advantages of this process: i) low temperature for carbonization, ii) the possibility of occurring in an aqueous phase, iii) it can be considered a non-expensive process because carbonaceous materials of crude biomass can be used as carbon source, iv) it has the capacity to incorporate other chemical structures such as nanoparticles or functional monomers, and v) the easy access to materials with functional surfaces that can be used and easily transformed allowing a simple post-chemical modification.

Similar to the HTC, the torrefaction process uses dry biomass as the substrate. It is a process where the crude biomass is heated under an inert atmosphere at a temperature range of 200 to 300°C, in which the hydroxyl groups are removed and a hydrophobic material is produced (Alonso et al., 2016; Wilk et al., 2015). However, in the case of HTC, studies have been carried out with different temperature ranges, pressures and proportions of initial biomass/water ratios in the reactor (Bach et al., 2017; Bach; Skreiberg, 2016; Wilk and Magdziarz, 2017), thus denoting the need for further improvements.

The terms HTC and wet torrefaction (WT) designate similar processes, both of which are currently used in the literature (Bach et al., 2013; Bach et al., 2017; Zhang et al., 2017) to define a process that occurs in a hydrothermal medium or compressed hot water at temperatures between 180–260°C (Bach et al., 2013; Bach et al., 2016; Chen et al., 2012). However, there is a distinction between both processes. According to Bach et al. (2013), the HTC process is mainly used to produce charcoal with high carbon content, which can not only be used as a fuel but also an activated carbon and soil conditioner, while the WT process aims to produce only solid fuels. Energy efficiency is more critical to the WT process when compared to HTC process, and therefore it is not uncommon to find records in the WT literature being performed at relatively lower temperatures than HTC (Bach et al., 2013). Therefore, studies with WT are important tools to consolidate the knowledge of the HTC process, however it is necessary that the distinctions between them be maintained.

Bach et al. (2017) carried out a study where they investigated the effect of wet torrefaction of *Chlorella vulgaris* (cultivated in a synthetic medium) on the productivity and combustion properties of the final product. WT was performed under different temperatures (160, 170 and 180°C) and durations of time (5, 10 and 30 min). The energy yield decreased with increasing temperature, with a 68% yield at a temperature of 160ºC. Moreover, in the thermogravimetry analysis first peaks were observed after a temperature of 200ºC; however, small peaks were observed at temperatures below 160ºC, indicating the possibility that WT of this biomass can be performed at lower temperatures.

Finally, it should be noted that there is a need for studies that use anaerobic digestion reactors at larger scales, since several studies have been carried out on a pilot scale and extrapolating the scale will promote an advance of this method in microalgae biomass use. In addition, more studies should be done using wet biomass as the raw material. Among them, HTC appears to be a potential process as it can be operated under lower temperatures than the other thermochemical processes because it does not require reactors that have a high cost to support high temperatures.

5.3.4. Final products

The subjects with the most publications within the theme "final products" were gaseous biofuels, value-added bioproducts and, lastly, liquid biofuels (Figure 5.7). These products correspond to the results of anaerobic digestion, production of sugar, lipids and proteins-rich biomass and biodiesel, respectively.

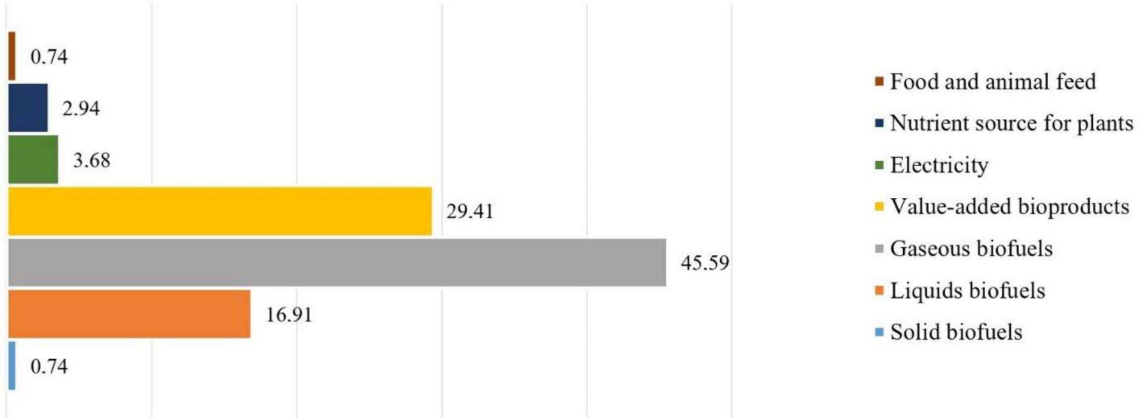

Figure 5.7. Percentage of subjects found within the theme "final products".

Solid biofuels had the lowest number of publications within this theme. The only article published referred to wastewater-grown algae biomass as a raw material in the pyrolysis process, for the production of biogas, biochar and bio-oil in a biorefinery (Sarkar et al., 2015). Biochar (solid biofuel) had demonstrated its potential as a soil conditioner that is capable of improving water retention and nutritional status (Lehmanne and Joseph, 2009). It also helps to adsorb toxic elements present in the soil, which results in improved crop productivity (Brennan et al., 2014). Furthermore, Agarwal et al. (2015) and Brennan et al. (2014) cited that pretreatment with biochar can be used as an adsorbent for the removal of wastewater pollutants.

In addition to pyrolysis, hydrochar from HTC can also be applied to the soil or even to be used as an adsorbent for the removal of wastewater pollutants. Also, there is no need to dry the product, reinforcing the potential of HTC as a method for use for microalgae biomass.

It is noteworthy that in the majority of the studies, no energy balance or life cycle analysis was performed with the objective of ascertaining the viability of the production of these products. Moreover, it is not clear how much each of these products would cost in the market.

When it comes to food and animal feed production, Ibekwe et al. (2017) evaluated the final composition of a HRAP biomass from livestock wastewater treatment in terms of nutrients and microorganisms, and suggested its use in animal feed production. Further studies still need to be carried out to prove the health safety of transforming biomass cultivated in effluents into animal feed. Zepka et al. (2010) cultivated microalgae in a parboiled rice effluent and indicated that this may be a potentially important source of protein.

Another important use of microalgae biomass that is still underexplored is its use as a nutrient source for plants. Castro et al. (2017) obtained favorable results regarding plant growth and soil quality improvement when they applied microalgae biomass to soil in comparison to a commercial chemical fertilizer. However, with the high requirements of the fertilizer industry in relation to standard fertilizer granules, the application of algal biomass as a liquid concentrated biomass may not be a viable process. Changes should be made regarding the application pattern and the logistics of this operation, and it is necessary to carry out studies that aim to standardize biomass for later application, such as granule production. The investment in biofertilizer production has a great chance of success, since the final product has a high price in the market, has a great resource demand and finally, has lower energy requirements for its processing when comparing it some energy routes. In addition, algae biomass presented in fertilizer granules may contribute to the improvement of the physical and chemical properties of soil over time. Kamyab et al. (2017) suggested that algal species should be used as a biofertilizer as an alternative to conventional synthetic fertilizers. This is due to the higher cost of chemical fertilizer and the problems related to soil and water contamination that are caused by them.

This option of nutrient recovery appears to be a potential use of the by-products from biorefineries. It can be applied to biomasses that are produced in effluent treatment, those that did not have favorable enough characteristics to be applied in the energy conversion process and the residual by-products of thermochemical and biological treatments that present enough nutrients to be used as fertilizers, soil conditioners or to be returned to the cultivation stage as nutrient source for biomass production.

5.3.5. Sustainability assessment

Alternatives that can help to improve the sustainability balance of a certain valorization route are: (i) the relation between the characteristics of the effluent and the characteristics of the final biomass; (ii) the use of software that demonstrates the environmental and economic feasibility of the route, as well as allowing it to infer the critical point of each step of the process; and (iii) the biorefinery approach as a way to optimize the use of biomass and thus to reduce costs and waste production. Regarding these alternatives, the first and third topics have been mentioned and discussed previously.

In the case of energy balance, economic and sustainable evaluation and LCA, 17 publications were registered, and only two of them (Udom et al., 2013, Takabe et al., 2017) were done based on primary data. The others were based on data from the literature (secondary data). This point needs to be carefully evaluated. Secondary data or pilot scale extrapolation can lead to values that overestimate or underestimate the actual impact of the product or process.

One tool that has been gaining importance in the study of environmental impacts that are caused by a product or process is LCA. Several studies have related LCA to microalgae valorization routes. Collota et al. (2017) used LCA to compare three alternatives of lipid extraction technologies from microalgae biomass. Carneiro et al. (2017) conducted a comparison of the production methods of fossil fuels, ethanol and biodiesel in Europe and Brazil through LCA. Wang et al. (2017) performed LCA in the pyrolysis process of the microalgae biomass. Gnansounou and Raman (2016) carried out a LCA of algae biodiesel and its co-products.

The purpose of LCA is to identify and quantify potential environmental impacts in all stages of a production chain, from raw material extraction to final product disposal, through processing, manufacturing, packaging, transportation, distribution and its use.

One of the advantages of LCA is that given the complexity and the amount of data needed to perform this analysis, it is possible to conduct a partial study, as long as it meets the proposed objectives. The main techniques used are: cradle-to-gate, that is, all processes after the manufacture of the product are excluded; gate-to-grave, where all processes prior to the manufacture of the product are excluded; and gate-to-gate, i.e. only the manufacturing stage is considered. In addition, product impacts may be related to different impact categories. In other words, impacts can be accounted for in different impact categories, e.g. global warming, depletion of the ozone layer, human toxicity, ecotoxicity in fresh water, marine, terrestrial and eutrophication, among others.

Despite being a versatile tool, LCA is still not used often to map the environmental impacts of wastewater-grown biomass and its valorization routes. Also, it is important to perform this analysis with primary data or with the least possible extrapolation.

5.4. Conclusions

The main obstacles within the theme "microalgae cultivation" are i) mixed biomass when the objective is to obtain raw material for lipid production and ii) a focus on better understanding and removal of EOPs in domestic sewage treatment. In addition, there is a need to study the effluent in order to obtain algae biomass with the desired characteristics.

Biomass harvesting is an area that still needs improvement, but drying, must be avoided when possible, leading to a choice of energy routes that use wet biomass, especially HTC. Regarding lipid extraction, it is questionable whether all of the small operations that are used to obtain high productivities will be economically and environmentally costly when scaled up.

It should be noted that there is a need for studies that use anaerobic digestion reactors at larger scales. In addition, further studies should be done using wet biomass as a raw material. Among them, HTC appears to be a potential process, as it can be operated at lower temperatures than other thermochemical processes. The use of biomass as a biofertilizer is still a little explored subject.

Among the alternatives that can help to improve the balance of sustainability of a given recovery route, is the use of software that demonstrates the environmental impact of the route. LCA is an important tool to support decision making and needs to be further explored in the universe of microalgae. It allows inferring about the environmental impacts of an entire route, in addition to determining the critical point of each stage of the process.

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6. ARTIGO II. Algal biomass from wastewater: soil phosphorus bioavailability and plants productivity

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Abstract: The cultivation of microalgae in wastewater allows to obtain a biomass concentrated in nutrients and organic material. This biomass added to phosphate fertilizers can promote a slow release of the nutrient and consequently a higher absorption of phosphorus (P). The objective of this study was to investigate P uptake by plants subjected to triple superphosphate (TSP) fertilization, added with microalgae biomass (MB) grown in wastewater. TSP was added with different MB proportions in order to verify if there would be a different behaviour in P release for millet (*Pennisetum glaucum* L.) plants. With the proportion that maximized P accumulation in plants, a second experiment was carried out to investigate whether MB exerts influence of P diffusion in the soil. Finally, a third trial was conducted in a greenhouse, where TSP and TSP + 12% MB were applied to the soil under different phosphorus doses in corn (*Zea mays* L.). The proportion of MB in TSP that maximized the increase of P content and concentration in plants was approximately 12 % MB. From this proportion, a reduction in the values of the variables analysed in the plant with the increase of the proportion of MB in the biofertilizer was observed. Similar behaviour was observed when evaluating P diffusion in sandy and clay soils. Fertilizers TSP and TSP + 12 % MB showed no difference in P diffusion in the soil, while the ratio of 30 % MB clearly impaired P diffusion. In a greenhouse, the P content presented significant difference for the tests carried out with TSP and TSP + 12% MB fertilizer, in which the latter provided higher P recovery rate by plants. Therefore, MB added to TSP had a positive influence on plant development and its P recovery capacity when applied in a proportion of 12 % MB to the fertilizer mass.

Keywords: microalgae; nutrient recycling; agricultural valorisation; circular economy; environmentally friendly fertilizers.

6.1. Introduction

In many situations, the soil is unable to meet all crop nutrient requirements, which requires the use of fertilizers to achieve higher agricultural productivity. Among the most used fertilizers are nitrogen, phosphate and potassium, which contain the main macronutrients for plants. The production of synthetic fertilizers contributes significantly to different impact categories, such as acidification potential, eutrophication potential and global warming potential (Murphy et al., 2014).

Among the main macronutrients used in agricultural cultivation, P stands out as

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presenting a peculiar management difficulty due to its rapid adsorption to soil particles. It is estimated that when a soluble source of P is applied as fertilizer in a soil, more than 90% of the element is adsorbed in the first hour of contact and is unavailable to plants (Novais et al., 2007).

Despite its importance, the efficiency of P use does not reach 20%, and the remainder ends in wastewater or reaches surface water through runoff from cultivated fields (Solovchenko et al., 2016). Due to these losses, which increase the cost, waste of energy and pollution of the environment (Chen et al., 2018), it is necessary to adopt technologies that increase the efficiency of the use of phosphate sources resulting in reduced fertilizer costs and lower use of non-renewable natural resources.

In the case of phosphate fertilizer, efforts are made to increase P bioavailability after its application to soil. For this purpose, technologies have been adopted and added to fertilizers, aiming to increase their efficiency. There are different types of fertilizer technologies, which have various names, such as slow-release fertilizers-compounds that have low water solubility (Chalk et al., 2015); controlled-release fertilizers (González et al., 2015), which consist of coating highly soluble granules with water-insoluble material (Chalk et al., 2015) and “enhanced efficiency fertilizers” (EEFs), a junction of groups called slow-release and controlled-release materials (Timilsena et al., 2015). Generally, all these groups can be called environmentally friendly fertilizers (EFFs), which is a name for fertilizers that aim to reduce environmental pollution by nutrient losses by slowing or even controlling the release of nutrients into the soil (Chen et al., 2018).

Among these technologies, the use of materials of synthetic or organic origin, mixed with the fertilizer mass, which reduce the speed of nutrient release to the soil, can be highlighted. The goal is to promote a synchronisation between nutrient release and plant demand, thus preventing the phosphorus provided by the fertilizer from being quickly adsorbed to the soil particle. The increase of organic material to fertilizers has been a recent target of research (Purnomo et al., 2018; Sharkaw et al., 2018).

Soil organic matter can correlate with P adsorption both positively, mainly due to the anionic character of organic matter, and negatively, blocking P adsorption sites in soil (Novais et al., 2007). Therefore, it should be stated that each material, whether animal waste, biochar or any biomass rich in organic material may, by virtue of its constitution, behave differently when added to the fertilizer mass.

Waste, especially biomass, is a large reservoir of nutrients that can be recovered through different technologies and used to manufacture fertilizers. In this aspect, a subject that has been widely studied is the cultivation of microalgae due to the countless possibilities of using this biomass in the production of biofuels, biofertilizer and animal feed (Javed et al., 2019; Zhang et al., 2019). Moreover, when this cultivation is carried out in wastewater, this practice allows the recovery of N, P and, therefore, an obtaining of a nutrient-concentrated algae biomass (Cai et al. 2013) and organic carbon (Costa et al., 2016). This practice, besides promoting the wastewater treatment, allows the obtaining of a MB with different potential for use. However, when recovered from wastewater by chemical or biological processes, P is often present in a form that does not meet specifications for agricultural use (Solovchenko et al., 2016), as it is in its organic form.

In this context, we highlight the possibility of microalgae biomass grown in wastewater to be added to phosphate fertilizers, in order to increase the efficiency of P uptake by plants. Although several works related to microalgae biorefinery cite the route of biofertilizers as potentially advantageous, there are few published studies referring to this subject. Favourable results regarding plant growth have already been found in the literature when applying moist microalgae biomass to the soil (Castro et al., 2017; Marks et al., 2017). However, data proving the increased efficiency of phosphate fertilizer in plants by the addition of dry microalgae biomass have not yet been obtained. Therefore, the objective of this study was to investigate, through different experiments, the bioavailability of P in soil, with the fertilizer source being TSP added from MB grown in wastewater.

6.2. Materials and Methods

6.2.1. Algal biomass production

The algal biomass was produced at the experimental wastewater treatment and biomass production plant of the Sanitation and Environmental Engineering Laboratory of the Federal University of Viçosa (UFV), Minas Gerais, Brazil (UTM co-ordinates 722924 E, 7702003 S, zone 23 K). The municipality of Viçosa, with an average altitude of 648 m above sea level, is characterised by an average annual rainfall of approximately 1,221 mm and an average annual temperature ranging between 19 °C and 20 °C. Relative humidity is, on average, 81%. The local climate, according to the Köppen classification, is Cwa type-tropical in altitude with hot and rainy summers and cold and dry winters. (Rocha et al., 2012).

The microalgae biomass was cultivated as a by-product of the wastewater treatment of a meat processing industry in high-rate algal ponds (HRAPs). The main activity of this industry is the production of sausages (salami and hams, among others) and the production of shredded desalted codfish. The wastewater of industrial origin is generated in the various stages of the production process, especially in the discarding of the cooking and cooling tanks of sausages and of the desalting tanks of cod and also the washing of the floors and equipment at the end of the production processes.

As an initial evaluation, the wastewater characterization should be carried out aiming at obtaining a biomass suitable for the intended use. Therefore, in this study, a nitrogen rich effluent from a primary flotation unit was chosen. Its characteristics are better described in previous studies (Castro et al. 2017 and Souza et al. 2019).

The pilot scale HRAPs used for biomass production have the following characteristics: width = 1.28 m, length = 2.86 m, total depth = 0.30 m, useful depth = 0.30 m, surface area = 3.30 m^2 and useful volume = 1.00 m^3 (Figure 6.1). The HRAPs were made of fibreglass and the pedals of stainless steel, with six blades. During operation, pedalwheels were powered by a 1 hp electric motor. The rotation was reduced by a reducer coupled to the motor and controlled by inverter frequency (series WEG CFW-08), which ensured a liquid velocity between 0.10 m s^{-1} and 0.15 m s^{-1}. Similar values were used in different research with HRAPs (Picot et al., 1991; Park et al., 2011) and ensured the necessary revolving.

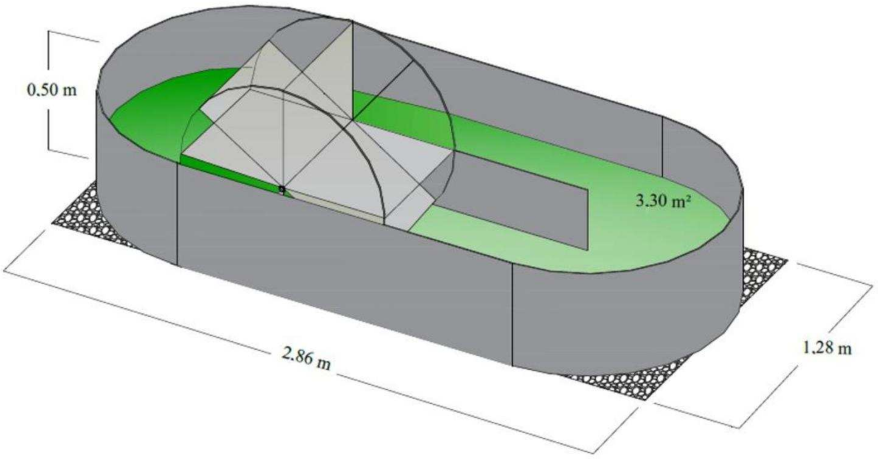

Figure 6.1. HRAP scheme used for biomass production.

The biomass was produced in a 14 days' batch in October 2017. In the operation, 40% of inoculum was used in relation to the total volume of the pond, which consisted of microalgae biomass previously adapted to the conditions of the wastewater. A CO_2 injection system was also used in order to control the pH of the pond, maintaining it between 7 and 8.

At the end of the operation, the biomass was concentrated by chemical flocculation, with the addition of 50% NaOH solution (m v^{-1}) to raise the pH to 12. Then, the wastewater in the HRAP was stirred with the aid of a plastic container for 3 minutes and the sedimentation performed in the HRAP itself during the night. The concentrated biomass at the bottom of the HRAP was collected manually using plastic containers after the wastewater disposal. After collection, the pH of the concentrated biomass was neutralised with the addition of 1:1 HCl solution (v v^{-1}). Then the biomass was concentrated by a high speed refrigerated centrifuge, at 10,000 rpm for 3 minutes, and finally dried in a forced circulation oven at 60 ºC. The characterisation of algal biomass obtained after drying is described in Table 6.1 (Costa et al., 2016).

Table 6.1. Chemical characterization of algal biomass after drying.

N	P	K	C	H	O	Ca	Mg	S	Na
				(%)					
5.59	1.18	0.14	38.39	6.51	28.55	9.76	0.36	0.95	0.83
Zn	Fe	Mn	Cu	B	Cd	Pb	Cr	Ni	
				(ppm)					
192.70	23415	158.20	37.00	2.89	ND	ND	23.20	15.10	

ND = Not detectable. Fonte: Costa et al. (2016).

6.2.2. Biofertilizer production

The production of fertilizer granules was carried out in a granulator plate, whose working principle is based on the formation of granules obtained from continuously fed powder material with the addition of liquid (H_2O). The rotary movement of the plate and its inclination, at an angle ranging from 45 and 60º, enables the formation of the granules. Fertilizers consisting of TSP were produced with the addition of different MB proportions.

The granules were initially separated according to their particle size using 2 to 3 mm sieves. In each experiment, the granules were previously weighed to obtain minimal variability between replicates. Finally, fertilizers were visually analysed using a JSM-6010LA scanning electron microscope (SEM).

6.2.3. Experimental Design

There were three trials carried out to evaluate the influence of MB on soil phosphorus adsorption/desorption and as a source of nutrients for plants. The first trial was conducted in a plant growth chamber. TSP was added with different MB proportions in order to verify if there would be a different behaviour in the phosphorus release to plants. The second test was performed to evaluate the phosphorus adsorption/desorption in TSP with 12 % (ratio that maximized dry matter mass production (mMS) from the previous experiment) and 30 % (maximum ratio tested in the previous experiment, tended to decrease phosphorus release to soil) MB added. And the third trial was conducted in a greenhouse in which TSP and TSP + 12 % MB were applied to the soil under different doses (0, 100, 200, 300 and 400 mg dm^{-3} P).

In all trials, the 2 dm^3 soil samples were placed in plastic bags for liming by the Al_3^+ neutralisation method and Ca_2^+ and Mg_2^+ elevation (Alvarez and Ribeiro, 1999), with commercial calcitic limestone (Ca:Mg ratio 4:1). Then, the soil moisture was adjusted to 60% of field capacity, determined by the porous plate extractor 10 kPa pressure (Reichardt, 1988). After 21 days of incubation, the soil was air dried, deforested and passed through a 2 mm sieve. Chemical and physical soil analyses were performed according to Nelson and Somers (1982), EMBRAPA (2009) and Almeida et al. (2012).

6.2.3.1. Phosphorus Absorption Under Different BM Concentrations—Trial 1

To set up this experiment, subsurface samples (20–40 cm) from a very clayey Red-Yellow Latosol, collected in the region of Viçosa, MG, were used (Table 6.2). The collected material was air dried, soil clusters were breaked up and passed through a 2.0 mm sieve to obtain air dried soil.

The soil samples were weighed (200 cm^3) and placed in 500 dm^3 pots. Fertilization with 300 mg dm^{-3} of P was made with TSP at MB proportions of 5, 10, 15, 20 and 30% (w/w). TSP without the addition of biomass was used as a control. The experimental design was randomised blocks (DBC), with six treatments and five replications, totalling 30 experimental units.

Table 6.2. Chemical and physical properties of the soil – Trial 1.

Parameter	Unit	Value
Sand[1/]	%	35
Silt[1/]	%	10
Clay[1/]	%	55
OM[2/]	%	1.52
pH – H_2O[3/]	-	5.73
H + Al^{3+}[6/]	cmol$_c$ dm^{-3}	2.50
Ca^{2+}[4/]	cmol$_c$ dm^{-3}	1.38
Mg^{2+}[4/]	cmol$_c$ dm^{-3}	0.28
K^{+}[5/]	mg dm^{-3}	13.00
P[5/]	mg dm^{-3}	1.40
Base saturation	%	40.30
Sum of bases	cmol$_c$ dm^{-3}	1.69
P_{rem}[5/]	mg L^{-1}	11.1
S[7/]	mg dm^{-3}	2.50
Zn[7/]	mg dm^{-3}	0.76
Effective CEC	cmol$_c$ dm^{-3}	1.69
Potential CEC	cmol$_c$ dm^{-3}	4.19

[1/]Pipette method (Almeida et al., 2012); [2/]Nelson and Somers (1982); [3/]Soil to water ratio 1:2.5 (EMBRAPA, 2009); [4/] Potassium chloride extraction method 1 mol L^{-1} (EMBRAPA, 2009); [5/]Mehlich^{-1} Extractor (EMBRAPA, 2009); [6/]Calcium acetate extraction method 0,5 mol L^{-1} - pH 7.0 (EMBRAPA, 2009); [7/]Monocalcium phosphate in acetic acid extraction method (EMBRAPA, 2009).

A basic fertilization with N, K, S and micronutrients was performed according to Novais et al. (1991) on all soil volume before planting. Then four pre-germinated millet seeds (*Pennisetum glaucum* L.), variety BRS 1501, were sown.

Pot irrigation was performed daily, keeping soil moisture at 70 % of field capacity. The assay was conducted in a controlled environment (temperature ± 25 °C) for 30 days.

At the end of the experiment, the plants were sectioned about 0.5 cm from the ground, placed in paper bags and placed in a forced air circulation greenhouse at 65 ºC, to determine the shoot dry mass matter (SDMM). Then, the samples were ground and, after nitroperchloric digestion, the P concentration in the extracts were determined according to EMBRAPA (2009). The nutrient content in the plant was calculated by multiplying the nutrient concentration by the respective dry matter weight values (NC = mMS x NT / 1000, where NC = nutrient content (mg pot^{-1}); DM = weight dry matter (g pot^{-1}) and NT = nutrient concentration (g kg^{-1}).

6.2.3.2. Phosphorus Diffusion Experiment—Trial 2

In this experiment, in addition to fertilizer sources, two soil types were tested, as soil texture is also an important factor in influencing phosphorus diffusion. Soils of two different textures were used: clay and sand.

The chemical and physical properties of the clay soil used in the experiment after acidity correction were shown in Table 6.3.

Table 6.3. Chemical and physical properties of the clay soil – Trial 2.

Parameter	Unit	Value
Sand$^{1/}$	%	19.30
Silt$^{1/}$	%	0.80
Clay$^{1/}$	%	79.90
OM$^{2/}$	%	10.60
pH – $H_2O^{3/}$	-	6.00
H + $Al^{3+6/}$	$cmol_c\ dm^{-3}$	1.30
$Ca^{2+4/}$	$cmol_c\ dm^{-3}$	1.23
$Mg^{2+4/}$	$cmol_c\ dm^{-3}$	0.35
$K^{+5/}$	$mg\ dm^{-3}$	10.00
$P^{5/}$	$mg\ dm^{-3}$	0.40
Base saturation	%	55.30
Sum of bases	$cmol_c\ dm^{-3}$	1.61
$P_{rem}{}^{5/}$	$mg\ L^{-1}$	10.00
$S^{7/}$	$mg\ dm^{-3}$	10.50
$Zn^{7/}$	$mg\ dm^{-3}$	0.44
Effective CEC	$cmol_c\ dm^{-3}$	1.61
Potential CEC	$cmol_c\ dm^{-3}$	2.91

$^{1/}$Pipette method (Almeida et al., 2012); $^{2/}$Nelson and Somers (1982); $^{3/}$Soil to water ratio 1:2.5 (EMBRAPA, 2009); $^{4/}$ Potassium chloride extraction method 1 mol L^{-1} (EMBRAPA, 2009); $^{5/}$Mehlich^{-1} Extractor (EMBRAPA, 2009); $^{6/}$Calcium acetate extraction method 0,5 mol L^{-1} - pH 7.0 (EMBRAPA, 2009); $^{7/}$Monocalcium phosphate in acetic acid extraction method (EMBRAPA, 2009).

The chemical and physical properties of the sandy soil used in the experiment after acidity correction were shown in Table 6.4.

Table 6.4. Chemical and physical properties of the sandy soil – Trial 2.

Parameter	Unit	Value
Sand[1/]	%	71.40
Silt[1/]	%	1.40
Clay[1/]	%	27.20
OM[2/]	%	14.60
pH – H_2O[3/]	-	6.30
H + Al^{3+}[6/]	$cmol_c\ dm^{-3}$	0.50
Ca^{2+}[4/]	$cmol_c\ dm^{-3}$	2.94
Mg^{2+}[4/]	$cmol_c\ dm^{-3}$	0.35
K^+[5/]	$mg\ dm^{-3}$	2.00
P[5/]	$mg\ dm^{-3}$	0.40
Base saturation	%	87.80
Sum of bases	$cmol_c\ dm^{-3}$	1.61
P_{rem}[5/]	$mg\ L^{-1}$	30.00
S[7/]	$mg\ dm^{-3}$	33.60
Zn[7/]	$mg\ dm^{-3}$	0.70
Effective CEC	$cmol_c\ dm^{-3}$	3.61
Potential CEC	$cmol_c\ dm^{-3}$	4.11

[1/]Pipette method (Almeida et al., 2012); [2/]Nelson and Somers (1982); [3/]Soil to water ratio 1:2.5 (EMBRAPA, 2009); [4/] Potassium chloride extraction method 1 mol L^{-1} (EMBRAPA, 2009); [5/]Mehlich^{-1} Extractor (EMBRAPA, 2009); [6/]Calcium acetate extraction method 0,5 mol L^{-1} - pH 7.0 (EMBRAPA, 2009); [7/]Monocalcium phosphate in acetic acid extraction method (EMBRAPA, 2009).

Petri dishes of 8 cm in diameter by 1 cm high were filled with the same amount of soil (approximately 80 g), and a fertilizer granule was placed in the centre of them. In all, 24 experimental units were conducted. The design was randomised blocks, with three blocks, two soil textures and three fertilizer sources (TSP, TSP + 12 % MB, TSP + 30 % MB), besides the control treatment (without application of fertilizer sources). The plates were incubated for 30 days, with soil moisture maintained at field capacity and room temperature (~24 ºC).

Evaluations were performed at 1, 10, 20 and 30 days after incubation. On each day of evaluation, the technique of filter paper impregnated with Fe oxide to evaluate the diffusion radius of P was used, after reaction with the malachite green dye, according to methodology proposed by Degryse and McLaughlin (2014). After the filter paper has completely dried, the papers were scanned and analysed using imaging software (GNU Image Manipulation Program, v. 2.6.11, Free Software Foundation, Boston, MA).

6.2.3.3. Greenhouse Experiment—Trial 3

The experiment was conducted in a greenhouse in pots containing 2.5 dm^3 of clay soil. The evaluated fertilizers were: TSP and TSP + 12% MB. The doses used were 0, 100, 200, 300 and 400 mg dm^{-3} of P. P fertilization occurred in a localised manner, using a cylinder as the basis for the arrangement of the granules in the centre of each pot. For this, the fertilizers were mixed to a volume corresponding to 20% of the soil of the pot. The soil (clay soil previously characterised in the phosphorus diffusion experiment, session 2.3.2) was moistened and six seeds of corn (*Zea mays* L.) were sown per pot. After germination, thinning was done to maintain two more uniform plants per pot.

Water was supplied daily to maintain soil moisture between 70 and 90 % of field capacity. Coverage fertilization was divided into three plots, performed at 6, 13 and 20 days, totalling 200 mg dm^{-3} N (urea and ammonium sulphate); 150 mg dm^{-3} K (KCl); 0.81 mg dm^{-3} B (Boric acid); 50 mg dm^{-3} of S (ammonium sulphate); 3.0 mg dm^{-3} Zn ($ZnSO_4.7H_2O$); 1.3 mg dm^{-3} Cu ($CuSO_4.\ 5H_2O$); 3.6 mg dm^{-3} of Mn ($MnSO_4.H_2O$) and 0.15 mg dm^{-3} of Mo ($(NH_4)_6Mo_7O_{24}.4H_2O$).

At the end of the experiment (30 days), the plants were sectioned at about 0.5 cm from the soil, placed in paper bags and placed in a forced air oven at 65 ºC to determine the SDMM. Then, the samples were ground, and after nitroperchloric digestion, the P concentration in the extracts determined according to EMBRAPA (2009).

The nutrient content in the plant was calculated by multiplying the nutrient concentration by the respective dry matter weight, according item 2.3.1. The growth rate of the trend line corresponding to the content, plotted in mg pot^{-1} x mg pot^{-1}, was analysed, and the P recovery rate was then obtained.

6.2.4. Data Analysis

Data regarding P concentration and content in the plants of the first trial were submitted to regression analysis with the aid of the software R© version 3.5.3 (R Core Team, 2016). The averages for the diffusion radii of the second test were subjected to analysis of variance and the means were compared by Tukey test at 5 % probability of error. Finally, the data regarding mMS and P content of the last test were submitted to regression analysis with the aid of the software R© version 3.5.3 (R Core Team, 2016). After regression analysis, the models were submitted to the model identity test according to Regazzi (1996) in order to verify if the models are statistically different.

6.3. Results

6.3.1. Characterization of biofertilizer

The MB in the fertilizer influenced the availability and absorption of P by millet plants. The results regarding the content and concentration of P in the shoots varied significantly ($p < 0.05$) with the increase of MB proportion following the quadratic regression model (Figure 6.2 and Figure 6.3). In the proportions of 12.42 % and 12.82 % of MB, the concentration and content of P, in the aerial part of the plant, reached the maximum (5.42 g kg^{-1} and 2.31 µg pot^{-1}, respectively). Up to these proportions, the organic material present in the fertilizer granule contributed to the availability of P in the soil solution. Above that, there was a reduction in this availability. In treatment without MB, P content was 10.39 % lower than that found for MB that maximizes absorption. For the maximum proportion of MB tested (30 %), the content was 20.35 % lower when compared to the one that provided the highest accumulation of P.

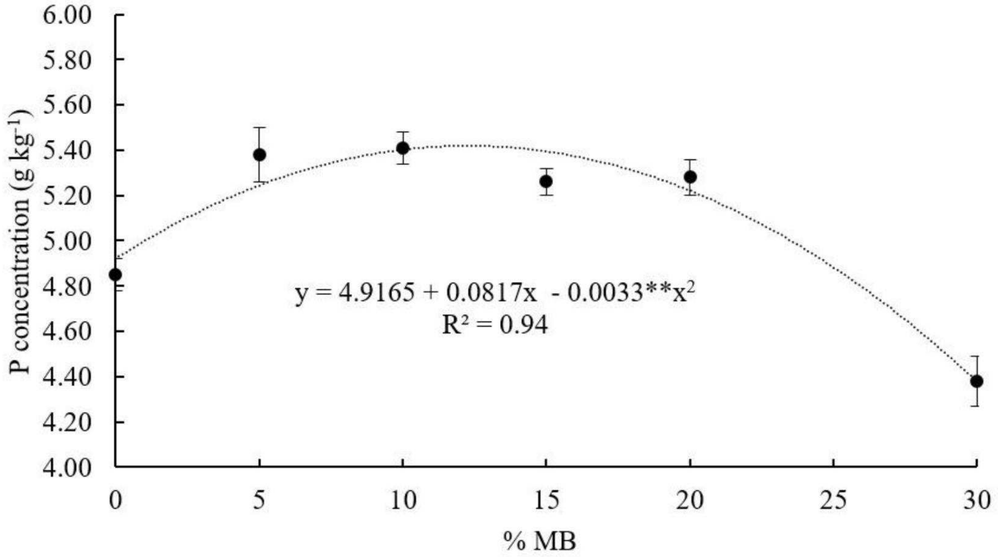

Figure 6.2. P concentration of aerial part of millet (*Pennisetum glaucum* L.) plants (g kg^{-1}), as a function of MB (%) doses with phosphate fertilizer (TSP). Error bars indicate standard deviation. ** significant at 5% probability by t-test.

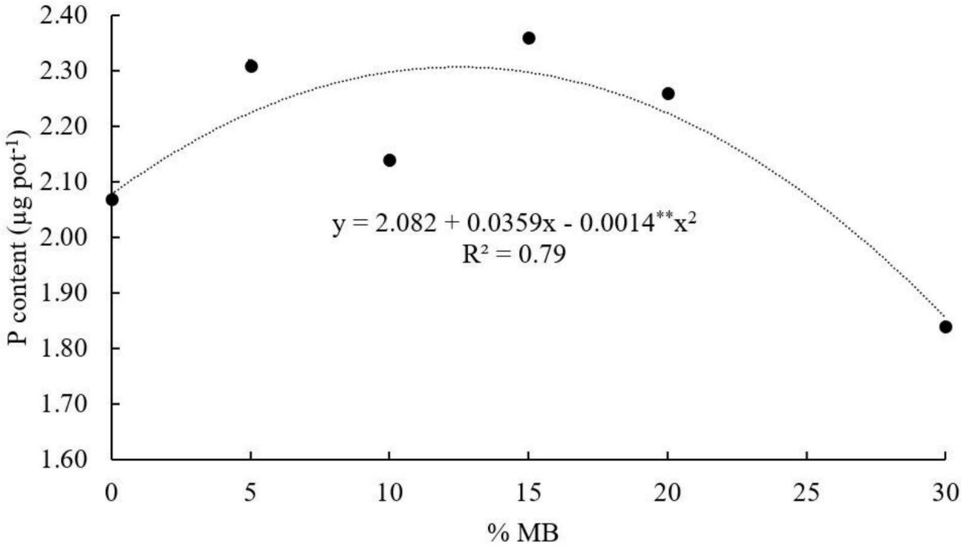

Figure 6.3. P (μg pot^{-1}) content of millet (*Pennisetum glaucum* L.) shoot, as a function of MB (%) doses with phosphate fertilizer (TSP). Error bars indicate standard deviation. **significant at 5% probability by t-test.

Some factors may be associated with different P availability with an increasing MB ratio. One of these factors is that the organic material may have formed a "coating" on the fertilizer granule acting as a physical barrier to nutrient release. As the proportion of algal biomass in the fertilizer increased, the less visible P crystals in the fertilizer became (Figure 6.4). Figure 4a, corresponding to the SEM image obtained for the SPT, shows the presence of larger and more visible crystals when compared to the other images. Following images 6.4b (MB5), 6.4c (MB10), 6.4d (MB15) and 6.4e (MB20), it is still possible to notice the presence of crystals, but they are smaller and more camouflaged in the fertilizer mass. Finally, in Figure 4f, it is not possible to observe them.

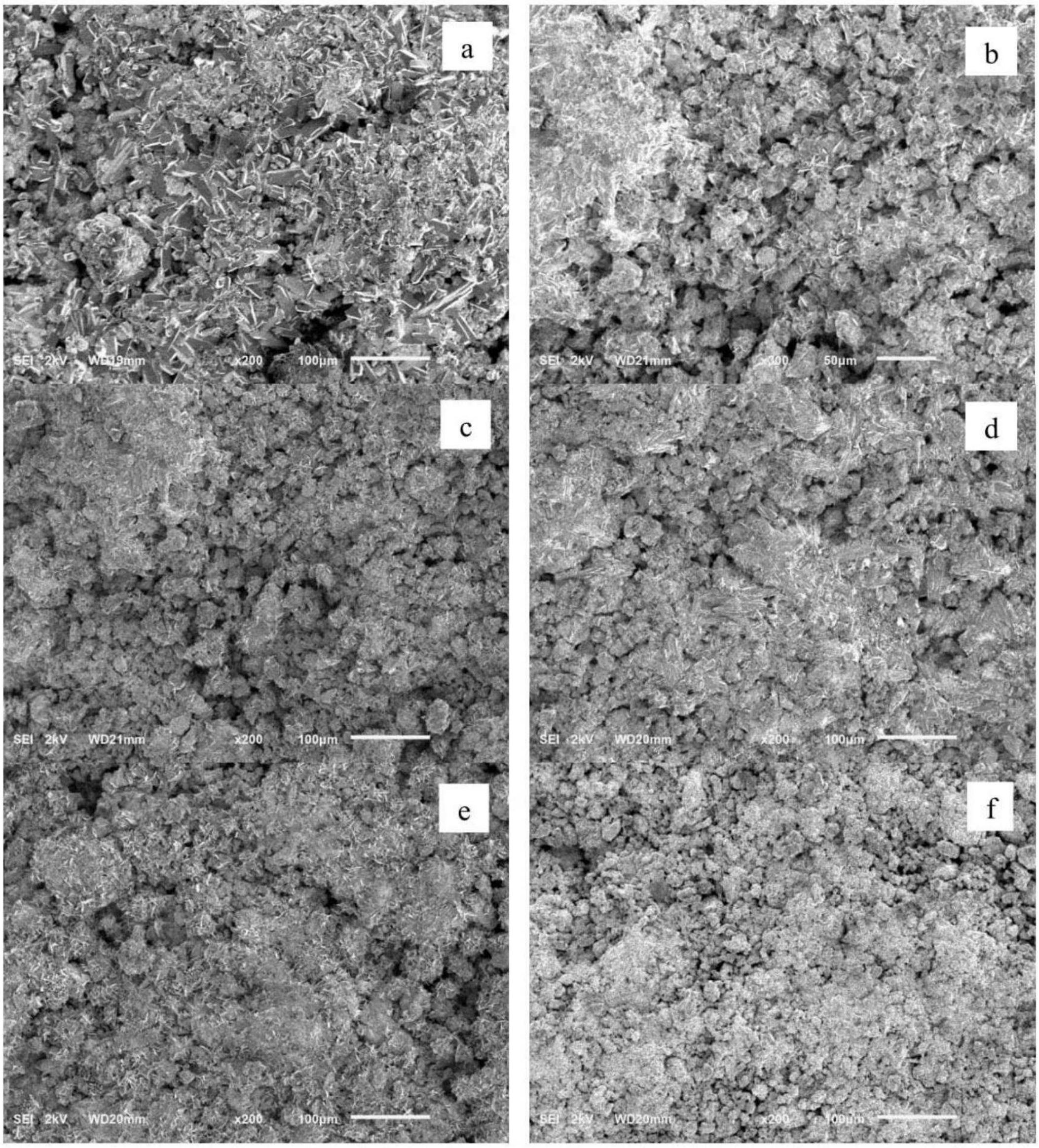

Figure 6.4. SEM image of granules of sectioned fertilizers. a) TSP; b) TSP+MB5; c) TSP+MB10; d) TSP+MB15; e) TSP+MB20 and f) TSP+MB30.

6.3.2. Phosphorus diffusion

In clay soil, P diffusion reached a smaller radius (up to 10.17 mm) when compared to sandy soil, which reached a maximum value of 12.02 mm (Figures 6.5, 6.6, 6.7 and 6.8). The values obtained for TSP in clay soil were approximate to those obtained by Lustosa Filho et al. (2019), who observed approximate values at 8.20 mm for TSP applied in clay soil.

It was observed that there was no statistically significant difference of TSP and TSP + 12 % MB ($p > 0.05$) fertilizer on any evaluation day for both soil textures. Fertilizer TSP + 30 % MB was different from the others on all evaluation days, except for the 30th day of the sandy soil, which, despite having a relatively smaller diffusion radius than the others, did not present statistical difference, probably due to greater variability of data ($\sigma = \pm 0.60$).

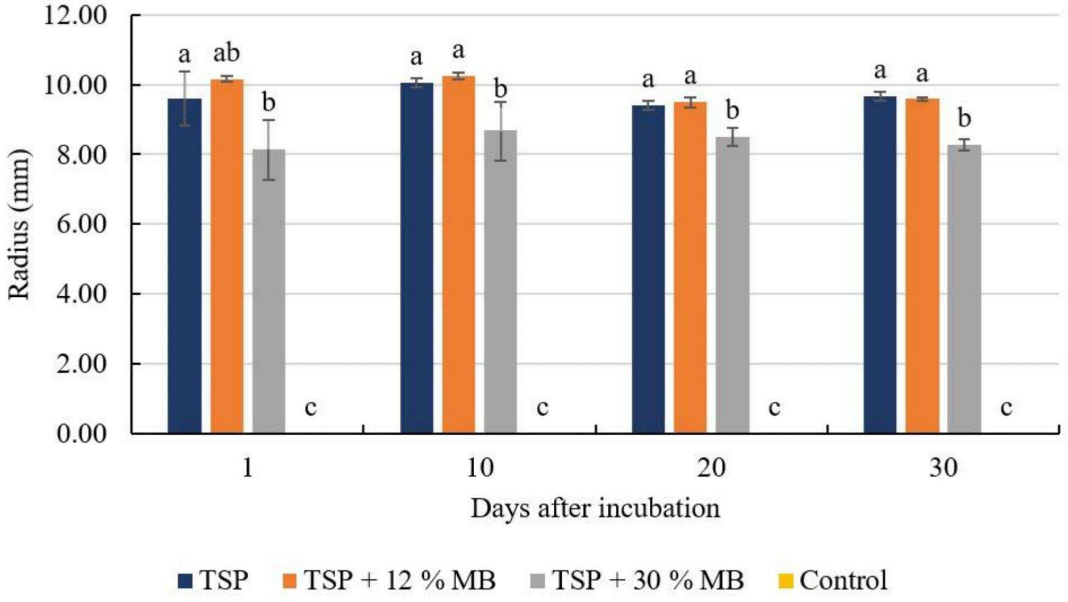

Figure 6.5. P diffusion radius in clay soil. * Means followed by the same letter do not differ significantly from each other by the Tukey test at 5 % probability.

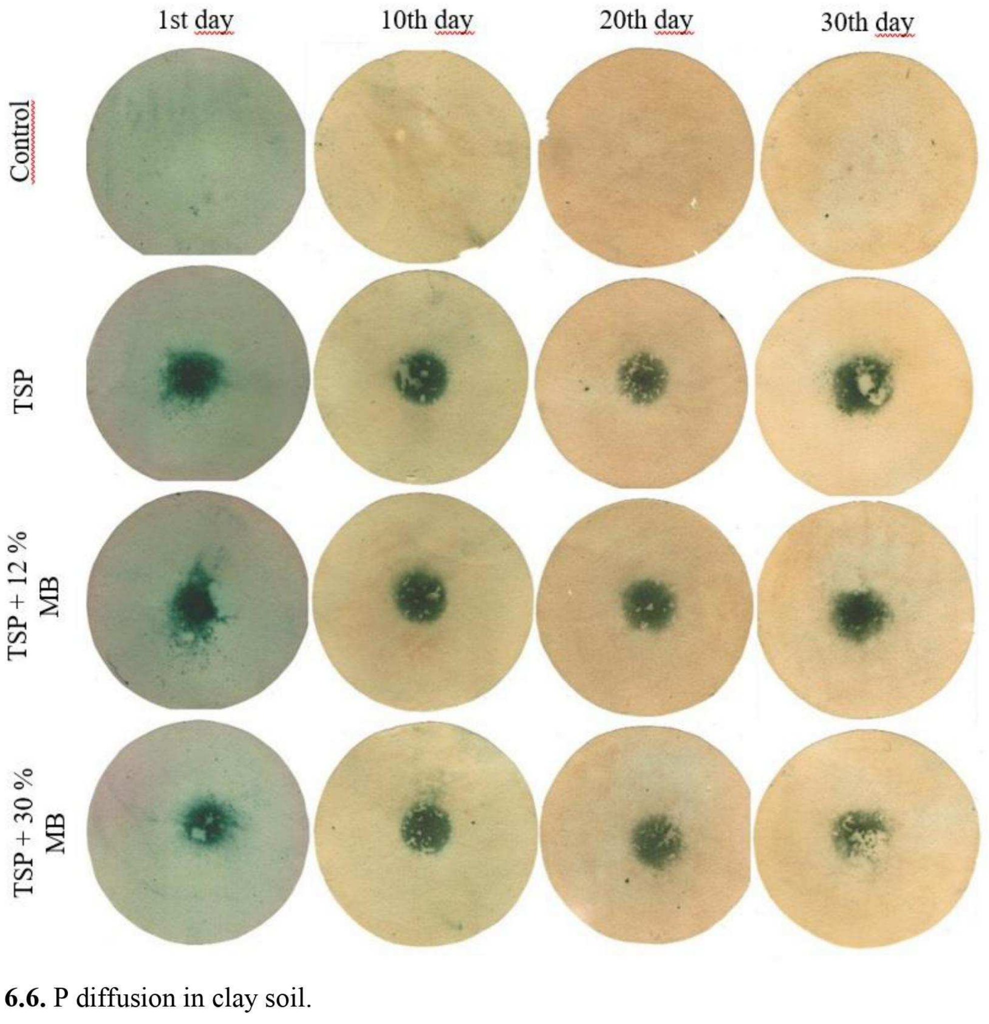

Figure 6.6. P diffusion in clay soil.

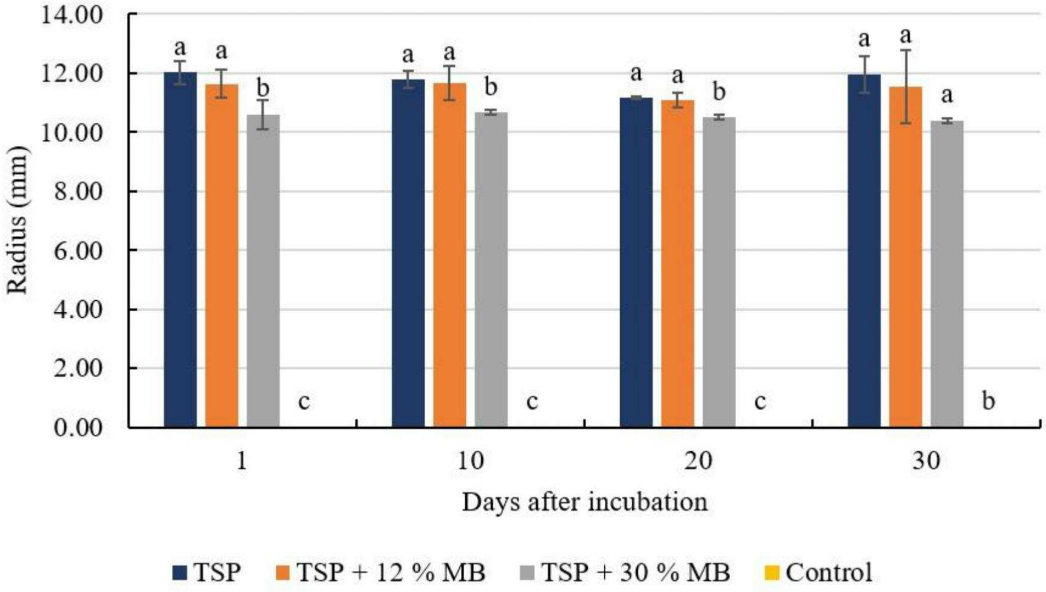

Figure 6.7. P diffusion radius in sandy soil. * Means followed by the same letter do not differ significantly from each other by the Tukey test at 5 % probability.

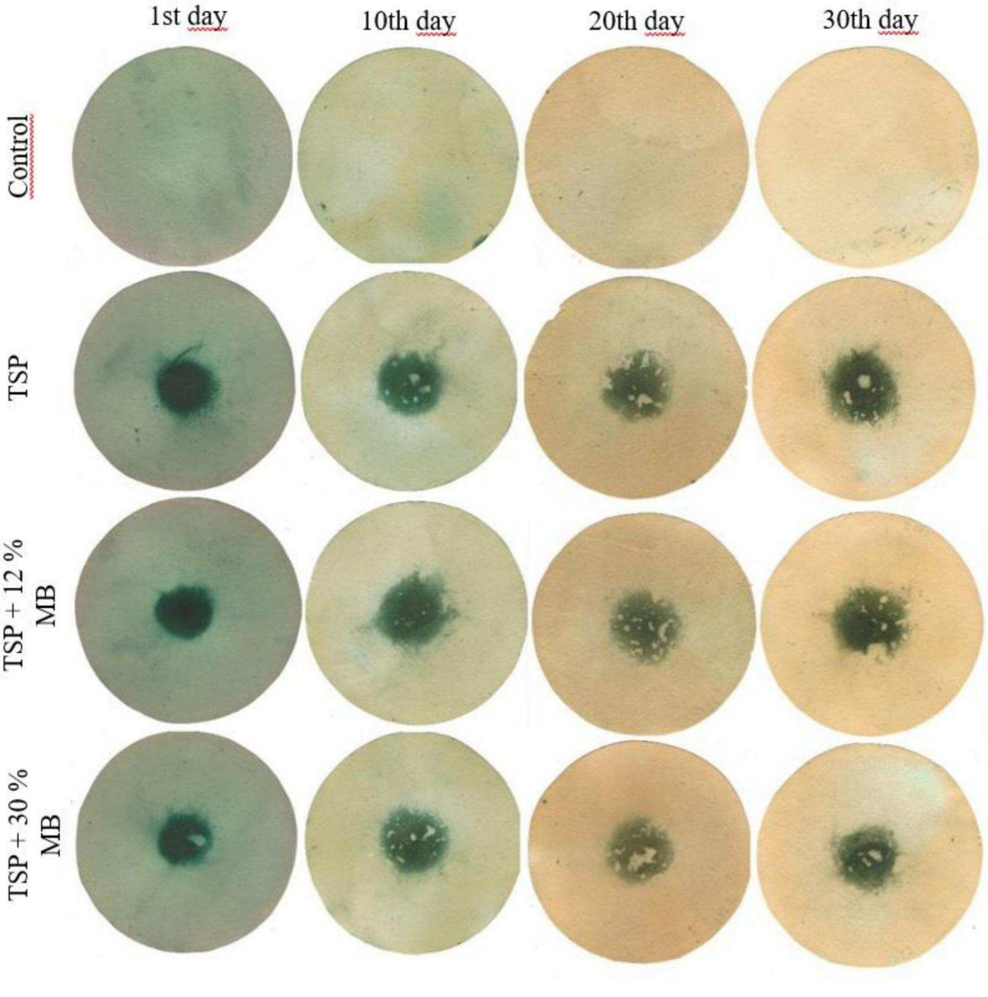

Figure 6.8. P diffusion in sandy soil.

The results are consistent with those obtained in the first trial, in which 30 % MB treatment delayed P release in the soil. Chojnacka et al. (2019) mention that the main advantage of organo-mineral fertilizers is the slow release of nutrients due to the competition mechanism of organic binders and phosphorus in the soil adsorption sites. However, slow release is an advantage when it promotes synchronisation between nutrient availability and plant uptake. Such a result was not obtained when mixing proportions greater than 12 % MB in TSP for this study.

Within each treatment, a tendency of P diffusion stabilisation was observed from day one, as observed in the initial radius of the P diffusion zone (Figures 6.6 and 6.8), which can be attributed to saturation of sorption sites they limit the movement of P in soil (Degryse et al., 2017).

6.3.3. Greenhouse Experiment

The models used to represent the SDMM data presented better fit in the square root function (Tabela 6.5). In this scenario, the dose that maximized function for TSP fertilizer was 354 mg dm^{-3} and for TSP + 12 % MB, 370 mg dm^{-3}, and the maximum dry matter yields were 11.44 g pot^{-1} and 12.58 g pot^{-1}, respectively (Figure 6.9).

Table 6.5. Regression models for description of SDMM and P content.

Fertilizer	Regression equation	R^2
	SDMM (g pot^{-1})	
TSP	$\hat{y} = 1.6314 + 1.0425^{***}\sqrt{[dose]} - 0.0277^{*}[dose]$	0.99
TSP + 12 % MB	$\hat{y} = 1.6825 + 1.1323^{***}\sqrt{[dose]} - 0.0294^{**}[dose]$	0.98
	P content (mg pot^{-1})	
TSP	$\hat{y} = 8.3522 + 0.0546^{***}[dose]$	0.93
TSP + 12 % MB	$\hat{y} = 0.8745 + 0.0740^{***}[dose]$	0.99

*significant at the 10 % probability of error level by the t-test; ** significant at the 5 % probability of error level by the t-test; *** significant at the 1 % probability of error level by the t-test.

When applying the model identity test for the square root function parameters, no significant difference (p-value> 0.05) was observed between the models.

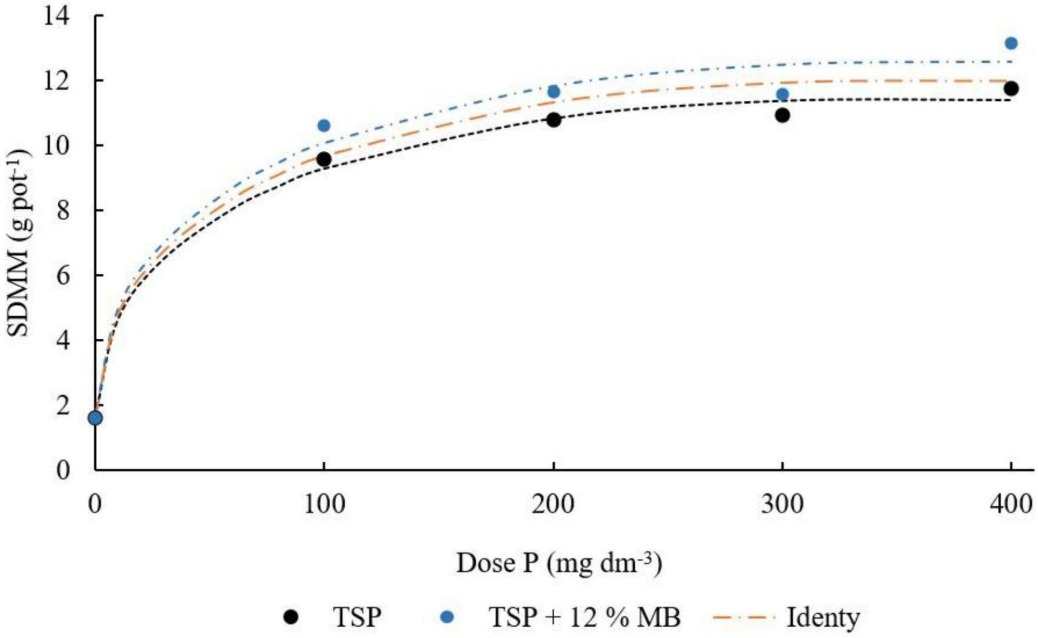

Figure 6.9. Production of SDMM according doses of P fertilizers and fertilizer sources.

Regarding the P content in plants, the data from both sources tested fit the linear model better (Figure 6.10). Up to the dose of approximately 160 mg dm^{-3}, the TSP fertilizer was superior in accumulated P content and, from then on, the TSP + 12 % MB started to give a higher accumulation of P.

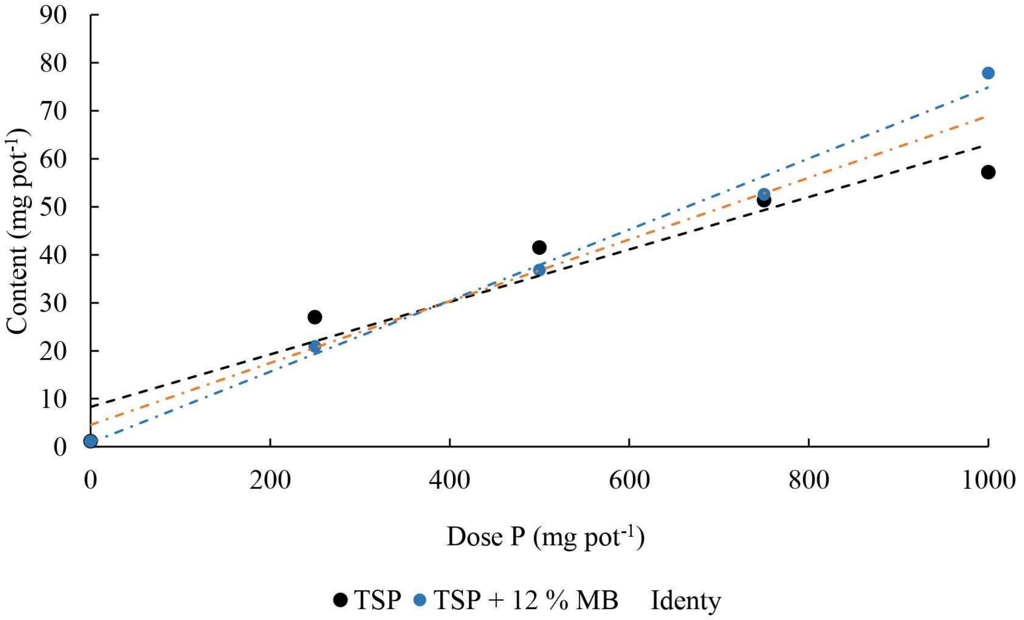

Figure 6.10. P content as a function of P doses and fertilizer sources.

Moreover, it can be inferred that TSP + 12% MB fertilizer has a P recovery rate of

7.40 %, with the addition of one unit of dose. TSP provides a 5.50 % recovery rate. When comparing the linear models in the model identity test, it can be inferred that there was no statistically significant difference at the 5 % level (p-value >0.05). The significant difference was observed at the 15% probability of error level.

Shoot dry weight results corroborated with values obtained in the literature (Table 6.6).

Table 6.6. Literature results of shoot dry weight of corn plants.

Fertilization method	N and P doses (mg kg^{-1} of soil)	Time until harvesting	Shoot dry weight ($plant^{-1}$)	Reference
TSP with microalgae biomass	200 N and 100 P – without MB	4 weeks	4.78 g	This study
	200 N and 100 P – with 12 % MB		5.29 g	
Inoculation with communities of whole rhizosphere microorganisms	71.43 N and 128.57 P – without inoculation	8 weeks	~3 g	Carmona et al., 2019
	71.43 N and 128.57 P – species obtained in Michoacán		~5 g	
	71.43 N and 128.57 P – species obtained in Guanajuato		~3 g	
	71.43 N and 128.57 P – species obtained in Estado do México		~6 g	
NPK and inoculated with biofertilizer microorganisms	187.50 N and 187.50 P – without inoculum	9 weeks	5.66 g	Larsen et al., 2017
	187.50 N and 187.50 P – inoculated with *Rhizophagys irregularis*		5.28 g	
	187.50 N and 187.50 P – inoculated with *Trichoderma harzianum*		5.56 g	
	187.50 N and 187.50 P – inoculated with *Azospirillum brasiliense*		5.36 g	
Organomineral inoculated with Mycorrhizal fungi	300 N and 92.3 P – inoculated with *G. mosseae*	~12 weeks	~7 g	Wu et al., 2005
	300 N and 92.3 P – inoculated with *G. intraradices*		~4.50 g	

6.4. Discussion

Although the purpose of the study was not to promote the formation of a coated fertilizer, this occurred when the TSP was added with ratios above 12% MB. Coatings are used to promote a synchronisation between nutrient release and absorption by plants. EEFs coatings are generally formulated so that nutrients are coated with environmentally friendly materials that can be degraded in the soil and converted to carbon dioxide, water, methane, inorganic compounds or microbial biomass (Chen et al., 2018). This is a more common and commercially available standard (Naz and Sulaiman, 2016).

In general, the coatings may be of synthetic or organic origin. Particles have advantages over synthetic ones because they are biodegradable, totally releasing the encapsulated nutrient (Schneider et al., 2016), as well as having less soil disposal time until its complete degradation.

More studies should be performed with MB biofertilizer before its classification as EEFs, since in the proportion of MB in TSP that maximizes the adsorption of P by plants, no effect on P diffusion was observed. That is, this source behaved similar to that in which MB was not applied. In contrast, higher proportions of MB (above 12%) showed a reduction in nutrient release as well as a decrease in its absorption by plants, but they did not promote synchronisation.

However, the results showed that the microalgae biofertilizer is desirable for a smart fertilizer, since the promoted phosphorus adsorption higher to conventional fertilizer. Experiments need confirmation with long-term field data. However, if this biofertilizer are proved of higher efficiency, they might be an alternative for organomineral fertilizer production, which help to recycle and add value to organic residues and reduce the pressure on finite reserves of phosphate rocks.

Regarding the adsorption of P by OM, present in biomass, the main reason is its anionic character, which, through cations such as Al, Fe and Ca adsorbed to it, would retain P. However, there are studies that show the negative participation of OM, reducing P adsorption by means of organic acids that block active adsorption sites and or solubilise these oxide oxides, reducing their adsorption surfaces (Novais et al., 2007). The addition of up to 12 % MB in the fertilizer seemed to block the phosphorus adsorption sites in the soil due to organic matter presence and allowed for greater availability of macro and micronutrients present in the microalgal biomass.

The recovery rate of P in TSP + 12 % MB may have been higher due to the relationship that macronutrients and micronutrients present in the biomass (Table 1) may have exerted in plant development and consequently in their ability to assimilate P in soil solution.

Micronutrients may also have influenced the highest concentration and content at 12% MB in the first experiment.

Among the essential micronutrients present in the biomass are Zn, Fe, Mn and B. At these low concentrations, micronutrients are critical for plant growth and development, acting as constituents of cell walls (e.g. B) and membrane integrity (e.g. B and Zn), redox systems in cells and enzyme activators (e.g. Fe and Mn), detoxification of superoxide radicals and synthesis of proteins and the phytohormone (Indole-3-acetic acid) (e.g. Zn) (Broadley et al., 2012). Despite their low concentrations in plant tissues and organs, micronutrients are just as important as macronutrients for their nutrition.

Another possibility is that phytohormones present in the biomass may have had a positive effect up to 12% MB in the fertilizer mass. Although the functional role of the phytohormones present in microalgae is still controversial, molecular evidence of the performance of these phytohormones begins to be evidenced in the literature (Lu and Xu, 2015).

6.5. Conclusion

The proportion of MB in the triple superphosphate that maximized P content and concentration in plants was approximately 12 % MB. From then on, a reduction in the values of the analysed variables was observed with the increase in the proportion of MB in the biofertilizer. Similar behaviour was observed when evaluating phosphorus diffusion in sandy and clay soils. At 30% MB, the diffusion was clearly impaired by the increase of OM in the fertilizer mass, which promoted the formation of a physical barrier in the granule.

It is suggested that the positive result exerted by MB in plants is related to the macro and micronutrients constituting the biomass. Among the macronutrients, there are N, P and K. Among the essential micronutrients present in the biomass stand out Zn, Fe, Mn and B, besides Na, which is a non-essential but beneficial micronutrient.

The addition of an organic source of nutrients produced from the treatment of an environmental liability, which even in small proportions produces positive effects on plants, is of extreme interest to what is expected from the circular economy. It is an effective strategy for nutrient enhancement that contributes to resource savings, as well as environmental and social benefits.

It is recommended to extend the temporal and spatial scale (experimental plots) to make more accurate inferences related to the technical and financial feasibility of using MB in addition with mineral fertilizers. Additionally, experiments that focus on the influence of micronutrients present in biomass in plants, as well as the characterisation of MB for the presence of phytohormones.

6.6. Acknowledgment

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7. ARTIGO III. Microalgae based biofertilizer: a life cycle approach

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Abstract: Waste, especially biomass in general, is a large reservoir of nutrients that can be recovered through different technologies and used to produce biofertilizers. In the present study, environmental impacts of the production of microalgae biomass-based phosphate biofertilizer compared to triple superphosphate through life-cycle assessment conducted in the Simapro® software were investigated. The functional unit of the analysis was 163 g of P for both fertilizers. Phosphorus was recovered from a meat processing industry effluent in a high-rate algal pond. Impacts related to the entire biofertilizer chain impacted mainly on climate changes (3.17 kg CO_{2eq}). Microalgae biofertilizer had higher environmental impact than conventional fertilizer in all impact categories, highlighting climate change and terrestrial ecotoxicity. An ideal scenario was created considering that: all energy used comes from photovoltaic panels; in the separation step a physical method will be used, without energy expenditure (i. e. gravimetric sedimentation) and; biomass will be dried in a drying bed instead of the thermal drying. In this scenario, the impact of biofertilizer approached considerably those of triple superphosphate. When impacts of biomass cultivation and concentration stages were disregarded, drying step was of great relevance, contributing to increase biofertilizer impacts. More research is needed to optimize the algae production chain and determine the possibility of obtaining higher added value products more environmental attractive.

Keywords: effluent treatment; algal biomass; high rate algal ponds; biofertilizer; nutrient recovery.

7.1. Introduction

Modern agriculture depends on the input of fertilizers for food, fiber and biofuel production. However, a significant portion of fertilizers are lost, increasing agricultural cost, wasting energy and polluting the environment (Chen et al., 2018). Phosphorus (P) is a key component of most fertilizers and essential for plant development, however efficiency of P usage barely reaches 20 % (Solovchenko et al., 2016).

This is aggravated by the fact that P is a non-renewable and limited resource. For large-scale production, its mineral form is found only in phosphate rock reserves, which are small and finite, while nitrogen (N) and potassium (K), important plant macronutrients, are exploitable by processing air and seawater respectively (Geissler et al., 2015; Owen et al.,

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2015). Thus, because the extraction rate exceeds the regeneration of reserves and the increased use of phosphate fertilizers with population growth, concerns are raised about the future scarcity of these reserves and their impact on global food security. Therefore, increasing the efficiency of fertilizer use poses a challenge for the sustainability of modern agriculture.

A strategy that has gained prominence is environmentally friendly fertilizers (EFFs), which contribute by delaying or controlling the release of nutrients into the soil, thereby reducing loss by leaching, volatilization (Chen et al., 2018) or adsorption by soil particles. It is noteworthy the use of materials, of synthetic or organic origin, mixed with the fertilizer mass, which reduces the speed of nutrient release to the soil. The goal is to promote synchronization between nutrient release and plant demand, thus preventing the nutrient provided by the fertilizer from being quickly adsorbed to the soil particle.

It is extremely important to explore any potential offer from P. P can be recovered or reused from various sources, including domestic, agricultural and industrial wastewaters. About 98 % of ingested P ends up in wastewater and accumulates in the sewage sludge (Kalmykova et al., 2015), making it an attractive resource for recovery.

Waste, especially biomass, is a large reservoir of nutrients that can be recovered through different technologies and used to manufacture fertilizers. In this aspect, a subject that has been widely studied is the cultivation of microalgae, due to the countless possibilities of using this biomass in the production of biofuels (Callegari et al., 2020), biochar (Bolognesi et al., 2019), drugs (Habibzadeh et al., 2018), food and animal feed (Javed et al., 2019; Zhang et al., 2019). When this cultivation is carried out in wastewater, this practice allows the recovery of N, P and, therefore, obtaining a nutrient (Cai et al., 2013) and organic carbon (Costa et al., 2016) concentrated algae biomass. This practice, besides promoting the wastewater treatment, achieves a microalgae biomass (MB) with diverse potential uses. However, the overall environmental impact of P recovery compared to rock phosphate extraction remains an unresolved issue (Pradel; Aissani, 2019).

Soil organic matter can correlate with P adsorption both positively, mainly due to the anionic character of organic matter, and negatively, blocking P adsorption sites in soil (Novais et al., 2007). Therefore, it should be stated that each material, whether animal waste, biochar or any biomass rich in organic material may, by virtue of its constitution, behave differently when added to the fertilizer mass.

In this context, the possibility of MB grown in wastewaters can be added to phosphate fertilizers in order to increase the absorption efficiency of P by plants. There are few published works referring to this theme (Castro et al., 2017; Marks et al., 2017), and surveys that evaluate the environmental impact of the use of this material as a source of nutrient are still incipient.

Amann et al. (2018) highlighted the importance of making assessments to ensure that alternative technologies cause less environmental impacts compared to conventional fertilizer production. Given this, life-cycle assessment (LCA) can be an important tool, since the methodology assesses environmental aspects and potential negative impacts of a product. LCA are based on the collection and quantification of materials and energy required in its production processes, as well as waste and emissions released into the environment (Souza et al., 2019; ISO 14040, 2006).

In this sense, data were obtained to prove the increase of P uptake by plants by the addition of 12 % MB in the triple superphosphate fertilizer mass, described in Castro et al. (2020). The data of the above-mentioned study were inventoried and used as input data for LCA. Therefore, this study aimed to investigate the environmental impacts of the production of a microalgae biomass-based phosphate biofertilizer compared to TSP through life-cycle assessment conducted in the Simapro® software (© PRé Consultants B.V., Amersfoort, The Netherlands - www.pre-sustainability.com).

7.2.Materials and Methods

Primary data were collected when carrying out experiments which results are detailed in Castro et al. (2020). Therefore, primary data and the technical viability of microalgae biofertilizers obtained in Castro et al. (2020) were used as modeling input data of SIMAPRO® software to verify the environmental viability of this route.

7.2.1. Algal biomass production

A high rate algal pond (HRAP) (area = 3.30 m^2 e volume = 1 m^3) was operated in batch mode (14 days of operation) to produce MB. A primary effluent collected after the flotation unit from the wastewater treatment plant of a meat processing industry was used as a microalgae culture medium.

The HRAP had a six-blade stainless steel paddlewheel, powered by a 1HP electric motor responsible for operating 12 ponds. Therefore, only twelve-tenths of all energy expended were computed in the life-cycle assessment. During the operation, the CO_2 supplementation was controlled from the pH variation in the units. In addition, it had a CO_2 injection system, in which a gas cylinder containing 99 % CO_2 and a pump were used to recirculate the effluent in the carbonation column.

After production, the biomass was harvested with 50 % m v^{-1} sodium hydroxide (NaOH), promoting a pH increase up to 12. Paddlewheel was moved for a short period of time (approximately two hours), generating a hydraulic gradient suitable for coagulation. Biomass was collected after resting the effluent inside the HRAP for 24 h. The main characteristics of the biomass at the end of the batch were as follows: volatile suspended solids = 571.80 mg L^{-1}; total phosphorus = 7.40 mg L^{-1} and total Kjeldahl nitrogen = 68.40 mg L^{-1}. Detailed information about effluent characteristics could be found in previous study (Souza et al. 2019; Costa et al., 2016).

Finally, the biomass was taken to a forced circulation greenhouse (3 kWh) for 2 days for drying. The greenhouse has been estimated to be able to dry up to 400 kg of biomass at a time. P content in dry biomass was 1.18 % (Costa et al., 2016 e Castro et al., 2020).

7.2.2. Biofertilizer production

The biofertilizer was produced through the granulation process, with the addition of 12% dry MB into triple superphosphate (TSP). This proportion was chosen, after previous experiment conducted by Castro et al. (2020). Authors tested several other addition proportions, and 12% of MB corresponds to the value that presented higher P content in the millet plant shoot (*Pennisetum glaucum* L.). The granulator plate was used in bench scale, therefore, for LCA, data from a real scale granulator were used.

Further details on the experimental methodology of biofertilizer production and application in agricultural culture can be found in Castro et al. (2020). As this work does not aim to evaluate the effect of microalgae on the plant, the methodological and results aspects were limited to the LCA content.

7.2.3. Life-cycle Assesment

The LCA was done using Simapro® software (© PRé Consultants B.V., Amersfoort, The Netherlands- www.pre-sustainability.com), and was conducted according to international standards (ISO 2006a; 2006b), which establish its structure consisting of four steps: goal and scope definition, inventory analysis, impact assessment and interpretation.

7.2.3.1. Goal and functional unit definition

The aim of this study was to evaluate the environmental impacts of the production of a biofertilizer, based on TSP and 12 % (m/m) MB, and to compare its impacts with those of the commercially available conventional fertilizer production (100 % TSP compound).

As the products have different P contents, the production of 163 g of P for both evaluated fertilizers was adopted as the functional unit. This value considers the application, performed by Castro et al. (2020), of 1 kg of granulated biofertilizer (880 g of TSP and 120 g of MB), based on the conversion value of P_2O_5 to P (conversion factor = 0.43642) and the proportion of P in MB.

7.2.3.2. Scope definition: description of the scenarios, assumptions made, boundaries and data quality requirements

The study was conducted with a cradle-to-gate approach, which does not consider the steps after product manufacture for both TSP and biofertilizer.

For LCA, the “phosphate fertilizer, as P_2O_5 {RoW}, triple superphospate production” scenario from Ecoinvent v.3 database (Ecoinvent, 2014) was used, which corresponds to the production of TSP. In this scenario, the phosphate rock is processed by wet beneficiated and the phosphoric acid is produced from wet phosphate rock with the dehydrate process in the United States and from dry phosphate rock with. The final product composition is 48 % P_2O_5.

Since the scenario adopted for TSP did not include granulation step, the present study considered the expansion with the addition of this step to allow the comparison between conventional fertilizer and biofertilizer, since the granulation step was part of the biofertilizer cycle.

The included granulation process was elaborated considering the data presented by Farahani et al. (2017), which addresses the granulation of NPK fertilizer. It corresponds to a process similar to TSP granulation and to what was done in the biofertilizer production. The authors estimated the amount of energy in the process considering the inputs of natural gas, electricity, human energies, machinery and diesel fuel. In this study, human energy was not considered as an input.

For the production of biofertilizer, the HRAP cultivation processes, concentration, MB drying and final product granulation were considered (Figure 1). This scenario also presents products avoided due to the use of effluent as a culture medium, besides the included inputs and outputs. The biomass produced during treatment corresponds to a mix of bacteria and microalgae. It was measured by the concentration of volatile suspended solids, method 2540E of the Standard Methods for the Examination of Water and Wastewater (APHA, 2012).

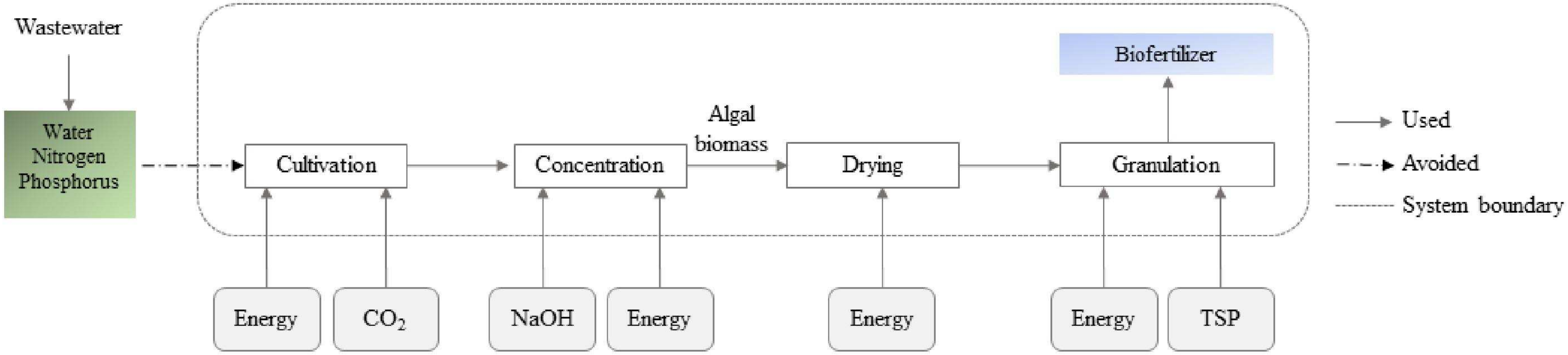

Figure 7.1. Biofertilizer product system.

In addition, the study was based on the view that biomass production corresponds to a stage in the operational process of a wastewater treatment plant (WWTP). Thus, the implementation stage of the WWTP was not taken into account, since its units have a useful life of approximately twenty-five years.

7.2.3.3. Life-cycle inventory

The biofertilizer scenario was modeled using primary data, equivalent to the operation of a HRAP, presented in Table 7.1. One operation obtained 55 L of harvested biomass.

Data were normalized to compose the process inputs and outputs that result in the desired amount of biomass (120 g MB) and thus compose the biofertilizer scenario inventory (Table 7.2). In the inventory, the input of TSP was in the form of phosphate (P_2O_5). Castro et al. (2020) used a TSP with 42 % P_2O_5 and the process used in the Ecoinvent v.3 library considers a TSP with 48 % P_2O_5. Therefore 0.370 g of P_2O_5 was used in the inventory to maintain the amount of P_2O_5 applied by the authors and, consequently, the amount of P.

Table 7.1. Primary data used as entrance in the LCA.

	Amount per operation	Time of operation	Power (kW)	Details
CO_2	0.270 kg	20 min (4 times during 5 min each)	-	CO_2 flow = 7 L/min 99% purity Density = 1.98 kg/m³
NaOH	1.515 kg	-	-	50% m/v Density = 1.515 kg/m³
Paddlewheels motor	-	24 h during 14 days	0.0613	-
Carbonation column pump	-	10 h during 14 days	0.02	-
Pump for biomass concentration	-	2 h	0.02	-
Greenhouse for biomass drying	-	24 h during 2 days	3	Useful capacity = 400 kg

Table 7.2. Biofertilizer life-cycle inventory.

Stage			Unit	Value
Cultivation	Input	Industrial wastewater	m^3	0.21
		CO_2	kg	0.057
		Electric energy	kWh	4.91
	Avoided products	Water	m^3	0.21
		Nitrogen	g	14.36
		Phosphorus	g	1.55
	Output	Treated effluent and algal biomass	m^3	0.21
Concentration	Input	Coagulant (NaOH)	kg	0.32
		Electric energy	kWh	0.0084
		Treated effluent and algal biomass	m^3	0.21
	Output	Wet biomass	m^3	0.012
Dyring	Input	Wet biomass	m^3	0.012
		Electric energy	kwh	4.32
	Output	Dry biomass	kg	0.12
Granulation	Input	Eletric energy	kwh	0.41
		Phospate fertiliser, as P_2O_5 {RoW} | triple superphospate production (48% P_2O_5)	g	0.370
		Dry algal biomass	kg	0.120
	Output	Biofertilizer	kg	0.891

The energy used as input in both scenarios (biofertilizer and TSP) was the Brazilian energy mix, made available by the Ecoinvent v.3 database from 2008 to 2014. Figure 7.2 shows the types and proportion of each energy source used to compose the Brazilian energy mix. Moreover, another scenario was used in the modeling, in order to replace the Brazilian energy mix with the Dutch energy mix. The predominant energy sources in both countries are very different from each other, justifying their choice. While in the Brazilian mix the hydroelectric energy is the main energy source, the Dutch has its highest percentage composed of natural gas (94.2%).

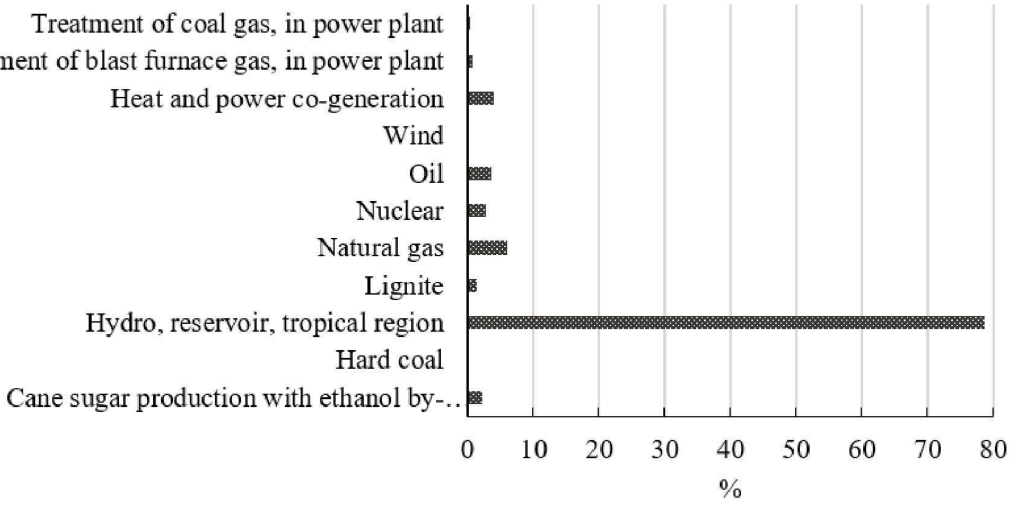

Figure 7.2. Brazilian Energy Mix (Ecoinvent 2014).

During the production stage, the inputs were the values of electric energy (kWh) used for the paddlewheels rotation and the effluent recirculation pump into the carbonation column to add the CO_2 into the HRAP. Water and nutrients were not assumed as inputs due to the use of the effluent.

The NaOH used was obtained from the Ecoinvent database, and its inventory includes the electrolysis process in a diaphragm cell, the following process steps are included into this process: brine production, brine purification, brine resaturation as well as the final handling of the electrolysis products, without the chlorine liquefaction (Ecoinvent, 2014).

Finally, for conventional fertilizers, impacts of 373 g of phosphate fertilizers (the same used in the biofertilizer scenario and presented in Table 2) were calculated, which is equivalent to 777 g of TSP (48 % P_2O_5) available from Ecoinvent. To granulate this amount of fertilizer, 0.36 kWh of energy demand was considered (Farahani et al., 2017).

7.2.3.4. Impact categories and LCA methodology

To quantify and evaluate life-cycle impacts, the SimaPro 8.2.3.0 software was used through the ReCiPe midpoint methodology, which is focused on environmental issues, making it, therefore, the most qualified method for this study (PRÉ, 2013). The impact categories evaluated were climate change, freshwater ecotoxicity, terrestrial acidification, freshwater eutrophication, particulate matter formation, terrestrial ecotoxicity and fossil depletion. These categories present as appropriate for the study and were also used in other works involving phosphate fertilizers (Pradel; Aissani, 2019; Silva; Kurlay, 2005; Silva; Kurlay, 2003) and microalgae biomass (Souza et al., 2019). In addition, for both scenarios, long-term emissions were disregarded.

2.3.1. Sensitivity analysis

Sensitivity analysis is a procedure used to estimate the effects of selected methods and data on the results of a study (NBR ISO 14041, 2004). Based on the LCA and the environmental impacts arising from each process, the sensitivity analysis was performed with the removal and/or replacement of the process inputs.

From this perspective, four different scenarios were created:

(i) Energy from cultivation to biofertilizer granulation was replaced by photovoltaic energy. The inventory for the photovoltaic panel was obtained from the Ecoinvent database (2014). The database considers electricity production with a grid-connected 3 kWp multi-Si (polycrystalline silicone cell) photovoltaic panel mounted on a tilted roof;

(ii) The NaOH used in the biomass concentration was removed as a way to simulate a gravitational physical process of harvesting the biomass via sedimentation, without the use of chemical reagents;

(iii) Drying energy was withdrawn to simulate other drying methods that do not require electrical energy for their execution (i. e. solar drying).

(iv) Disregarding production/cultivation and concentration stages from algal biomass, considering that impacts from these stages should be reallocated to the WWTP as wastewater treatment is compulsory by law.

7.3. Results and discussion

Figure 7.3 presents a summary of the life-cycle inventory used for biofertilizer production, as well the phosphorus mass balance throughout the process.

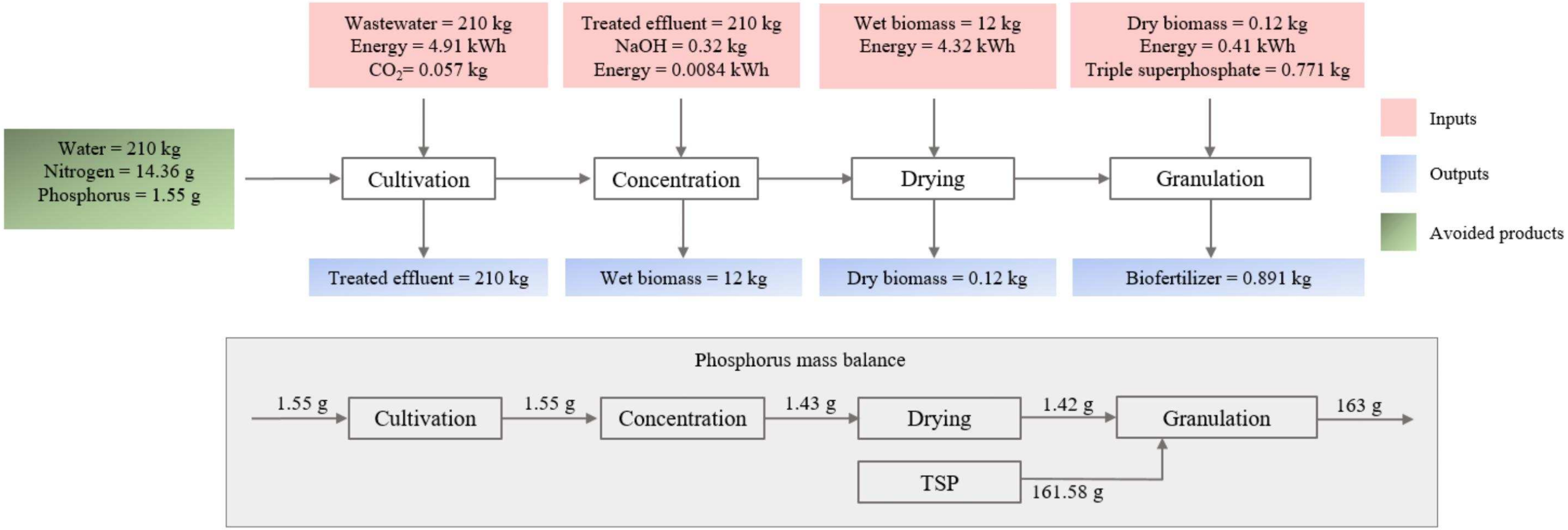

Figure 7.3. Life-cycle inventory of the biofertilizer production.

P losses during the treatment and use of microalgae biomass as biofertilizer were mainly due to the handling and transport of the biomass throughout the process, which can be considered insignificant. In the production of biomass, CO_2 injection was made in order to provide a greater P removal, since it allows the control of pH and prevents the chemical precipitation of phosphorus.

Scenarios were modeled considering the Dutch energy mix to replace the Brazilian energy mix. Of the seven impact categories, the climate change category was the only one in which the Brazilian energy mix had more negative impact (12%). This result can be explained, since the Ecoinvent database considers the change in land use to construct and install the hydroelectric plant to be more harmful than the extraction and use of natural gas. In the categories terrestrial acidification, freshwater eutrophication and particulate matter formation the impacts were the same for both mixes. In the terrestrial ecotoxicity, freshwater ecotoxicity and fossil depletion categories, the impact of the Dutch mix was greater (33%, 25% and 29%, respectively).

7.3.1. Comparison between microalgal biofertilizer and triple superphosphate impacts

The environmental impacts comparison, in relative percentage, of the biofertilizer and TSP scenarios are presented in Figure 7.4. Biofertilizer presented the greatest environmental impacts in all categories, especially in the terrestrial ecotoxicity (87.33 % higher) and climate change (75.28 % higher) categories, where the largest discrepancies occurred.

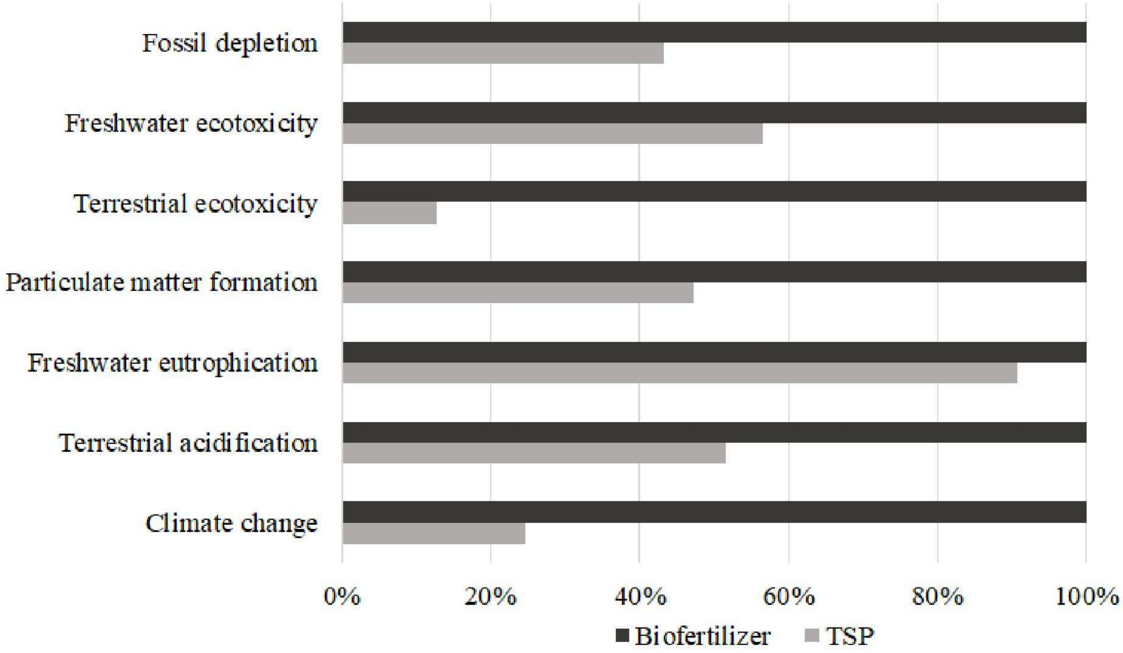

Figure 7.4. Percentage comparison of impacts between TSP and biofertilizer.

The climate change category contributed with the highest total impact for both scenarios (3.17 kg CO_2 eq. and 0.78 kg CO_2 eq. for biofertilizer and TSP, respectively). Also, when analyzing the contribution of impacts by processes in the biofertilizer scenario, this category also stands out, having a greater impact on all discriminated processes (Table 7.3).

Tabela 7.3. Impacts generated in the different phases of the process.

Product	Impact category	Climate change	Terrestrial acidification	Freshwater eutrophication	Particulate matter formation	Terrestrial ecotoxicity	Freshwater ecotoxicity	Fossil depletion
Biofertilizer	Unity	kg CO_2 eq	kg SO_2 eq	kg P eq	kg PM10 eq	kg 1,4-DB eq	kg 1,4-DB eq	kg oileq
	Cultivation	0.9530	0.0025	0.0000	0.0014	0.0015	0.0008	0.1220
	Concentration	0.4610	0.0029	0.0000	0.0011	0.0000	0.0004	0.1190
	Drying	0.9640	0.0030	0.0000	0.0015	0.0013	0.0009	0.1390
	Granulation	0.7880	0.0090	0.0007	0.0036	0.0004	0.0026	0.2870
	Final product	3.1660	0.0174	0.0007	0.0076	0.0032	0.0047	0.6670
TSP	Final product	0.7820	0.0090	0.0007	0.0036	0.0004	0.0026	0.2880

Among the processes, in general (except for climate change and terrestrial ecotoxicity) granulation was the stage that most contributes to impact generation, because it covers, besides the impact of energy consumption to granulate the biofertilizer, the impacts from TSP production.

Climate change is cited as the most important category when working with LCA for chemical fertilizer production (Hasler et al., 2015). For phosphate fertilizers, this is due to greenhouse gas (GHG) emitted in the phosphorus extraction steps and reactions between phosphate rock and sulfuric acid (Skowronska; Filipek, 2014). However, since the scenarios presented close TSP inputs (777 g and 771 g) and biofertilizer is about 75 % more impactful in this category, much of this impact is associated with the algal biomass production and processing steps.

Arashiro et al. (2018) compared the HRAP system associated with fertilizer production with the activated sludge system (technology conventionally used for wastewater treatment) using LCA. The authors pointed out that the first scenario offers lower impacts on the categories of climate change, ozone layer depletion, photochemical oxidant formation and fossil depletion. This result was attributed to the lower energy consumption required for HRAP operation when compared to the activated sludge system. In contrast, they found higher impacts for the terrestrial acidification, particulate matter formation and terrestrial ecotoxicity categories caused by NH_4^+ volatilization in HRAPs and the heavy metal content in the biofertilizer.

Thus, greater efforts are needed so that the recovery of P, through the cultivation and processing of microalgae, contributes to the preservation of this mineral resource without negative impacts of greater magnitude. Therefore, based on what was presented, it is essential to investigate strategies so that the steps consume less energy and/or make use of a less impacting energy source. Energy consumption is pointed as the main contributor to the environmental impacts of microalgae production. (Pérez-López et al., 2014; Souza et al., 2019). In this study, this was verified considering that energy was the most used input, requiring 6.51 kWh to produce 1 g of P (includes cultivation, concentration, and drying). Thus, the increase in impacts for the biofertilizer scenario in the climate change category can be justified.

Souza et al. (2019) investigated, through LCA, the environmental impacts of applying a microalgae-based biofertilizer for nitrogen (N) recovery and also identified climate change as the most impacting category. According to the authors, these impacts were mainly attributed to the microalgae cultivation stage and are justified by the high electricity consumption for low microalgae productivity, due to their competition with other microorganisms (fungi, bacteria and protozoa). This argument also justifies the present study, considering that the cultivation system was similar to that of above-mentioned authors. When considering P recovery, this is even more critical, since P is available in effluents in smaller quantities than N and is required in agriculture in similar quantities compared to nitrogen fertilizer.

The avoided products reduced the impacts related to the cultivation stage, which reflected in the benefit of using effluent as microalgae cultivation medium. However, this was not sufficient to offset the impacts (Figure 7.5).

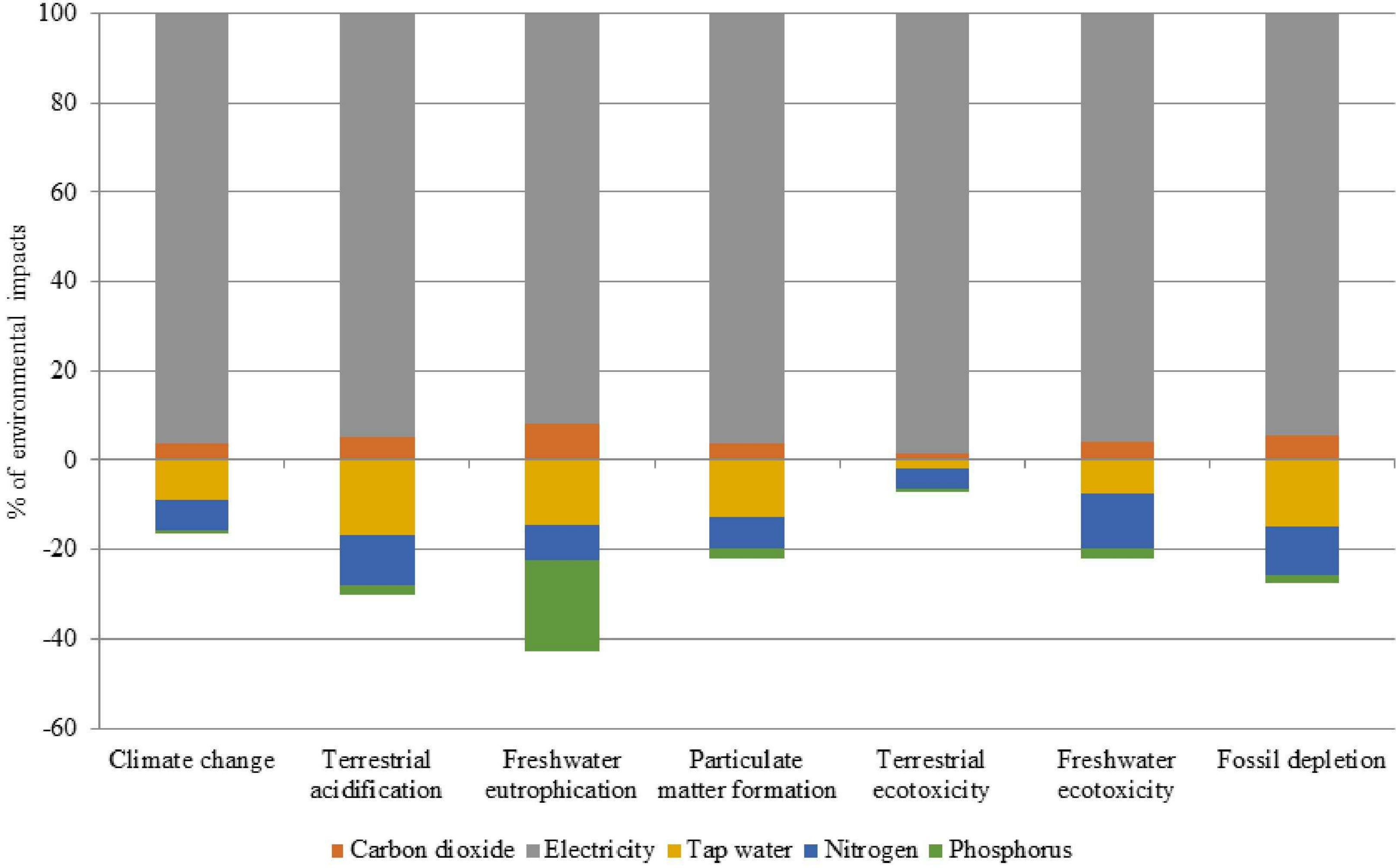

Figure 7.5. Environmental impacts related to inputs and avoided products, in percentage, at the cultivation stage.

Energy consumption also represents a barrier to other eco-friendly P recovery alternatives. Pradel; Aissani (2019), studying four different sludge-based fertilizer scenarios, pointed out that the required energy and other factors disfavor sludge-based products, which require about 99.40 % to 97.90 % more electricity. Chiew et al. (2015) found more favorable results regarding energy use, comparing the environmental impacts of a fertilizer produced from anaerobic digestion of food residues, combined with biogas reuse with the use of chemical fertilizer plus incineration of food residues. Both scenarios presented negative net primary energy balance, i.e., they avoided more than consumed primary energy. Even so, the digestate fertilizer proved to be more advantageous in dispensing with the use of nonrenewable phosphate rock, among all categories evaluated (primary energy use, global warming potential, potential acidification, potential eutrophication, cadmium flow to farm land and use of phosphate rock). Global warming potential was the category with the largest amount of impacts generated and, according to the authors, it is still necessary to investigate improvements in the digestion system so that the digestate fertilizer has better results than the conventional fertilizer in this category.

7.3.2. Identifying room for improvement for decreasing environmental impacts of microalgae biofertilizer

7.3.2.1. Source of energy

In this scenario, energy from the Brazilian energy mix, available in the Ecoinvent database from 2008 to 2014 (~ 80 % of the hydroelectric source) was replaced by photovoltaic energy. Results are shown in Table 7.4.

Table 7.4. LCI comparing biofertilizer production using hydroelectric energy with the photovoltaic energy.

Impact category	Unity	Biofertilizer	Biofertilizer (panel)	Reduction (%)
Climate change	kg CO_2 eq	3.1700	1.6700	47.3186
Terrestrial acidification	kg SO_2 eq	0.0175	0.0154	12.0000
Freshwater eutrophication	kg P eq	0.0007	0.0008	-14.2857
Particulate matter formation	kg PM10 eq	0.0077	0.0060	22.0779
Terrestrial ecotoxicity	kg 1.4-DB eq	0.0033	0.0016	51.5152
Freshwater ecotoxicity	kg 1.4-DB eq	0.0046	0.0034	26.0870
Fossil depletion	kg oil eq	0.6670	0.5350	19.7901

In the freshwater eutrophication category, the energy source change resulted in increased environmental impact, as can be seen in Table 7.4, where the impact reduction for this category was negative. This is mainly due to the presence of various carcinogenic heavy metals involved in the construction of photovoltaic panels. Carcinogenicity is one of the main parameters used in the impact calculation and therefore has considerable weight (Goedkoop et al., 2009). For other categories, impacts were reduced, especially the climate change category, since this scenario does not use hydroelectric energy or other energy sources from fossil fuels.

In general, the replacement of the energy source brings more favorable results for biofertilizer. Impacts were reduced in most of the categories investigated and in the climate change category (approximately 47 % reduction), which has the largest contribution to environmental impacts, as discussed earlier. Still, the reductions presented do not make biofertilizer less impactful in any category yet, compared to conventional fertilizer.

Photovoltaic panels have been the target of LCA studies and less impactful scenarios have been found. Among the strategies being studied, the use of emerging photovoltaic panel technologies stands out (Tsang et al., 2016), different panel installation processes (Antonanzas et al., 2019; Tsang et al., 2016) and recycling proposals (Del Pero et al., 2019; Klugmann-Radziemska; Kuczyńska-Łażewska, 2020; Pandoan et al., 2019; Rocchetti; Beolchini, 2015). Thus, advances in this direction can be investigated to reduce impacts on biofertilizer production and, more specifically, the production and processing of microalgal biomass.

7.3.2.2. Biomass dewatering, harvesting and drying methods

It was found that the use of an alternative (i.e. gravimetric sedimentation) separation method that dispenses the use of NaOH could contribute to a more optimistic biofertilizer scenario, considering all impact categories (Figure 7.6). This scenario avoided all impacts associated with NaOH production, such as sodium chloride depletion (Pradel; Aissani, 2019), gaseous emissions of SO_4^{2-} chlorinated waste generation to water and air, and the open-air deposition of hazardous waste.

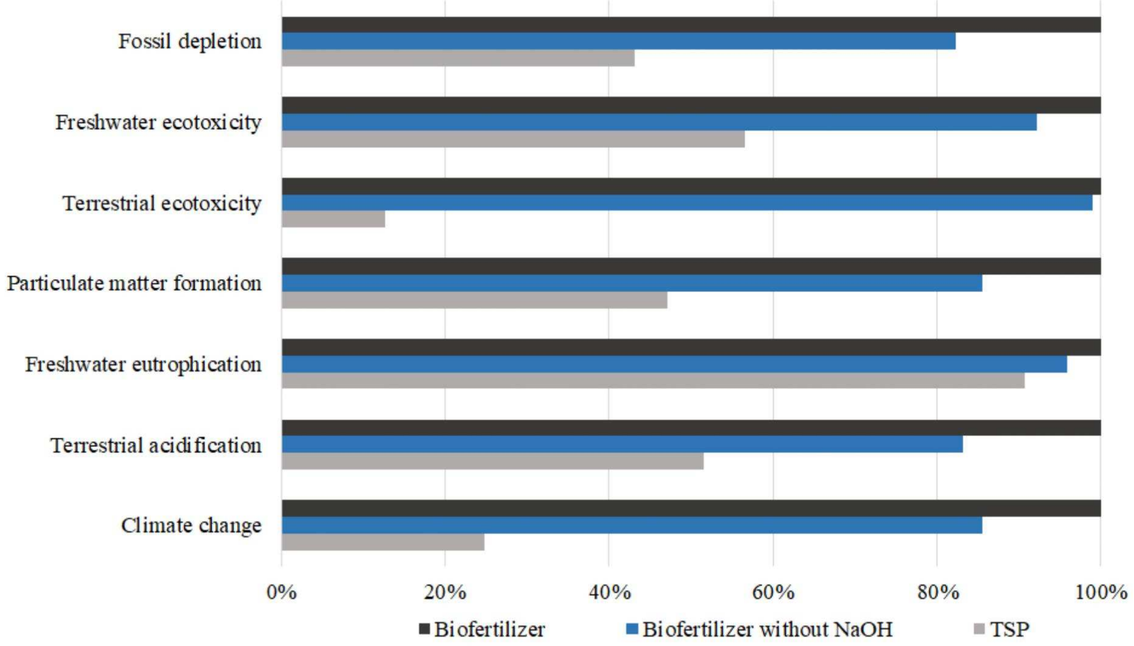

Figure 7.6. Percentage comparison of impacts between biofertilizer and biofertilizer without NaOH.

Although the biofertilizer without NaOH did not become less impacting than the TSP, it presented a more favorable scenario than the one in which NaOH was used. The fossil depletion category had the highest impact reduction (about 18%). Souza et al. (2019) point out that the amount of NaOH is considerably greater than the required amount of a conventional coagulant for this process, however, the use of coagulants such as ferric chloride and aluminum hydroxide causes the bioaccumulation of metals and alters the chemical characteristics of biomass to be used subsequently as fertilizer. Thus, further investigation is needed to overcome this barrier.

In the case of harvesting/concentration of microalgae biomass, there are several methods cited in the literature to perform this process. Among them the centrifugation (Mennaa et al., 2015); filtration (Milledge; Heaven, 2013); ozoflotation (Velasquez-Orta et al., 2014); coagulation (Udom et al., 2013); flocculation/coagulation (Drira et al., 2016). As can be seen, such methods include the addition of chemicals or the use of mechanical equipment that increases costs and process-related impacts. Udom et al. (2013) evaluated the cost-benefit and environmental impact of common harvesting techniques, namely: centrifugation, sun bed drying and belt presses. The authors concluded that centrifugation has the highest energy consumption in the life-cycle (700 MJ/ton of dried seaweed) and GHG emission (50 kg CO_2eq/ton of dried seaweed), while solar drying beds have the lowest energy consumption life-cycle energy (2.80

MJ/ton of dried algae) and the lowest GHG emissions (0.20 kg CO_2eq/ton of algae). However, the authors mentioned that the solar drying beds a can allow a solid content of approximately 40 %. Alternatively, some research mention bioflocculation or spontaneous flocculation as a mean of harvesting (Gutierrez et al., 2016; Úbeda et al., 2017; Yunos et al., 2017).

Still, the possibility of using support for attached growth of biomass in the cultivation reactors has been studied (Assis et al., 2019; Assis et al., 2017; Eliseus et al., 2017). This technology has proven effective in microalgae harvesting as it ensures complete biomass retention and offers an economical option (Bilad et al., 2012 a, b). In this regard, studies on the positioning of the membranes, the material constituting them and the evaluation of the life-cycle of the materials used in their constitution is of paramount importance to advance the theme. Such approaches, although not dispensing the drying step, could possibly contribute by avoiding the use of chemicals and offering better results regarding the concentration of harvested biomass. An effective harvesting method that minimizes biomass moisture is important, since post-harvest thermal drying is a very costly process (Sharma et al., 2013).

With respect to biomass drying, when the energy used in the biofertilizer scenario was removed, the impacts were considerably reduced, except for the climate change and terrestrial ecotoxicity categories (Figure 7.7).

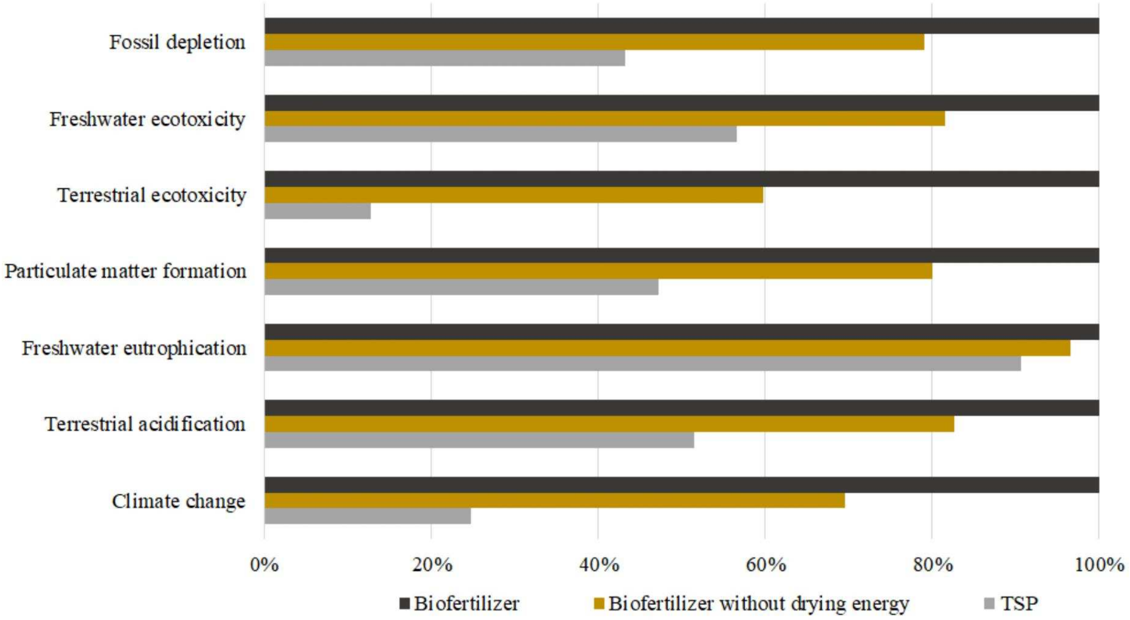

Figure 7.7. Percentage comparison of impacts between biofertilizer, biofertilizer without drying energy and TSP.

Various processes can be used for biomass drying, such as: solar, drum, atomization, spray and lyophilization drying. Solar drying requires no energy from fossil sources and is, therefore, a less expensive option. On the other hand, it depends on the climate, demands a very large area and can cause considerable denaturation of organic compounds (Milledge; Heaven, 2013). Among the cited processes, lyophilization tends to cause less damage to organic materials than spray drying, but is a more costly option (Brennan et al., 1969).

Therefore, it is believed that the best option is to invest in a more efficient dewatering process during harvesting. Milledge; Heaven (2013) suggest minimizing the water content of the harvested microalgae biomass before drying.

7.3.3. Best Scenario

In order to delineate an ideal scenario for microalgae biofertilizer, an analysis was performed that considers the use of photovoltaic panel, the biomass concentration without the use of NaOH and the natural drying of the biomass through drying bed. The result obtained is presented in Figure 7.8.

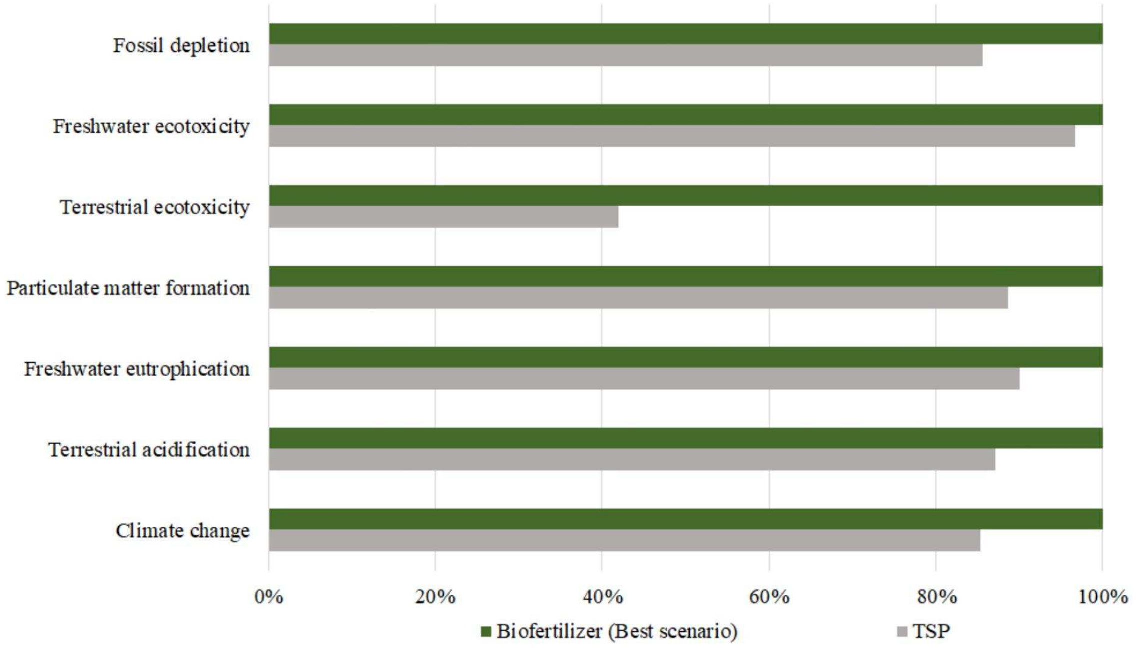

Figure 7.8. Percentage comparison of impacts between the best scenario of the biofertilizer and TSP.

A significant reduction in impact discrepancies was found when comparing biofertilizer and TSP scenarios, except for terrestrial ecotoxicity (the difference was still approximately 58

%). For the climate change category, there was a significant reduction of impacts through this scenario, showing a possible optimization of the production process through this ideal scenario proposal.

If 100 % of P from MB was used, this would result in a decrease of plant productivity and possible reduction in P recovery rate by plants, as mentioned by the experiment conducted by Castro et al. (2020), indicating that a higher percentage of MB is technically unfeasible. Although biofertilizer was still more impactful considering the best scenario, the above authors reported that TSP + 12 % MB fertilizer had a P recovery rate of 7.40 % whereas TSP provided a P recovery rate of 5.50 %. Therefore, over a longer period of time, this increase of soil P can bring environmental benefits that outweigh the impact.

In addition, the effects related to the production/cultivation and concentration stages of microalgae biomass were disregarded in the biofertilizer cycle and this scenario was compared to TSP impacts (Fig. 9). In this scenario, the impacts of the biofertilizer were reduced, but the drying stage had a significant impact on the biofertilizer production. This result highlighted the bottleneck of using dry microalgae biomass, unless drying is carried out naturally, without energy extensive consumption.

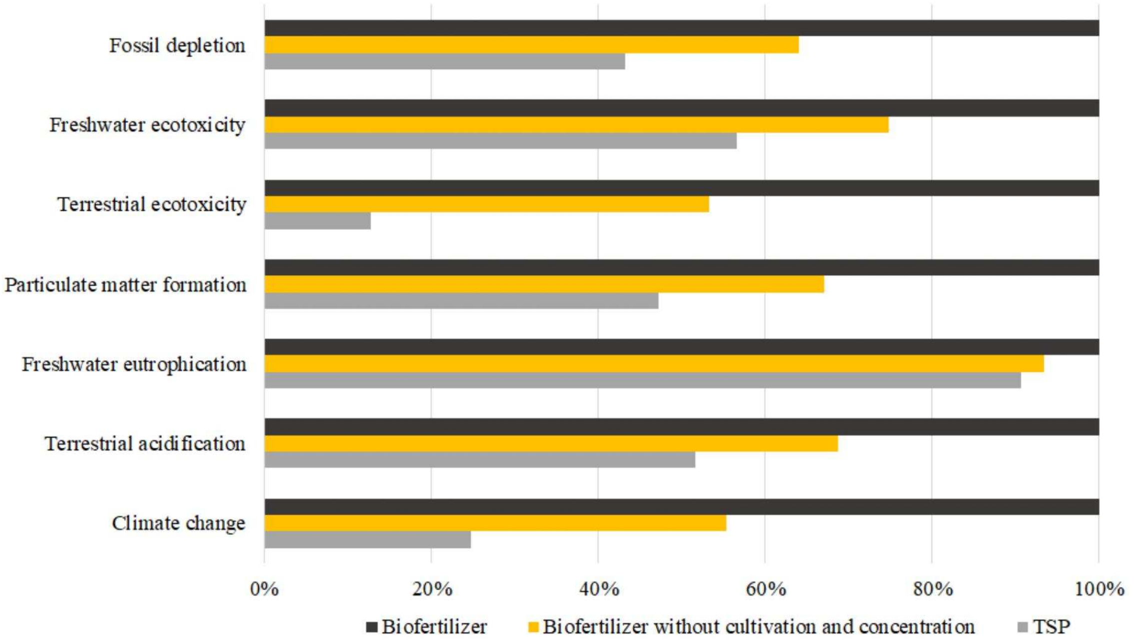

Figure 7.9. Percentage comparison of impacts between the scenario of disregarding impacts of the production/cultivation and concentration stages of algal biomass production for the biofertilizer obtaining and TSP.

Souza et al. (2019) performed a LCA of microalgae biomass compared to urea as fertilizing sources. In a first scenario that considered the stages of cultivation, concentration and application of wet biomass in the soil, the authors observed that algae biomass as a nutrient source was more impactful than urea. However, by removing the cultivation step, algae biomass became more attractive from an environmental point of view. Therefore, the use of more sustainable energy source in the production/cultivation stage is highlighted or even other methods of drying, as already mentioned throughout this manuscript.

In order to evaluate this impact, it is suggested to extend the temporal and spatial scale of the experiment with plants in experimental plots to make more accurate inferences related to the technical, economic and mainly environmental viability of using MB in conjunction with mineral fertilizers. All the benefits of MB can culminate in reduced environmental impact and can make this route viable.

7.4. Conclusions

The main contribution of this study was the comparison of biofertilizer with 12 % MB with conventional fertilizer, from the environmental point of view, with the use of the LCA tool to identify the main gaps involved in this technology. Results can assist in advancing technology that is still under development, in order to make it more competitive.

Microalgae biofertilizer had higher environmental impact than TSP in all impact categories, highlighting climate change and terrestrial ecotoxicity. As the scenarios presented very close TSP inputs (777 g and 771 g) and biofertilizer was about 75 % more impactful in this category, much of this impact was associated with the algal biomass production and processing steps.

An ideal scenario was created considering that: all energy used comes from photovoltaic panels; in the separation step a physical separation medium (i.e. gravimetric sedimentation) and; the biomass will be dried in a drying bed instead of the thermal drying. In this scenario, the impact of biofertilizer approached considerably those presented in the TSP, except in the terrestrial ecotoxicity category, where the biofertilizer still had approximately 58 % greater impact. The impacts of microalgae fertilizer remained greater in all impact categories. A scenario disregarding the biomass production/cultivation and concentration stages was considered in LCA of microalgae biofertilizers, so that impacts of the production/cultivation stage should be reallocated to the WWTP. However, drying step was of great relevance, contributing to increase biofertilizer impacts compared to TSP. Less energy consumption method should be considered, making the biofertilizer production process from algal biomass more environmentally attractive.

More research is needed to optimize the algae production chain and determine the possibility of obtaining higher added value products. In addition, experiments that focus on the influence of micronutrients on plant biomass are of great relevance. All the benefits of MB can culminate in reducing negative environmental impact making this route viable.

7.5. Acknowledgements

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8. ARTIGO IV. Hydrothermal carbonization of microalgae biomass produced in agro-industrial effluent: products, characterization and applications

Abstract: Hydrothermal carbonization is a thermochemical treatment whose objective is to convert carbohydrate components of a given biomass into carbon-rich material in an aqueous medium. Biomass of microalgae grown in wastewater is among the potential biomasses for this route. However, operational parameters of hydrothermal carbonization for different types of biomass are still being investigated. In general, larger temperature ranges (180–260 °C) are applied to woody biomass, which have fibrous and/or ligneous structures and, therefore, are more thermally stable than algae biomass. This study presents the hydrothermal carbonization of microalgae biomass grown in effluents. For this purpose, a Parr reactor was operated at different temperatures (130, 150 and 170 °C) and retention times (10, 30 and 50 min). The results show improvements in the properties of the hydrochar, mainly energy yield and carbon concentration, after the thermochemical treatment such as energy recovery and increased hydrophobicity of the carbonized material. It was observed that in the retention time of 10 minutes, the increase in temperature provided an increase of 7.53% in the yield of solids. In the retention times of 30 and 50 minutes, when the temperature was increased, the solid yield decreased 6.70% and 0.92%, respectively. Thus, the highest yield of solids (77.72%) and energy (78.21%) was obtained at a temperature of 170 °C and a retention time of 10 minutes. There was a high ash content in the raw biomass (32.99%) and an increase of approximately 3% in the carbonized material, regardless of the treatment applied. With the exception of potassium and sodium, the other macro and micronutrients were concentrated in the hydrochar after thermochemical treatment, indicating the potential of the material for agriculture application, in addition to energy use. Results showed that the retention time was the most significant parameter of the process.

Keywords: Hydrochar; Bioenergy; Biorefinery; Thermochemical processes.

8.1. Introduction

Microalgae are unicellular eukaryotic organisms that use sunlight, CO_2, water and nutrients to produce biomass. They are recognized as a valuable resource for transforming biomass into bioenergy (Arashiro et al., 2019; Cancela et al., 2019; Couto et al., 2018; Miyawaki et al., 2021) and bioproducts, as well as biofertilizer (Castro et al., 2017; Castro et al., 2020) and hydrochar (Chu et al., 2020b; Khoo et al., 2020). However, one of the main challenges for the industrial cultivation of microalgae is the high environmental and financial costs due to the necessary inputs for their production (Belete et al., 2019). In the search for the solution of this challenge, several studies address the use of effluents for the cultivation of a mixed biomass, composed of microalgae and other microorganisms (Couto et al., 2018; Assis et al., 2019; Assemany et al., 2020). The use of effluents as a culture medium aims to make use of the water and nutrients naturally present in the waste, thus reducing production costs. In the end, there is the treated effluent and a biomass that can be used for different recovery routes.

The processes of thermochemical conversion of algae biomass into biofuels or bioproducts are a route that has gained prominence in recent years. These processes include hydrothermal carbonization (HTC), hydrothermal liquefaction (HTL) and hydrothermal gasification (HTG), which occur in the presence of water (Barreiro et al., 2013). The advantage of these processes is to avoid the biomass drying step, which considerably increases the energy consumption required to remove moisture. Of the processes occurring in an aqueous medium, the HTC has shown potential, especially for acting in milder conditions of temperature and pressure compared to the others, demanding less energy and material resources for operation.

The HTC is defined as a thermochemical treatment, the purpose of which is to convert carbohydrate components of a biomass into carbon-rich material in an aqueous medium. The HTC occurs in hydrothermal media or hot compressed water at temperatures in the range of 180–260 °C (Cao et al., 2019; Chen et al., 2012; Bach et al., 2013; Bach et al., 2016; Khoo et al., 2020; Leng et al., 2020b). For microalgae biomass, the first study reported in the literature was carried out by Heilmann et al. (2010), and since then, the HTC of this biomass produced in synthetic medium has been investigated by several other authors (Heilmann et al., 2011; Levine et al., 2013; Lu et al., 2015; Ekpo et al., 2016; Smith et al., 2016; Bach et al., 2017; Lee et al., 2018; Cao et al., 2019; Zhao et al., 2019; Leng et al., 2020b). Among the most researched subjects, the main focus is given to the search for studying and/or optimizing the operational parameters related to this thermochemical process, aiming to concentrate energy at low temperatures without losing organic material.

The hydrochar (solid phase) is the product resulting from HTC. This product can be used for several purposes, depending on its chemical and physical characteristics. Among the possible applications of this material are: solid fuel (Bach et al., 2013; Bach et al., 2017; Liu et al., 2019); low cost adsorbent (Flora et al., 2013; Takaya et al., 2016; Zheng et al., 2017); soil amendments (Chu et al., 2020a; Chu et al., 2020b; Chu et al., 2020c); use as a substrate for removing trace metals (Xue et al., 2012; Sun et al., 2015); and for removing organic pollutants (Chung et al., 2015; Chung et al., 2016). The use of hydrocarbons applied in the soil has attracted attention. For example, the application of the bentonite hydrochar promoted some benefits for rice culture. It increased the rice yield and plant N use efficiency, and reduced NH_3 volatilization (Chu et al., 2020a). According to the authors, these results occurred due to the acidic property, larger pore volume and specific surface area of the bentonite hydrochar, which contributes to soil NH_4^+-N retention. On the other hand, Chu et al. (2020b) verified that *Chlorella vulgaris*-derived hydrochar, despite increasing the grain yield of the rice, it also increased the ammonia volatilization and N_2O emissions. Therefore, efforts are needed when concern applying algae biochar for agricultural purposes.

During the carbonization, there is also the production of aqueous phase (AP) in large amounts. The AP, although there are few studies that characterize it, needs to be treated and/or reused given the concentration of nutrients that remain in solution after thermochemical treatment. (Belete et al., 2019; Leng et al., 2020b). The treatment and utilization of AP are becoming a bottleneck for the industrialization of HTC (Leng et al., 2020a). In addition, recirculation, whether for the carbonization reactor itself (Leng et al., 2020b) or for the biomass cultivation reactor (Leng et al., 2020b; Khoo et al., 2020), is a way to reduce costs and negative environmental impacts in the process of effluent treatment, production and use of microalgae biomass. As the AP is rich in carbon and nutrients (N and P), its recirculation to the culture medium may enhance the biomass production. When recirculated to nutrient-poor wastewater, microalgae can be allowed to develop. In this case, AP represents an alternative source of low-cost nutrients for the cultivation of microalgae. In addition, the bioenergy recovery from AP by anaerobic digestion, supercritical water gasification, microbial fuel cell and microbial electrolysis cell has been studied (Leng et al. 2020a).

There are still few studies that report the HTC of wastewater grown microalgae biomass (Lee et al., 2019; Liu et al., 2019; Marin-Batista et al., 2019). Marin-Batista et al. (2019) investigated the HTC coupled with anaerobic digestion for thermochemical treatment of microalgal biomass (grown in 10% diluted centrifuged pig manure) and AP digestion. The authors studied HTC at 180, 210 and 240 ºC during 1 hour of reaction time and reported the highest levels of carbon (40.7% and 41.8%) and higher heating value (HHV) (18 and 18.6 MJ kg^{-1}) in the treatment of 180ºC and 210 ºC, respectively. Hydrochar's HHV was comparable to HHV of the lignite. There is a need for further research with different culture media, operational parameters, as well as the possibility of inserting this thermochemical process in the context of microalgae biorefinery.

Therefore, this study aimed to investigate the effect of HTC from microalgae biomass grown in agro-industrial effluent, in relation to fuel efficiency and properties. HTC was carried out under different temperatures and retention times. The main indicators of HTC performance were solid yield, higher heating value (HHV) and energy yield. Analyzes of the fuel structure were performed by Fourier-transform infrared spectroscopy (FT-IR), scanning electron microscope (SEM) and X-ray diffraction (XRD), and changes in its structure due to the process conditions were discussed. The thermal stability of raw and carbonized biomass was studied using thermogravimetric analysis (TG and DTG). Finally, the AP resulting from HTC was characterized with the aim of supporting decision making about its possible application.

8.2. Materials and methods

8.2.1. Algae biomass production

The microalgae biomass was cultivated as a by-product of the wastewater treatment of a meat processing industry in high-rate algal ponds (HRAPs). The main activity of this industry is the production of sausages (salami and hams, among others) and the production of shredded desalted codfish. The wastewater of industrial origin is generated in the various stages of the production process, especially in the discarding of the cooking and cooling tanks of sausages and of the desalting tanks of cod and also the washing of the floors and equipment at the end of the production processes. In this research, the primary effluent from the flotation stage was used.

The pilot-scale HRAPs used for biomass production have the following characteristics: width = 1.28 m, length = 2.86 m, total depth = 0.30 m, useful depth = 0.30 m, surface area = 3.30 m^2 and useful volume = 1.00 m^3. The HRAPs were made of fiberglass with pedals of stainless steel, with six blades. During operation, the paddle wheels were powered by a 1 hp electric motor. The rotation was reduced by a reducer coupled to the motor and controlled by inverter frequency (series WEG CFW-08), which ensured a liquid velocity between 0.10 and 0.15 $m\ s^{-1}$. Similar values were used in different research with HRAPs (Picot et al., 1991; J. B. K. Park et al., 2011) and ensured the necessary revolving.

The biomass was produced in a batch system lasting 17 days (April 2019). In the operation, 40% of inoculum was used in relation to the total volume of the pond, which consisted of microalgae biomass previously adapted to the conditions of the wastewater. At the end of the operation, the biomass was concentrated by chemical flocculation, with the addition of 50% NaOH solution ($m\ v^{-1}$) to raise the pH to 12. Then, the wastewater in the HRAP was stirred with the aid of a plastic container for 3 min, and the sedimentation occurred in the HRAP itself during the night. The concentrated biomass at the bottom of the HRAP was collected manually using plastic containers after the wastewater disposal. After collection, the pH of the concentrated biomass was neutralized with the addition of 1:1 HCl solution ($v\ v^{-1}$).

8.2.2. Algae biomass characterization

The wet biomass was submitted to phytoplankton analysis. The samples were collected and then preserved in a 4% formaldehyde solution (1:10 ratio between sample and solution (m v^{-1})). The main species within each genus were identified based on Parra et al. (1982) and Komarek and Fott (1983), according to Utermöhl methodology (1958); Wetzel and Likens (1991).

Subsequently, the samples were lyophilized, macerated and analyzed. The determination of protein content was carried out by the method of total Kjeldahl nitrogen (TKN), using a conversion factor of 6.25 g g^{-1} of the nitrogen content in protein (AOAC, 2000). The determination of the neutral lipid content was carried out following the AOAC method (Method 920.39; AOAC, 2000) using a Soxtherm 2000 extractor and petroleum ether as a solvent. The content of inert material was determined by the incineration process for 2 hours in a muffle at 600 °C (Method 942.05; AOAC, 2000). The carbohydrate content was determined from the quantitative acid hydrolysis of the biomass (Hoebler et al., 1989) followed by the phenol-sulfuric method (Dubois et al., 1956). Quantification was performed by spectrophotometry (490 nm) using the standard glucose curve.

The biochemical and taxonomic composition of algae biomass obtained after drying is shown in Table 1.

Table 8.1. Biochemical and taxonomic composition of raw microalgae biomass.

Biochemical composition (%)[a]		
Carbohydrates	7.42	
Neutral lipids	2.49	
Proteins	27.40	
Others (inert material)	32.99	
Organisms		
Chlorophyta	D (org mL^{-1})[1]	RA (%)[2]
Chlorella vulgaris	5.11×10^6	58.47
Didymocystis inermis	1.02×10^5	1.17
Tetradesnus obliquus	2.56×10^5	2.93
Bacillariophyta		
Navicula sp.	3.27×10^6	37.43
Total	8.74×10^6	100

[1] D = Density (org mL^{-1}); [2] RA = Relative abundance (%).

[a] Dry basis.

For the ultimate analysis (carbon, hydrogen, nitrogen, sulfur and oxygen) and of the contents of macro and micronutrients and trace metals, the samples were first dried in an oven at 60 °C (until constant weight), crushed and then standardized in sieves with opening of 100 mesh. The levels of carbon, hydrogen and nitrogen were determined via dry combustion, using a Perkin Elmer elementary analyzer, PE-2400, series II. The method consists in promoting the combustion of the sample at 925 °C and analyzing the gases released by gas chromatography. The sulfur content was obtained by turbidimetry, with the aid of a spectrophotometer, at a wavelength of 440 nm. The oxygen content was determined by adding the ash with C, H, N and S subtracted by 100.

To determine macro and micronutrients and trace metals, the samples were submitted to nitric-perchloric digestion (4:1, v v^{-1}) determining the total elements: P, quantified by colorimetry; K, by flame photometry; Ca and Mg, by atomic absorption; S, by turbidimetry, total N determined by the Kjeldahl method; Mn, Cu, Cd, Fe and Zn, by atomic absorption; B, by the colorimetric method with the reagent azomethine-H. Except for the total N, the other determinations were performed according to Embrapa (2009) procedures.

For the determination of the HHV, ash content and thermogravimetric analysis (TG and DTG), the biomass was classified in sieves with an opening of 0.42 mm (40 mesh) and 0.25 mm (60 mesh) collecting the fraction that was retained in the 60 mesh sieve. The ash content was determined according to the standard NBR 8112 (ABNT, 1986). The HHV was obtained experimentally through the method of the adiabatic calorimetric pump, according to the ASTM D 2015 standard (ASTM, 1982).

For the TG analysis, the DTG-60H device (Shimadzu) was used in a nitrogen gas atmosphere, at a constant flow rate of 50 mL min^{-1}, using approximately 2 mg of biomass placed in an open alumina capsule. Thermogravimetric curves were obtained from 100 °C to a maximum temperature of 600 °C, with a heating rate of 10 °C min^{-1}. Temperature values adoption were based in Bach et al. (2017), Liu et al. (2019) and Khoo et al. (2020), who performed TG on microalgae biomass and used the same range or reached a passive combustion reaction zone below it. The thermogravimetric curve was obtained to assess the loss of mass through temperature.

Fourier-transform infrared spectroscopy of the samples was performed using an FT-IR spectrometer (Perkin Elmer Spectrum 100). The main wavelengths were identified according to Driver et al. (2015) and Khoo et al. (2020): (i) 3600–3000 cm^{-1}, related to chemical bonds O–H of water; (ii) 2957, 2920, 2872, 2852 cm^{-1}, of the radicals $–CH_2$ and $–CH_3$ related to fatty acids; (iii) 1655, 1545 cm^{-1}, related to chemical bonds C=O and N–H of the starch associated with protein; (iv) 1455 cm^{-1}, of the radicals $-CH_2$ and $-CH_3$ associated with lipids and proteins; (v) 1200–900 cm^{-1}, of the radical C–O–C of the polysaccharides; and (vi) 1160, 1086, 1050, 1036 cm^{-1}, of the C–O radical of carbohydrates.

Finally, the biomass was visually analyzed using a JSM-6010LA scanning electron microscope (SEM) and submitted to the X-ray diffraction (XRD) analysis (D8 DISCOVER (BRUKER)) Cu-kα radiation ($\lambda = 1.5418$ Å) with angular variation of 1–50° (2θ).

8.2.3. Hydrothermal carbonization

Temperature and retention time are two important parameters for HTC of any biomass (Bach et al., 2017). Therefore, the effects of these two parameters on the yield of solids and energy were investigated, as well as on the properties of solid and aqueous products for wastewater grown microalgae biomass. Three temperatures were maintained over three retention times.

The operating conditions in this study were less severe than normal HTC for three reasons: (i) the range of 180–260 °C is applied to woody biomass, which has fibrous and/or ligneous structures and, therefore, is more thermally stable than algae biomass (Bach and Skreiberg, 2016; Bach et al., 2017; Ji et al., 2017); (ii) the temperature range (130–170 °C) was tested in previous experiments and demonstrated an improvement in the characteristics of the hydrochar in milder conditions of temperature (data not shown) and; (iii) studies available in the scientific literature that carried out HTC with microalgae biomass proposed shorter retention times than conventional ones and obtained satisfactory results (Bach et al., 2017; Lee et al., 2019).

The dry biomass was mixed with distilled water in a proportion of 1:10 m v^{-1}, until completing 220 mL. For the tests, a compact Parr 5500 Series reactor was used, with temperature control and agitation. Initially, the reactor was purged with nitrogen gas (N_2) for 5 min. Tests were carried out under temperatures of 130, 150 and 170 °C for 10, 30 and 50 minutes. Operating at these temperatures, the working pressure was 1, 3 and 6 bar, respectively, to decrease the heat transfer effect in the reaction medium. The agitation was adjusted to 500 rpm. The retention time started to be timed when the desired temperature was reached. In total, 20 experiments were carried out. For each combination of temperature and retention time, a reactor training operation (auto-tuning) and subsequently the definitive experiment of HTC were carried out to collect the desired information.

At the end of the experiment, the entire sample was removed from the reactor. Sample was centrifuged at 3,000 rpm for 1 minute, to separate the solid and liquid phases. The solid phase was dried in a forced circulation oven at 40 °C (until constant weight) and conditioned (under refrigeration) until the analysis was carried out and the AP was immediately submitted to analysis (Section 8.2.5).

8.2.4. Fuel characterization and carbonization performance

The following analyses were performed on solid fuel: HHV, ash, ultimate analysis, thermogravimetric analysis, Fourier-transform infrared spectroscopy (FT-IR) analysis, SEM and XRD according to the methodology mentioned in Section 2.2 for the characterization of raw biomass.

The solid yield (Ys) was determined according to Eq. (1) and Bach et al. (2017); Belete et al. (2019); Lang et al. (2019); Lee et al. (2019) and Xu et al. (2020):

$$Ys\ (\%) = \frac{m\ solids}{m\ raw\ biomass}\ x\ 100 \tag{1}$$

Where *m solids* denotes the mass of hydrochar produced after HTC, and *m raw biomass* denotes the amount of raw biomass submitted to HTC, on a dry basis. The percentage of energy yield was determined according to Eqs. (2) and (3):

$$De = \frac{HHV\ biochar}{HHV\ raw\ biomass} \tag{2}$$

$$Ey\ (\%) = Ys\ \times De \tag{3}$$

Where *De* and *Ey* are the energy enhancement factor and energy yield, respectively. *HHV hydrochar* and *HHV raw biomass* stand for the higher heating values of solid product and raw biomass, respectively.

8.2.5. Aqueous phase characterization

The supernatant, followed after centrifugation, was analyzed in order to determine the effect of HTC without displacing molecules from the solid phase to aqueous phase. The pH variable was measured using a Hach probe, model HQ40d, after reaching room temperature. The analyses of total phosphorus (TP) and total nitrogen Kjeldahl (TKN) were determined following the Standard Methods for the Examination of Water and Wastewater (APHA, 2012), based on the 4500PC and 4500-NorgB procedures, respectively. In addition, the total organic carbon (TOC) was determined using the Shimadzu TOC 5000 equipment.

8.2.6. Statistical analysis

The experimental planning and statistical analysis were performed using the Minitab® 17 software (trial version). The experiment followed a central composite design with a centered face, with a replicate at the central point and four axial points, totaling ten HTC experiments. With the energy yield data, it was possible to obtain an adjusted model to represent the energy yield data as a function of temperature and retention times.

8.3. Results and discussion

8.3.1. Hydrochar

8.3.1.1. Solid yield

The effects of temperature and retention time on solid yield are shown in Figure 8.1. In the retention time of 10 minutes, the increase in temperature provided an increase in the solid yield. On the other hand, in the retention times of 30 and 50 minutes, when the temperature increases, the solid yield decreased. Thus, the highest solid yield was obtained at 170 °C and retention time of 10 minutes. At the highest temperature tested (170 °C), the solid yield decreased by 9% when the retention time was from 10 to 50 minutes. The reduction in solids with the increase in the severity of HTC has been reported previously when studying microalgae biomass (Bach et al., 2017; Liu et al., 2019).

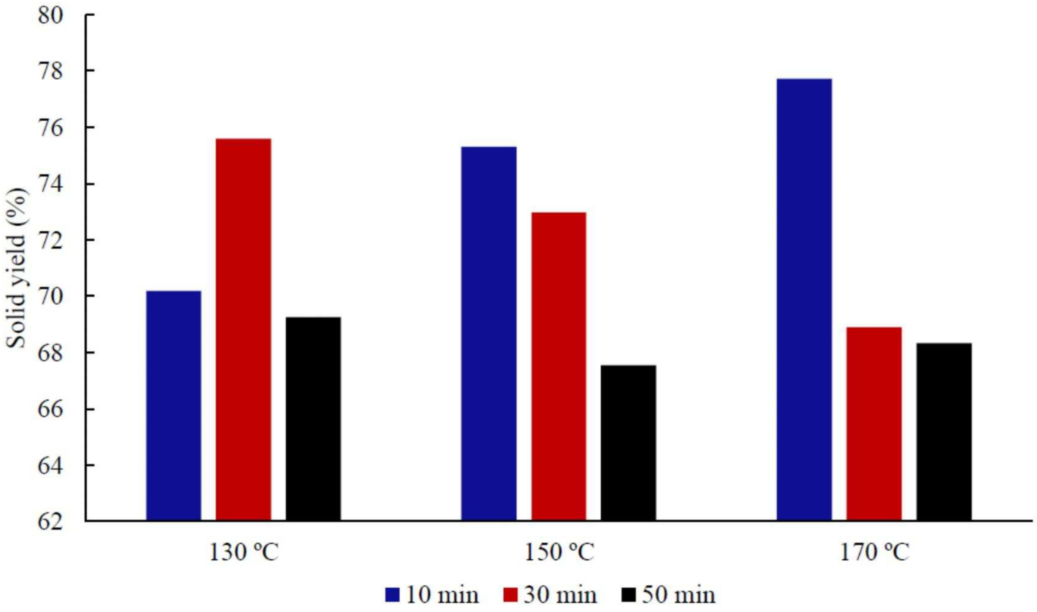


Figure 8.1. Solid yield at temperatures and retention times tested.

Bach et al. (2017) carried out wet torrefaction (a treatment similar to HTC) of microalgae biomass grown in synthetic medium (160–180 °C; 5–30 minutes) and observed that with the increase in temperature and retention time, there was a reduction in solid yield by approximately 10%. The yield reported in the present study (67% and 77%) was 15% higher than that reported by the authors previously mentioned.

Liu et al. (2019) performed HTC tests in microalgae biomass at temperatures of 180, 240 and 260 °C and retention time of 240 minutes. Results showed a reduction of approximately 12% in the solid yield from the lowest to the highest tested temperature. Authors explained that at relatively low temperatures, the polymers are partially decomposed into smaller compounds through hydrolysis and depolymerization reactions, resulting in high hydrochar yield. As the temperature increases, the decomposition of the previously formed solid phase began to occur. More activation energy is provided to break the chemical bonds of the components into their corresponding monomers, through the dehydration or decarboxylation reaction (Liu et al., 2019).

Possibly in the retention time of 10 minutes, the molecules have gone through a process of reorganization of the polymeric structure with increasing temperature, presenting a more organized structure resulting in greater solid yield at 170 °C. In the retention times of 30 and 50 minutes, there was enough time for the macromolecular components to be broken down into their corresponding monomers as the temperature increased. The decrease in solid mass was caused by dehydration and decarboxylation reactions (Danso-Boateng et al., 2013).

8.3.1.2. Energetic characterization of hydrochar

The ash content, elemental composition, HHV, energy density and energy yield of raw biomass and hydrochar are shown in Table 8.2.

Table 8.2. Ash content, ultimate analysis, higher heating value and energy yield of raw biomass and hydrochar.

HTC parameters	Ash (%)[a]	Ultimate analysis (%)[b]					HHV (MJ/kg)	Energy density[c]	Energy yield[d] (%)
		C	H	N	O[e]	S			
Raw biomass	32.99	50.44	8.51	6.03	34.50	0.52	16.99	NA[f]	NA[f]
130 ºC – 10 min	34.16	53.58	9.09	5.63	31.23	0.46	17.34	1.02	71.60
130 ºC – 30 min	34.26	53.01	9.13	5.70	31.68	0.48	17.09	1.01	76.03
130 ºC – 50 min	39.56	58.54	9.98	6.11	24.93	0.45	17.47	1.03	71.17
150 ºC – 10 min	35.44	53.31	9.08	5.45	31.69	0.47	17.03	1.00	75.45
150 ºC – 30 min	34.62	53.36	8.60	5.20	32.37	0.46	17.07	0.99	72.90
150 ºC – 50 min	35.87	58.07	9.71	5.77	26.01	0.43	17.02	1.00	67.57
170 ºC – 10 min	36.94	54.28	9.12	4.74	31.45	0.41	17.10	1.01	78.21
170 ºC – 30 min	37.33	54.11	9.10	4.74	31.61	0.44	17.16	1.01	69.58
170 ºC – 50 min	34.76	53.48	8.81	4.31	32.99	0.41	16.99	1.00	68.30

HHV: higher heating value (MJ/kg).

[a] Dry basis; [b] Ash-free dry base; [c] energy density = $HHV_{hydrochar}$ / $HHV_{raw\ biomass}$. [d] energy yield = energy density * hydrochar yield. [e] Determined by difference. [f] NA: not available.

Ash contents in carbonized materials were approximately 3% higher than that of raw biomass, regardless of the treatment. Lee et al. (2019) performed HTC of sewage sludge and *Chlorella sp*. grown in wastewater, in a temperature range of 180 to 270 °C, and reported that the ash content increased gradually up to 16%, with increasing temperature. It is important to highlighted that, in the present study, there was a concentration of carbon in the hydrochar, which is the objective of HTC, in all the performed treatments.

Liu et al. (2019), working with wastewater grown microalgae biomass, also observed the increase in ash content (11%) with the increase of HTC temperature. Authors also observed that when the ash content in the raw biomass decreased from 44.66% to 14.45%, the HHV increased from 9.51 to 24.23 MJ kg^{-1}. This suggests a considerable effect of the high ash content on the hydrothermal behavior of microalgae. High ash content is a characteristic of products resulting from the carbonization of sewage sludge, as reported by S. J. Park et al. (2011) and Park and Jang (2011). It is noteworthy that, in the present study, the ash content of the raw biomass can be considered high (32.99%), in relation to other biomasses used in the HTC process. For example, tree leaves presented an ash content of approximately 6% (Xu et al., 2020) and algae biomass grown in synthetic medium, with an ash content of 9% (Bach et al., 2017).

For HHV no difference was observed due to the application of treatments. Energy density represents the ratio between the HHV of raw biomass and hydrochar. Thus, it allows to identify the variation of this parameter. Energy density of the hydrochar produced in all treatment was low. Despite this, as solid yields were high, energy yields were 67.67 – 78.21%. Energy yield can be interpreted as the energy recovery efficiency of raw biomass (Leng et al., 2020b), therefore, it is possible to consider that there was a high energy recovery in the present study. The EY found was higher than that reported by Leng et al. (2020b) for a *Chlorella* hydrochar with less ash content and using AP recirculation as a strategy to increase the hydrochar yield. Still, it is essential to investigate biomass pretreatment methods to reduce ash content and thereby increase HHVs. Marin-Batista et al. (2019) investigated the removal of ash from the hydrochar produced from microalgae (grown in 10% diluted centrifuged pig manure) by removing inorganic compounds from its surface by washing with 96% ethanol (4 mL g^{-1} hydrocarbon), 1 M HCl (50 mL g^{-1} hydrocarbon) and 3% (v/v) H_2O_2 (50 mL g^{-1} hydrocarbon). Authors reported that ethanol and H_2O_2 decreased the carbon content of the hydrochar. On the other hand, HCl increased the carbon content by 1.40x.

For biochar produced from wastewater grown microalgae, the feasibility depends on the energy consumption required during carbonization. Therefore, the cultivation stage should be inserted in a wastewater treatment system, where the microalgae biomass would be considered a waste. Considering the treatment with the highest energy yield (170 °C and 10 minutes), the HTC stage should consume less than 17.1 MJ per kg of produced hydrochar to be advantageous.

Regarding the elemental composition, it was observed that the percentages of C and H increased, up to 8.10 and 1.47 %, respectively, and the levels of O, N and S decreased in relation to the raw biomass (up to 9.57, 1.72 and 0.11 %, respectively), regardless of the treatment applied. The results of C, O and H occurred mainly as a result of dehydration and decarboxylation processes, which remove H (the difference for raw biomass does not vary considerably) and O from the solid phase, in the forms of H_2O and CO_2 (Funke and Ziegler, 2010).

The percentages of N and S decreased as temperature increased for HTC, due to dissolution of N and S oxides formed during HTC in the processing liquid (Fang et al., 2018). This reduction is important, as it reduces the emission of polluting gases during the burning of the solid material. This trend was also recorded by Lang et al. (2019), when carrying out HTC of swine effluent.

Comparing the atomic ratio of H/C (related to aromaticity) and O/C (related to polarity) in products, using the van Krevelen diagram (Van Krevelen, 1950), changes in the degree of coalification were obtained during HTC treatment (Figure 8.2). The lower the respective proportions, the greater the degree of coalification and the energy potential of the products (McKendry, 2002).

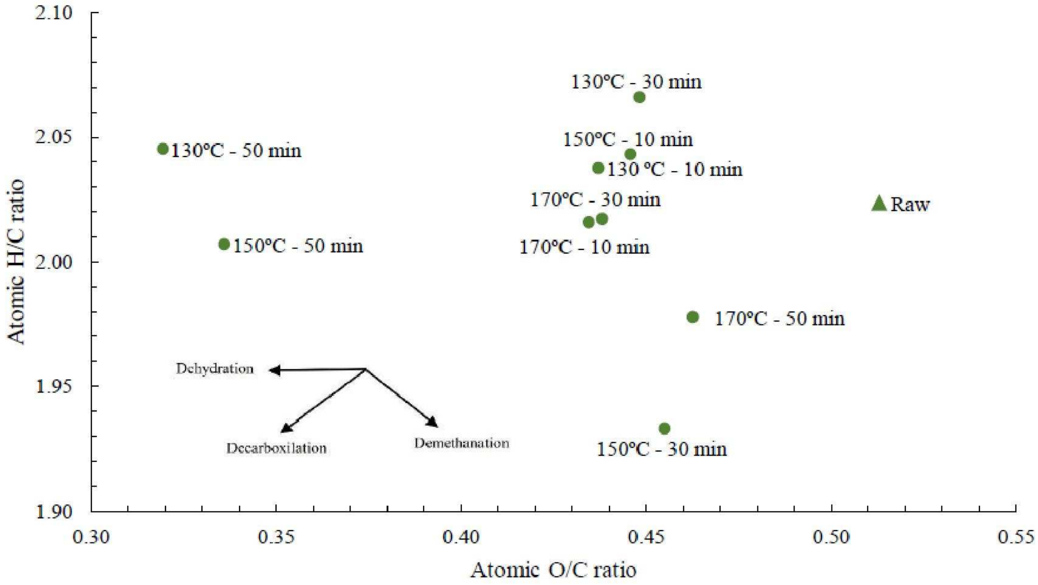


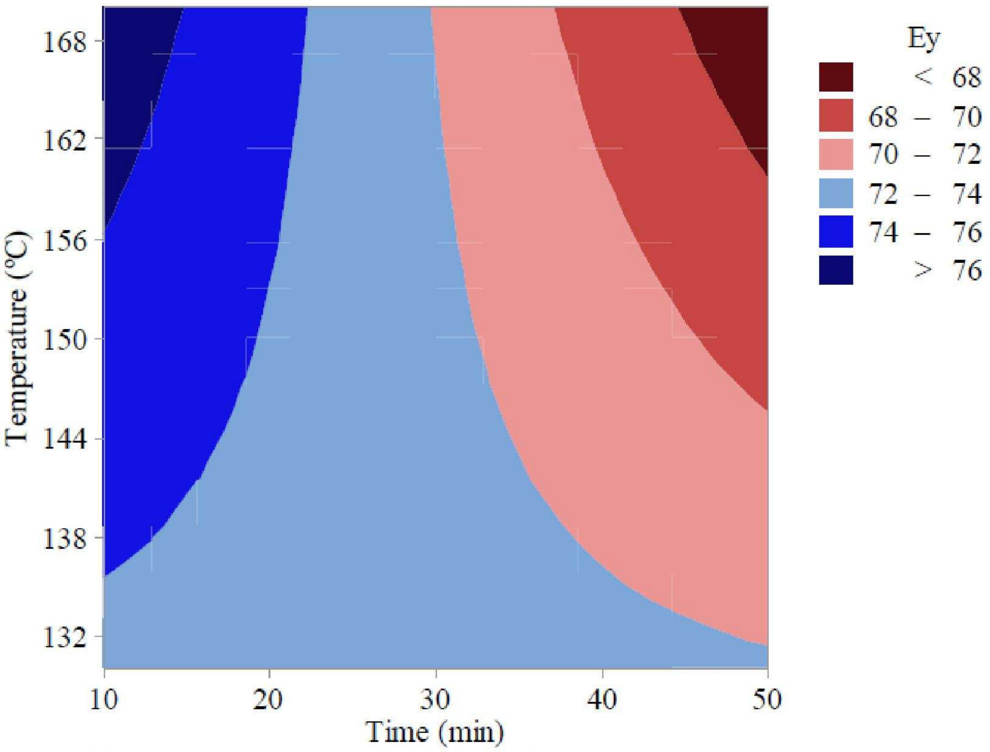
Figure 8.2. Van Krevelen diagram for raw and carbonized biomass.

The O/C atomic ratio is an important criterion for estimating the degree of deoxygenation that occurred during the HTC biomass process. The H/C ratio provides answers about the aromatic content of hydrothermal coals (Tekin et al., 2014). In comparison with other biomasses, the H/C ratio may be considered high for the hydrochar of the present study. Atta-Obeng et al. (2017) and Wiedner et al. (2013) reported that the atomic ratio H/C is considered high when H/C is ≥ 0.7, indicating that there are no condensed aromatic structures (Atta-Obeng et al., 2017). Bach et al. (2017) and Lee et al. (2019) recorded maximum values of approximately 2.00 and 1.60 for biomass of the microalgae *Chlorella sp.* (grown in synthetic medium) and *Chlorella vulgaris* (grown in municipal effluent), respectively.

Regarding the atomic ratio O/C, there was a considerable reduction in the hydrochar in relation to raw biomass. It is noteworthy that since the oxygenated functional groups tend to be hydrophilic, the reduction in the atomic O/C ratio means the loss of oxygenated functional groups on the surface, increasing the hydrophobicity of the chars (Yao; Ma, 2018). This result is interesting when the focus of the thermochemical treatment is the use of hydrochar as a fuel.

The result of the thermochemical treatment was interesting because it reduced the O/C ratio of the hydrochar in relation to the raw biomass, making the values close to that of peat and lignite (Marin-Batista et al., 2019; Leng et al., 2020b). However, the results for the Y-axis, which represents the H/C ratio, did not show such significant reductions. The hydrochar was not comparable with other types of coal, which would be around 1.6.

The adjusted model for representing energy yield data as a function of temperature and retention times was significant at the level of 5% probability of error, and the determination coefficient was 74% (Figure 3). Based on this result, it can be concluded that time was the most significant variable in the model, and the retention time of 10 minutes was sufficient to promote carbonization and retention of the greatest amount of energy in the hydrochar. It is important to note that, as will be reported in Section 8.3.2, temperatures above 180 °C may result in the release of toxic compounds for AP.



$$Ey = 72.38^{***} - 0.452Temperature - 3.02^{**}Time - 2.37^{*}Temperature \times Time$$

Figure 8.3. Adjusted surface model of the interaction between the parameters adopted at HTC in the energy yield of the hydrochar. ***Significant at the level of 1% probability of error; **Significant at 5% probability of error and *Significant at the level of 10% probability of error.

The results obtained demonstrated that as the energy performance is dependent on the HHV and the solid yield, although the HHV did not present significant differences with the application of the different treatments, for the high solid yield in the shortest retention time and highest temperature tested, the energy yield was maximized.

In a more specific interpretation of the response surface, we have that the shorter the retention time, the greater the energy efficiency, especially at temperatures above 156 °C. The temperature started to exert more influence on the model only at higher retention times, when there was a tendency to reduce energy efficiency with its increase.

8.3.1.3. Thermal stability of the hydrochar

The thermal stability of raw biomass and hydrochar was studied thermogravimetrically. The TG and DTG curves at 50 minutes of retention time at all temperatures and at 170 °C at all tested retention times are shown in Figure 8.4. From the TG curves presented, it can be seen that carbonized biomass was more reactive than raw biomass. From approximately 250 °C, it becomes even more pronounced that carbonized biomasses are more thermally stable than raw biomass.

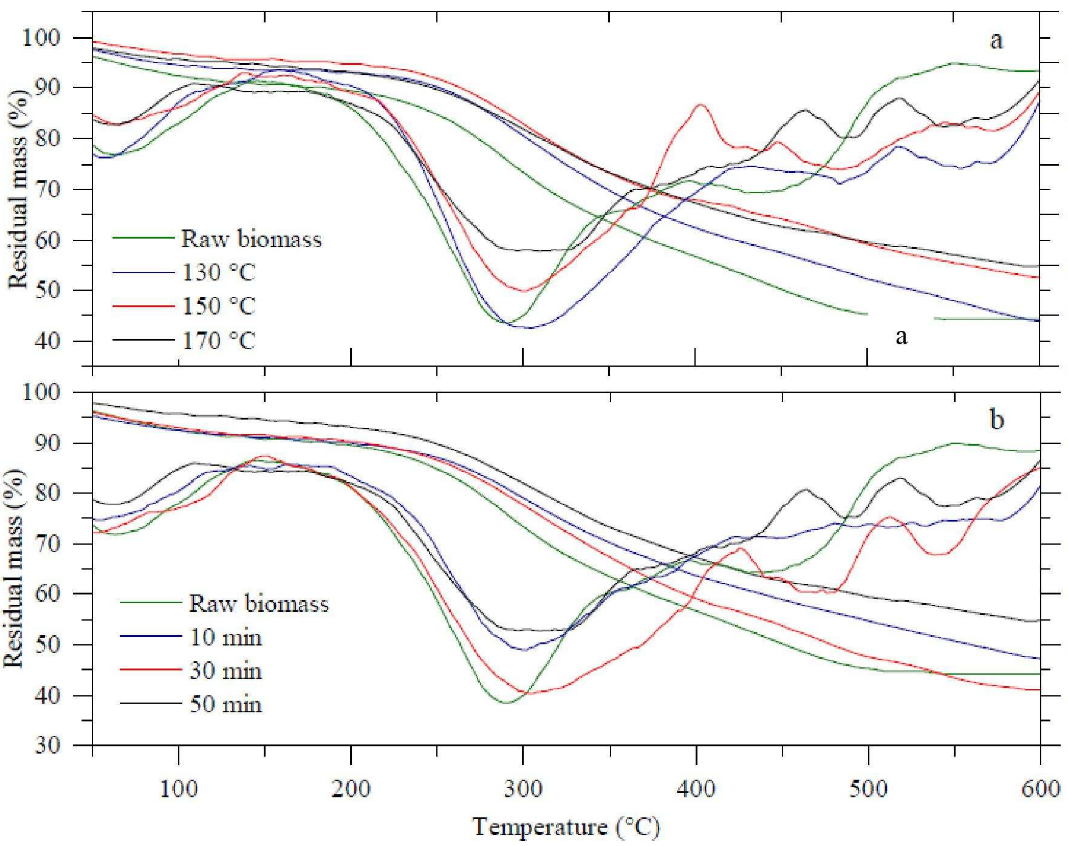


Figure 8.4. TG and DTG curves for raw biomass and hydrochar. (a) 50 minutes; (b) 170°C.

More detailed behavior regarding the temperature increase can be observed in the DTG curves, from which three different stages can be easily highlighted, according to Bach et al. (2017): the first stage is between 105 and 350 °C, showing the devolatilization and oxidative reactions of components as carbohydrates and proteins with low thermal stability. The second stage is in the range of 350 to 500 °C for carbonized biomass, representing the devolatilization and burning of components as lipids, more thermally stable. The third stage is a consequence of the combustion of components produced at higher temperatures.

Regarding residual mass, in Figures 4a and 4b, it can be observed more specifically that the increase in temperature and retention time promoted the achievement of a more stable biomass. In DTG curve for temperature of 170 °C (Figure 4b), the retention times of 10 and 50 minutes showed a closer trend, different from the 30 minutes condition. It is worth mentioning that some instantaneous peaks occurred in the second stage, and this may be related to several depolymerization reactions that occur when the biomass is hydrothermally degraded (Sabio et al., 2016, Bach et al., 2017). Posteriorly, the compounds produced from these reactions can recombine to form several new species through repolymerization reactions (Bach et al., 2017).

8.3.1.4. Functional groups on hydrochar surface

The three main components of microalgae, carbohydrates, lipids and proteins, have been described from the characteristic peaks in the FT-IR spectra. It was decided to present, in Figure 8.5, the spectra corresponding to the most severe conditions evaluated, since the behavior of the other spectra was similar, changing only the intensity.

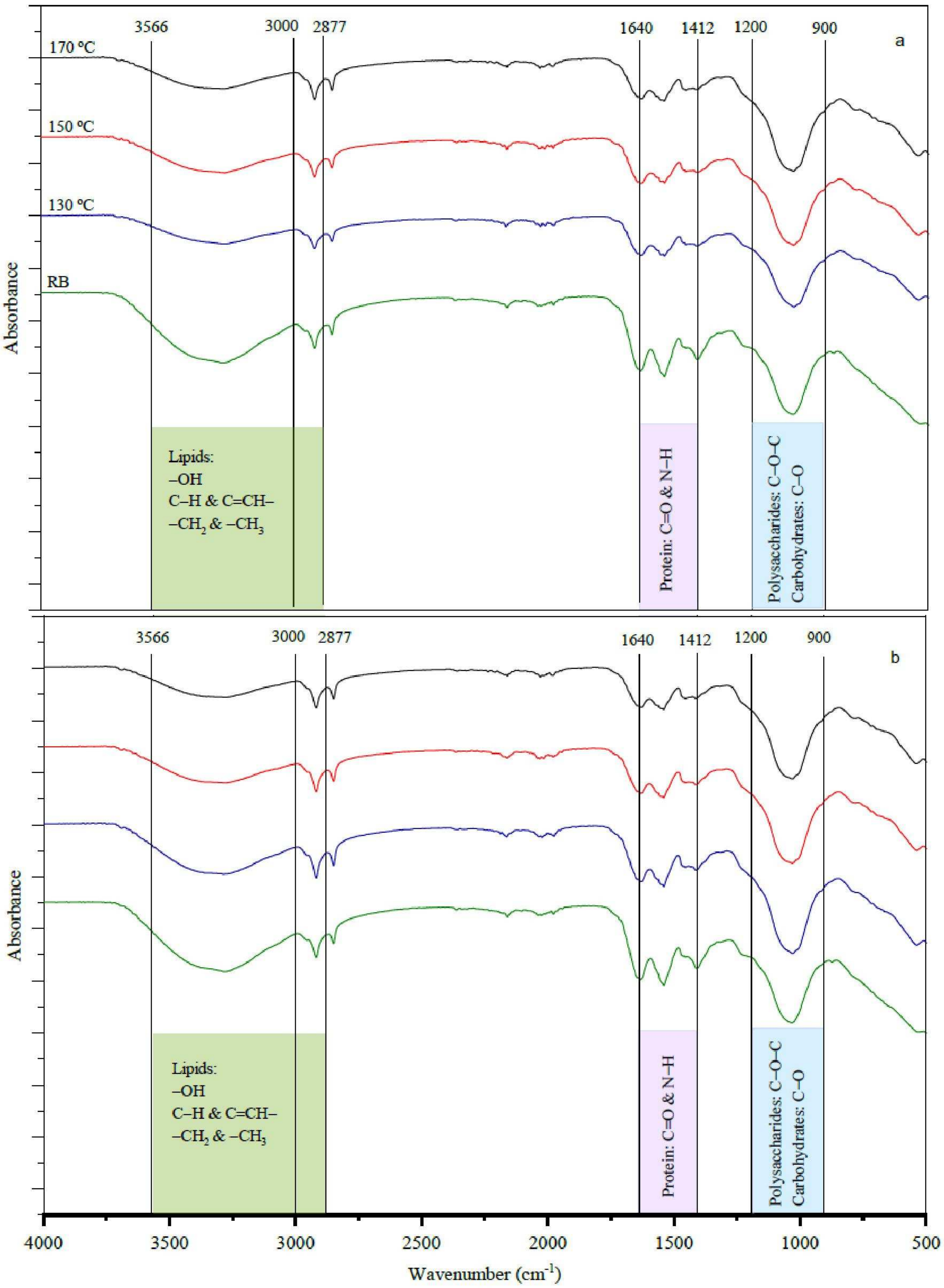


Figure 8.5. FT-IR spectra for raw biomass and carbonized biomass: (a) 50 min; b) 170 °C.

Figures 8.5a and 8.5b show that when the carbonized material is compared with the raw biomass, the carbohydrate content tends to increase. This is because the peaks around 1200 to 900 cm^{-1}, representing carbohydrates and polysaccharides, have lightly grown, both in terms of temperature and retention time. This result was confirmed by the ultimate analysis, when the increase in temperature caused an increase in the carbon content.

The peaks around 1640–1412 cm^{-1} (representing proteins) for the hydrochar showed a downward trend, regardless of the severity of HTC. That means there was protein consumption during HTC. This behavior is related to the N content that reduces with the application of the thermochemical treatment. It is noteworthy that due to the balance of mass and energy, a small percentage of N was also lost in the gaseous form.

Lipids, represented in the curves between 3000–2877 cm^{-1}, showed a slight tendency to increase with HTC. The concentration of lipids in the raw biomass was already small, and for this reason, it may not have shown much difference in this analysis. As for the peak around 3300 cm^{-1}, which corresponds to the OH bond in the water, the spectra showed that the carbonized microalgae absorb less water during storage than the raw microalgae. This result indicated dehydration of biomass during HTC reaction (Bach et al., 2017; Khoo et al., 2020), corroborating the result of the Van Krevelen diagram, presented in Section 8.3.2.3.

SEM images (Figure 8.6) showed that raw biomass presents irregular and poorly defined particles and, when subjected to initial reaction temperatures and times (130 ºC and 150 ºC; 10 and 30 min), there was a trend in forming more defined and larger structures in relation to raw biomass. On the other hand, when the reaction severity increased (170 ° C and 50 min), there was a clear reduction in the size of the biomass structures.

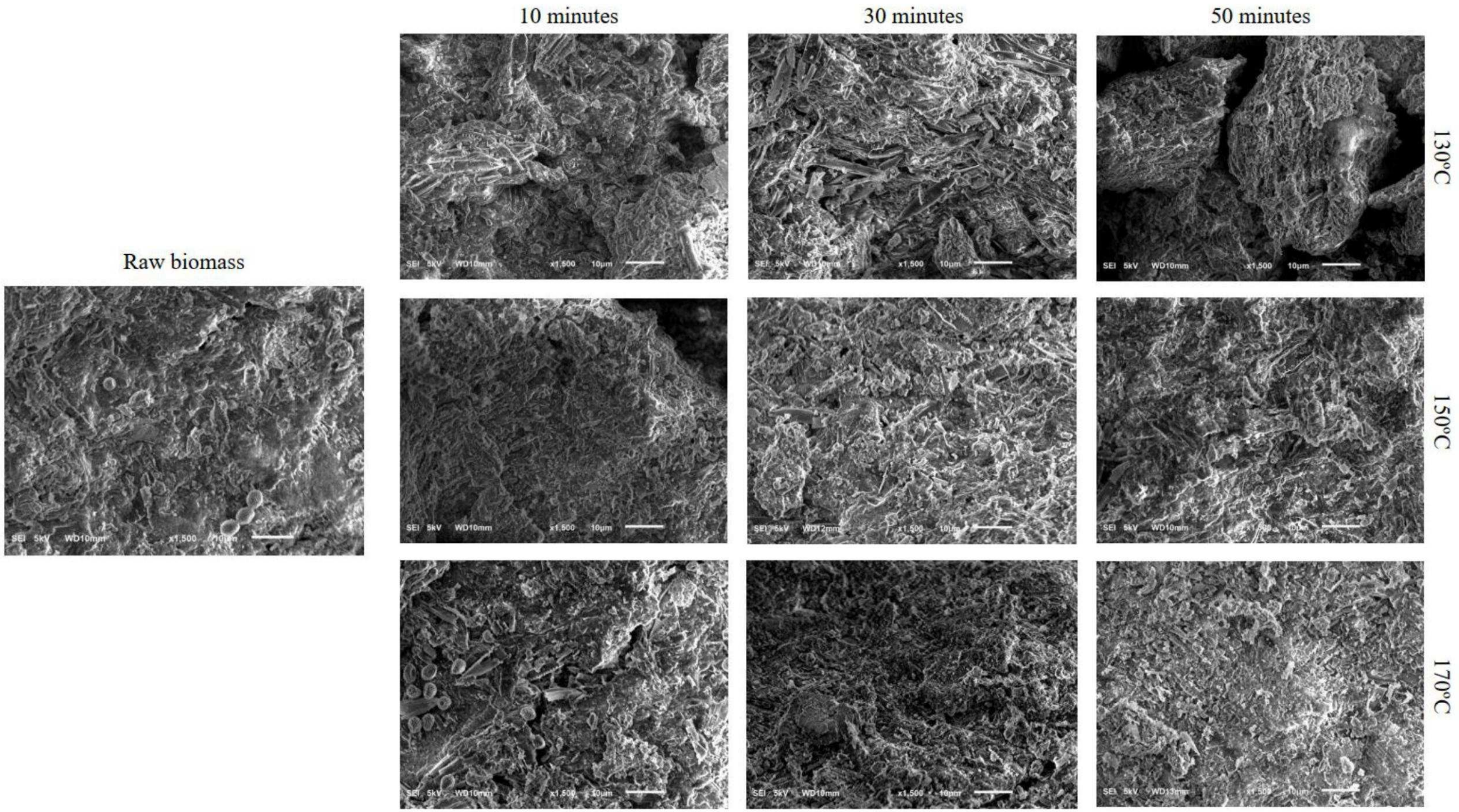


Figura 8.6. SEM images of the raw biomas and hydrochars.

The results obtained with the XRD analysis are shown in Figure 8.7 and corroborate those presented in the SEM analysis. In the intermediate temperatures and retention time treatments, there was a tendency for the material to organize itself in better defined structures with a predominance of carbon. At the highest temperature and retention time treatment, peaks returned to show lower intensities, expressing a tendency to undo the structures produced in the mildest conditions.

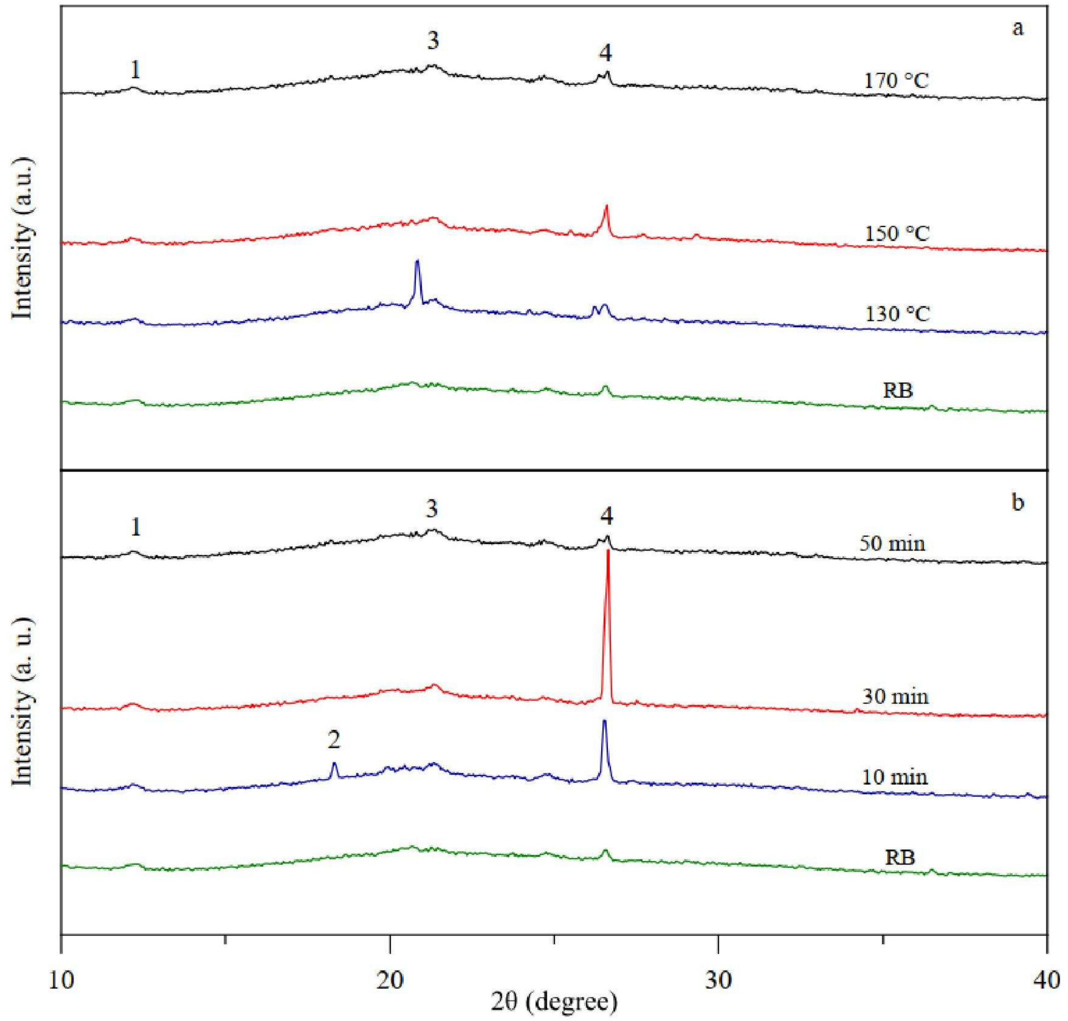


Figure 8.7. X-ray chromatograms of raw biomass and hydrochars (a) 50 min and (b) 170 °C. (1) Iron oxide hydrate – 1-622; (2) Silicon oxide – 36-63; (3 e 4) Carbon - 20-258.

The peaks found in the XDR analysis basically refer to iron oxide hydrate, presented in peak 1, which did not show any difference with the increase in temperature and retention time. At peak 2, clearly shown in the hydrochar chromatogram formed at 170 °C and 10 minutes, was observed the silicon oxide, related to the presence of ash in the biomass. Finally, carbon, represented in peaks 3 and 4, is the predominant constituent of biomass which, as presented in the elementary analysis (Table 2).

Peaks were less intense for conditions of 50 minutes of retention time and temperature increase (Figure 6a), suggesting that in this retention time, most of the compounds returned to solubilize. On the other hand, fixing 170 ºC of temperature condition and increasing retention time (Figure 6b), could be observed that peaks were greater in the intermediate times. This results suggested that in these operating conditions there was a tendency in the formation of more chemically organized structures.

8.3.1.5. Macro and micronutrients and trace metals

In order to allocate the hydrochar in the most appropriate way based on its constitution, analyses of macro and micronutrients (except those that have already been mentioned in the ultimate analysis) and trace metals that make up the raw and carbonized biomass were performed and are shown in Table 8.3. With the exception of K and Na, which were reduced in the solid material after carbonization, for the other nutrients there was a tendency to increase in hydrochar, regardless of temperature and retention time. This behavior indicates that the HTC process was efficient in concentrating most of the analyzed constituents.

Table 8.2. Macro and micronutrients * and trace metals in raw biomass and hydrochar.

HTC parameters	P	K	Ca	Mg	Na	Cu	Fe	Zn	Mn	Ni	Pb	Cr
			%						mg kg^{-1}			
Raw biomass	1.87	0.38	2.66	0.61	1.49	27.91	4462.22	367.32	74.70	11.05	5.10	25.58
130ºC – 10 min	2.22	0.26	3,48	0.75	1.30	32.69	6148.20	478.02	153.92	16.80	7.25	41.58
130ºC – 30 min	2.31	0.27	3,47	0.77	1.30	32.97	5905.18	485.35	156.05	15.15	7.10	39.00
130ºC – 50 min	2.33	0.25	3.46	0.78	1.30	34.97	5935.81	490.44	159.64	15.40	8.45	41.18
150ºC – 10 min	2.32	0.24	3.64	0.76	1.29	34.46	6285.49	499.07	155.40	16.95	6.20	39.28
150ºC – 30 min	2.38	0.28	3.90	0.79	1.40	36.04	6655.66	525.70	166.06	17.95	6.45	45.20
150ºC – 30 min	2.38	0.26	3.81	0.79	1.33	39.38	6742.88	529.33	169.77	18.25	8.70	48.41
150ºC – 50 min	2.21	0.25	3.64	0.72	1.33	35.24	6322.70	485.36	148.32	16.85	8.05	44.85
170ºC – 10 min	2.36	0.28	3.49	0.75	1.27	37.50	5921.57	464.75	152.59	14.45	7.45	38.61
170ºC – 30 min	2.37	0.23	3.56	0.75	1.32	35.88	6021.23	500.91	152.20	16.90	7.60	42.54
170ºC – 50 min	2.57	0.18	4.21	0.82	1.27	40.02	7244.16	594.88	181.90	23.15	8.10	39.30

* Boron and cadmium resulted in values below the detection limits.

In the literature, several possible uses for the char resulting from HTC are investigated as an alternative to energy use. In the agricultural field, it can be used for soil correction, as it has stable carbon and other nutrients (McGaughy and Reza, 2018), and as an adsorbent for the removal of Cu and Cd (Regmi et al., 2012). In addition, the application of the product as an alternative source of phosphorus and/or nitrogen as fertilizer to crops is noteworthy.

Char has the potential to improve agricultural profitability by increasing crop productivity. Arif et al. (2017) observed that the incorporation of biochar into the soil increased the production of corn and wheat grains by 24% and 18%, respectively, compared with similar plots without biochar. This increase is related to the effects of char on the physicochemical properties of the soil, such as greater water retention capacity, increased cation exchange capacity and the supply of nutrients to plants. In this sense, there is the beneficial effect of the application of biochar in the short-term soil as a potential source of nutrients and in the long term, as a soil conditioner. The authors mentioned an increase in phosphorus absorption, by plants submitted to biochar fertilization, of up to 18%, a value considered high when it comes to this nutrient that has a high capacity for adsorption to soil particles, making it unavailable to plants. Castro et al. (2020) observed absorption of 7.4% of P by plants submitted to fertilization with organomineral fertilizer (a mixture between triple superphosphate plus 12% of microalgae biomass grown in effluent).

In this sense, it is necessary to evaluate whether the most advantageous option would be to granulate an organomineral fertilizer, which carries the impacts of the energy involved in drying the biomass and granulation of the fertilizer, or to subject the biomass to a thermal treatment at low temperatures, with the main impacts being related to the energy spent on HTC. A life cycle assessment for both routes would contribute to decision making.

Chu et al. (2020b) investigated the application of *Chlorella vulgaris*-derived hydrochars that employed water or citrate acid solution as the reaction medium on rice paddy soil. The authors reported an increase in volatilization of NH_3 and N_2O due the low C/N ratio (8.44 and 7.79, respectively for water and citrate acid solution) and H/C ratio (0.61 and 0.49, respectively), that stimulated the nitrification and denitrification, and inhibited the N_2O oxidation in soil. In the present study, higher H/C (1.97 - 2.07) and C/N (8.37 - 11.45) ratios were found. Thus, it is believed that possibly the hydrochar found here may offer less volatilization and, consequently, less environmental impacts. However, in order to verify whether the benefits regarding the grain yield and the efficient use of N by the platform will be maintained, further studies need to be carried out and should include other analyzes not performed here, such as determination of the porous volume and diameter, and specific surface area.

Regarding the concentrations of trace metals (Cu, Zn, Ni, Pb and Cr), the obtained hydrochar showed concentrations below the ones mentioned by the United States Environmental Protection Agency (USEPA, 1995). Therefore, it may have no impediment regarding the application in the soil. For the elements Fe and Mn, the hydrochar obtained at temperature of 170 °C and 50 minutes of retention time had the highest Fe content (7244.16 mg kg^{-1}). It is recommended that only 0.21 g of hydrochar/kg of soil should be applied, following the recommendation of 1.55 mg Fe/kg of soil (Embrapa, 1991).

Regarding the levels of Ca, Mg and Na, although the protection agencies have not suggested limits as to the amount to be applied to the soil, the recommendation is that the hydrochar should be subjected to analysis and that its application in the soil should be monitored frequently. Embrapa (1991) mentions that if the Ca:Mg ratio is not a control variable of the study, the stoichiometric ratio around 4:1 between them meets the need of most cultures. The stoichiometric relationship in the present study ranged from 4.36 to 5.13, therefore, being in a desirable range for application to the soil.

One of the most relevant factors for the use of organic material in agriculture is the concentration of salts, mainly Na. The excess of Na in the soil can cause problems of salinity and sealing through the dispersion of clays. It affects the hydraulic conductivity of the soil and, therefore, the proper development of the exposed crop. Thus, the behavior of this soil must be assessed with respect to the Na concentration, and, if necessary, changes in management must be made.

8.3.2. Aqueous phase

The AP characteristics obtained after the HTC reactions are shown in Table 8.4. It was observed that in the most severe conditions of temperature and retention times, there was an increase in TKN and TOC in the AP compared to the milder conditions tested.

Table 8.4. Characterization of AP after HTC.

HTC parameters	TKN	TP	TOC	pH
		(mg)		
130ºC – 10 min	177.6	29.8	1349.0	7.3
130ºC – 30 min	233.7	26.0	1547.7	7.4
130ºC – 50 min	246.4	25.6	1597.7	7.4
150ºC – 10 min	295.9	31.2	1808.5	7.9
150ºC – 30 min	289.4	32.6	1965.6	7.5
150ºC – 30 min	289.4	32.9	1984.3	7.4
150ºC – 50 min	321.4	28.4	2042.1	7.3
170ºC – 10 min	322.7	21.5	1880.9	7.3
170ºC – 30 min	358.2	25.5	2157.0	7.3
170ºC – 50 min	358.8	22.2	2147.5	7.3

TP showed a tendency to concentrate on the carbonized material with higher percentages of P than that of the raw biomass, regardless of the temperature and retention time. This is because phosphorous can be found in multiple forms and longer retention times may promote its precipitation into the solid phase (McGaughy; Reza, 2018). The TP values in the AP recorded in this study corroborate those recorded in the literature (Belete et al., 2019), and, as expected, there was no gas loss of this element.

An inversely proportional relationship between the amount of nitrogen in the hydrochar and the AP was recorded. While at higher temperatures the nitrogen content decreased in hydrochar, in AP this content increased. The highest concentration of N in AP, at the highest temperature, was probably the result of the deamination of amino acids from organic material (Belete et al., 2019). It is estimated that the N lost by gas was around 0 and 1.5%, being higher at 170 °C.

The resulting AP from HTC consists of residual water and inorganic and organic matter resulting from the degradation of the solid phase (Berge et al., 2011). In this case, the carbon present in the AP is entirely of organic origin, and the results for inorganic carbon showed practically zero values for all treatments. It has been previously reported that organic carbon can include toxic organic molecules, for example, phenol derivatives (Wang et al. al., 2012; Wiedner et al., 2013; Poerchmann et al., 2014). Merzari et al. (2019) stated that to produce hydrochar and subsequently use the AP, the temperature and retention time should not be higher than 180–200 °C and 15–30 min, respectively, because higher temperatures and longer retention times enable the formation of undesirable compounds.

The high concentration of organic carbon in AP was observed in other studies that carried out HTC under more severe conditions of temperature and retention time. As an example, Belete et al. (2019) recorded a concentration of dissolved organic carbon (DOC) of

33,625 mg L^{-1} in HTC of the sewage sludge under a temperature of 210 °C and retention time of 240 minutes.

Danso-Boateng et al. (2015) reported the presence of toxic and non-biodegradable compounds in the AP resulting from HTC, which compromises its discharge and use in other biological processes, such as anaerobic digestion. Poerschmann et al. (2014) reported that the AP resulting from HTC from malt bagasse showed characteristics of substrate with potential for the production of biogas. Biogas production was higher than the anaerobic digestion of low-value biomass raw materials, such as corn silage.

Although the anaerobic digestion of the AP is cited as a potential route, the AP in the present study was not fully adequate to be submitted to anaerobic digestion. The C/N ratio of the AP varied between 6 and 7.5:1, being a ratio considered unbalanced for anaerobic digestion. According to Speece (1996), C/N ratios less than 20 are considered unbalanced for anaerobic digestion, leading mainly to the release of ammonia and consequently to the inhibition of digestion, indicating the need for co-digestion with a richer carbon substrate.

More recently, Belete et al. (2019) studied the possibility of using the resulting activated sludge AP from HTC as a cultivation medium for microalgae. Authors obtained nutrient levels high enough to allow a dilution of 354x, resulting in a total nitrogen/total phosphorus ratio of 23:1. During the growth of *Chlorella sp.* and *Coelastrella sp.*, authors observed a DOC removal of approximately 5x, obtaining an approximate final value of approximately 20 mg L^{-1}.

Du et al. (2012) obtained favorable results of biomass production in concentrations of P between 1.96 and 6.23 mg L^{-1} and a N:P ratio of 28:1 when using the resulting AP from HTC of green algae. Cultivation. N:P ratio varying from 5:1 to 28:1 is recommended for microalgae cultivation (Du et al., 2012; Xin et al., 2010). Costa (2016) performed the production of microalgae biomass using effluent from the meat processing industry. Of the five batches operated with effluent from the flotation unit, the N:P ratios varied between 5 and 17:1. In the present study, the N:P ratios varied from 6 to 16:1, which makes the AP ideal for the cultivation of microalgae.

Leng et al. (2020b) studied the effect of AP recirculation on HTC, using biomass of microalgae and soy straw as raw material for carbonization. Due to the AP recirculation, the hydrochar yield was increased by about 6% for these two biomasses, and the energy yield increased by about 10%. AP recirculation is a practical approach to improve HTC performance. Also, the authors cultivated *Chlorella* sp. in a medium with different dilutions (0.5-2%) of *Chlorella* HTC derived AP and soybean straw HTC derived AP. The authors found that the growth of microalgae biomass was higher in less concentrated culture medium for *Chlorella* HTC derived AP. When using soybean straw HTC derived AP, optimum condition was a more concentrated culture medium. According to the authors, concentrations of inhibiting factors in medium with 2% *Chlorella* HTC derived AP was too high and inhibited the growth of *Chlorella* sp.. On the other hand, the concentration of nutrients in the medium containing 0.5% soybean straw HTC derived AP seemed to be too low to support the microalgae growth. AP reported by Leng et al. (2020b) presented higher concentrations of TOC and TP than the AP of the present study, so it is believed that the recirculation of this byproduct can be used in the cultivation of microalgae biomass in smaller dilutions.

Additionally, Krylova and Zaitchenko (2018), referring to the use of AP resulting from HTC from plant biomass as a fertilizer source, reported that the mineral substances contained in plants, such as phosphorus and ammonia ion, remain dissolved in water after HTC, and therefore it has the potential to be used as fertilizer. However, it is important to highlight the need for a more detailed characterization of AP, regarding the presence of compounds with greater potential for contamination, such as phenols and benzenediols (Poerschmann et al., 2014). The presence of these compounds may indicate a treatment for toxic compounds removal, avoiding the contamination of surface or groundwater, as well as soil and plants. The need for an additional treatment of AP before discarded or use can increase the costs related to the thermochemical process (Becker et al., 2014). Further knowledge about its composition, as well as regarding N and P transformations, are necessary. Also, compounds considered toxic to the environment must be taken into account in an assessment of the life cycle of this route. The presence of these materials, when assessing the life cycle, can make the conventional HTC route unfeasible from an environmental point of view.

8.4. Conclusions

In this study, the HTC of microalgae biomass grown in agro-industrial effluent at three temperatures and three retention times was explored. The main results found showed that:

- Conditions of 10 minutes of retention time and 170°C of reaction temperature provided the highest productivity of solids and the highest energy efficiency;

- Carbonized biomass was more thermally stable compared to raw biomass. In the shortest retention times, more organized carbon structures were observed;

- The thermochemical treatment contributed to the increase in the hydrophobicity of the carbonized material in relation to the raw biomass;

- With the exception of K and Na, which were reduced in the solid material after carbonization, for the other macro and micronutrients there was an increasing trend in hydrochar, regardless of temperature and retention time.

- The aqueous phase increased the concentrations of nitrogen and organic carbon, with an increase in retention time and temperature. On the other hand, there was a tendency to reduce the concentration of phosphorus.

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9. ARTIGO V. Carbonização hidrotérmica da biomassa de microalgas cultivada em efluente agroindustrial: uma abordagem do ciclo de vida

Resumo: Entre as rotas de valorização energéticas da biomassa de microalgas estão em ascenção, nos últimos anos, os tratamentos hidrotérmicos. Como essas rotas ocorrem em meio aquoso, o impacto relacionado a secagem da biomassa é evitado. Nesse aspecto foram realizadas investigações quanto à temperatura e tempo de reação para a CHT de biomassa de microalgas cultivada em efluente agroindustrial. Os resultados obtidos foram utilizados neste estudo, com vistas a avaliação de impactos ambientais dessa rota. O objetivo deste estudo foi avaliar os impactos ambientais da CHT de microalgas cultivadas em efluentes, bem como identificar o processo que requer melhorias e determinar os impactos do ciclo de vida de diferentes tecnologias no nível do sistema. A etapa de cultivo foi o processo mais impactante em seis das oito categorias investigadas. Para a etapa de carbonização, as categorias mais impactantes foram eutrofização de água doce e eutrofização marinha, representando 9,41% e 8,13% do impacto, respectivamente. Esse resultado está relacionado à disposição de N e P resultante da fase aquosa em corpos hídricos que são os principais elementos relacionados a eutrofização. Uma forma de redução do impacto da etapa de carbonização seria a recirculação da fase aquosa no sistema. Os impactos nas categorias de eutrofização de água doce e eutrofização marinha poderiam ser reduzidos em aproximadamente 55% caso o lançamento da fase aquosa não fosse realizado diretamente em corpos hídricos.

Palavras-chave: ACV; hidrochar; bioenergia; tratamento termoquímico.

9.1. Introdução

A biomassa é considerada a fonte de energia mais abundante e renovável do mundo, podendo ser convertida em várias formas de energia úteis, como eletricidade, calor e biocombustíveis (Paredes-Sánchez et al., 2019; Ubando et al., 2019). Diferentes tipos de biomassa são referenciados como sendo matérias primas com potencial para conversão em energia, tais como biomassas lignocelulósicas, lodo de esgoto e resíduos industriais (Wilk et al., 2019; Wiedner et al., 2013).

Entre as inúmeras matérias primas que vem ganhando destaque cita-se a biomassa de microalgas. As microalgas são organismos eucarióticos e procarióticos que usam a luz solar, CO_2, água e nutrientes para produzir biomassa. As microalgas podem ser cultivadas de duas maneiras principais. Uma é o meio de cultivo sintético, em que fertilizantes comerciais são adicionados à água, onde são inoculadas as espécies de microalgas desejadas na biomassa final. O outro meio de cultivo é constituído pelas águas residuárias que contém além de outros elementos, água, nutrientes e microrganismos como algas, bactérias, fungos e protozoários. Em condições ambientais propícias, o crescimento dos microrganismos ali presentes se dará, levando à obtenção de uma biomassa mista, contendo microalgas e demais organismos.

A principal vantagem do cultivo em efluentes é a possibilidade de geração de biomassa com potencial para utilização dentro de diversas rotas energéticas e agrícolas devido à sua constituição, ao mesmo tempo em que se obtém um efluente tratado diminuído o passivo ambiental da disposição desse resíduo, rico em nutrientes, em corpos hídricos, principalmente.

Por outro lado, uma desvantagem é que o cultivo em efluentes sob condições pouco apropriadas e com competição com outros microrganismos propicia a obtenção de uma biomassa com características diferentes daquelas cultivadas em meio sintético. Cita-se, como exemplo, o reduzido acúmulo lipídico da biomassa de microalgas cultivada em efluentes (Assemany et al., 2016; Couto et al., 2018). De acordo com Mehrabadi et al. (2015), a principal razão para o baixo conteúdo de lipídios da biomassa cultivada em LAT com águas residuárias é a mistura de algas e bactérias. Como o conteúdo lipídico das bactérias é tipicamente inferior a 10% (Brown et al., 1996), o conteúdo global lipídico é reduzido e consequentemente o conteúdo energético da biomassa.

Nesse contexto, várias tecnologias de conversão de biomassa de microalgas cultivadas em efluentes em bioenergia, incluindo, entre outras, transesterificação (Zhu et al., 2013; Olkiewicz et al., 2016) e digestão anaeróbica (Park et al., 2013), foram extensivamente investigadas. Esses processos de conversão exigem diferentes matérias-primas e recursos, tais como reagentes, combustíveis e energia, o que resulta em diferentes graus de impacto ambiental.

Entre as rotas de valorização energéticas em ascensão, destacam-se os tratamentos hidrotérmicos da biomassa de microalgas. Como essas rotas ocorrem em meio aquoso, o impacto relacionado a secagem da biomassa é evitado. Dos processos que ocorrem em meio aquoso, o CHT tem apresentado potencial, principalmente por atuar em condições mais amenas de temperatura e pressão com relação aos demais, demandando assim menos recurso material para operação. Porém, essa rota ainda carece de investigações tanto na parte técnica quanto ambiental.

Uma ferramenta que vem ganhando espaço no estudo de impactos ambientais causados por um produto ou processo é a análise de ciclo de vida (ACV). Diversos trabalhos relacionados à ACV das rotas de aproveitamento de microalgas têm sido realizados. Collota et al. (2017) utilizaram ACV para comparar três alternativas de tecnologias de extração de lipídios da biomassa de microalgas. Carneiro et al. (2017) realizaram uma comparação das rotas de produção de combustíveis fósseis, etanol e biodiesel na Europa e no Brasil através da ACV. Wang et al. (2017) realizaram ACV no processo de pirólise da biomassa de microalgas.

Gnansounou e Raman (2016) realizaram uma ACV do biodiesel de algas e seus co-produtos. Esses estudos permitem inferências sobre a performance ambiental das rotas de valorização, bem como os principais pontos a serem melhorados em cada processo. Além destes, cita-se pesquisas que realizaram ACV de CHT de diferentes biomassas foram realizadas. Benavente et al. (2017) realizaram CHT de resíduos de moinho de azeitona em comparação com métodos químicos e biológicos de aproveitamento dessa matéria-prima. Os autores alertam para o impacto causado pela disposição inadequada da fase aquosa pós carbonização.

Nesse aspecto foram realizadas investigações quanto à temperatura e tempo de reação para a CHT de biomassa de microalgas cultivada em efluente agroindustrial (artigo IV desta tese). Os resultados obtidos foram utilizados neste estudo, com vistas a avaliação de impactos ambientais dessa rota. O objetivo deste estudo foi avaliar os impactos ambientais da CHT de microalgas cultivadas em efluentes, bem como identificar o processo que requer melhorias e determinar os impactos do ciclo de vida de diferentes tecnologias no nível do sistema.

9.2. Materiais e métodos

9.2.1. Produção de biomassa de microalgas

A biomassa de microalgas foi cultivada como subproduto do tratamento do efluente de uma indústria de beneficiamento de carnes em lagoas de alta taxa (LAT). As LATS estão localizadas na Unidade Experimental de Tratamento de Efluentes e Produção de Biomassa, Universidade Federal de Viçosa (UFV), Minas Gerais, Brasil (Coordenadas UTM 722924 E, 7702003 S, zona 23 S) Essa indústria que tem como principal atividade, a produção de embutidos e a produção de bacalhau dessalgado desfiado. O efluente de origem industrial é gerado nas diversas etapas do processo produtivo, em especial nos descartes dos tanques de cozimento e resfriamento dos embutidos e descartes dos tanques de dessalga do bacalhau e também da lavagem dos pisos e equipamentos ao final dos processos produtivos. Salienta-se que nesta pesquisa foi utilizado o efluente do flotador (efluente primário).

A LAT em escala piloto utilizada para a produção da biomassa possui as seguintes características: largura = 1,28 m, comprimento = 2,86 m, profundidade total = 0,5 m, profundidade útil = 0,3 m, área superficial = 3,3 m² e volume útil = 1 m³. A LAT foi confeccionada em fibra de vidro e foi equipada com um conjunto de pás giratórias em aço inoxidável, com seis lâminas. Durante seu funcionamento, as pás foram movimentadas por motor elétrico de 1cv. A rotação foi reduzida por redutor acoplado ao motor e controlada por inversor de frequência (marca WEG série CFW-08), que garantiu velocidade entre 0.10 e 0.15 m/s. Valores semelhantes foram utilizados em diferentes pesquisas com LAT (Picot et al., 1991; Park et al., 2011a) e asseguraram o revolvimento necessário.

A biomassa foi produzida em sistema de batelada com duração de 17 dias. Foi utilizado 40 % de inóculo, em relação ao volume total da lagoa, que consistia em biomassa de microalgas adaptada previamente às condições deste efluente. Após a produção, a biomassa foi colhida com hidróxido de sódio (NaOH) a 50% mv^{-1}, promovendo um aumento de pH até 12. As pás foram movidas por curto período de tempo (aproximadamente duas horas), gerando um gradiente hidráulico de velocidade adequado à coagulação. A biomassa foi coletada após repouso do líquido no interior da LAT por 24 h. Para colheita utilizou-se uma bomba submersa pequena para auxílio na remoção do sobrenadante.

O efluente utilizado para o cultivo de microalgas e a biomassa obtida ao final da batelada foi caracterizada seguindo metodologia APHA (2012). As principais características do efluente foram as seguintes: fósforo total (4500 PC) = 10,26 mg L^{-1} e nitrogênio total Kjeldahl = 55,10 mg L^{-1}. A biomassa ao final do tratamento apresentou um teor de sólidos suspensos voláteis (2540–SSV E) de 183 mg L^{-1}.

9.2.2. Carbonização hidrotérmica

A biomassa seca foi misturada com água destilada em uma proporção de 1:10, até completar 220 mL. Para os testes foi utilizado um reator compacto *Parr 5500 Series*, com controle de temperatura e agitação. Inicialmente o reator foi purgado com nitrogênio gasoso (N_2) durante 5 min. Foram realizados testes sob as temperaturas 130ºC, 150ºC e 170 ºC (pressão de 1, 3 e 6 Bar, respectivamente), durante 10, 30 e 50 minutos. A agitação foi ajustada para 500 rpm. O tempo de reação começou quando a temperatura desejada foi atingida. Ao final da reação, toda a amostra foi retirada do reator, sendo a mesma centrifugada a uma rotação 3000 rpm por 1 minuto, para separação das fases sólida e líquida. A fase sólida foi seca em estufa de circulação forçada a 40ºC e acondicionada até a realização das análises e a fase aquosa foi imediatamente analisada. Mais detalhes sobre os procedimentos adotados no tratamento termoquímico podem ser obtidos no artigo 3 (item 8) desta tese.

9.2.3. Análise do ciclo de vida

A ACV foi conduzida de acordo com padrões internacionais (ISO 2006a; 2006b), que estabelece a sua estrutura composta por quatro fases: objetivo e definição do escopo, análise do inventário, avaliação de impacto ambiental e interpretação.

9.2.3.1. Objetivo e unidade funcional

O objetivo deste estudo foi avaliar os impactos ambientais da produção do biochar, parte sólida resultante da carbonização hidrotérmica de biomassa de microalgas. Além disso, buscou-se identificar lacunas no processo e melhorias que possam culminar em redução do impacto ambiental dessa rota.

A unidade funcional adotada foi de 15,55 g de biochar. Essa unidade foi proposta com base em trabalho realizado previamente, no qual um reator de CHT foi operado com 220 mL de biomassa de microalgas e concentração de 100 g L^{-1}, ou seja, 20 g de biomassa e 200 mL de fase aquosa. Na temperatura de 170ºC e tempo de reação de 10 minutos, a recuperação energética foi de 77% e o biochar produzido apresentou um poder calorífico superior de 17,10 MJ kg^{-1}. Esse tratamento foi escolhido por ser o ponto que maximizou o rendimento energético no experimento supracitado.

9.2.3.2. Escopo: definição da fronteira do sistema, descrição dos cenários e suposições feitas

O estudo foi conduzido com abordagem do berço ao portão, que não considera as etapas após a manufatura do produto. Foram criados dois cenários de avaliação de impactos ambientais, nos quais os consumos e as produções de materiais e energia foram considerados. As etapas dos cenários, bem como as entradas e saídas podem ser observados na Figura 9.1. Destacam-se, além das entradas e saídas, os produtos evitados devido ao uso de efluente como meio de cultivo.

O cenário 1 considera que no final do ciclo de vida da CHT a fase aquosa será diretamente disposta em corpos hídricos. Assim, nesta etapa foram incluídas emissões para água, sob a forma de nitrogênio (N) e fósforo (P) presente na fase aquosa. Já cenário 2 considera que a fase aquosa não será disposta em corpos hídricos, podendo ser reutilizada no processo ou disposta no solo como fonte de N e P. Nesse caso, adotou-se o N e o P como subprodutos e foi feita alocação com base no critério de massa. Esse critério assume que os impactos são alocados para cada produto e subproduto produzidos, com base em sua massa.

A biomassa cultivada durante o tratamento de efluente corresponde a um mix de bactérias e microalgas. Esta foi mensurada através da concentração de sólidos suspensos voláteis, método (2540E) do Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Nesta etapa, considera-se o consumo energético para a movimentação das pás.

A etapa de concentração da biomassa inclui o uso de hidróxido de sódio (NaOH) e energia elétrica para fazer com que a biomassa da LAT seja concentrada até aproximadamente 20 g kg^{-1} de sólidos suspensos voláteis. Na etapa de secagem, inclui-se a energia consumida pela estufa para que a biomassa atinja a concentração necessária para entrar no reator de CHT.

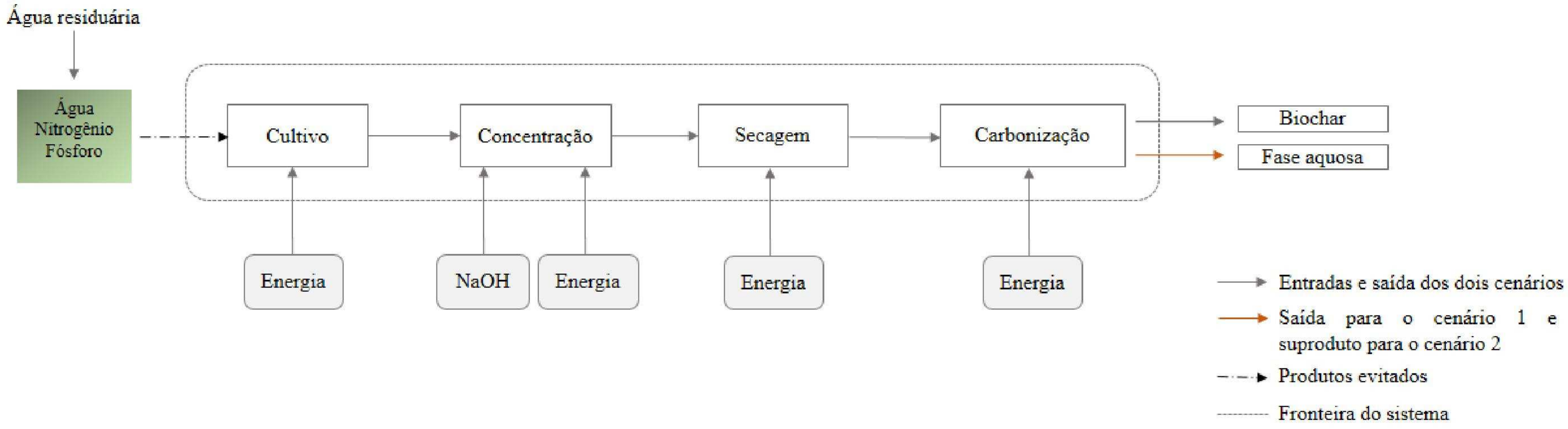

Figura 9.1. Sistema de produto do biochar.

Além disso, o estudo se deu sob a ótica de que a produção da biomassa corresponde a uma etapa do processo operacional de uma estação de tratamento de águas residuárias (ETAR). Assim, não foi levado em consideração a etapa de implantação da ETAR, dado que suas unidades possuem vida útil aproximadamente de vinte e cinco anos.

9.2.4. Inventário do ciclo de vida

O cenário do biochar foi modelado usando dados primários, equivalentes a operação de 4 LATs. Os dados são apresentados na Tabela 9.1. Na etapa do cultivo considerou-se que a densidade do efluente é de 1 kg dm^{-3}. Assim, para a obtenção de 220 mL de biomassa algal a uma concentração de 100 g L^{-1} na entrada do processo de carbonização, o cenário foi modelado com vistas ao tratamento de 109 kg de efluente.

Assim, os dados foram normalizados para compor as entradas e saídas dos processos que resultem na quantidade de biomassa que foi utilizada no *Parr reactor* (20 g de BM) e assim compor o inventário do cenário do biochar (Tabela 9.2).

Tabela 9.1. Dados primários usados como entrada na ACV.

	Quantidade por lagoa	Tempo por operação	Potência (kW)	Detalhes
Motor para rotação da pá da LAT	-	24 h durante 17 dias	0,0833	Para 1 LAT
NaOH	0,5 kg	-	-	50% m/v Densidade = 0,5 kg/m^3
Bomba para concentração da biomassa	-	2 h	0,0200	Para 1 LAT
Estufa para secagem da biomassa	-	24 h durante 2 dias	2,2900	Capacidade útil = 400 kg
Reator Parr	-	30 minutos	0,7000	20 minutos até atingir a temperatura estabelecida

Tabela 9.2. Inventário de ciclo de vida do biochar.

Processos			Unidade	Valor
Cultivo	Entradas	Água residuária industrial	kg	0,109
		Energia elétrica	kWh	3,70
	Produtos evitados	Água	kg	0,109
		Nitrogênio	g	6,01
		Fósforo	g	1,12
	Saídas	Efluente tratado e biomassa algal	kg	0,109
Concentração química	Entradas	Coagulante (NaOH)	kg	0,0545
		Energia elétrica	kWh	0,00436
		Efluente tratado e biomassa algal	kg	109
	Saídas	Biomassa algal úmida	kg	0,95
Concentração térmica	Entradas	Biomassa algal úmida	kg	0,95
		Energia elétrica	kWh	0,34
	Saídas	Biomassa algal (100 g L^{-1})	kg	0,22
CHT[1]	Entradas	Energia elétrica	kWh	0,350
		Biomassa algal (100 g L^{-1})	g	220
	Saídas	Biochar	g	15,55

[1] No cenário 1 considerou-se o nitrogênio (322,62 mg) e fósforo (21,55 mg) presentes na fase aquosa resultando da etapa de CHT como emissões para água, enquanto no cenário 2 esses nutrientes foram considerados como produtos evitados.

A energia utilizada como entrada em todos os processos foi o mix energético brasileiro, disponibilizado pela base de dados da Ecoinvent v.3 no período de 2008 a 2014. Na Figura 7.2 do capítulo 7, pode-se observar os tipos e a proporção da fonte utilizada para compor o mix. Durante a etapa de produção, as entradas foram a energia elétrica (kWh) utilizadas para rotação da rotação da pá e recirculação do efluente. As entradas de água e nutrientes se deram exclusivamente pela água residuária.

Na etapa de concentração química, o NaOH utilizado foi obtido na base de dados do Ecoinvent, e seu inventário inclui, além do processo de eletrólise em célula de diafragma, as seguintes etapas durante a sua produção: produção de salmoura, purificação de salmoura, ressaturação de salmoura, assim como o manuseio final dos produtos de eletrólise, sem a liquefação do cloro (Ecoinvent, 2014).

Na etapa de concentração térmica foi utilizado um equivalente energético necessário para que a biomassa seja concentrada até a concentração de 100 g L^{-1}. Isso porque, mesmo com a concentração química a biomassa possui uma umidade muito elevada para ser inserida no reator de CHT.

9.2.5. Categorias de impacto e metodologia de avaliação de impacto do ciclo de vida

Para quantificar e avaliar os impactos do ciclo de vida, utilizou-se o software SimaPro 8.2.3.0, através da metodologia ReCiPe midpoint, focado em questões ambientais, o que o torna o método mais qualificado para este estudo (PRE, 2013). As categorias de impacto avaliadas foram mudanças climáticas, acidificação terrestre, eutrofização de água doce, eutrofização marinha, toxicidade humana, formação fotoquímica de oxidantes, ecotoxicidade de água doce. Estas categorias se mostraram mais apropriadas para o estudo e também foram empregadas em outros trabalhos envolvendo tratamentos termoquímicos e estudos envolvendo biomassa de microalgas (Benavente et al., 2017; Souza et al., 2019).

Ressalta-se que, para ambos os cenários, foram desconsideradas as emissões a longo prazo.

9.3. Resultados e discussão

9.3.1. Análise do inventário do ciclo de vida

A Figura 9.2 apresenta o inventário do ciclo de vida da produção do biochar. Devido à concentração de nutrientes (N e P), a FA poderia ser aproveitada de diversas formas, sendo: cultivo de microalgas, recirculação na CHT ou mesmo disposta no solo como fonte de nutrientes para plantas.

No inventário do ciclo de vida, os quantitativos de N e P constituintes da FA foram discriminados. No cenário 1 considerou-se que a FA será lançada diretamente em corpos hídricos, sem a recirculação para qualquer fim. No cenário 2, foi proposta uma alternativa para reduzir os impactos do biochar, onde a produção no N e P foi considerada como um subproduto do ciclo de vida do biochar, sendo os impactos alocados por massa.

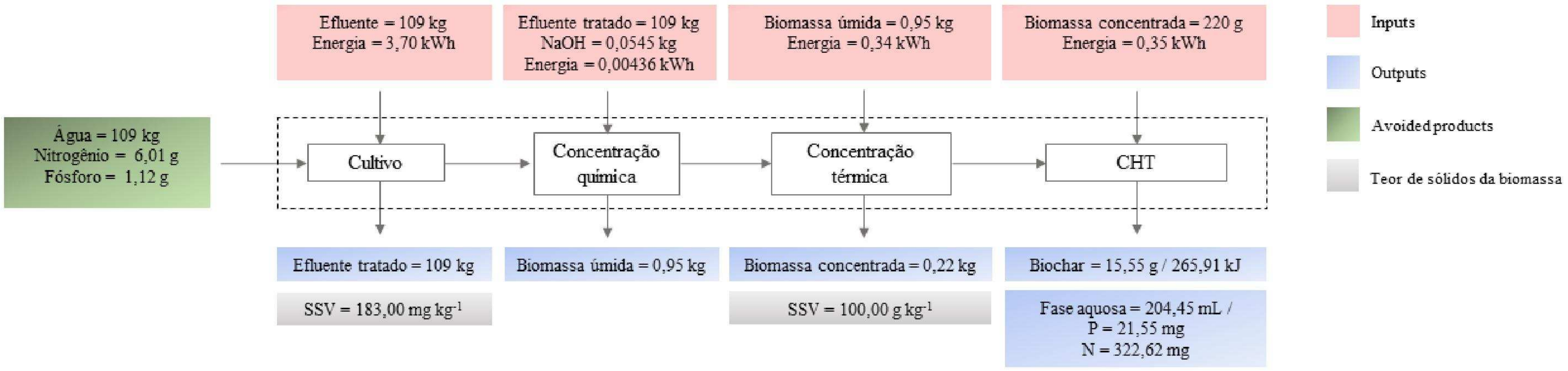

Figura 9.2. Inventário de ciclo de vida da produção do biochar.

Na Tabela 9.3 são apresentados os impactos de cenário 1, segundo as categorias anteriormente definidas. Observa-se que a categoria mudança climática foi a mais relevante. Este resultado reforça o que foi encontrado no capítulo 7 e o que foi reportado por outros autores que trabalharam com ACV envolvendo biomassa de microalgas (Souza et al., 2019).

Tabela 9.3. Impacto ambiental do cenário 1.

Categoria de impacto	Unidade	Valor
Mudanças climáticas	kg CO_2 eq	1,02
Acidificação terrestre	kg SO_2 eq	0,00314
Eutrofização de água doce	kg P eq	5,40E-05
Eutrofização marinha	kg N eq	0,000481
Toxicidade humana	kg 1,4-DB eq	0,0378
Formação de oxidante fotoquímico	kg NMVOC	0,00221
Ecotoxicidade de água doce	kg 1,4-DB eq	0,000835
Ecotoxicidade marinha	kg 1,4-DB eq	0,000538

A Figura 9.3 apresenta o percentual de contribuição de cada processo até a produção do biochar do cenário 1 em cada uma das categorias de impacto analisadas. A etapa de cultivo foi o processo mais impactante em seis das oito categorias investigadas. Isso ocorre porque essa etapa possui um gasto energético elevado e resultou em pouca produção de biomassa. Uma estratégia para melhorar o desempenho energético seria aumentar a produtividade de biomassa. O suprimento de CO_2 contribui para o aumento da produção de microalgas. A suplementação de CO_2 ao meio de cultivo supera a limitação de carbono enfrentada pela produção de microalgas em águas residuárias através de sistemas abertos.

Como exemplo cita-se os resultados obtidos no capítulo 7, no qual a biomassa também foi produzida em efluente agroindustrial. Considerando somente a etapa de cultivo o impacto para produzir 1 g de biomassa utilizando injeção de CO_2 foi de 0,0068 kgCO_2eq/g de biomassa. Em contrapartida no efluente em que não foi utilizado CO_2, o impacto para mudanças climáticas foi de 0,038 kgCO_2eq/g de biomassa. Sendo assim, em termos de impacto ambiental o uso de CO_2 compensa devido a maior produtividade de biomassa.

Além disso, algumas alternativas podem ser adotadas para melhorar ainda mais o cenário de utilização de CO_2. Assis et al. (2019) investigaram a adição de gases de escape da combustão de gasolina e CO_2 em alta concentração (comercialmente disponível) durante o cultivo de microalgas e tratamento de esgoto doméstico em LATs. Os autores verificaram que a fonte alternativa de CO_2 não influenciou na eficiência do tratamento e rendimento de biomassa. Logo, as emissões atmosféricas garantem elevada eficiência, podendo ainda, contribuir para a mitigação das emissões de gases de efeito estufa e, consequentemente, para diminuição dos impactos associados a categoria mudanças climáticas. Adicionalmente, como mencionado no capítulo 7, o uso de energia fotovoltaica pode ser uma forma de reduzir os impactos ambientais associados à produção de biomassa de microalgas.

Para a etapa de CHT no cenário 1, as categorias mais impactantes foram eutrofização de água doce e eutrofização marinha. Esse resultado está relacionado à disposição de N e P resultante da fase aquosa em corpos hídricos que são os principais elementos relacionados a eutrofização. Ressalta-se que não estão sendo considerados os impactos de outros compostos que podem estar presentes na FA, tais como fenóis e benzenodióis.

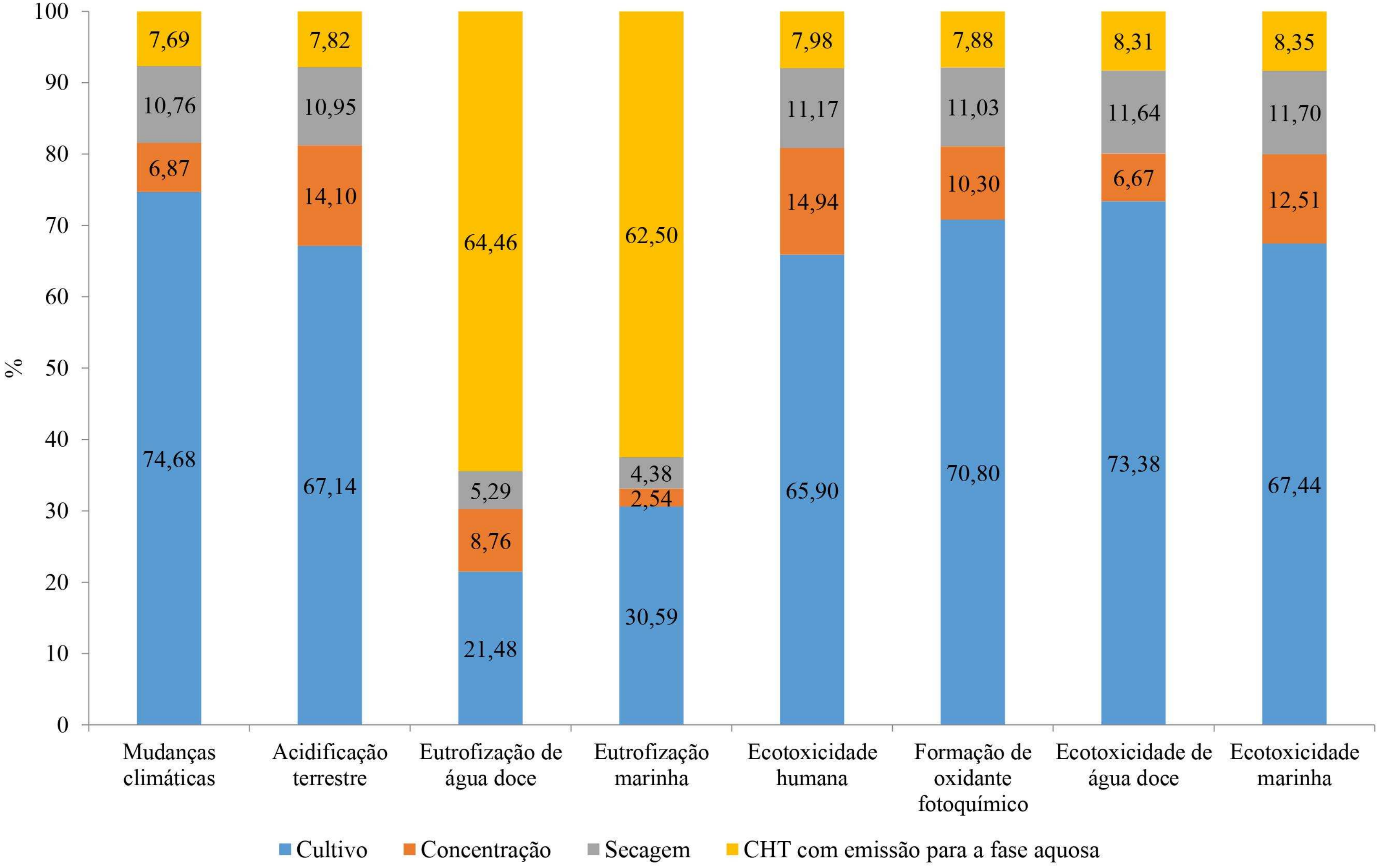

Figura 9.3. Percentual de contribuição de cada processo nas categorias de impacto considerando o lançamento da FA diretamente em corpos hídricos.

Sendo assim, a FA precisa de tratamento adicional antes de ser descartada, o que pode tornar esse processo termoquímico oneroso (Becker et al., 2014). Para este tratamento, faz-se necessário o conhecimento mais aprofundado sobre sua composição. Ainda, os compostos considerados tóxicos para o ambiente devem ser levados em consideração em uma avaliação mais detalhada do ciclo de vida dessa rota. A presença desses materiais, quando da avaliação do ciclo de vida, pode tornar a rota da CHT convencional inviável do ponto de vista ambiental.

9.3.2. Disposição da fase aquosa

A FA da CHT é composta pela água residual e pela matéria inorgânica e orgânica resultante da degradação dos resíduos sólidos (Berge et al., 2011). Conforme observado no capítulo 8, o carbono presente na FA deste estudo é totalmente de origem orgânica. Já foi reportado anteriormente que o carbono orgânico pode incluir moléculas orgânicas tóxicas (por exemplo, derivados do fenol) (Wang et al. al., 2012; Weidner et al., 2013; Poerchmann et al., 2014).

A Figura 9.4 apresenta a comparação percentual entre os impactos do cenário 1, modelado com a etapa de CHT com emissão para a fase aquosa, e o cenário 2, que considera o N e P como subprodutos da CHT. As categorias de impacto que apresentam diferenças significativas são eutrofização de água doce e eutrofização marinha (aproximadamente 60% menor). Não foi contabilizado o impacto positivo da recirculação da FA no sistema.

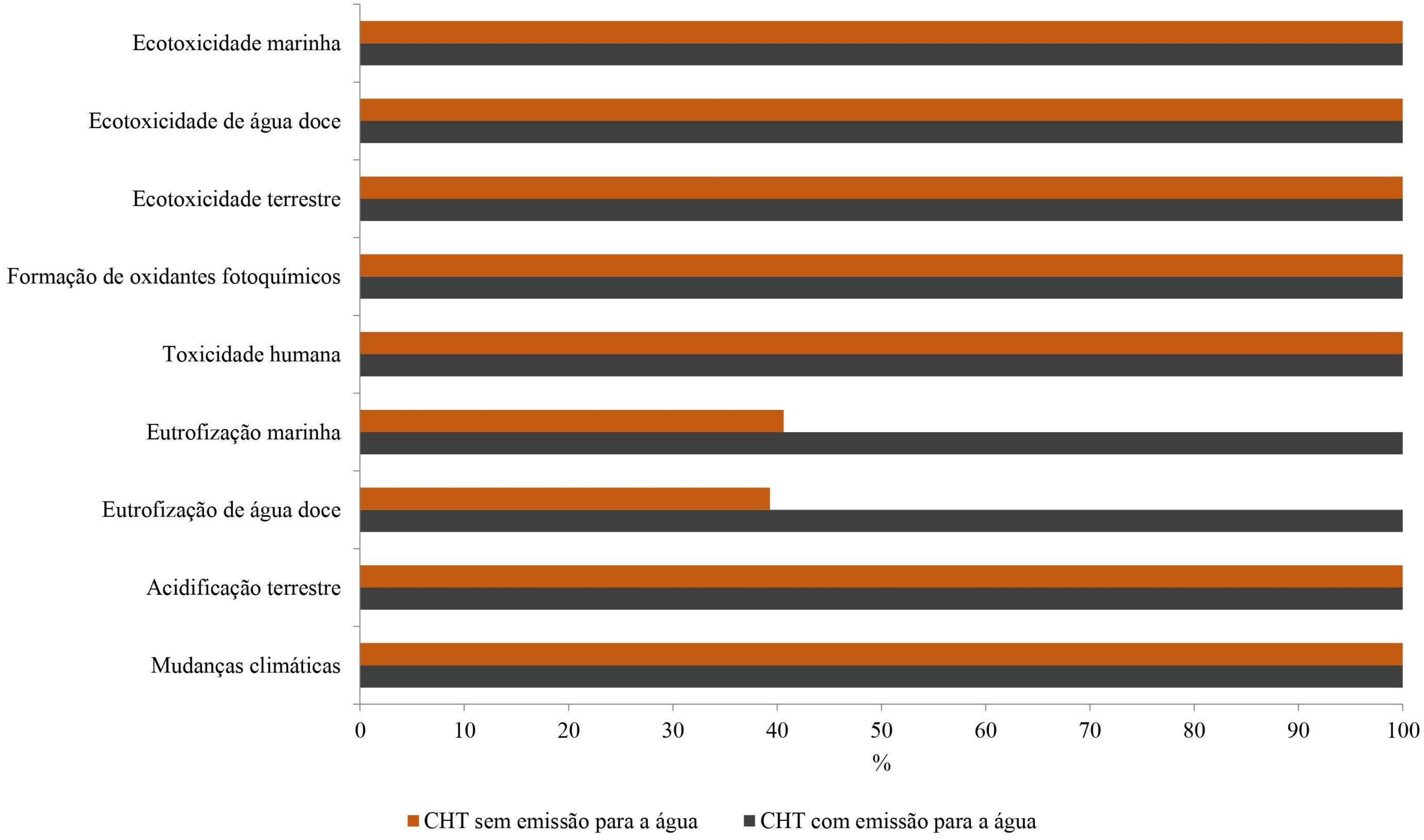

Figura 9.4. Comparação percentual entre os impactos da CHT com emissão para a fase aquosa e adotando o N e P como subprodutos da CHT.

Benavente et al. (2017) avaliaram os impactos ambientais associados a CHT de resíduos do moinho de azeitonas. Os resultados indicaram que a disposição da FA do processo CHT influencia significativamente nos impactos ambientais do sistema, indicando que são necessárias pesquisas que investigam alternativas de tratamento. Além do tratamento do resíduo, a recirculação da FA pode contribuir para redução dos impactos da disposição no ambiente.

Belete et al. (2019) investigaram a viabilidade do uso da fase aquosa da CHT como fonte alternativa de nutrientes para o cultivo de microalgas. A CHT do lodo de esgoto foi realizada e a fase aquosa foi caracterizada sendo considerada adequada para o crescimento de microalgas. Taxas de crescimento na fase aquosa de CHT e no meio controle foram semelhantes, indicando que a fase aquosa de CHT não inibiu o crescimento de microalgas. As concentrações de nitrogênio e fósforo foram reduzidas em mais de 90 % e o carbono orgânico dissolvido reduziu em 80 % após 6 dias de cultivo, resultando em qualidade da água adequada para descarga.

Leng et al. (2020) investigaram o efeito da recirculação de FA nas propriedades do hidrochar de diferentes tipos de biomassa. Uma biomassa de *Chlorella* e palha de soja foram carbonizados hidrotermicamente a 220°C por 240 min com recirculação da FA por quatro ciclos, respectivamente. O rendimento de biochar foi aumentado de 20,5% para 26,7% para a biomassa de *Chlorella* e de 47,7% para 54,7% para a palha da soja. A recuperação de energia pela CHT aumentou entre 10% e 12%.

O cenário que considera a produção de N e P como subprodutos da produção do biochar e aloca, pelo critério de massa, os impactos relacionado à produção dos mesmos é apresentado na Figura 9.5. Nesse cenário, a disposição da FA em corpos hídricos deixaria de ser um problema, pois a fase aquosa poderia ser recirculada no sistema, sendo que o maior percentual de impactos em todas as categorias seria para a etapa de cultivo.

O impacto foi reduzido principalmente pelo fato desses nutrientes não serem lançados em corpos hídricos. O impacto da produção dos mesmos é muito reduzido em relação ao biochar. Como a quantidade produzida é muito pequena, e o critério de alocação foi por massa, os impactos relacionados a esses nutrientes também foi baixo.

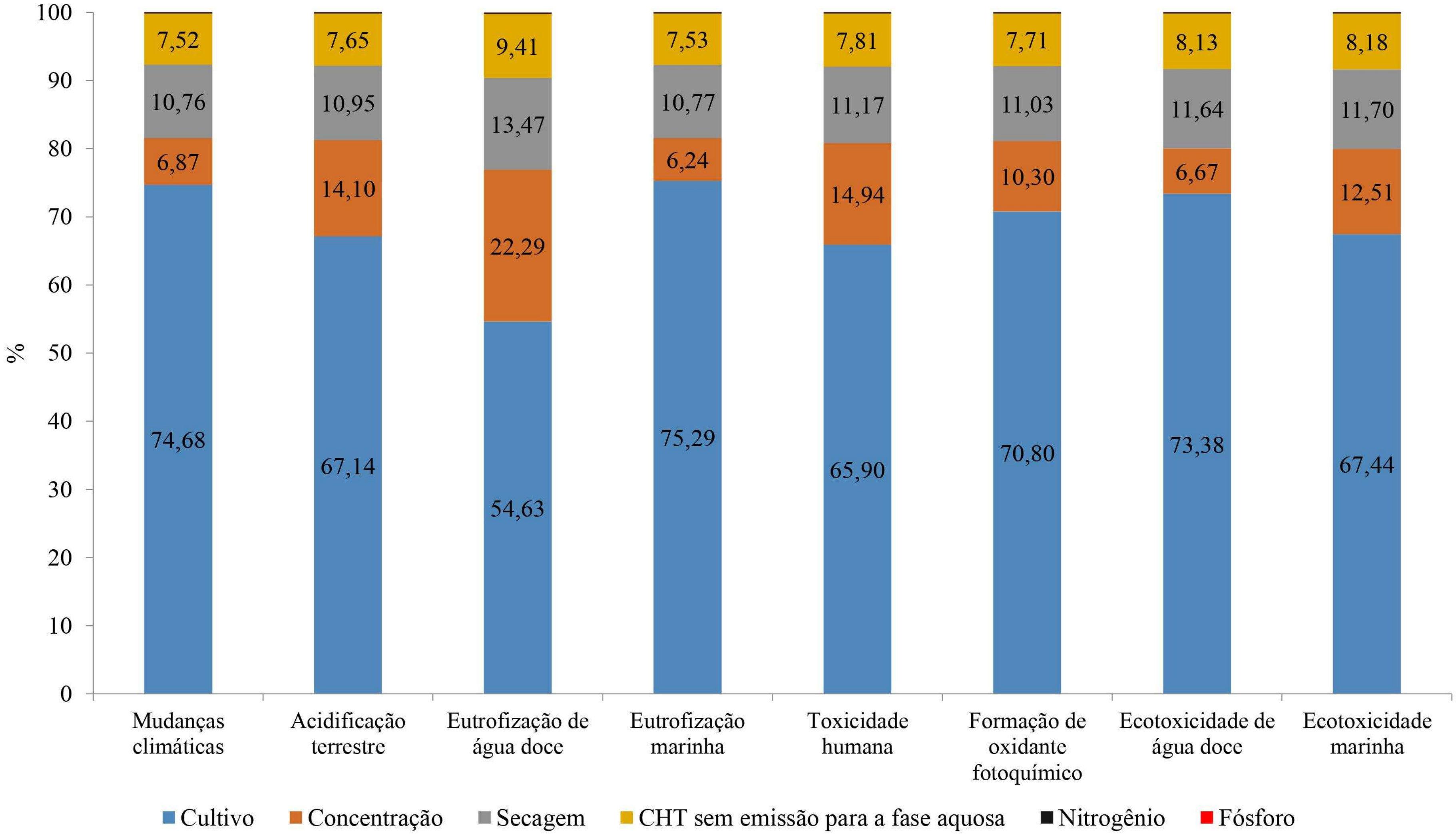

Figura 9.5. Percentual de contribuição de cada processo nas categorias de impacto considerando o lançamento da FA diretamente em corpos hídricos.

Benavente et al. (2017) reportaram que a CHT apresentou impacto ambiental maior que processos de tratamento biológico nas categorias de impacto eutrofização de água doce, eutrofização marinha e ecotoxicidade de água doce. As duas primeiras categorias coincidem com as obtidas neste estudo. A categoria ecotoxicidade de água doce foi avaliada por esses autores, com auxílio do método USEtox (Rosenbaum et al., 2008). Por esse motivo, essa categoria tenha sido tão sensível no trabalho desses autores quando se trata da disposição da FA em corpos hídricos.

Mais estudos são necessários para determinar o cenário ideal de reutilização da fase aquosa resultante da CHT, bem como a comparação desse tratamento com outras rotas de aproveitamento da biomassa de microalgas.

9.4. Conclusão

A etapa mais impactante no ciclo de vida do biochar foi o cultivo. Considerando somente a etapa de CHT no cenário 1, as categorias eutrofização de água doce e eutrofização marinha são as mais impactantes. Uma fonte alternativa de energia poderia ser utilizada na tentativa de reduzir o impacto da energia elétrica no cultivo da biomassa de microalgas atrelado ao aproveitamento de CO_2 a partir de emissões gasosas.

O tratamento ou destinação adequada da FA também é uma forma de reduzir os impactos associados à CHT. Os impactos nas categorias eutrofização de água doce e eutrofização marinha foram reduzidos em até 60 %, na etapa de CHT, considerando a não disposição da FA em corpos hídricos.

Recomenda-se aumentar a escala da CHT para proposições mais assertivas a respeito dessa rota de aproveitamento, bem como a comparação dessa rota, com outras rotas de valorização da biomassa de microalgas.

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10. CONCLUSÃO GERAL

No cultivo de algas, as principais lacunas no conhecimento são os impactos dos poluentes nos reatores de crescimento que afetam as características finais da biomassa. A colheita de biomassa é uma área que ainda precisa de muitas adequações e a secagem deve ser evitada, sempre que possível, levando a uma escolha de rotas de valorização que usam biomassa úmida. O biochar e biofertilizante são produtos com potencial para valorização energética e agrícola da biomassa de algas cultivadas em efluentes. A ACV é uma ferramenta importante para apoiar a tomada de decisões e precisa ser mais explorada no universo das microalgas.

No âmbito da valorização agrícola, a proporção de MB no superfosfato triplo que maximizou o conteúdo e a concentração de P nas plantas foi de aproximadamente 12% MB. A partir de então, observou-se uma redução nos valores das variáveis analisadas com o aumento da proporção de MB no biofertilizante. Comportamento semelhante foi observado na avaliação da difusão de fósforo em solos arenosos e argilosos. A 30% MB, a difusão foi claramente prejudicada pelo aumento da MO na massa de fertilizante, o que promoveu a formação de uma barreira física no grânulo. Sugere-se que o resultado positivo exercido pelo MB nas plantas esteja relacionado aos macro e micronutrientes que constituem a biomassa.

O biofertilizante de microalgas apresentou impacto ambiental maior que o SFT em todas as categorias de impacto analisadas na ACV e grande parte desse impacto esteve associado às etapas de produção e processamento da biomassa algal. No cenário ideal criado, no qual foi considerado o uso do painel fotovoltaico como fonte de energia, a concentração de biomassa sem o uso de NaOH e a secagem natural da biomassa através do leito de secagem, o impacto do biofertilizante se aproximou consideravelmente daqueles apresentados no SFT, exceto na categoria ecotoxicidade terrestre, na qual o biofertlizante ainda apresentou impacto aproximadamente 64 % maior.

No âmbito da valorização energética, a CHT da biomassa de microalgas mostrou-se como uma rota viável tecnicamente. Os resultados mostraram que o rendimento de sólidos e a recuperação energética apresentaram uma tendência em aumentar no menor tempo de reação testado (10 minutos) com o aumento da temperatura.

A biomassa carbonizada pode ser utilizada como biocombustível embora uma melhoria significativa nas suas características pode ser obtida com a remoção prévia das cinzas que, devido a seu alto teor na biomassa bruta, pode ter ocasionado uma redução no poder calorífico. Frente às suas características, a fase aquosa pode ser utilizada como meio de cultivo para microalgas ou para recirculação na própria CHT.

A etapa mais impactante no ciclo de vida do biochar foi o cultivo da biomassa de microalgas. As categorias de impacto mais afetadas na etapa de CHT são eutrofização de água doce e eutrofização marinha. O tratamento ou destinação adequada da FA é uma forma de reduzir os impactos associados à CHT. Os impactos nas categorias eutrofização de água doce e eutrofização marinha foram reduzidos em até 60 % considerando a não disposição da FA em corpos hídricos.

11. SUGESTÕES PARA PESQUISAS FUTURAS

Em relação aos biofertilizantes recomenda-se estender a escala temporal e espacial (parcelas experimentais) para que inferências mais precisas relacionadas à viabilidade técnica e financeira do uso de biomassa de microalgas, além de fertilizantes minerais. Além disso, experimentos que enfocam a influência de micronutrientes presentes na biomassa em plantas, bem como a caracterização de BM para a presença de fitohormônios. Todos os benefícios da BM podem culminar em redução de impacto ambiental podendo tornar essa rota viável.

No que tange a rota do biochar, recomenda-se que sejam realizados mais testes relacionados à remoção prévia das cinzas que afeta diretamente, entre outras características, o poder calorífico do biochar. Frente às suas características, a fase aquosa pode ser utilizada como meio de cultivo para microalgas ou para recirculação na própria CHT. Mas também carece de caracterização e experimentação mais aprofundada. Além disso, a escala da análise da CHT deve ser aumentada para resultados mais precisos a respeito do impacto ambiental dessa rota.

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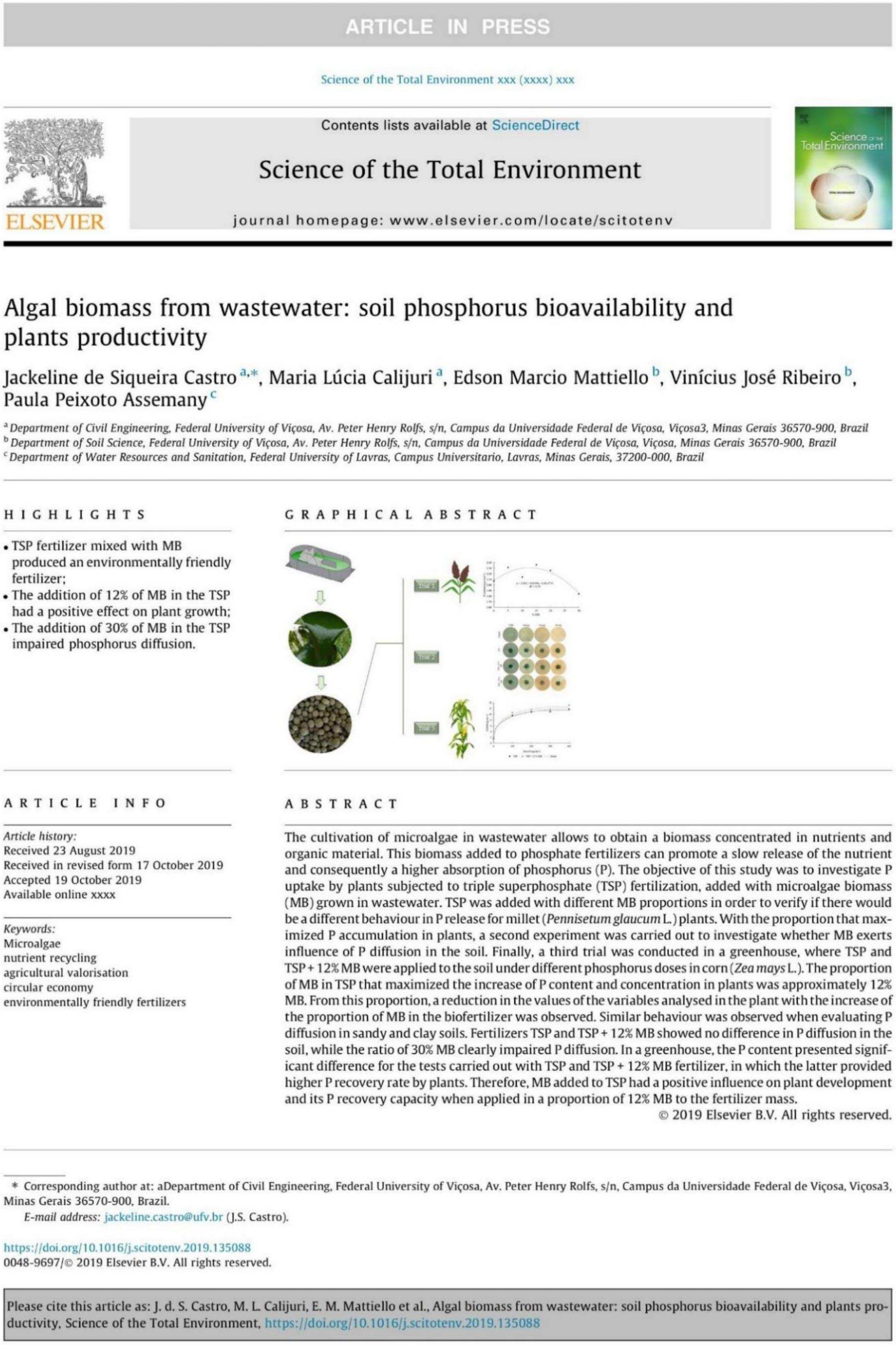
12. ANEXO I



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Algal biomass from wastewater: soil phosphorus bioavailability and plants productivity

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HIGHLIGHTS

- TSP fertilizer mixed with MB produced an environmentally friendly fertilizer;
- The addition of 12% of MB in the TSP had a positive effect on plant growth;
- The addition of 30% of MB in the TSP impaired phosphorus diffusion.

GRAPHICAL ABSTRACT

Trial 1
Trial 2
Trial 3

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ABSTRACT

The cultivation of microalgae in wastewater allows to obtain a biomass concentrated in nutrients and organic material. This biomass added to phosphate fertilizers can promote a slow release of the nutrient and consequently a higher absorption of phosphorus (P). The objective of this study was to investigate P uptake by plants subjected to triple superphosphate (TSP) fertilization, added with microalgae biomass (MB) grown in wastewater. TSP was added with different MB proportions in order to verify if there would be a different behaviour in P release for millet (*Pennisetum glaucum* L.) plants. With the proportion that maximized P accumulation in plants, a second experiment was carried out to investigate whether MB exerts influence of P diffusion in the soil. Finally, a third trial was conducted in a greenhouse, where TSP and TSP + 12% MB were applied to the soil under different phosphorus doses in corn (*Zea mays* L.). The proportion of MB in TSP that maximized the increase of P content and concentration in plants was approximately 12% MB. From this proportion, a reduction in the values of the variables analysed in the plant with the increase of the proportion of MB in the biofertilizer was observed. Similar behaviour was observed when evaluating P diffusion in sandy and clay soils. Fertilizers TSP and TSP + 12% MB showed no difference in P diffusion in the soil, while the ratio of 30% MB clearly impaired P diffusion. In a greenhouse, the P content presented significant difference for the tests carried out with TSP and TSP + 12% MB fertilizer, in which the latter provided higher P recovery rate by plants. Therefore, MB added to TSP had a positive influence on plant development and its P recovery capacity when applied in a proportion of 12% MB to the fertilizer mass.

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13. ANEXO II

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Microalgae based biofertilizer: A life cycle approach

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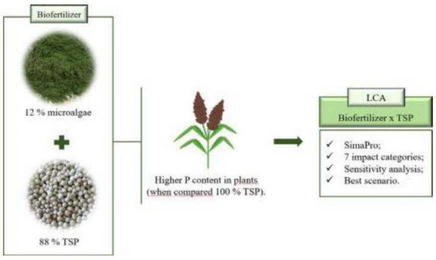
HIGHLIGHTS

- Environmental impacts of the production of microalgae-based biofertilizer were compared to triple superphosphate.
- Biofertilizer presented the greatest environmental impacts in all categories investigated.
- The biggest differences occur in the climate change and terrestrial ecotoxicity categories.
- Dewatering and drying steps are improvement opportunities in the microalgae-based biofertilizer.

GRAPHICAL ABSTRACT



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ABSTRACT

Waste, especially biomass in general, is a large reservoir of nutrients that can be recovered through different technologies and used to produce biofertilizers. In the present study, environmental impacts of the production of microalgae biomass-based phosphate biofertilizer compared to triple superphosphate through life-cycle assessment conducted in the Simapro® software were investigated. The functional unit of the analysis was 163 g of P for both fertilizers. Phosphorus was recovered from a meat processing industry effluent in a high-rate algal pond. Impacts related to the entire biofertilizer chain impacted mainly on climate changes (3.17 kg CO_2eq). Microalgae biofertilizer had higher environmental impact than conventional fertilizer in all impact categories, highlighting climate change and terrestrial ecotoxicity. An ideal scenario was created considering that: all energy used comes from photovoltaic panels; in the separation step a physical method will be used, without energy expenditure (i.e. gravimetric sedimentation) and; biomass will be dried in a drying bed instead of the thermal drying. In this scenario, the impact of biofertilizer approached considerably those of triple superphosphate. When impacts of biomass cultivation and concentration stages were disregarded, drying step was of great relevance, contributing to increase biofertilizer impacts. More research is needed to optimize the algae production chain and determine the possibility of obtaining higher added value products more environmental attractive.

1. Introduction

Modern agriculture depends on the input of fertilizers for food, fiber and biofuel production. However, a significant portion of fertilizers are lost, increasing agricultural cost, wasting energy and polluting the

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14. ANEXO III

PRÉ-TESTES: Carbonização hidrotérmica da biomassa algal cultivada em águas residuárias

Resumo: Os parâmetros utilizados na carbonização hidrotérmica da biomassa de algas apontados na literatura são muito variáveis de um trabalho para outro. Além disso, não existem registros dsse tratamento utilizando como matéria-prima a biomassa de algas cultivadas em efluente. Diante disso, optou-se por realizar uma análise exploratória de faixas de temperatura e tempo de espera que propiciassem melhores resultados na carbonização hidrotérmica de biomassa cultivada em efluentes. Foram então realizados dois experimentos como pré-testes. No experimento I foram realizados dois tratamentos: (i) carbonização hidrotérmica a 134 ºC e (ii) torrefação seca a 200 ºC, ambos com tempo de espera de 60 minutos. Observou-se que o poder calorífico superior do biochar da torrefação seca foi superior em relação ao da carbonização hidrotérmica; Quando comparados os valores de poder calorífico superior de biochar de biomassas algais registradas na literatura, os valores encontrados nesse teste apresentaram-se abaixo do esperado. Portanto, com base nos resultados do experimento I, optou-se por realizar uma segunda etapa de testes com menor tempo de espera e com temperaturas maiores para a carbonização hidrotérmica. No experimento II, foram realizados três testes sob as temperaturas 130 ºC, 150 ºC e 170 ºC durante tempo de espera de 30 minutos. O poder calorífico superior foi maximizado à temperatura de 150 ºC. Os resultados obtidos para poder calorífico superior foram condizentes com resultados reportados na literatura o que levou a concluir que o tempo de espera testado pode ser considerado bom, apesar de não terem sido testados tempos de espera menores. Portanto, o experimento final de carbonização hidrotérmica que comporá a tese, contará com delineamento experimental baseado em experimento composto central, e terá como pontos centrais da análise a temperaura de 150 ºC e tempo de espera de 30 minutos. O objetivo é obter uma superfície de resposta com os pontos ótimos de temperatura e tempo de espera que propiciem a obtenção de um produto com mais alta recuperação de energia.

Palavras-chave: biochar; microalgas; processo termoquímico; valorização energética; superfície de resposta.

1. Introdução

No caso da biomassa de microalgas, o primeiro trabalho reportado na literatura sob a conversão por tratamento hidrotérmico foi realizado por Heilmann et al. (2010) e desde então, a CHT da biomassa de microalgas produzida em meio sintético tem sido investigada por diversos outros autores (Bach et al., 2017a; Heilmann et al., 2011; Levine et al., 2013; Lu et al., 2015; Smith et al., 2016). Esses trabalhos buscaram estudar/otimizar os parâmetros operacionais relacionados a esse tratamento termoquímico, visto que cada tipo de biomassa e a forma com que a mesma é produzida reflete diretamente em suas características finais e nos parâmetros de operação a serem adotados.

Apesar de vários trabalhos terem sido realizados com biomassa obtida em meio de cultivo sintético, a biomassa mista cultivada em efluente apresenta características diferentes daquelas cultivadas em meio sintético e não foram encontrados estudos que utilizaram essa matéria prima na literatura pesquisada. Portanto, há que se destacar a necessidade de pesquisas relacionadas à CHT da biomassa de microalgas cultivadas em efluentes.

Apesar de todas as vantagens citadas, ainda há muito o que se investigar na CHT da biomassa de microalgas cultivadas em efluentes. Entre os fatores a serem investigados estão os parâmetros operacionais, tais como temperatura de operação e tempo de reação, o uso de catalisadores e/ou aditivos que contribuem com a eficiência do processo. As características da biomassa a ser submetida à CHT é fator determinante na escolha desses parâmetros.

Quando se trata da biomassa de microalgas, de maneira geral, a temperatura de operação não tem superado 215 ºC (Bach et al., 2017a; He et al., 2013; Levine et al., 2013). No trabalho realizado por Bach et al. (2017a), a biomassa de microalgas foi cultivada em meio sintético e a TU foi operada sob diferentes temperaturas (160, 170 e 180 ºC). Quando a temperatura de operação foi aumentada de 170 ºC para 180 ºC houve uma redução considerável na produtividade de sólidos e o poder calorífico superior (PCS) praticamente se manteve igual, além disso quanto mais severas as condições da TU, menor foi o conteúdo final de lipídios e proteínas.

Um dos parâmetros operacionais mais variáveis conforme trabalhos citados na literatura é o tempo de espera que variam de 5 minutos até 720 minutos. Se comparados os trabalhos relacionados à CHT do lodo de esgoto realizados por He et al. (2013) e Afolabi et al. (2017) no qual o primeiro trabalhou com tempo de reação que variaram de 240 a 720 minutos a 200 ºC e o segundo com tempo de reação de 30 min a 180 e 200 ºC, observa-se que ambos obtiveram valores muito próximos de PCS nos produtos finais. Os grupos funcionais resultantes dessas reações precisam ser melhores estudados para que se possa de fato entender se há a necessidade de tempos de reação tão elevados, o que elevaria a demanda energética do processo.

Diante da inexistência de trabalhos relacionados à CHT da biomassa de microalgas cultivadas em efluentes e devido às diferenças dessa biomassa com as demais reportadas na literatura, o objetivo deste estudo foi realizar uma busca com experimentos exploratórios testando a biomassa cultivada em efluentes quanto à sua possível aplicação na CHT. Buscou-se compreender o comportamento dessa biomassa frente à diferentes temperaturas e tempos de espera, além de comparar este tratamento com um tratamento de torrefação via seca (TS) da mesma biomassa. Com base nos dados obtidos nessa análise exploratória pretende-se realizar um planejamento experimental, com tratamentos que possam oferecer resultados mais consistentes com relação ao uso desse tipo de biomassa na CHT.

2. Materiais e métodos

2.1. Experimentos

A produção da biomassa se deu na unidade experimental de tratamento de efluentes e produção de biomassa situada na área externa do Laboratório de Engenharia Sanitária e Ambiental (LESA), na Universidade Federal de Viçosa (UFV), no estado de Minas Gerais, Brasil (20°45’14”S, 42°52’54”W). O município de Viçosa, com altitude média de 648 m em relação ao nível do mar, caracteriza-se por uma precipitação média anual de aproximadamente 1221 mm, e temperatura média anual que oscila entre 19 ºC e 20 ºC. A umidade relativa do ar é, em média, de 81%. O clima local, segundo a classificação de Köppen, é do tipo Cwa, tropical de altitude com verões quentes e chuvosos e invernos frios e secos (Rocha et al., 2012).

Os testes de CHT do experimento I foram realizados no Laboratório de Painéis e Energia da Madeira (LAPEM) no Departamento de Engenharia Florestal (DEF-UFV) e os testes do experimento II foram realizados no Laboratório de Simulação de Processos Químicos (LaSip) no Departamento de Engenharia Química (DEQ-UFV).

2.2. Produção da biomassa algal

A biomassa de microalgas foi cultivada como subproduto do tratamento do efluente de uma indústria de beneficiamento de carnes em lagoas de alta taxa (LAT).

Essa indústria que tem como principal atividade, a produção de embutidos (salames do tipo mortadela, presuntos, apresuntados, linguiças diversas, salsichões, entre outros) e a produção de bacalhau dessalgado desfiado. O efluente de origem industrial é gerado nas diversas etapas do processo produtivo, em especial nos descartes dos tanques de cozimento e resfriamento dos embutidos e descartes dos tanques de dessalga do bacalhau e também da lavagem dos pisos e equipamentos ao final dos processos produtivos. Salienta-se que nesta pesquisa foi utilizado o efluente do flotador (efluente primário).

A LAT em escala piloto utilizada para a produção da biomassa possui as seguintes características: largura = 1,28 m, comprimento = 2,86 m, profundidade total = 0,3 m, profundidade útil = 0,3 m, área superficial = 3,3 m² e volume útil = 1 m³. A LAT foi confeccionada em fibra de vidro e os pedais em aço inoxidável, com seis lâminas. Durante seu funcionamento, os pedais foram movimentados por motor elétrico de 1cv. A rotação foi reduzida por redutor acoplado ao motor e controlada por inversor de frequência (marca WEG série CFW-08), que garantiu velocidade entre 0,10 e 0,15 m/s. Valores semelhantes foram utilizados em diferentes pesquisas com LAT (Picot et al., 1991; Park et al., 2011) e asseguraram o revolvimento necessário.

A biomassa utilizada no experimento I foi produzida em batelada com duração de 14 dias em outubro de 2015. A biomassa utilizada no experimento II e também a ser utilizada nos demais experimentos foi produzida em batelada com duração de 21 dias em março/abril de 2018. Em ambas as operações foi utilizado 40 % de inóculo, em relação ao volume total da lagoa, que consistia em biomassa de microalgas adaptada previamente às condições deste efluente.

2.3. Preparação e caracterização da biomassa

Após a produção da biomassa, a mesma foi concentrada por meio de floculação química, com adição de solução de NaOH 50 % (m v^{-1}) para elevar o pH até 12. Em seguida, o efluente na LAT foi agitado com auxílio de recipiente de plástico durante 3 minutos e a sedimentação realizada na própria LAT durante o período noturno. A biomassa concentrada no fundo da LAT foi coletada de forma manual, após o descarte do efluente. Após a coleta, o pH da biomassa concentrada foi neutralizado com adição de solução de HCl 1:1 (v v^{-1}).

A biomassa úmida utilizada no experimento II foi submetida à análise fitoplanctônica. As amostras foram coletadas e posteriormente conservadas em solução de formol 4 % (razão de 1:10 entre solução e amostra). Após esse procedimento, as amostras foram enviadas por correio para o Laboratório de Microbiologia Ambiental, da Universidade Federal de São Carlos (UFSCAR) para a identificação. Foram identificadas as principais espécies dentro de cada gênero com base em Parra et al. (1982) e Komarek; Fott (1983), segundo metodologia Uthermöl (1958); Wetzel e Likens (1991); APHA (2012).

Para as análises da composição química elementar (carbono, hidrogênio, nitrogênio, enxofre e oxigênio) e dos teores de macro e micronutrientes e metais pesados, primeiramente as amostras foram secas em estufa a 60 °C, trituradas e depois classificadas em peneiras com abertura de 100 mesh. Os teores de carbono, hidrogênio e nitrogênio foram determinados via combustão seca, utilizando-se analisador elementar Perkin Elmer, PE-2400, série II. O método consiste em promover a combustão da amostra a 925ºC e analisar os gases liberados por cromatografia gasosa. O teor de enxofre foi obtido por turbidimetria, com o auxílio de um espectrofotômetro, no comprimento de onda de 440 nm. O teor de oxigênio foi determinado pelo somatório das cinzas com C, H, N e S decrescido de 100.

Para determinação dos elementos totais, macro, micronutrientes e metais pesados das biomassas algais, de forma individual, foram submetidas à digestão nítrico-perclórica (4:1, v/v) determinando-se os elementos totais: P, quantificado por colorimetria; K, por fotometria de chama; Ca e Mg, por absorção atômica; S, por turbidimetria, N total determinado pelo método Kjeldahl; Mn, Cu, Fe e Zn, por absorção atômica,; B, pelo método colorimétrico com o reagente azometina-H. Excetuando o N total, as demais determinações foram realizadas conforme procedimentos da EMBRAPA (1997).

Para a determinação do poder calorífico superior (PCS), análise química imediata (AQI) (materiais voláteis, carbono fixo e teor de cinzas) e análise termogravimétrica (TGA) a biomassa foi classificada em peneiras com abertura de 0,42 mm (40 mesh) e 0,25 mm (60 mesh) recolhendo-se a fração que ficou retida na peneira de 60 mesh. A composição química imediata (matérias voláteis, carbono fixo e teor de cinzas) foi determinada de acordo com a norma NBR 8112 (ABNT, 1986). O poder calorífico superior foi obtido experimentalmente por meio do método da bomba calorimétrica adiabática, de acordo com a norma ASTM D 2015 (ASTM, 1982).

Para análise TGA utilizou-se o aparelho DTG-60H, Shimadzu, em atmosfera de gás nitrogênio, a uma vazão constante de 50 mL min^{-1} empregando-se aproximadamente 2 mg de biomassa colocadas em cápsula de alumina aberta. As curvas termogravimétricas foram obtidas a partir de 100ºC até temperatura máxima de 600ºC, com taxa de aquecimento de 10 ºC min^{-1}. A curva termogravimétrica foi obtida para avaliar a perda de massa através da temperatura e a partir destas procederam-se os cálculos de perda de massa nos seguintes intervalos de temperatura: 100-200ºC, 200-300ºC, 300-400ºC, 400- 500ºC e 500-600ºC.

2.4. Experimento I

No experimento I foram realizados dois testes: (i) CHT e (ii) torrefação seca (TS). Para o teste de CHT foram pesadas 10 g de biomassa seca, à qual foi misturada 30 mL de água deionizada, obtendo uma relação final de biomassa:água de 1:3. As amostras foram levadas para uma autoclave a 134 ºC com um tempo de espera de 60 minutos. Após 60 minutos, as amostras foram então levadas para uma estufa de circulação forçada a 100 ºC durante 48h horas até sua completa secagem. As amostras foram pesadas para obtenção do rendimento e posteriormente preparadas para as análises de PCS, AQI e TGA, como descrito no item 5.2.3.

A torrefação seca foi realizada em mufla a 200 ºC com um tempo de espera de 60 minutos. As amostras foram posteriormente pesadas para obtenção do rendimento e preparadas para as análises de PCS, AQI e rendimento.

As etapas e análises realizadas no experimento I podem ser observadas na Figura 1.

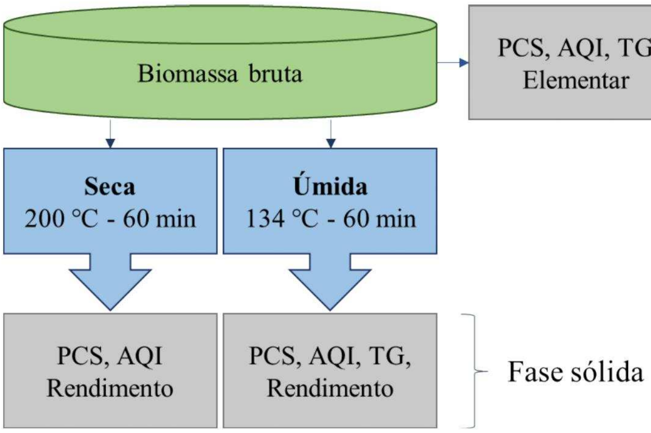

Figura 1. Representação esquemática dos tratamentos (azul) e das análises realizadas em cada etapa (cinza) no experimento I.

2.5. Experimento II

A biomassa seca foi misturada com água destilada em uma proporção de 1:20, até completar 200 mL. Para os testes foi utilizado um reator compacto *Parr 5500 Series*, com controle de temperatura e agitação (Figura 2).

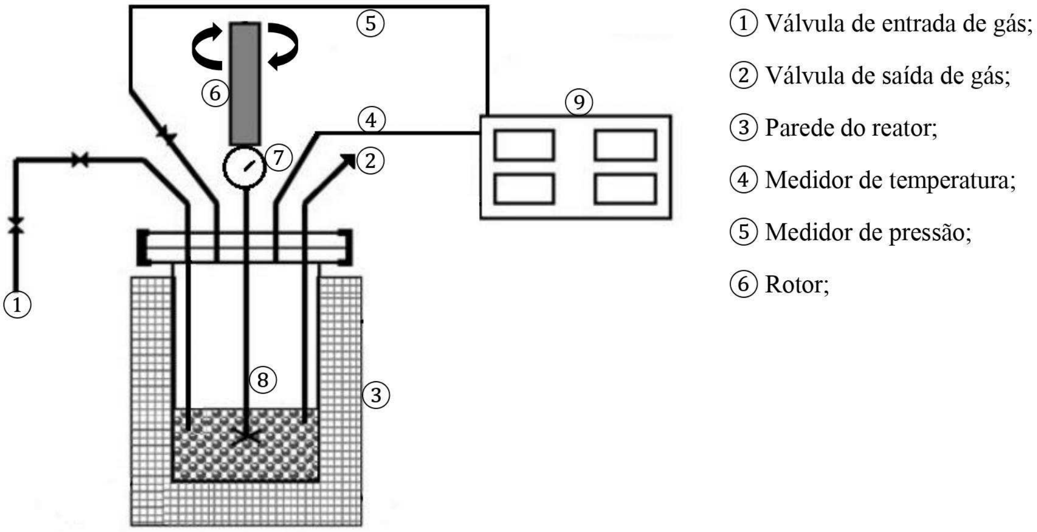

Figura 2. Diagrama esquemático do reator *Parr 5500 Series* fechado.

Inicialmente o reator foi purgado com nitrogênio gasoso (N_2) durante 10 min. Foram realizados três testes sob as temperaturas 130 ºC, 150 ºC e 170 ºC durante 30 minutos. A agitação foi ajustada para 500 rpm.

O tempo de reação começou quando a temperatura desejada foi atingida. No final do tempo, retirou-se uma amostra do reator, sendo a mesma centrifugada para separação das fases sólidas e líquida. A fase sólida foi seca em estufa de circulação forçada a 60 ºC e posteriormente a amostra foi pesada para obtenção do rendimento e então levada para realização do PCS.

As etapas e análises realizadas no experimento II podem ser observadas na Figura 3.

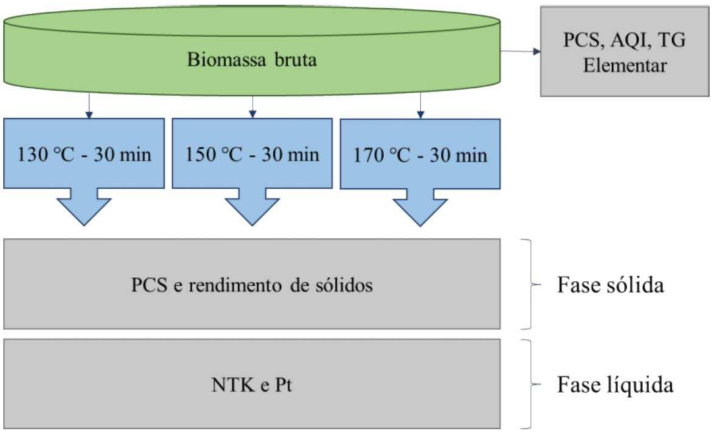

Figura 3. Representação esquemática dos tratamentos (azul) e das análises realizadas em cada etapa (cinza) no experimento II.

A produtividade de sólidos (Ps) foi determinada de acordo com a equação 5.1:

$$Ps\ (\%) = \frac{m\ char}{m\ biomassa}\ x\ 100 \tag{1}$$

Em que: *m char* é a massa de char produzida após a CHT e *m biomassa* é a quantidade de biomassa bruta submetida à CHT, em base seca. O percentual de recuperação de energia foi determinado de acordo com as equações (5.2 e 5.3):

$$Fator = \frac{PCS\ char}{PCS\ biomassa} \tag{2}$$

$$RE\ (\%) = Ps\ \times Fator \tag{3}$$

Em que: Fator e RE são o fator de intensificação de energia e recuperação de energia, respectivamente; *PCSchar* e *PCSbiomassa* representam o poder calorífico superior do char e biomassa bruta, respectivamente.

2.6. Delineamento experimental

O experimento I foi instalado segundo delineamento inteiramente casualizado (DIC) com dois tratamentos, em duplicata, totalizando 4 unidades amostrais, sendo que as mesmas foram comparadas com a biomassa bruta ao final do experimento. Os dados foram submetidos à análise de variância (ANOVA) e posteriormente os tratamentos foram comparados entre si, por meio do teste de Tukey, a 95% de probabilidade no software R versão 3.4.3. No experimento II não foi realizado delineamento estatístico.

3. Resultados e discussão

3.1. Experimento I

A biomassa algal utilizada no experimento I, apresentou uma relação C:N de 6,87:1 (Tabela 1) (Costa el al., 2016). Esse resultado é semalhante aos encontrados por Couto et al. (2018) e Afolabi et al. (2017), embora esses autores tenham trabalhado com efluente doméstico como meio de cultivo para produção de suas biomassas. O resultado de PCS da biomassa bruta também corroborou com os resultados encontrados por esses autores.

Tabela 1. Caracterização química da biomassa algal após secagem.

N	P	K	C	H	O	Ca	Mg	S	Na
(%)									
5,59	1,18	0,14	38,39	6,51	23,27	9,76	0,36	0,95	0,83
Zn	Fe	Mn	Cu	B	Cd	Pb	Cr	Ni	
(ppm)									
192,70	23415	158,20	37,00	2,89	ND	ND	23,20	15,10	

ND = Não detectável; Adaptado de Costa et al. (2016).

Um combustível é tanto melhor quanto mais alto for seu poder calorífico, seu teor de carbono fixo e de material volátil e menor for seu teor de cinzas. Observa-se (Tabela 2) que a biomassa TS apresentou PCS significativamente maior ($p<0,05$) que a biomassa bruta (BB) e CHT ao final do experimento. Esse tratamento apresentou o menor rendimento da biomassa, porém sua porcentagem de carbono fixo ficou mais elevada que os demais tratamentos o que pode ter influenciado na elevação do poder calorífico superior.

Tabela 2. Resultados da caracterização imediata nas biomassas bruta (BB), CHT e TS.

Amostra	PCS	Voláteis	Cinzas	Carbono	Rendimento
	kcal kg^{-1}		(%)		
BB	4871b	67,30a	25,29a	7,41b	100,00a
CHT	4971b	68,98a	23,37b	7,65ab	91,30b
TS	5091a	66,43a	23,72b	9,85a	82,67c
CV(%)	0,52	1,85	3,72	6,76	0,88

[1] Médias seguidas da mesma letra, na coluna, não diferem entre si a 5% de significância pelo teste de Tukey.

O poder calorífico é um excelente parâmetro para se avaliar a potencialidade energética de combustíveis e é definido como a quantidade de energia liberada na combustão completa de uma unidade de massa do material combustível (Protásio et al., 2011). Os valores de PCS para o char da biomassa de microalgas submetida à CHT apresentou uma média de 4971 kcal/kg. Em trabalho realizado por Bach et al. (2017) que realizou CHT de biomassa de algas cultivadas em meio sintético, o maior valor de PCS observado foi de 6214,77 kcal kg^{-1} (170 ºC, 30 min). Levine et al. (2013) em seus trabalhos com *Nannochloropsis oculata* cultivada em meio sintético obtiveram resultado de PCS máximo de 6697,23 kcal kg^{-1} (210 ºC, 30 min). Denota-se que há possibilidade, caso aumentada a temperatura da CHT com a biomassa de microalgas, de o PCS aumentar consideravelmente, tendo como base os demais trabalhos mencionados. Há ainda que se destacar que diferentemente dos trabalhos acima citados, que operaram com tempo de espera de 30 minutos, o tempo de espera utilizado foi de 60 minutos o que pode ter favorecido a obtenção de valores de PCS menores quando comparados com outras biomassas de microalgas.

O percentual de carbono fixo refere-se à fração de carvão que se queima no estado sólido. Combustíveis com teores mais elevados de carbono fixo são preferíveis por queimarem mais lentamente (Sturion et al., 1988). As três biomassas apresentaram baixo teor de carbono fixo. Ressalta-se que o teor de carbono fixo e o teor de cinzas possuem elevado índice de correlação com o poder calorífico, entretanto, nesse estudo, apesar do baixo teor de carbono fixo e alto teor de cinzas, a biomassa apresentou alto poder calorífico. Levando-se em consideração que o percentual de C H estão abaixo dos valores registrados na literatura para biomassas de microalgas (Bach et al. 2017a; Bach et al. 2017b), o que pode estar elevando o poder calorífico, são os grupos funcionais presentes no material volátil.

Os materiais voláteis são importantes durante a ignição e as etapas iniciais do processo de combustão (Cortez et al., 2008). Os tratamentos realizados não influenciaram significativamente no aumento ou redução do material volátil presente na biomassa de algas. Os valores registrados neste experimento estão em conformidade com outros trabalhos quanto ao conteúdo de material volátil da biomassa (Bach et al., 2017a). Porém, os grupos funcionais desse material volátil podem ser diferentes das biomassas algais cultivadas em meio sintético.

Para o estudo dos grupos funcionais, sugere-se para os próximos testes, a análise de espectroscopia no infravermelho por transformada de Fourier (FT-IR), que é uma técnica experimental para a caracterização de polímeros, em termos de identificação e/ou determinação de características estruturais, principalmente no que se refere a grupos funcionais e ligações presentes na amostra.

Na Figura 4 estão apresentadas as curvas termogravimétricas para as biomassas (BB e CHT), na faixa de temperatura de 100 a 700 ºC.

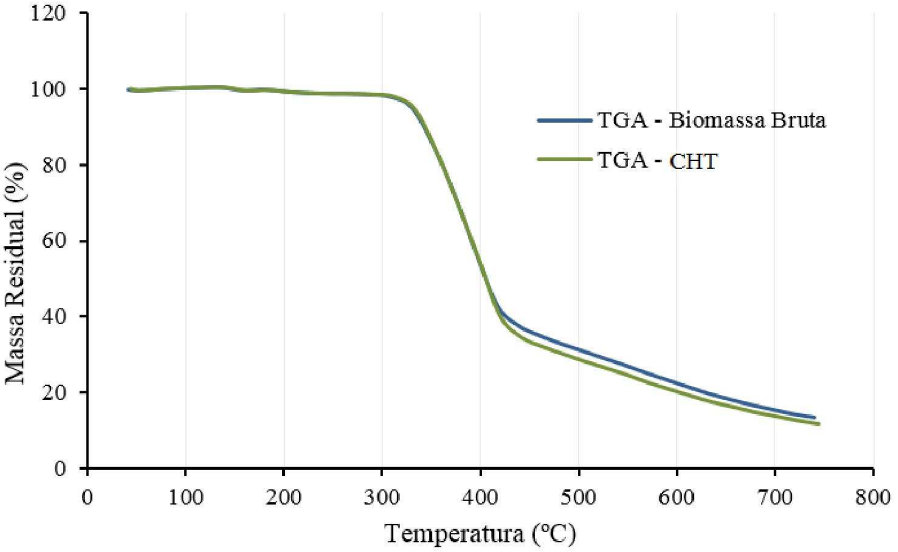

Figura 4. Curvas TGA para BB e CHT (Atmosfera de nitrogênio, taxa de aquecimento de 10 ºC min^{-1}).

Por meio da análise termogravimétrica é possível verificar em que temperatura é iniciada a decomposição térmica e ainda, em que faixa de temperatura a decomposição térmica da biomassa é mais pronunciada (Oliveira, 2003). Comparando-se as curvas de degradação térmica para as biomassas, verifica-se que os resultados foram muito semelhantes entre a BB e CHT, denotando que modificações mais abrutas podem ser realizadas na CHT com o objetivo de influenciar de forma positiva a biomassa submetida ao processo de carbonização, como por exemplo o aumento da temperatura.

Na Figura 5, são apresentadas as curvas de termogravimetria derivadas (DTG) para a BB e para a CHT. Novamente, as curvas não se diferiram entre si, mostrando que o tratamento ao qual a biomassa foi submetida não foi eficiente em apresentar diferença desta para a biomassa bruta.

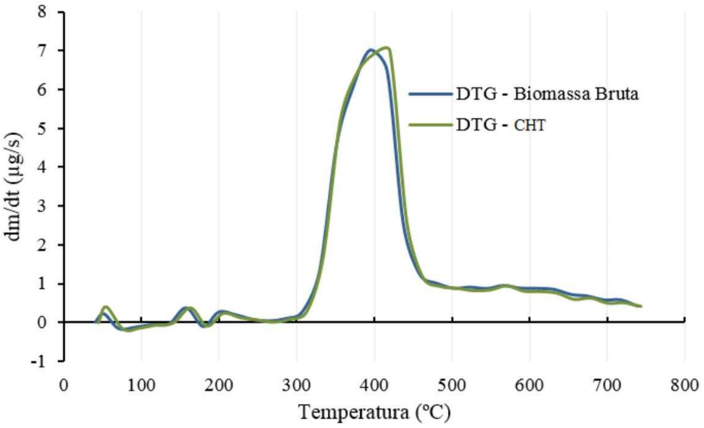

Figura 5. Curvas de DTG para BB e CHT.

O comportamento da combustão pode ser observado mais detalhadamente a partir das curvas DTG, a qual apresentou basicamente três estágios diferentes, os quais também foram encontrados por Bach et al. (2017a): o primeiro estágio está entre 105 °C e 300 °C, mostrando as reações oxidativas de componentes com baixa estabilidade térmica (por exemplo, os carboidratos e proteínas); o segundo estágio está na faixa de 300-460 °C, mostrando a queima de componentes mais termicamente estáveis (por exemplo, lipídios). E a terceira etapara é uma conseqüência da combustão dos char produzidos em altas temperaturas.

Sugere-se portanto, para o próximo experimento, a utilização de temperaturas mais elevadas e tempos de espera mais reduzidos com o objetivo de otimizar o tratamento termoquímico em estudo.

3.1. Experimento II

A biomassa algal utilizada no experimento II possuía as seguintes características (Tabela 3):

Tabela 3. Caracterização da biomassa algal após secagem.

N	P	K	C	H	O	Ca	Mg	S	Na
				(%)					
3,50	0,62	0,22	62,61	10,33	12,45	1,64	0,28	0,50	1,25
Zn	Fe	Mn	Cu	B	Cd	Pb	Cr	Ni	
				(ppm)					
396,20	2239,25	40,00	9,55	13,00	ND	ND	12,30	5,70	

PCS	Voláteis	Cinzas	Carbono fixo
kcal kg^{-1}		(%)	
6628,50±47,38	82,86±0,32	10,61±0,01	6,52±0,32

ND = Não detectável

A relação C:N dessa biomassa é de 17,89:1, valor muito superior aos comumente registrados na literatura para biomassas produzidas em efluente. Além disso, o PCS foi semelhante ao de biomassas de cultivo sintético (Bach et al., 2017a).

De acordo com a análise DTG (Figura 6), o primeiro estágio está entre 125 °C e 270 °C, que são, provavelmente, as reações oxidativas de componentes com baixa estabilidade térmica (por exemplo, os carboidratos e proteínas); o segundo estágio está na faixa de 270 e 410 °C, mostrando a queima de componentes mais termicamente estáveis (por exemplo, lipídios). E a terceira etapa é uma conseqüência da combustão dos biochars produzidos em altas temperaturas.

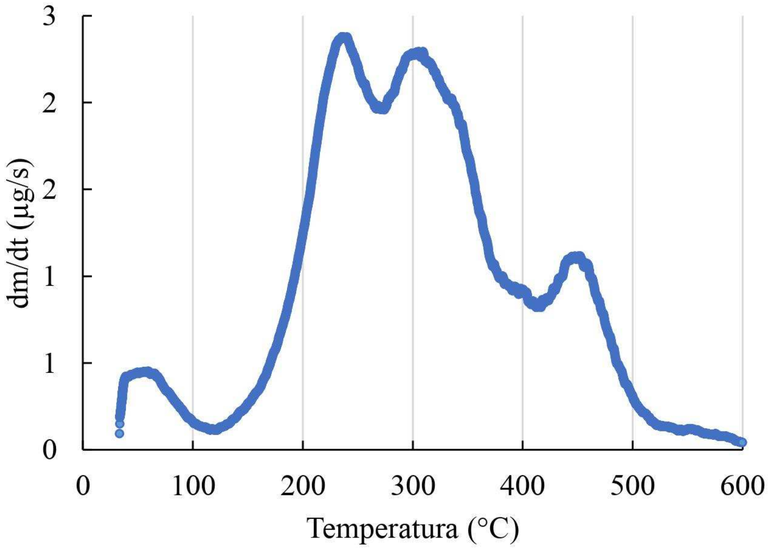

Figura 6. Curva DTG da biomassa bruta utilizada no experimento II.

Comparando-se a análise TGA obtida no experimento I com a obtida no experimento II observa-se que neste, os compostos se degradaram a uma temperatura mais baixa, começando aos 200 ºC e terminando aproximadamente a 400 °C (Figura 7).

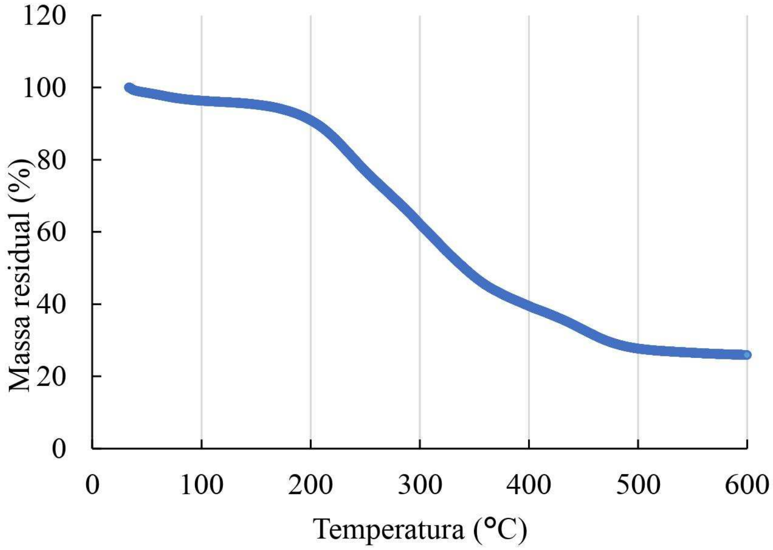

Figura 7. Curva TGA da biomassa bruta utilizada no experimento II.

A biomassa mista, utilizada nos testes de CHT do experimento II, era composta por 99,4 % da clorofícea *Chlorella vulgaris,* como pode ser observado na tabela 4.

Tabela 4. Análise fitoplanctônica no efluente bruto (com inóculo) e no efluente pós batelada.

Organismos	Efluente Bruto		Efluente Tratado	
Cianobactéria	D[1]	AR[2]	D	AR
Leptolyngbya sp.	14613	0,4 %	-	0,0 %
Microcystis aeruginosa[1]	336101	8,7 %	17049	0,3 %
Clorofíceas				
Chlorella vulgaris	3192961	82,5 %	5285069	99,4 %
Desmodesmus communis	175357	4,5 %	-	0,0 %
Desmodesmus opoliensis	138824	3,6 %	-	0,0 %
Scenedesmus acuminatus	14613	0,4 %	17049	0,3 %
Total	3872470	100 %	5319167	100 %

Grupos	Biovolume[3]	AR	Biovolume	AR
Clorofíceas	$56,1 \times 10^6$	28 %	$69,2 \times 10^6$	88 %
Cianobactérias	$143,1 \times 10^6$	72 %	$9,6 \times 10^6$	12 %
Total	$199,3 \times 10^6$	100 %	$78,8 \times 10^6$	100 %

[1] D = Densidade (org mL^{-1}); [2] AR = Abundância Relativa (%) e [3] Biovolume ($\mu m^3\ mL^{-1}$).

Bach et al. (2017a) realizaram CHT de biomassa de *Chlorella vulgaris* cultivada em meio sintético e obtiveram valores de PCS e produtividade de sólidos muito próximos dos obtidos neste trabalho. Muito embora o PCS do char tenha sido pouco diferente da biomassa bruta, o tratamento termoquímico promoveu uma redução considerável no volume de sólidos (~ 40 %) (Tabela 5).

Tabela 5. Resultados do PCS dos sólidos resultantes da CHT sob diferentes temperaturas.

Amostra	PCS (kcal kg^{-1})	Ps (%)	RE (%)
Biomassa bruta	6628,50		
130 ºC - 30 min	6789,50	63,04	64,57
150 ºC - 30 min	6830,50	62,71	64,62
170 ºC - 30 min	6726,00	61,75	62,66

O percentual de recuperação de energia (RE) foi muito próximo para os três tratamentos, sendo o maior à temperatura de 150 °C (64,62 %). Esse resultado indica que aproximadamente dois terços da energia disponível na biomassa foi convertida para o char. Os valores obtidos se assemelham aos encontrados por Bach et al. (2017a) quando operaram a 160 ºC, 170 ºC e 180 ºC e obtiveram 68,05 %, 66 % e 61,49 % de recuperação de energia. Os autores citam que quanto mais abruptas forem as condições da CHT da biomassa de microalgas menores serão os rendimentos energéticos. Portanto, concluem que os valores que maximizaram o tratamento termoquímico são 170 ºC/30 minutos ou 180ºC/10 minutos.

Os autores acima citados, trabalharam com uma biomassa de *Chlorella vulgaris* cultivada em meio sintético com um teor de cinzas de 9,02 %. A biomassa utilizada no presente trabalho possui um teor de cinzas de 10,61±0,01 %. Denotando mais uma vez a similaridade das biomassas estudadas.

A biomassa algal utilizada no experimento II possui teores de carbono e hidrogênio mais elevados que os registrados no experimento I. Acredita-se ser esse o fator determinante para elevação do PCS do char resultante. Rassalta-se, a necessidade do estudo dos grupos funcionais na biomassa mista.

Devido a necessidade de separação das fases sólidas e líquidas para que o char seja usado como combustível, vê-se a necessidade de avaliação da parte líquida resultante da separação sólido-líquido pós CHT. Isso será realizado nos próximos experimentos, conforme a Figura 3 deste anexo.

Conclui-se que à temperatura de 150 ºC o PCS foi maximixado e o tempo de espera de 30 minutos pode ser considerado bom se comparados os resultados com os encontrados na literatura. Portanto, o delineamento experimental para o próximo estudo deve contar com esses valores como ponto central.

4. Conclusão

A biomassa mista, contendo microalgas, pode ser submetida à CHT à temperaturas mais baixas que a faixa citada como parâmetro para esse tratamento termoquímico;

Temperaturas e tempos de espera elevados podem reduzir os resultados de recuperação de energia;

A temperatura de 150 ºC o PCS foi maximixado e o tempo de espera de 30 minutos pode ser considerado bom se comparados os resultados com os encontrados na literatura. O delineamento experimental para o próximo estudo deve contar com esses valores como ponto central.

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15. ANEXO IV

Hydrothermal carbonization of wastewater grown algal biomass

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Keywords: microalgae · hydrochar · thermochemical carbonization · energy recovery

Introduction

The hydrothermal carbonization (HTC) can be defined as a thermochemical treatment, that has the objective to convert carbohydrates into a rich carbon material in an aqueous medium. The treatment occurs in a hydrothermal medium or compressed hot water with temperatures between 180-260 ºC (Bach et al., 2013; Bach et al., 2016; Chen et al., 2012).

Among the advantages of HTC, Kubo (2011) highlights: i) low temperature for carbonization; ii) possibility to occur in aqueous phase; iii) can be considered a non-expensive process, since carbonaceous materials of crude biomass can be used as carbon source; iv) has the capacity to incorporate other chemical structures such as nanoparticles or functional monomers; and v) the easy access to materials with functional surfaces that can be used and easily transformed allowing a simple post-chemical modification. Despite all these advantages, there is still much to investigate regarding HTC process, especially in terms of operational parameters, such as temperature. The temperature can be highlighted, as it significantly influences the characteristics of the final product, as well as the type of material used in the carbonization reactor. When it comes to microalgae biomass, in general, the operating temperature has not exceeded 215 °C (Bach et al., 2017; He et al., 2013; Levine et al., 2013). The aim of this study was to understand the behavior of algal biomass cultivated in effluent at different HTC temperatures.

Materials and Methods

The microalgae biomass was cultivated in a high rate algal pond during the treatment of a meat processing industry effluent.

The HTC tests were performed in the Laboratory of Simulation of Chemical Processes of the Chemical Department, UFV. The dry biomass was mixed with distilled water in a proportion of 1:20, until 200 mL. A *Parr 5500 Series* reactor with agitation and temperature control was used for the tests. Three tests were realized with temperatures of 130 ºC, 150 ºC e 170 ºC, under 30 minutes.

At the end of the reaction time, the samples were withdrawn from the reactor and centrifuged for separation of the solid and liquid phases. The solid phase was dried in a forced circulation oven at 60 °C and then the sample was weighed to obtain the solid yield (Ys), the energy yield (Ye) and the higher heating value (HHV) (kcal kg^{-1}). The HHV was experimentally obtained through the adiabatic calorimetry method, according to the ASTM D 2015 standard (ASTM, 1982). The yield of solids was calculated using Equation (1):

$$Ys\ (\%) = \frac{m\ char}{m\ biomass}\ x\ 100\ (1)$$

Where: m_{char}: mass of solid products (g); $m_{biomass}$: mass of the biomass used in the test.

The energy yield was determined according to the following Equations (2) e (3):

$$Factor = \frac{HHV\ char}{HHV\ biomass}\ (2)$$

$$Ye\ (\%) = Ys\ \times Factor\ (3)$$

Where: Factor e Ye are the energy enhancement factor and energy yield, respectively; HHV_{char} and $HHV_{biomass}$ stand for the higher heating values of solid product and raw microalgae, respectively.

The immediate chemical composition (volatile material, fixed carbon and ash content) was determined according to the NBR 8112 (ABNT, 1986). Nitrogen content was obtained through

16. ANEXO V

CERTIFICADO

Apresentação Oral

Declara-se para os devidos efeitos que

Maria Lúcia Calijuri

apresentou o trabalho com o título

"INFLUÊNCIA DA BIOMASSA DE MICROALGAS NA BIODISPONIBILIDADE DE FÓSFORO"

Jackeline de Siqueira Castro, Maria Lúcia Calijuri, Edson Marcio Mattiello, Vinícius José Ribeiro, Paula Peixoto Assemany

no **18.º Encontro de Engenharia Sanitária e Ambiental (ENASB) e do 18.º Simposium Luso-Brasileiro de Engenharia Sanitária e Ambiental (SILUBESA)**, com o tema geral **"A Engenharia Sanitária no Desenvolvimento Sustentável"**, que se realizou de 10 a 12 de setembro de 2018, na Fundação Dr.º António Cupertino Miranda, Porto.

O Presidente da Comissão Organizadora do 18.º ENASB, 18.º SILUBESA

Paulo J. Ramisio

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