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Alberto Meireles dos Santos

CICLAGEM DE NUTRIENTES E ENERGIA POR PROCESSOS BASEADOS EM MICROALGAS A PARTIR DAS ÁGUAS RESIDUAIS DO ABATE E PROCESSAMENTO DE AVES E SUÍNOS

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

Orientador: Prof. Dr. Eduardo Jacob Lopes

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RESUMO

Tese de doutorado
Programa de Pós-graduação em Ciência e Tecnologia dos Alimentos
Universidade Federal de Santa Maria

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AUTOR: ALBERTO MEIRELES DOS SANTOS
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A possibilidade de ciclagem de nutrientes a partir de processos microalgais é uma rota tecnológica potencial a ser aplicada em estações de tratamento de águas residuais. Em face disto, o trabalho teve por objetivos: (a) desenvolver um biorreator aplicável à conversão de matéria orgânica, nitrogênio e fósforo em produtos do metabolismo microalgal; (b) avaliar a cinética de produção de biomassa; (c) avaliar a cinética de consumo de matéria orgânica e nutrientes (nitrogênio e fósforo); (d) realizar balanços de material e análises de potencial econômico do processo e dos bioprodutos; (e) determinar a composição quantitativa e qualitativa da biomassa e produção de bioprodutos; (f) determinar a composição centesimal da biomassa; (g) determinar o perfil de ácidos graxos da biomassa; e (h) caracterizar o biodiesel a partir de biomassa microalgal. A ciclagem de nutrientes por *Phormidium autumnale* em águas residuais apresentou altas eficiências de remoção de 97,6%, 85,5% e 92,4% para DQO, N-TKN e P-PO_4^{-3}, respectivamente, paralelamente a uma produtividade de lodo microalgal de 0,27 kg/m^3/d. A análise econômica demonstrou um custo de US$ 2,66/m^3 de águas residuais industriais tratadas e, como consequência desse processo, o custo de produção do lodo microalgal foi de US$ 0,03/kg de biomassa desidratada. O lodo microalgal gerado mostra predominância de ácidos graxos saturados, indicando uma fonte potencial para produção de biodiesel. A biomassa microalgal extraída de lipídios apresentaram alto teor de aminoácidos, minerais e pigmentos livres, sendo uma potencial fonte para alimentação animal. Portanto, o cultivo de microalgas heterotróficas em águas residuais demonstrou ser capaz de remover poluentes e, ao mesmo tempo, produzir biocombustível e ser uma fonte potencial de alimentação animal a partir de lamas de microalgal, podendo ser obtida uma margem de lucro de 94%.

Palavras chave: ciclagem de nutrientes, microalga/cianobactéria, água residual, análise de custo, biodiesel, alimentação animal.

ABSTRACT

Master Thesis
Post-Graduate Program in Food Science and Technology
Federal University of Santa Maria, RS, Brazil

NUTRIENT CYCLING AND ENERGY BY MICROALGAE-BASED-PROCESSES FROM WASTEWATER OF POULTRY AND SWINE SLAUGHTERHOUSE

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The nutrient cycling from microalgae-based processes is a potential technological route to be applied in wastewater treatment plants. Therefore, the objectives of this research are: (a) develop a bioreactor applicable to conversion of organic matter, nitrogen and phosphorus into products of microalgal metabolism; (b) evaluate the kinetics of biomass production; (c) evaluate the kinetics of organic matter and nutrients consumption (nitrogen and phosphorus); (d) carry out material balances and analyzes of economic potential of the process and bioproducts; (e) determine the quantitative and qualitative composition of biomass and production of bioproducts; (f) determine biomass composition of biomass; (g) determining fatty acid profile of biomass; and (h) characterize biodiesel from microalgal biomass. The nutrient cycling by *Phormidium autumnale* in agroindustrial wastewater indicate high removal efficiencies of 97.6%, 85.5% and 92.4% for COD, N–TKN and $P–PO_4^{-3}$, respectively, in parallel to a microalgal sludge productivity of 0.27 kg/m^3/d. The economic analysis demonstrated a cost of USD 2.66/m^3 of treated industrial wastewater, and as consequence of this process, the production cost of microalgal sludge was USD 0.03/kg of dehydrated biomass. The generated microalgal sludge shows predominance of saturated fatty acids, indicates the potential of its use as a suitable lipid input for biodiesel synthesis. The lipid-extracted microalgae (LEM) showed high content of free amino acids, minerals and pigments, being a potential source for animal feed. Thus, the heterotrophic microalgae cultivation in wastewater has demonstrated to be capable of removing pollutants and, simultaneously, producing biofuel and being a potential source for animal feeding from microalgal sludge, a profit margin of 94% can be obtained.

Keywords: nutrient cycling, microalgae/cyanobacteria, wastewater, cost analysis, biodiesel,

animal feed

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CAPÍTULO 1

INTRODUÇÃO

INTRODUÇÃO

A biotecnologia de microalgas tem-se centrado no crescimento autotrófico ou fotossintético destes microrganismos onde o CO_2 e a luz do sol servem como fonte de carbono e energia para a proliferação celular. Porém, é difícil chegar a uma elevada densidade de biomassa de microalgas, devido a desvantagens desse modo de cultivo, como por exemplo, a penetração da luz é inversamente proporcional à concentração de células e o sombreamento mútuo das células pode causar insuficiência luminosa, o que leva a uma concentração muito baixa de biomassa microalgal e, consequentemente, o rendimento muito baixo de produtos. A baixa concentração de biomassa também aumenta o custo de separação de biomassa do meio de cultura (LIANG, 2013; PANCHA et al., 2014). Tendo isso em vista, outras formas de cultivo devem ser utilizadas para produção de bioprodutos a partir de biomassa de microalgas.

A ciclagem de nutrientes por microalgas emerge como uma tecnologia promissora porque equilibra vetores sustentáveis por poluentes reutilizados, como carbono, nitrogênio e fósforo, presentes nas águas residuais geradas pela indústria. Assim, eles criam uma biomassa que pode extrair uma grande variedade de bioprodutos com valor agregado substancial (SANTOS et al., 2016).

O cultivo heterotrófico tem sido usado com êxito para geração de biomassa microalgal e metabólitos. Neste processo as microalgas são cultivadas em substratos orgânicos, tais como glicose, em biorreatores heterotróficos. O crescimento da microalga é independente do fator luz, o que permite a possibilidade de escalonamento mais simples pois uma menor superfície do reator em relação ao volume pode ser utilizada. Estes sistemas proporcionam um alto grau de controle do crescimento e também menores custos de separação da biomassa, devido às altas densidades celulares obtidos, e os custos de instalação são mínimos (BRENNAN & OWENDE, 2010; TURON et al., 2015). No entanto, os obstáculos para a cultura em escala comercial de microalgas heterotróficas ainda são econômicos. A utilização de efluentes industriais como fontes de baixo custo ou valor zero de substrato orgânico para suportar o crescimento de microalgas heterotróficas é necessário para que a obtenção de bioprodutos seja economicamente viável.

Os efluentes industriais contêm carbono orgânico, nitrogênio, fósforo e outros componentes menores. Isto faz com que a composição das águas residuais adequado para o cultivo de microalgas. Além do crescimento da biomassa microalgal para a extração de bioprodutos, as águas residuais podem ser tratadas simultaneamente (ZHOU et al., 2014; KHEMKA & SARAF, 2017).

Apesar das vantagens em relação ao cultivo autotrófico, o crescimento heterotrófico pode possuir algumas limitações como baixa tolerância a altas concentrações de carbono, poucas espécies de microalgas podem crescer na ausência de luz, alguns compostos orgânicos inibem o crescimento, competição com outros microrganismos, como bactérias e leveduras, e a falta de produção de metabólitos fotossintéticos (PEREZ-GARCIA et al., 2011; KHALID et al., 2016).

Assim sendo, com o intuito de alcançar um bom desempenho e eficiência, biorreatores exigem procedimentos avançados de regulação para garantir o desempenho e a eficiência de bioprocessos. Em geral, um processo biológico é uma rede de reações bioquímicas complexas manipuladas por enzimas (MAILLERET et al., 2004). De fato, tais redes cinéticas originam dinâmicas altamente complexas e não-lineares de enzimas, nutrientes e concentração de produto em biorreatores (ABDOLLAHI & DUBLJEVIC, 2012). Por isso é necessário a otimização de biorreatores heterotróficos microalgais, para um próximo passo após escala laboratorial.

OBJETIVO GERAL

Considerando a necessidade atual do desenvolvimento de novos processos e produtos com foco no desenvolvimento sustentável das atividades industriais, o projeto objetiva desenvolver processos de biorrefinaria microalgal suportados na agroindústria do abate e processamento de aves e suínos, com ênfase na obtenção de bioprodutos que agreguem valor a cadeia produtiva.

Objetivos específicos

(a) desenvolver um biorreator aplicável à conversão de matéria orgânica, nitrogênio e fósforo em produtos do metabolismo microalgal;
(b) avaliar a cinética de produção de biomassa;
(c) avaliar a cinética de consumo de matéria orgânica e nutrientes (nitrogênio e fósforo);
(d) realizar balanços de material e análises de potencial econômico do processo e dos bioprodutos;
(e) determinar a composição quantitativa e qualitativa da biomassa e produção de bioprodutos;
(f) determinar a composição centesimal da biomassa;
(g) determinar o perfil de ácidos graxos da biomassa;
(h) caracterizar o biodiesel a partir de biomassa microalgal;

CAPÍTULO 2

REVISÃO BIBLIOGRÁFICA

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2.1 Microalgas

O termo microalga não apresenta nenhum valor taxonômico. No entanto, define seres microscópicos diversos presentes em sistemas aquáticos, fotossintetizantes em sua grande maioria e apresentam estrutura vegetativa conhecida como talo, cuja diferenciação celular é caracteristicamente pequena ou nula (Lourenço, 2006). Dez grandes grupos fazem parte da classificação geral moderna de microalgas de acordo com Graham e Wilcox (2000), são eles: Cyanophyta, Chlorarachniophyta, Glaucophyta, Euglenophyta, Cryptophya, Prymnesiophyta, Dinophyta, Ochrophyta, Rhodophyta e Chlorophyta.

Alguns grupos se destacam sob o aspecto biotecnológico, o que inclui as cianobactérias (Cyanophyta), clorofíceas (Chlorophyta) e as diatomáceas (Ochrophyta) (PAHL et al., 2010; PHUKAN et al., 2011; TANG et al.; 2011, LAM & LEE, 2012; MATA et al.; 2012; MARKOU et al., 2012).

As cianobactérias constituem um grupo bem definido de eubactérias, sendo as únicas bactérias capazes de produzir oxigênio como produto colateral da fotossíntese. Clorofila *a* e diversos pigmentos acessórios de proteção e ampliação da captação de luz (ficobilinas e carotenoides) estão presentes, associados à tilacóides membranosos. Dentre os organismos autotróficos, as cianobactérias são singulares por apresentarem organização celular procariótica e pela ausência marcante de flagelos além da maioria das organelas celulares (KÜHL et. al., 2005). Sob o ponto de vista de aplicação biotecnológica, são considerados os organismos mais versáteis, uma vez que podem mediar até três metabolismos em paralelo para a obtenção de energia e manutenção dê suas estruturas (QUEIROZ et al., 2013).

Em relação às Chlorophytas que englobam as clorofíceas, formas unicelulares predominam na maioria das espécies. São dotadas de talos multicelulares e juntamente com as cianobactérias, as clorofíceas levam ao extremo os habitats possíveis para sua existência, mas apesar dessa grande variabilidade, cerca de 90% do total de espécies (sobretudo as formas microscópicas) ocorrem em água doce. Numerosas clorofíceas são capazes de suplementar a aquisição de carbono por meio da fotossíntese, pela utilização de matéria orgânica dissolvida na forma de açúcares, ácidos aminados e outras moléculas orgânicas, caracterizando um quadro de osmotrofia e mixotrofia (JOHN, 2003).

O grupo Ochrophyta, onde se inclui as diatomáceas, é o grupo mais heterogêneo, com espécies que variam desde células microscópicas até algas gigantes, com diferenciação de tecidos. A clorofila *a* está presente na maior parte das ocrófitas, mas há também algumas formas heterotróficas não pigmentadas. Os produtos de reserva consistem em gotículas de gordura no citoplasma e/ou um polissacarídeo solúvel derivado de glicose formado por ligações glicosídicas do tipo β-1,3, denominado de crisolaminarina. Há usualmente dois flagelos heteromórficos, e a cobertura das células varia consideravelmente entre as espécies, no caso das diatomáceas, as estruturas de sílica são as de ocorrência mais abrangente (REVIERS, 2006).

O gênero *Phormidium autumnale* tem sido estudada devido a sua ampla tolerância a concentrações elevadas de amónio (54 mg de NH_4^+/L), a sua resistência à contaminação com outros grupos de microalgas e a sua capacidade de autofloculação que permite a recirculação da biomassa semelhante ao processo de lodo ativado. Também é muito competitivo a valores de pH elevados (8,5-10), e tem um grande potencial no tratamento terciário de águas residuais urbanas (RICHMOND ET AL., 1982; RICHMOND & BECKER, 1986; BUELNA ET AL., 1988; DE LA NOÜE & BASSÉRES, 1989; TALBOT & DE LA NOÜE, 1993; PROULX et al., 1994).

2.2 Metabolismo das microalgas

Algumas espécies de microalgas podem apresentar até três tipos de metabolismo, a fotossíntese, a respiração e a fixação de nitrogênio. A fotossíntese é a forma de obtenção de energia mais utilizada pelas microalgas, e em casos que não há contato com a luz, algumas obtêm energia através da respiração. Há microalgas que desenvolveram organelas capazes fixar nitrogênio da atmosfera quando este se encontra escasso no meio (LOURENÇO, 2006).

A formação de cada composto no interior da célula de microalga é regulada por complexos mecanismos metabólicos, sendo a fotossíntese a forma metabólica mais utilizada em cultivos microalgais. Em microalgas fotossintéticas, o complexo sistema coletor de luz ligado à clorofila *a* e aos carotenoides captura energia solar na forma de fótons. Esta energia é utilizada pelo fotossistema II na oxidação catalítica da água, liberando prótons, elétrons e moléculas de O_2. Os elétrons com baixo potencial são transferidos através da cadeia de transporte de elétrons fotossintéticos até o fotossistema I que levam à redução da ferredoxina para a formação de NADPH. Um gradiente eletroquímico é formado devido à liberação de prótons após a oxidação da água para o lúmen do tilacóide, o qual é utilizado para conduzir a produção de ATP via ATP sintase. Os produtos fotossintéticos NADPH e ATP são os substratos para o ciclo de Calvin-Benson, onde o CO_2 é fixado em moléculas de três átomos de carbono que são assimilados em açúcares, amido, lipídios, ou outras moléculas exigidas para o crescimento celular. Já o substrato para a hidrogenase, íon hidrogênio e elétron, são supridos tanto via cadeia de transporte de elétrons fotossintéticos como via fermentação do carboidrato armazenado (BEER et al., 2009).

Outra rota metabólica utilizada por algumas microalgas é a respiração. Em um sentido amplo, todos os organismos, incluindo microalgas, usam as mesmas vias metabólicas para a respiração. Como esperado, o metabolismo das microalgas geralmente se assemelha, com apenas pequenas diferenças ao de plantas superiores. A respiração aeróbia é a mais completa e eficiente, e conta com três etapas fundamentais: formação de piruvato a partir de açúcares, ácidos graxos ou proteínas (via de Embden-Meyerhof), ciclo do ácido tricarboxílico e cadeia de transporte de elétrons (SANTOS et al., 2016). Durante todo o processo respiratório com a presença de compostos que podem ser oxidados e reduzidos de forma reversível, estes formam uma cadeia de transporte de elétrons, onde se forma ATP mediante um mecanismo denominado fosforilação oxidativa. Algumas espécies de cianobactérias, diatomáceas e clorofíceas são capazes de assimilar compostos orgânicos de baixo peso molecular, e podem subsistir inteiramente deles na ausência de luz através da respiração. Esta forma de aproveitamento é extremamente vantajosa em ambientes com elevada turbidez e baixa penetração de luz, fatores que limitam a fotossíntese (PAHL et al., 2010).

Finalmente, alguns grupos de microalgas formam uma célula especial denominada heterócito, responsável pela fixação de nitrogênio na forma gasosa (N_2) quando não há concentração mínima de nitrogênio na forma iônica assimilável na água, proporcionando-lhe vantagem competitiva em relação às outras espécies microalgais, que não possuem esta adaptação. O heterócito se desenvolve a partir de uma célula vegetativa normal, que se torna maior, com parede espessa e conteúdo claro. Nestas microalgas o nitrato e nitrito sofrem uma redução com o auxílio de nitrato redutase e nitrito redutase, respectivamente. Nitrato redutase utiliza a forma reduzida do dinucleotídeo de adenina nicotinamida (NADH) para transferir dois elétrons, o que resulta na conversão do nitrato em nitrito. O nitrito é reduzido a amônio por nitrito redutase e ferredoxina, com transferência de um total de seis elétrons na reação. Assim, todas as formas de nitrogênio inorgânico são finalmente reduzidas a amônio, antes de ser incorporado em aminoácidos dentro do fluido intracelular. Finalmente, utilizando-se o glutamato (Glu) e trifosfato de adenosina (ATP), a glutamina sintase facilita a incorporação de amônio ao aminoácido glutamina (CAI et al., 2013).

2.3 Tratamento de efluentes agroindustriais

As águas agroindustriais são resultado de diversas operações unitárias do processamento industrial. A indústria de processamento de carne utiliza aproximadamente 62 milhões de m³ por ano de água no mundo. No entanto, apenas uma pequena quantidade torna-se componente do produto final, e a fração restante converte-se em águas residuárias com concentrações elevadas de material em suspensão, elevada carga de matéria orgânica e rica também em nitrogênio e fósforo (SROKA et al., 2004). O lançamento destes efluentes em corpos hídricos proporciona alterações na concentração de oxigênio dissolvido, além de possibilitar a eutrofização de corpos hídricos (DEMIRBAS, 2011).

O tratamento de resíduos líquidos agroindustriais baseia-se normalmente em três operações: primária (remoção de sólidos), secundária (remoção da matéria orgânica) e terciária (remoção de poluentes específicos como nitrogênio, fósforo e metais pesados). No tratamento primário, tem-se por objetivo a remoção de sólidos em suspensão, através da separação física das partículas (ABDEL-RAOUF et al., 2012). O tratamento secundário é a fase onde há a remoção dos compostos orgânicos que não foram removidas no tratamento primário. A matéria orgânica é removida através de processos biológicos, sendo as lagoas de estabilização, os reatores anaeróbicos e o processo de lodos ativado os mais utilizados (LIU, 2003; AGUNWAMBA et al. 2004; OLUKANNI & DUCOSTE, 2011). O tratamento terciário, por sua vez, baseia-se na remoção de poluentes mais específicos que não foram removidos apropriadamente no tratamento secundário, principalmente nitrogênio e fósforo. A remoção de nitrogênio ocorre usualmente através da nitrificação e desnitrificação (DINÇER & KARGI, 2000). A remoção de fósforo ocorre normalmente através da precipitação química, empregando coagulante a base de alumínio ou ferro, além de cal, sendo este conjunto eficiente na remoção de fosfatos (REITZEL et al., 2006; MILLER et al., 2011).

As três etapas do tratamento de resíduos líquidos agroindustriais apresentam certas limitações que acabam onerando sua utilização no setor agroindustrial. Em processos de tratamento secundário, as lagoas de estabilização, além de baixa eficiência de remoção de matéria orgânica, ocupam uma grande área física da agroindústria. As diversas configurações de reatores anaeróbios, embora eficazes na remoção de material orgânico, apresentam operação marcadamente instável em função da variação das condições ambientais. Em processos que apresentam grande eficiência de remoção de matéria orgânica, como os processos de lodos ativados, o intensivo consumo de oxigênio é considerado a maior limitação, pois está diretamente associado ao custo de operação do sistema (VON SPERLING & CHERNICHARO, 2002). Já em processos de tratamento terciário, como a precipitação de fósforo, a adição de químicos ocasiona geralmente a geração de poluição secundária (TRAN et al., 2012) e finalmente os processos de nitrificação-desnitrificação, a perda de nitrogênio para atmosfera, é normalmente a questão mais criticada, uma vez que as diversas formas do elemento poderiam ser usadas na obtenção de blocos construtores agregadores de valor (PEREZ-GARCIA et al., 2011). Neste sentido, são necessárias novas tecnologias em processos alternativos com baixo custo e eficiência adequada de conversão de poluentes no setor agroindustrial.

2.4 Tratamento de efluentes baseado em microalgas

As microalgas possuem substancial capacidade de bioconversão de material orgânico e nutrientes presentes em águas residuárias. Neste sentido, sua utilização em efluentes agroindustriais seria uma alternativa em relação às formas convencionais de tratamento, baseadas em consórcios microbianos com base em bactérias e leveduras (QUEIROZ et al., 2007; MARKOU & GEORGAKAKIS, 2011).

Nos processos autotróficos de tratamento de efluentes com microalgas, as lagoas de alta taxa são as mais utilizadas. Elas são dispostas em forma de circuitos ovais rasos (0,2 - 1m) com paredes divisórias centrais, em canais simples ou múltiplos canais, ocorrendo à mistura através de uma roda de pás. Apesar de ser um processo de baixo custo capital e operacional, ocupam um grande espaço físico, há problemas de contaminação e são dependentes exclusivamente de luz solar e clima local (PARK et al., 2011).

Os fotobiorreatores também podem ser utilizados no tratamento de efluentes industriais. Basicamente, consistem em sistemas fechados, constituídos por um tubo transparente, na forma tubular ou helicoidal. O sistema possui normalmente uma unidade de troca de gás para adição de CO_2 e remoção do O_2 produzido fotossinteticamente. Apresentam maior produtividade em relação às lagoas de alta taxa, embora sejam fortemente limitados por fatores relacionados à engenharia de escalonamento (HO et al., 2011).

Biorreatores heterotróficos, por sua vez, apresentam substancial potencial de aplicação em estações de tratamento de efluentes industriais, particularmente agroindústrias, cujas águas residuárias apresentam elevadas concentrações de material orgânico, nitrogênio e fósforo, que resultam normalmente em relações C/N e N/P adequadas para suportar o desenvolvimento heterotrófico microalgal (QUEIROZ et al., 2011). Segundo estes autores, a principal vantagem desses arranjos está relacionada à independência da energia luminosa durante os processos de bioconversão de poluentes, simplificando os aspectos de engenharia de dimensionamento e construção dos reatores. Desta forma, os sistemas heterotróficos permitem a utilização de fermentadores convencionais amplamente distribuídos a nível industrial (PEREZ-GARCIA et al., 2011, TABERNERO et al., 2012). Adicionalmente, Queiroz et al., (2013), citam ainda como um importante diferencial destes biorreatores, a possibilidade do tratamento simultâneo de matéria orgânica, nitrogênio e fósforo, em um único estágio, com obtenção de elevadas eficiências de remoção, o que permitiria o emprego de um único reator para o tratamento de três poluentes, que ocorre na maioria das estações de tratamento em até três estágios distintos.

2.5 Potencial econômico tratamento de efluentes baseado em microalgas

Os estudos técnico-econômicos dos processos baseados em microalgas mostraram-se cenários economicamente inviáveis (LEE, 2001; NORSKER et al., 2011; ACIÉN et al., 2012). Essa inviabilização está relacionada principalmente à reduzida escalabilidade do fotossintético e aos altos custos operacionais dos processos heterotróficos. Segundo Wijffels et al. (2010), as rotas tecnológicas são imaturas e precisam ser plenamente desenvolvidas, implicando a necessidade de um grande esforço em pesquisa e desenvolvimento (P&D). Esses autores relataram que a biotecnologia de microalgas será competitiva e comercialmente atraente até 2020.

Além das substâncias conhecidas, a quantidade de compostos de interesse comercial que podem ser obtidos das microalgas parece ser imprevisível. Em nível mundial, há um crescente interesse em tecnologias limpas, sustentáveis e orgânicas, na obtenção de produtos para o consumo humano, demanda uma contínua busca por espécies e/ou variedades capazes de sintetizar grandes quantidades de compostos específicos e de como é possível potencializar a biossíntese destes (condições de cultivo, melhoramento genético etc.). Essas pesquisas, também, se fazem necessárias à identificação dos produtos que podem ser extraídos das microalgas, da possível atividade biológica (estudos metabológicos e toxicológicos) e do desenvolvimento de mercados específicos para estes. Igualmente, há a necessidade de pesquisas visando ao desenvolvimento e, principalmente, ao aperfeiçoamento dos sistemas de produção em escala comercial, a fim de tornar comercialmente viáveis alguns dos sistemas conhecidos (ABINANDAN & SHANTHAKUMAR, 2015; BATISTA et al., 2015; ZHANG et al., 2016).

Na análise e estimativa de custo para projetar um novo processo, quase todas as decisões são impactadas pelos fatores econômicos e, portanto, é fundamental estudar a economia do processo. Os principais critérios para avaliar a viabilidade são o projeto preliminar e a estimativa do potencial econômico a serem atingidos, e o conhecimento do preço do produto final é necessário para cobrir os custos envolvidos. A viabilidade desses processos foi determinada com base na análise técnico-econômica do processo simultâneo de tratamento de águas residuárias e produção de biomassa, que é conduzida com base em uma relação custo-benefício. Indicadores de viabilidade como equilíbrio econômico (EE), lucratividade, rentabilidade e período de retorno sobre o investimento são os principais parâmetros em uso (SALEHI et al., 2014).

2.6 Bioprodutos

O emprego de microalgas como biocatalisadores em reações de conversão resulta em bioprodutos de natureza intracelular, além de metabólitos extracelulares, passíveis de utilização como insumos intermediários ou produtos finais de uma série de consumíveis (SPOLAORE et al., 2006). De acordo com estes autores, proteínas, lipídios e pigmentos são os principais contituintes intracelulares de interesse, ao lado de compostos orgânicos voláteis, exopolissacarídeos e lipídios exocelulares de natureza extracelular.

As microalgas para consumo humano são comercializadas em diferentes formas, tais como comprimidos, cápsulas e líquidos. São utilizadas devido ao seu elevado teor de proteínas e seu valor nutritivo, além de ser rica em ácidos graxos poliinsaturados (EPA, DHA e ω-3) (PEREZ-GARCIA et al., 2011).

Em relação ao uso na alimentação animal, são consideradas importantes fontes de proteína com respostas zootécnicas associadas a ganho de peso, melhora na resposta imunológica e melhora na fertilidade (PULZ & GROSS, 2004). Adicionalmente, a biomassa microalgal é também utilizada para refinar os produtos de aquicultura, como por exemplo a coloração característica de salmonídeos (LORENZ & CYSEWSKI, 2000).

Outro aspecto de relevância na exploração destes bioprodutos está associado ao pigmentos. Além daclorofila a, as microalgas contêm pigmentos auxiliares ficobiliproteínas e carotenóides (WANG & PENG, 2008). Extratos de pigmentos microalgais incluem β-caroteno, astaxantinae ficocianina que posuem uma vasta gama de aplicações em industrias de alimentos e farmacêuticas (VISKARI & COLYER, 2003, DEL CAMPO et al., 2007).

A incorporação de isótopos estáveis (^{13}C, ^{15}N e ^{2}H) a partir de moléculas inorgânicas relativamente baratas ($^{13}CO_2$, $^{15}NO_3$, $^{2}H_2O$) para compostos orgânicos mais valorizados (por exemplo, aminoácidos, carboidratos, lipídios e ácidos nucleicos) é outra possibilidade de aplicação de células microalgais. Estes isótopos estáveis têm finalidades de incorporação em macromoléculas para facilitar a sua determinação estrutural em nível atômico e estudos metabólicos (FERNÁNDEZ et al., 2005).

Finalmente, o fracionamento da biomassa em biocombustíveis é uma opção emergente em biotecnologiade microalgas. É atrativa principalmente em espécies de microalgas que podem aumentar significativamente acumular lipídios intra e extracelulares (CHISTI et al., 2007; PARMAR et al., 2011).

Independente do bioproduto a ser explorado, a associação de um processo de tratamento de efluentes com a produção de insumos que apresentam valor comercial poderá contribuir efetivamente para a sustentabilidade das atividades de transformação industrial, possibilitando em qualquer segmento o estabelecimento de verdadeiras biorrefinarias (QUEIROZ et al., 2013).

2.7 Biorrefinarias microalgais

O biorrefino é um conceito de produção baseado no desenvolvimento sustentável. Neste sentido, as biorrefinarias são sistemas que combinam as tecnologias necessárias entre a concepção, exploração das matérias primas biológicas (biomassa) e a produção de insumos intermediários e produtos finais, isto é conseguido usando uma combinação sinérgica entre conversões biológicas e químicas. Através da produção de vários produtos, uma biorrefinaria pode maximizar o valor derivado da matéria-prima da biomassa (SINGH & GU, 2010).

A biomassa como matéria prima fundamental para o desenvolvimento e exploração de uma biorrefinaria pode ser obtida de origem diversa, o que inclui cultivares agrícolas, resíduos agrícolas, florestais e industriais, além de biomassas microbianas de origem bacteriana, fúngica e microalgal (KAMM & KAMM, 2004).

Desta forma quatro grandes grupos de processos de conversão estão envolvidos em sistemas de biorrefinaria, sendo eles bioquímicos, químicos, termoquímicos e mecânicos (GHATAK, 2011).

Atualmente a principal limitação no sentido de viabilizar biorrefinarias em escala industrial está associado a disponibilidade e custo de produção das biomassas. Esta problemática está associada ao relativo baixo custo do petróleo e seus derivados, o que em uma análise comparativa, normalmente restringe as rotas de produção renováveis (LIU et al., 2012). Adicionalmente, o petróleo como matéria-prima não apresenta outro uso que não a refinação. Por outro lado, as biomassas, principalmente de origem agrícola, podem ser usadas para outros fins que não o refino, levando invariavelmente a conflito de interesses que resultam em processos regulatórios de mercado (YANG, 2007).

Neste sentido, biomassas microbianas oriundas de estações de tratamento de efluentes podem representar uma importante fonte de insumos passíveis de fracionamento por biorrefino. Isso dependerá evidentemente da composição adequada, que permita a exploração de compostos de valor comercial (SINGH & GU, 2010).

Jones & Mayfield, (2012) relatam que a sustentabilidade industrial para a exploração de processos microalgais dependerá da criação de sistemas com aproveitamento global, completamente otimizado e eficiente, que use todos os componentes da biomassa microalgal, o que somente poderá ser obtido sob o escopo de uma biorrefinaria.

CAPÍTULO 3

MANUSCRITO 1

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Nutrient cycling in wastewater treatment plants by microalgae-based processes

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Chapter 2

NUTRIENT CYCLING IN WASTEWATER TREATMENT PLANTS BY MICROALGAE-BASED PROCESSES

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ABSTRACT

Conventional wastewater treatment processes are expensive and require complex operations and maintenance. They also generate large volumes of secondary waste that must be appropriately disposed. Besides there is a loss of nutrients, where is necessary, in an environmental standpoint, the nutrient cycling for a sustainable industrial development. Nutrient cycling may be defined as the transformation of nutrients from one chemical form to another, and/or the flux of nutrients between

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defined control volumes. In most systems, microorganisms are important agents of nutrient cycling. In this sense, the possibility of nutrients cycling from microalgae-based processes is a potential technological route to be applied in wastewater treatment plants. Microalgae play critical roles in energy fluxes and nutrient cycles by incorporating and redistributing dissolved organic matter and inorganic nutrients in the environment. Several species are known to live in limiting environments. These microorganisms show wide potential for use as biocatalysts in environmental biotechnology processes due to their robustness and simple nutritional requirements. Divided into six discreet parts, the chapter covers topics on nutrient cycling in wastewater treatment plants, the characteristics of industrial pollution, the industrially consolidated processes for wastewater treatment, the concept of process intensification applied to wastewater treatment, the emerging processes to wastewater treatment and the concept of biorefinery applied to wastewater treatment, summarizing a range of useful techno-economic opportunities to be applied on wastewater treatments plants.

Keywords: global cycle, microalgae/cyanobacteria, pollutants, waste, industrial

INTRODUCTION

The necessity to relieve current industrial wastewater treatment plants environmentally and economically has been pushing the creation of technologies that effectively contribute to industrial sustainable development. In this sense, the establishment of waste treatment processes that balance the vectors environment, economy and society will be imperative in treatment plants in the medium term.

On the one hand, environmental preservation is a necessity, and rigidly controlled by regulatory standards; on the other hand, the economic impact of implementing pollution control systems makes global food supply chains often inefficient. The search for sustainable industrial processes is currently a decisive criterion in the choice of technological routes of industrial processing.

The inability of wastewater treatment processes to eliminate pollutants, as a result of the principle of conservation of matter,hashistorically geared the development of technologies through management of compounds, and volatilizationor physical separation of pollutants has been the basis of most environmental containment processes. The advancement of knowledge through the establishment of more robust methods of analysis, such as life cycle analysis (LCA) [1], have shown the weaknesses of these systems, by pressing the engineering processes for the development of sustainable solutions for application in production chains.

Nutrient cycling by microalgae emerges as a promising technology because it balances sustainable vectors by reuse of pollutants, like carbon, nitrogen and phosphorus, present in wastewater generated by the industry and, thus, form a biomass where one can extract a variety of bioproducts with high added value.

These forward-looking technologies, which are in the process of research and development (R and D) or in an escalation phase are genuinely based on some form of reuse of agroindustrial waste aiming to create opportunities for amortization of substantial costs of treatment processes. In parallel to this first stage of development, which seeks a balance between revenue and expenditure, in the long-term wastewater treatment plants will be consolidated as revenue-generating, through the excessive use and exploitation of resources [2].Thus, the objective of this chapter is to evaluate nutrient cycling in wastewater treatment plants by microalgae-based processes**.** The focus is directed to nutrient cycling, characteristics of agroindustrial pollution, industrially consolidated processes for treatment of industrial wastewater, process intensification applied to waste treatment, emerging processes for treatment of industrial wastewater and waste utilization and recovery through establishment of industrial biorefineries.

NUTRIENT CYCLING

Life on our planet is dependent upon nutrient cycling in the biosphere. Atmospheric carbon dioxide would be exhausted in a year or so by green plants if it were not for the the fact that the atmosphere is continually recharged by CO_2 generated by respiration and fire [3]. Also, it is well-known that life requires constant cycles of nitrogen, oxygen, and water. These cycles include a gaseous phase and have self-regulating feedback mechanisms that make them relatively perfect. Any increase in movement along one path is quickly compensated for by adjustments along other paths [4, 5].

This life-essential movement of nutrients, from the environment into plants and animals and back again, is a vital function of the ecology of any region. In any particular environment, the nutrient cycle must be balanced and stable if the organisms that live in that environment are to flourish and keep a constant population [6].

Therefore, nutrient cycling may be defined as the transformation of nutrients from one chemical form to another, and/or the flux of nutrients between organisms, habitats, or ecosystems. In most ecosystems, microorganisms are important agents of nutrient cycling [7].

Human impact on nutrient cycles has fundamentally changed the regulation of ecosystem processes. The combustion of fossil fuels has released large quantities of nitrogen and sulfur oxides into the atmosphere and increased their inputs to ecosystems. Fertilizer use and cultivation of nitrogen-fixing crops have further increased the fluxes of nitrogen in agricultural ecosystems. The disposal inadequate of industrial wastewater in water bodies is the main cause of eutrophication, and has severe negative effects on biodiversity. Together, these human impacts cause imbalance in nutrient cycling [8].

Microalgae play critical roles in marine energy fluxes and nutrient cycles by incorporating and redistributing dissolved organic matter and inorganic nutrients in the oceans. Because of their capacity for rapid growth, those microorganisms are a major component of global nutrient cycles. Understanding what controls their distributions and their diverse suite of nutrient transformations is a major challenge. What is emerging is an appreciation of the previously unknown degree of complexity within the microbial community [9].

Microalgae have various forms of nutrient cycling, as shown in Figure 1. Carbon, in the form of carbon dioxide, may be fixed from the atmosphere and industrial exhaust gases through the photosynthetic activity of autotrophic microalgae.

Some microalgae display heterotrophic behavior, using organic forms of carbon present, for example, in wastewater, where it is metabolized through respiration to generate ATP. After carbon, nitrogen and phosphorus are elemental constituents with a very important contribution in microalgal cells; they are assimilated and found in a variety of biological substances.

Photosynthesis can be defined as the synthesis of organic compounds through the assimilation of CO_2 with the use of light as an energy source. CO_2 is incorporated into a 5-carbon acceptor, ribulose-1,5-bisphosphate (RuBP) in an energy requiring reaction catalyzed by the primary carboxylating enzyme, RuBP carboxylase. The product splits into two molecules of a 3-carbon compound, phosphoglyceric acid (PGA), and the reduction of PGA, mediated by the electrons carrier NADPH (nicotinamide adenine dinucleotide phosphate) leads to formation of a series of sugar phosphate intermediates and, finally, glucose.

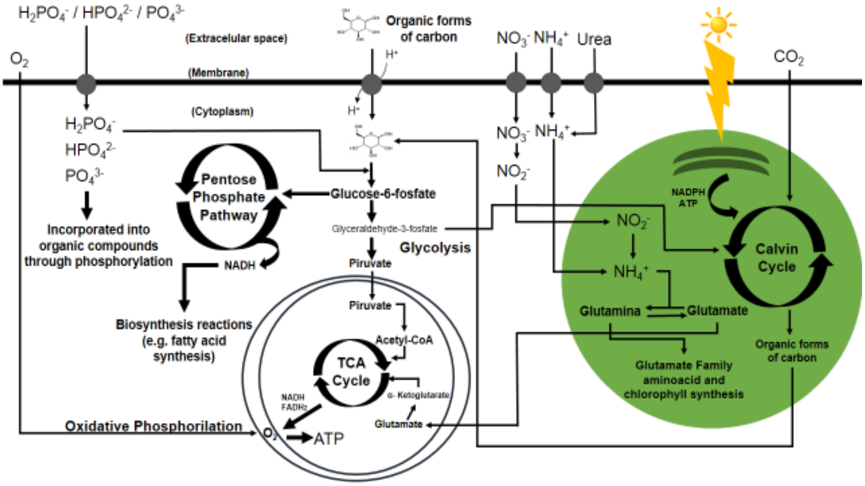

Figure 1. Nutrient cycling by microalgae-based processes.

During this sequence of metabolic transformations, known as the Calvin cycle, the acceptor RuBP is regenerated, ready to accept another CO_2 molecule. The requirements for energy (in the form of ATP) and reductant (NADPH), however, render the transformations during the Calvin cylce fully dependent on the primary photochemical act, which takes place in the organelle membranes. It is where where light energy, absorbed by the highly organized assemblies of photosynthetic pigments and electron carriers, called Photosystem I and Photosystem II, excites the chlorophyll molecule in the reaction center. This leads to the expulsion of high energy electrons and their flow down a redox potential gradient which results in the formation of strongly electronegative electron carriers, like ferredoxin and NADPH. Part of the released energy is incorporated during transport of this electron into ATP in the process of photophosphorylation.

Another form of nutrient cycling of organic forms of carbon used by some microalgae is respiration. Oxidative assimilation of glucose begins with a phosphorylation of hexose, yielding glucose-6-phosphate, which is readily available for storage, cell synthesis, and respiration. Of the several pathways used by microorganisms for aerobic glycolysis (breakdown of glucose), apparently only two - glycolysis (Embden-Meyerhof Pathway) and pentose phosphate pathway - have been shown in microalgae. When the ATP/ADP ratio is high, microalgae use Pentose Phosphate pathways for generating NADPH used in reactions of biosynthesis; for example, biosynthesis of fatty acids. Otherwise, when the ATP/ADP ratio is low, organic forms of carbon are metabolized through glycolysis into pyruvate. Then, pyruvate is converted into acetate. After that, acetate (carried by coenzyme A) is metabolically oxidized into the tricarboxylic acid (TCA) cycle to provide reductive molecules (NADH and $FADH_2$) for respiratory electron transport chain (oxidative phosphorylation), and electrons are transferred from electron donors (NADH and $FADH_2$) to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP.

Nitrogen is a critical nutrient required in the growth of all organisms. Microalgae play a key role in converting inorganic nitrogen into its organic form through a process called assimilation. In addition, cyanobacteria are capable of converting atmospheric nitrogen into ammonia by means of fixation. Assimilation, which is performed by all eukaryotic algae, requires inorganic nitrogen to be only in the forms of nitrate, nitrite, urea and ammonium. As shown in Figure 1, translocation of the inorganic nitrogen occurs across the plasma membrane, followed by the reduction of oxidized nitrogen and the incorporation of ammonium into aminoacids. Nitrate and nitrite undergo reduction with the assistance of nitrate reductase and nitrite reductase, respectively. Nitrate reductase uses the reduced form of nicotinamide adenine dinucleotide (NADH) to transfer two electrons, resulting in the conversion of nitrate into nitrite. Nitrite is reduced to ammonium by nitrite reductase and ferredoxin (Fd), transferring a total of six electrons in the reaction. Thus, all forms of inorganic nitrogen are ultimately reduced to ammonium prior to being incorporated into aminoacids within the intracellular fluid. Finally, using glutamate (Glu) and adenosine triphosphate (ATP), glutamine synthase facilitates the incorporation ammonium into the aminoacid glutamine or chlorophyll synthesis.

Phosphorus is also a key factor in the energy metabolism of microalgae and is found in nucleic acids, lipids, proteins, and the intermediates of carbohydrate metabolism. Inorganic phosphates play a significant role in algae cell growth and metabolism. During algae metabolism, phosphorus, preferably in the forms of $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}, is incorporated into organic compounds through phosphorylation, much of which involves the generation of ATP from adenosine diphosphate (ADP), accompanied by a form of energy input. Phosphates are transferred by energized transport across the plasma membrane of the algal cell.

Therefore, the ability of microalgae to assimilate nutrients mainly in removing pollutants in agroindustrial wastewater is extremely important for global nutrient cycling. It shows an efficient ability to incorporate macronutrients (carbon, nitrogen and phosphorus) in a variety of biological substances such as energy transfer molecules (ADP, ATP), genetic materials (RNA, DNA), proteins, carbohydrates, lipids and pigments through metabolism.

CHARACTERISTICS OF AGROINDUSTRIAL POLLUTION

Industrial waste, by definition, is the set of fractions of raw materials, which were not incorporated into the final product. In this way, the ideality of a technological process of the industry could be achieved in processes whose inputs are fully incorporated in the final products. In these ideal cases, waste would not be linked to global supply chains [10].

As technological processes and process engineering are not aligned with this profile, what is verified in practice is the generation of industrial waste proportionally to process efficiency. As a consequence of this state of the art, the massive generation of liquid, solid and gaseous waste is ordinarily seen in the industrial sector.

Another factor to be considered is associated with the scales of the processes. Due to low profitability of some industrial products, the economic viability of these processing units occurs only in greatly enlarged scale, which results in substantial amounts of waste to be managed.

Regarding forms of waste, the current legislation is ineffective as far as gaseous waste is concerned, with concrete initiatives toward the particulate pollution and in the medium to long-term perspective for greenhouse gases. Solid waste, in turn, has been effectively transformed into co-products associated with the main proceedings, where reuse as animal feed, fertilizer and energy is already consolidated.

On the other hand, the rigor of the current legislation as regards management of liquid waste requires greater efforts in the development and establishment of technologies [11].

The most commom form of industrial pollution is abundant in organic matter and nutrients (nitrogen and phosphorus) of nature suspended and dissolved, beyond biological contaminants, normally expressed by thermotolerant coliforms and specific inorganic and organic pollutants, which are a function of the raw material and technological route taken.

These parameters are controlled according to their concentration in wastewater (C) associated with the industry operating flow (F), resulting on pollutant loading, which is the amount of given pollutant released in a receiver ecosystem, expressed in units of mass by time (Eq. 1).

$$\text{Pollutant loading} = F \times C \tag{1}$$

In this sense, large industries are required to have waste with lower concentrations of pollutants when compared to small-sized industries. In addition, one must consider the possibility of attendance to fixed minimum efficiency in cases which do not reach the emission standard, expressed as concentration (mg/L), and regulatory agencies allow the disposal of effluent above the fixed value provided that there is proven removal efficiency (%), typically 75% for total nitrogen and total phosphorus and 95% for thermotolerant coliforms [12]. This additional possibility stems from the currently existing lower technological level to mitigate these polluting forms.

INDUSTRIALLY CONSOLIDATED PROCESSES FOR WASTEWATER TREATMENT

Originally, industrial wastewater treatment plants have been support by stabilization ponds, in a process of natural symbiosis of photosynthetic and heterotrophic organisms. Low capital and operating cost can be characterized as the main advantage of these processes, despite slow removal performance of organic material, results in long hydraulic retention times and therefore tanks with very high volumes. The area occupied by these systems is not currently available in modern industries. Some variants of conventional stabilization ponds, such as aerated lagoons, were developed, but the gain in efficiency was not able to overcome the major obstacles, making this obsolete technology. The stabilization ponds still in operation are gradually being disabled or adapted in other arrangements for wastewater treatment [13]. The main use is aimed at application in polishing operations for previously treated wastewater, especially when there is the presence of low biodegradability compounds.

In a second step, the industry adopted more intensive treatment processes based on natural anaerobic and aerobic technologies. Anaerobic reactors of various settings have been extensively applied to organic material conversion. The main benefits of this technology are related to the low operating costs associated with the possibility of energy reuse of the resulting methane gas.

Moreover, significant hydraulic retention times, formation of unpleasant odors, the need for mesophilic temperatures, difficult sludge sedimentation and sensitive to shock loads have limited its consolidation [14]. Thus, activated sludge process has become technology largest potential application. This aerobic stabilization process is based on a microbial consortium of bacteria, protozoa, fungi, algae and rotifers that efficiently converts organic material from wastewater, and part of the nitrogen and phosphorus material, contributing to the partial removal of nutrients from wastewater, dishonoring subsequent operations to remove these pollutants. Regardless of these advantages, this technology is limited by massive dissolved oxygen supply required by the process, resulting in large pumping stations air and especially the cost of energy required to move the air blower. Additionally, high substrate yield coefficients ($Y_{X/S}$), usually at about 0.3 kg_{sludge}/kg_{DBO} result in a sludge mass production that requires suitable treatment and disposal, further increasing process cost [15].

As feedstocks are characteristic of some industries, the removal of nitrogen and phosphorus has become a necessity in treatment plants.

The removal of nitrogen by the nitrification-denitrification process, which consists in the oxidation of ammonia to nitrate in two serial reactions and subsequent reduction of nitrate to nitrogen gas, the process became more extensively used for this purpose. The use of two bioreactors in a row, or a single reactor with nitrification and denitrification zones, or the adaptation of activated sludgereactors have been the main operating arrangements of this system. The complexity of the operation associated with nitrogen loss is considered the main disadvantage of this technology [2]. Furthermore, a technology based on volatilization of nitrogen in gaseous form (N_2) is severely challenged by virtue of the value of this element, which is used as basic building blocks of many consumables used by societies.

Phosphorus removal in industrial wastewater, in turn, has been considered the highest level of operating complexity. Numerous biological processes (bacterial systems, use of macroalgae and microalgae), chemical processes (chemical precipitation and adsorption) and also the use of fertigation techniques have been tentatively used, due to the fragility of these technologies. Chemical precipitation through coagulating the basis of iron or aluminum is the best technology established in terms of removal efficiency, although the formation of metal salts of difficult disposal limits its technological consolidation [16].

In addition to these exclusively biotechnological processes, physical-chemical techniques have gained substantial importance in treatment plants, due to the advance in the development of flocculants substances with more effective action than conventional mineral flocculants based on iron and aluminum. Anionic, cationic or nonionic polymers, and synthetic polyelectrolytes have been extensively used in forced sedimentation processes, because of their effectiveness in removing particulate and colloidal pollutants, contributing to the overall reduction of the pollution load of wastewater, thus helping the biological treatment systems [17].

The diagnosis of current treatment processes evidences the high demand for human resources, chemicals and energy, due to the current extension of treatment plants. Moreover, the massive generation of secondary pollution should be considered, through biological sludge and chemicals resultant of the transformation processes that contribute to further encumbering the process. In this sense, a new approach in the development and optimization of technological processes and process engineering should be introduced aiming to overcome current limitations.

PROCESS INTENSIFICATION APPLIED TO WASTE TREATMENT

The process intensification is an engineering concept associated with substantial improvements in industrial processing plants. This approach is related to the overall increase in efficiency of the production process, by reducing the relative size of the equipment/production capacity, energy consumption and waste generation. This new paradigm seeks to drastically consolidate more sustainable industrial processes [18].

Intensive processes reduce the number of unitary operations of process, require lower power density during operation, do not generate secondary pollution and allow the reuse/recovery of biomass formed during bioconversion processes.

Figure 2 lists the principal elements of process intensification, in which strategies can be divided in the intensification of equipment (hardware) and/or methods (software). In this perspective, some previously consolidated initiatives in the area of industrial processes and other fields in research and development phase (R and D) are being proposed. Some direct applications of this approach in the treatment of agroindustrial wastewater mainly include the development of multifunctional reactors, simultaneously capable of converting more than one kind of pollutant [19]; development of equipment for solid-liquid separation, to reduce the water content of biological sludge [20]; development of separation membranes for better control of the biological phase of the bioreactor [21]; development of inert supports for cell immobilization [22]; besides the chemical and energetic utilization of the generated biomass and co-products [23, 24]. These technologies are being referred to as emerging treatment processes.

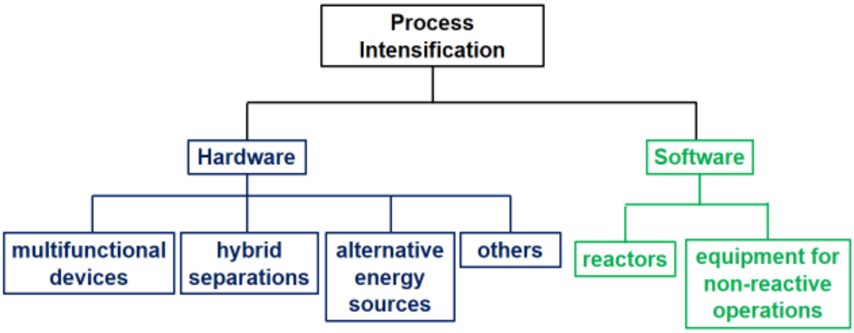

Figure 2. Process Intensification Elements.

EMERGING PROCESSES FOR INDUSTRIAL WASTEWATER TREATMENT

The intensification of the classic systems of anaerobic and aerobic nature has been conducted in order to obtain reactors with high flow rates. This increase has been made possible through the uncoupling of hydraulic retention time and sludge retention time. In addition to economic benefits, these adjustments allow an increase in the conversion capacity of conventional pollutants and micropollutants. Fluidised bed reactor (FBR) and membrane bioreactor (MBR) are the most stable arrangement, technologically. In particular the use of membranes for micro and ultrafiltration lead to numerous benefits to processes such as increased COD removal efficiency and micropollutants, due to improved properties of the microbial biomass, because high cell densities enhance the conversion of pollutants by retaining slow-growing microorganisms. In addition there is a reduced production of excess sludge, better operational reliability, facility of scale and size reduction of process equipment [25, 26].

Furthermore, technologies combining anaerobic and aerobic treatment processes in a single bioreactor should be introduced in the near future.

Arrangements without physical separation of the aerobic and anaerobic areas, sequencing batch reactors (anaerobic and aerobic), in addition to combined anaerobic and aerobic systems supported by granular sludge or biofilm [27].

At the level of removal of nutrients, particularly nitrogen, Anammox process [28], which consists of the direct oxidation of nitrite and ammonium into nitrogen gas under anaerobic conditions, has considered the most promising technology. This process reduces the number of unit operations required in conventional processes of nitrification-denitrification, wiping the process under capital and operational perspective.

In addition to exclusively biological processes, physico-chemical and biological combined processes have been considered, due to the ineffectiveness of bioprocesses on emerging pollutants such as pesticides, drug residues, dyes, solvents, polycyclic aromatic hydrocarbons and corrosion inhibitors that are massively being linked ace on agroindutrial production chains, and consequently being dragged through the wastewater. Thus, integrated use of biotechnological processes with photochemical processes or advanced oxidation processes have gained importance in the tertiary wastewater treatment. Chemical oxidation technologies are based on the use of oxidizing agents such as ozone, hydrogen peroxide or chlorinated species [29]. The advanced oxidation processes generate hydroxyl radicals with high reactivity, being extremely effective in oxidizing a broad range of organic compounds. Photochemical oxidation processes that include the direct photolysis and photocatalysis (heterogeneous or homogeneous) are included in this group of technologies [30]. Emission standards of increasingly rigid pollutants, which lead to control of emerging pollutants, will certainly require this type of technology, although, energy consumption and chemical reagents are currently considered the main barriers to be overcome in the consolidation of techniques.

Phytoremediation [31] is another group of emerging techniques for the treatment of industrial waste. These techniques are based on the use of plants in situ aiming at decontamination of soils, sediments and wastewater. Among the many possible arrangements, flooded, artificially constructed areas, known as wetlands, have been potentially used for industrial decontamination purposes. Maintaining high performance in cold climates has been regarded as the main advantage of these techniques. The management of biomass, however, is a limiting aspect to the spread of this technology [32], although the use of species with energy potential has been considered a strategy to overcome this obstacle.

The high electricity demand of most aerobic treatment processes, according to the requirements of aeration, have pressured the development of sustainable systems of production that form energy. Bioelectrochemical systems [33, 34] like microbial electrolysis cells and microbial fuel cells are potential initiatives in this direction. These technologies are based on the conversion of chemical energy contained in organic compounds dissolved in wastewater directly into electricity. In addition to electricity, these systems can produce, in parallel, co-products that can add value to the process, as hydrogen, butanol and bioplastics. Several factors limit, however, its immediate application, which includes reducing microbial activity in the oxidation of the fuel at the anode or reducing the oxidant at the cathode, reduced electron transfer rates in both the anode and the cathode, mass transfer limitations in relation to protons and substrates in the electrolyte and protons across the membrane, besides ohmic resistance of the reactor.

Enzymatic catalysis [35] is another potentially attractive technological route for the treatment of industrial wastewater with targeted applications, mainly the degradation of specific recalcitrant organic compounds. Although environmentally balanced, this technique needs solid development in the engineering of process escalation, improved stability in relation to environmental conditions (temperature, pH and inhibitors), as well as substantial reduction in operational cost, mainly with regard to biocatalysts.

Finally, microalgae-based processes have gained increasing relevance in the treatment of agroindustrial wastewater [36]. The first developments were based on the removal of nutrients (nitrogen and phosphorus) through the photosynthetic treatment system [37], and more recently, the use of heterotrophic routes [38] in complete darkness, have enabled the simultaneous removal of organic material, nitrogen and phosphorus in single stage. The main advantage associated with this type of process is the conversion of pollutants in a chemically attractive biomass with reuse potential as an intermediate and/or final product of a series of consumables. However, many biological aspects andengineering of scale require solution for large-scale applications, although of all emerging listed techniques, this is considered the one that gathers the most attractive features, and establishes the best adherence to concept of process intensification. Microalgal heterotrophic bioreactors, for example, are multifunctional devices capable of removing organic matter, nitrogen and phosphorus in one step. These devices show high performance in simultaneous conversion of pollutants, causing capital reductions (size of the main unit) and operational reduction (electricity) by 25% [39]. Figure 3 illustrates a pilot system for the simultaneous conversion of BOD, N-TKN and

$P\text{-}PO_4^{3-}$ of wastewater from rice parboiling.Inherent in this pollutant conversion process, microalgal biomass is formed, on average values for substrate yield coefficients ($Y_{X/S}$) varying between 0.3 to 0.5 $kg_{biomass}$ / kg_{BOD}, thus resulting in a massive generation of biomass.

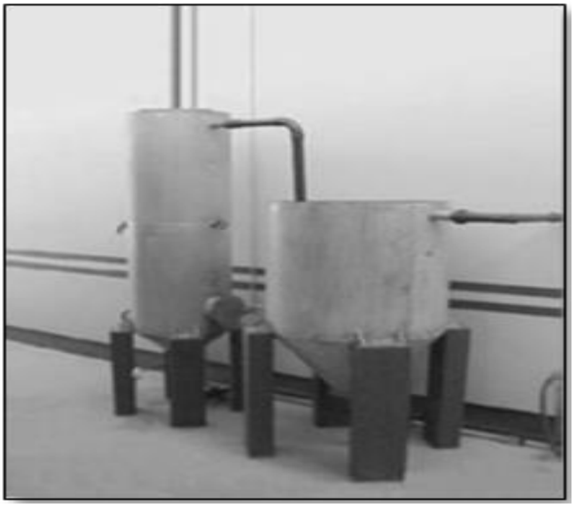

Figure 3. Microalgal heterotrophic bioreactor coupled to sedimentation tank installed in a rice parboiling industry in Pelotas city, RS, Brazil.

As opposed to the bacterial processes or other microbial consortia employed in treatment plants, microalgal biomass is high commercial potential input [40], due to individual fractions that it is composed of, represented for the content of proteins, lipids, carbohydrates, minerals, pigments and phenolic compounds. These compounds, when recovered and purified, can be fed in the industry itself or marketed in other industrial sectors [41], adding value to the production chain.

UTILIZATION AND WASTE RECOVERY - INDUSTRIAL BIOREFINERIES

The harnessing of industrial waste toward its valuation has been the target of multiple efforts of production chains. The economic characteristics of this type of activity, because of the low profit margin normally achieved with products, press for the rational use of raw materials.

Successful examples can be checked in the sugar and ethanol mills, where solid waste is used in co-generation of electrical and thermal energy and wastewater is used as a bio-fertilizer [42].

At the same time, soy agroindustry splits the grain of the oilseed in multiple products and co-products, making it difficult to identify the main product of activity [43].

In terms of raw materials of animal origin, blood waste, bones, and other less noble fractions of carcasses have been successfully incorporated into animal feed formulation [44].

These first initiatives are consolidated in the current scenario of these industrial activities, and represent opportunities for easier implementation. The industry, however, has developed the most advanced technology for reuse processes in an attempt to recover inputs lost during processing procedures. In this sense, the concept of biorefining is gaining more space in the sector. Biorefineries combine synergistically biological and chemical conversions, directed to the recovery and purification of biomass fractions. These processing units, although well established in theory, are not economically viable primarily because of the high cost of plant biomass. Thus, biosolids resulting from wastewater treatment processes, particularly microbial biomasses, have unlimited potential for exploitation, depending obviously on the chemical characteristics of the biomass.

Figure 4 shows a conceptual diagram of a biorefinery, indicating the conversion of biomass into multiple products through a sequence of unit operations that characterize the biorefinery process.

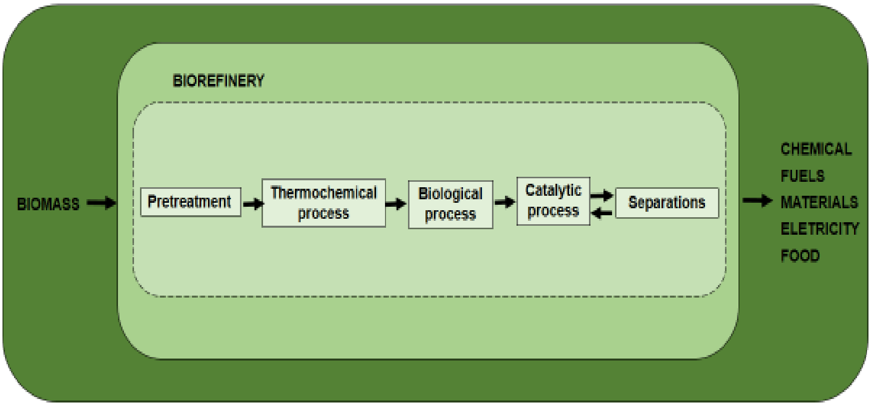

Figure 4. Conceptual diagram of a biorefinery.

Within this perspective, and corroborating with global standards [45, 46] which classify solid waste from industrial sources, which include microbial biomasses resulting from wastewater treatment plants, it can be concluded that the sustainable use of this type of material may represent an attractive productive that is a competitive advantage in the industrial sector.

Table 1. Biosolids composition resulting from agroindustrial waste treatment processes

Composition	Activated sludge 1	Activated sludge 2	Microalgal Sludge 3
General components (g/100g of dried biosolids)			
Protein	37.5	40.0	52.5
Lipids	6.0	7.4	13.8
Carbohydrates	15.0	10.0	27.1
Minerals	32.2	15.0	6.5
Aminoacids (g/100g of dried biosolids)			
Alanine	7.9	7.0	2.5
Glycine	7.1	4.6	2.8
Valine	5.8	3.8	2.0
Threonine	5.4	4.2	2.0
Serine	5.1	3.3	1.6
Leucine	6.6	5.8	2.7
Isoleucine	3.7	2.5	1.9
Proline	4.3	3.2	1.2
Methionine	1.5	-	0.7
Aspartic acid	9.9	8.5	3.7
Phenylalanine	4.4	3.4	1.6
Glutamic acid	12.2	8.9	7.5
Lysine	3.9	3.9	2.0
Tyrosine	3.4	2.1	0.8
Arginine	4.8	3.4	2.3
Histidine	1.7	0.9	0.8
Cystine	2.7	3.6	1.7
Tryptophan	0.7	-	0.5
Pigments (g/100g of dried biosolids)			
Chlorophyll a	-	-	0.08
Phycocyanin	-	-	7.15

1: Brewing [47]; 2: Cattle slaughterhouse [47], 3: fish processing [38].

The necessity of proper disposal of massive volumes of these biosolids in landfills substantially burdens the overall cost of wastewater treatment operations, generating, in parallel, the wastage of important parts of commercial value. Table 1 shows the composition of microbial biomass derived from conventional treatment processes (activated sludge) and emerging processes (heterotrophic microalgal systems), in which the potential for exploitation is evident when considering these biomass fractions alone or combined.

Although nutritionally attractive, one must consider the possibility of accumulation of toxic compounds along the production chain, often limiting the direct application of biosolids as single-cell protein in animal feeds. In this sense, the compounds of decontamination of toxic sludge, as well as extraction and purification of value-added components, may be alternatives to rationalize the use of these resources [48].

On the other hand, due to these restrictions, often regulated by environmental legislation, the use of biosolids in non-food products can be a strategy for rapid implementation. The production of biofuels, especially biodiesel, is considered a technological route of high exploration potential, since microbial biomass present composition of lipids and fatty acids that allow the technical and economic exploitation of the process [49]. In this case, treatment processes mediated by microalgae are considered as real options towards conversion of waste associated with the production of third generation biofuels. An example of the exploitation of the potential of this technology can be shown in Figure 5, establishing an average production for soybean oil in the order of 0.00046 g_{oil}/m^2.day. The oil extracted from microalgae sludge resulting from treatment of slaughterhouse wastewater, in turn, has a production potential estimated at 0.50 kg/m^3.day, when compared to the same annual soybean production cycle (120 days/year) suggests the need for a bioreactor operated continuously at 6 L/m^2 for the occurrence of production equivalence.Additionally, considering the possibility of expanding the annual production cycle as a unique possibility of bioprocesses, this theoretical equivalence can be estimated in a system at 2 L/m^2 (336 days/year of operation), clearly demonstrating the potential of exploiting this technological route for the production of oleaginous raw materials.

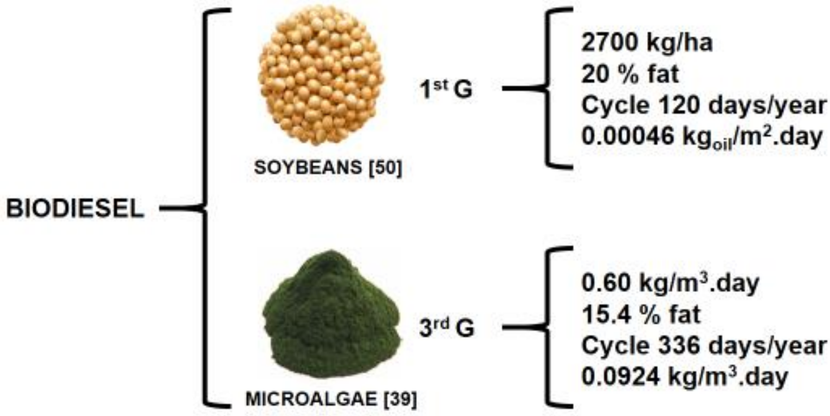

Figure 5. Comparison of the productivity of first (1^{st}G) and third generation biodiesel (3^{rd}G).

Besides, the quantitative aspects should be considered in the comparison of the initial requirements for both production processes, since soybean oil has its production based on high fertility land areas, while oil microalgal can be exclusively supported by agroindustrial waste.

Regardless of the biorefining route being considered, this approach aims to balance the treatment process, since it will reduce or eliminate the need for solids management, to be disposed in sanitary landfills, while generating economically attractive products and valuing the global production chain.

In this chapter, we discussed nutrient cycling within the context of microalgae metabolism, beyond treatment and harnessing of industrial waste within the context of technological processes and process engineering, in which we can summarize the need for consolidation of the following aspects for advancement of industrial engineering:

- Intensification of performance of existing technologies
- Minimization of biosolids training that are not suitable for reuse
- Reduction in the number of unit processes operations
- Reduction in the dimensions of equipment
- Reduction of capital and operating costs of processes

The elements which made these advances and that require the most effort in Research and Development (RandD) are:

- Consolidation in the use of molecular biology techniques
- Advances in implementation of analytical instrumentation
- Development of materials and membrane separation processes
- Optimization of enzymatic preparations and enzyme immobilization techniques
- Development of biofilms carriers

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CAPÍTULO 4

MANUSCRITO 2

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The bioeconomy of microalgal heterotrophic bioreactors applied to agroindustrial wastewater treatment

Alberto Meireles dos Santos, Gabriela Rigo Roso, Cristiano Ragagnin de Menezes, Maria Isabel Queiroz, Leila Queiroz Zepka, Eduardo Jacob-Lopes

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The bioeconomy of microalgal heterotrophic bioreactors applied to agroindustrial wastewater treatment

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ABSTRACT

This paper presents a techno-economic analysis of microalgal heterotrophic bioreactors applied to the treatment of poultry and swine slaughterhouse wastewater. The process is based on a multifunctional bioreactor used to simultaneously convert organic matter (chemical oxygen demand [COD]), nitrogen (N–TKN) and phosphorus ($P–PO_4^{-3}$) into microalgal biomass. The experimental data, obtained from a bench-scale facility, were used to estimate the costs of an industrial scale (16,000 m^3/d). The results indicate removal efficiencies of 97.6%, 85.5% and 92.4% for COD, N–TKN and $P–PO_4^{-3}$, respectively, in parallel to a microalgal sludge productivity of 0.27 kg/m^3/d. The economic analysis demonstrated a cost of USD 2.66/m^3 of treated industrial wastewater, and as consequence of this process, the production cost of microalgal sludge was USD 0.03/kg of dehydrated biomass.

Keywords: Microalgae/cyanobacteria; Industrial effluent; Heterotrophic cultivation; Cost analysis

1. Introduction

Society has been demanding a sustainable industrial development outlined by environmental responsibility, renewable energy use and higher energy efficiency [1]. It is believed that there can be a transformation in the industrial sector with less impact on the environment, and therefore, industries have invested in process intensification, through the development of innovative apparatuses and techniques that offer drastic improvements in manufacturing and processing, substantially decreasing equipment volume, energy consumption, or waste formation, and ultimately leading to cheaper, safer, sustainable technologies [2]. One of the basic components of process intensification is the so-called multifunctional reactors, which are described as reactors combining at least one more function, usually a unit operation [3].

Currently, Brazil has high competence and competitiveness in the production and productivity of poultry and swine meat; it is the third largest producer and the largest exporter of poultry meat and the fourth largest producer and exporter of swine [4]. The industry of poultry and swine slaughterhouses generates a large volume of wastewater with a high pollutant load. It is estimated that this industrial process demands an average water volume of 10 m^3 per ton of final product, leading to a high volume of wastewater to be treated [5].

In the wastewater treatment facilities, although conventional methods can be used, the high energy consumption and the generation of secondary pollution limit the techno-economic feasibility of the main wastewater treatment systems, such as activated sludge, nitrification-denitrification, and phosphorus precipitation.

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* Corresponding author.

In this sense, processes with high efficiency, cost effectiveness, and environmental friendliness should be developed to make the global production chain sustainable [6–9].

Heterotrophic microalgal bioreactors are a potential technology to be applied in industrial wastewater treatment facilities. One characteristic of heterotrophic microalgae metabolism is the simultaneous conversion of pollutants present in wastewater in a single step, thereby reducing capital and operational costs. In addition, substantial amounts of microalgae biomass with a high potential of exploitation as industrial feedstocks are formed, and they are inherent in the process of treatment [10,11].

Phormidium is a genus of single-cell blue green algae, belonging to the phylum cyanobacteria. It is filamentous, unbranched in shape and about 3–4 μm in diameter. Several species live in limiting environments such as thermal springs, desert soils, and polluted sites. These blue green algae show considerable potential for use as biocatalysts in environmental biotechnology processes because of their robustness and simple nutritional requirements [12,13].

The techno-economic studies of the microalgae-based processes have been shown to be economically infeasible scenarios [14–16]. This infeasibility is related mainly to the reduced scalability of the photosynthetic and the high operational costs of the heterotrophic processes. According to Wijffels et al. [17], the technological routes are immature and need to be fully developed, implying the need for a large effort in research and development (R&D). These authors reported that microalgal biotechnology will be competitive and commercially attractive by 2020.

In the analysis and cost estimate for designing a new process, almost all the decisions are impacted by the economic factors, and therefore, it is critical to study process economics. The major criteria to judge feasibility are preliminary design and economic potential estimation to be attained, and knowledge of the price of the final product is necessary for covering the costs involved. The feasibility of these processes has been determined based on the techno-economic analysis of the simultaneous process of wastewater treatment and biomass production, which is conducted based on a relationship of a benefit-cost ratio. Feasibility indicators such as economic equilibrium (EE), profitability, rentability, and period of return on investment are the main parameters in use [18].

In this regard, the aim of this study is to evaluate the techno-economic modeling of microalgal heterotrophic bioreactors when applied to wastewater treatment in poultry and swine slaughterhouses.

2. Material and methods

2.1. Microorganism and culture conditions

The microalgae used was *Phormidium* sp., originally isolated from the Cuatro Cienegas desert (26°59′N, 102°03′W, Mexico) [19]. Stock cultures were propagated and maintained in solidified agar-agar (20 g/L) containing synthetic BG11 medium [20]. The incubation conditions used were 25°C, light intensity of 1,000 lux, and a photoperiod of 12 h. To obtain the inoculums in liquid form, 1 mL of sterile synthetic medium was transferred to slants; the colonies were scraped and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

2.2. Wastewater

The poultry and swine slaughterhouse wastewater used in the experiments was obtained from an industry located in Santa Catarina, Brazil (27°14′02″S, 52°01′40″W). It was collected from the discharge point of an equalization tank over a period of 1 year, and analyzed for pH, chemical oxygen demand (COD), N–TKN, P–PO_4^{-3}, TS, SS, VS, and FS following the Standard Methods for the Examination of Water and Wastewater [21]. Table 1 shows the average composition of the wastewater, in a 1 year of sampling. The C/N ratio and N/P ratio were calculated through COD, N–TKN, and P–PO_4^{-3}.

2.3. Description of the process

The unit operations of the process were based on a patent application developed by Jacob-Lopes et al. [22]. The core of the process is one heterotrophic microalgal bioreactor that is used to simultaneously convert COD, N–TKN, and P–PO_4^{-3} into microalgal biomass. A primary treatment composed by a fine screen, Parshall flume, rotary sieve, and equalization tank was used. After the biological treatment, the microalgal sludge was processed by a decanter, a belt filter, and a drum dryer. Fig. 1 shows the flow diagram of the process.

The bench-scale bioreactor was made of polyvinyl chloride and had an external diameter of 12.5 cm and a height of 16 cm, resulting in a height/diameter (h/D) ratio equal to 1.28 and a nominal working volume of 2.0 L. The dispersion system of the reactor consisted of a 1.5 cm diameter air diffused device located inside the bioreactor. In addition to the bioreactor, the bench-scale facility is fitted with all the necessary ancillaries to convert the pollutants of the agroindustrial wastewater into dried microalgal biomass.

The operational conditions of the continuous process were previously optimized in order to define a pH adjusted to 7.6, temperature of 20°C, volumetric airflow rate per volume unit of 1 VVM (volume of air per volume of wastewater per minute), absence of light, and a dilution rate of 0.6/d [23]. The loading rates of COD, N–TKN, and P–PO_4^{-3} were 2,460.0 ± 524.4, 77.1 ± 7.2, and 1.7 ± 0.12 mg/L/d, respectively.

Table 1
Average composition of the wastewater

Parameter	Value
pH	5.9 ± 0.05
COD (mg/L)	4,100 ± 874
N–TKN (mg/L)	128.5 ±12.1
P–PO_4^{-3} (mg/L)	2.84 ± 0.2
TS (mg/L)	3.8 ± 2.7
FS (mg/L)	0.9 ± 0.3
VS (mg/L)	2.9 ± 1.4
SS (mg/L)	1.9 ± 0.8
C/N	31.9
N/P	45.2

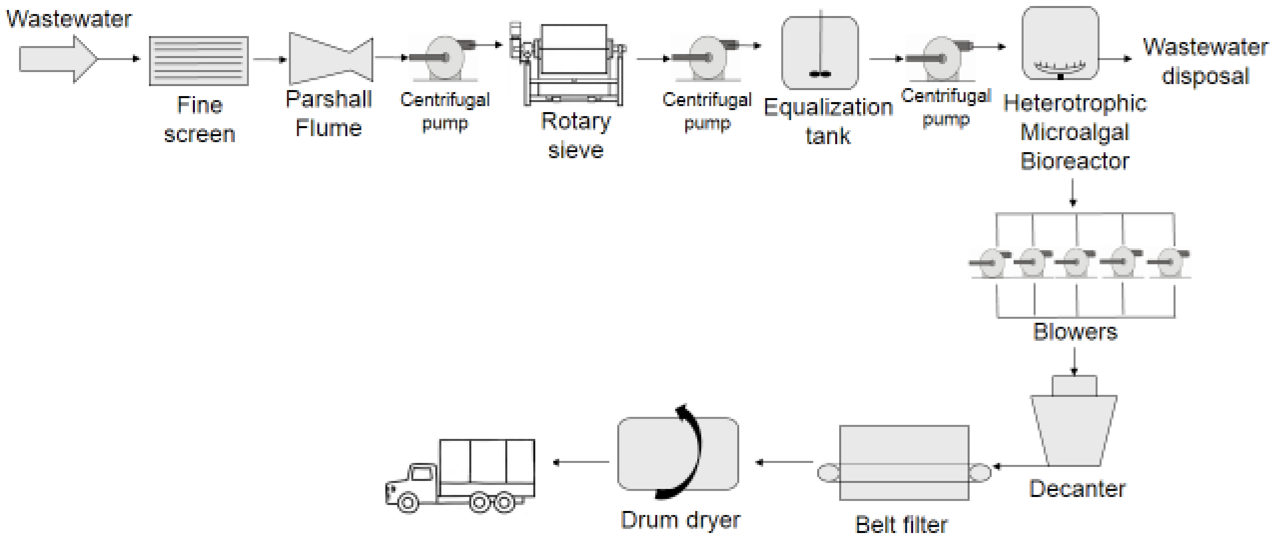

Fig. 1. Process flow diagram of the agroindustrial wastewater treatment.

The steady-state was considered to have been established after at least 3 volume charges, with a variation of cell dry weight less than 5%.

2.4. Sampling and analytical methods

Samples were collected at regular intervals of 24 h and characterized for COD, N–TKN, P–PO_4^{-3}, cell biomass, and dissolved oxygen concentration. The COD, N–TKN, and P–PO_4^{-3} were determined according to the methodology previously defined in section 2.2. Cell biomass was gravimetrically evaluated by filtering the wastewater through a 0.45-µm membrane filter (Millex-FG®, Billerica, MA, USA), drying at 60°C until constant weight. The dissolved oxygen concentration in the wastewater was determined by a polarographic oxygen sensor (Mettler-Toledo, Zurich, Switzerland). The analysis was performed in triplicate, and data refer to the average of six repetitions.

2.5. Scale-up and sensitivity analysis of the wastewater treatment process

The theoretical scale-up of the process was performed using the criteria of constant oxygen transfer rate, through the constant volumetric mass transfer coefficient (KL_a) method [24]. The volumetric mass transfer coefficient (KL_a) was estimated by Eq. (1):

$$\ln\left(\frac{C^*-C}{C^*-C_0}\right) = -KL_a\left(t_1-t_2\right) \tag{1}$$

where C^* is the oxygen concentration in saturation (mg/L); C is the oxygen concentration at time $t = t_i$ (mg/L); C_0 is the critical oxygen concentration (mg/L); KL_a is the volumetric mass transfer coefficient (min^{-1}); and t is the time (min).

The scale-up sought to keep the geometric similarity of the bench-scale bioreactor (Eq. (2)). The constant volumetric oxygen transfer coefficient (KL_a) was determined, and the new operating conditions were found that allegedly reproduce the same conditions on a bench-scale (Eq. (3)) [25]:

$$\frac{d_1}{H_1} = \frac{d_2}{H_2} \tag{2}$$

$$\frac{Q_2}{V_2} = \frac{Q_1}{V_1}\left(\frac{H_1}{H_2}\right)^{2/3} \tag{3}$$

where d_1 is the diameter of the bench-scale reactor (m); d_2 is the diameter of the full-scale reactor (m); H_1 is the height of the bench-scale reactor (m); and H_2 is the height of the full-scale reactor (m); Q_2 is the air flow rate of the full-scale reactor (m^3/min); Q_1 is the air flow rate of the bench-scale reactor (m^3/min); V_1 is the reactor volume at the bench-scale; and V_2 is the reactor volume at the full-scale.

The power density demand was directly obtained by the correlation between the volumetric airflow rate per volume unit used in the bioreactor and the capacity of blowers.

The estimation of the large-scale process was based on an industrial plant operating at a wastewater flow rate of 16,000 m^3/d, working 24 h/d, and 336 d/year.

2.6. Cost analysis methodology

To assess the wastewater treatment cost and the production cost of microalgal sludge in the described facility, the flowchart of the process had to be described in detail, including a list of equipment, its size, and the consumables of the process.

The used methodology to determine the total capital investment (TCI) is shown in Fig. 2 [25]. The TCI was based on estimation of the TCI, which is the sum of the fixed capital investment (FCI) and the working capital (WC). Manufacturing fixed-capital investment represents

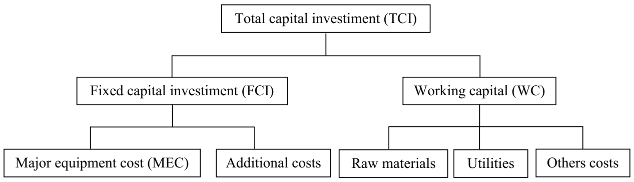

Fig. 2. Representation of the cost methodology.

the capital necessary for the installed process equipment with all auxiliaries that are needed for the complete process operation.

In keeping with standard bioprocess engineering practice, the fixed costs were estimated as factors of the major equipment costs (MEC). The total fixed capital was calculated after MEC determination, using appropriate factors (Lang factors), by multiplying the corresponding factor according to the nature of the item. The estimate cost for each piece of equipment was obtained from a website that estimates engineering the prices in free on board (FOB) in USD [26].

The WC estimated to the proposed industrial plant consisted of the total amount of money invested in raw materials and supplies, utilities, labor costs, and others (supervision, payroll charges, maintenance, operating supplies, general plant overheads, tax, and contingency). A percentage method was employed to calculate the different items. The amount of the raw materials was supplied per unit of product and determined from process material balances according to the direct quotations from market prices whereas the consumption of utilities was estimated from the power consumption of the process, which considered a value of 2% of the plant's capital for an overall utility cost [16,27].

The direct labor costs were calculated by estimating five workers, three shifts a day, working 8 h/d, and earning USD 8.50/h. This value was multiplied by two to include labor charges, totaling the costs.

2.7. Feasibility analysis of process

To determine the techno-economic feasibility of the process, an overall economic analysis was conducted based on a relationship of benefit/cost ratios, represented by feasibility indicators such as EE (EE = total fixed cost/index contribution margin), index contribution margin (ICM = total revenue – [total variable cost/total revenue]), profitability (P = net profit/total investment), rentability (R = net profit/total revenue), and period of return on investment (PRI = total investment/net profit) [18].

3. Results and discussion

3.1. Wastewater treatment and microalgal sludge production

The bioreactor performance parameters are shown in Table 2 and Fig. 3. A simultaneous conversion at high rates of organic matter (0.75 kg/m^3/d), total nitrogen (0.02 kg/m^3/d), and total phosphorus (0.001 kg/m^3/d) was evidenced, resulting in removal efficiencies of 97.6%, 85.5%, and 92.4% for COD, N–TKN, and P–PO$_4^{-3}$, respectively. In terms of microalgal growth, maximum specific growth rates of 0.6 d^{-1} and average microalgal sludge productivity of 0.27 kg/m^3/d were obtained. Moreover, this wastewater treatment process showed a biomass yield coefficient of 0.34 kg$_{\text{sludge}}$/kg$_{\text{COD}}$ and a hydraulic detention time of 1.67 d. In terms of oxygen transfer, a volumetric mass transfer coefficient (KL_a) of 0.002 min^{-1} was evidenced in the bioreactor, in parallel to a power density demand of 9.7 W/m^3.

Table 2
Bioreactor performance parameters

Parameter	Value
$r_{\text{S (COD)}}$ (kg/m^3/d)	0.75 ± 0.01
$r_{\text{S (N–TKN)}}$ (kg/m^3/d)	0.02 ± 0.00
$r_{\text{S (P–PO4-3)}}$ (kg/m^3/d)	0.001 ± 0.00
RE$_{\text{(COD)}}$ (%)	97.6 ± 1.64
RE$_{\text{(N–TKN)}}$ (%)	85.5 ± 2.37
RE$_{\text{(P–PO4-3)}}$ (%)	92.4 ± 0.22
$\mu_{\max}$ (d^{-1})	0.60 ± 0.00
P_X (kg/m^3/d)	0.27 ± 0.01
$Y_{X/COD}$ (kg$_{\text{sludge}}$/kg$_{\text{COD}}$)	0.34 ± 0.00
HDT (d)	1.67 ± 0.00
KL_a (min^{-1})	0.002 ± 0.00

Note: $r_{\text{S (COD)}}$: COD consumption rate; $r_{\text{S (N–TKN)}}$: N–TKN consumption rate; $r_{\text{S (P–PO4-3)}}$: P–PO$_4^{-3}$ consumption rate; RE$_{\text{(COD)}}$: COD removal efficiency; RE$_{\text{(N–TKN)}}$: N–TKN removal efficiency; RE$_{\text{(P–PO4-3)}}$: P–PO$_4^{-3}$ removal efficiency; $\mu_{\max}$: maximum specific growth rate; P_X: average cellular productivity; $Y_{X/COD}$: biomass yield coefficient; HDT: hydraulic detention time; and KL_a: volumetric mass transfer coefficient.

The system performance complies with the main wastewater discharge standards [28] and could be an alternative to conventional wastewater treatment processes such as activated sludge, nitrification-denitrification, and chemical phosphorus precipitation, usually employed in the meat processing industry. Besides the wastewater treatment occurring in a single step, in a multifunctional reactor, the partial conversion of the pollutants in a microalgal biomass with a large potential of commercial exploitation is the differential of this technology.

Based on scale-up of the process (16.000 m^3/d), an air flow rate of 360 m^3/min was theoretically estimated. In these conditions, this process has the potential to generate 503,967.7 ton of microalgal biomass per year from the treatment of 5,376,000 m^3 of wastewater.

3.2. Determination of cost analysis

The cost estimate of wastewater treatment facility was determined using the basis description of the equipment in use, including its size and type (Table 3). The most costly equipment was the bioreactor, followed by the drum-dryer and then the belt filter used to dry the microalgae sludge. The total cost of the major equipment sums up to USD 25,968,800.00.

Table 4 shows the installation costs, including the deployment, instrumentation, piping, and other elements necessary that resulted in a total FCI of USD 70,894,824.00. Considering a lifetime of 10 years, the annual fixed capital per year, required to keep the facility in operation, was estimated at USD 8,112,393.40.

Within the WC, direct production costs such as raw materials, utilities, and labor were the main entries.

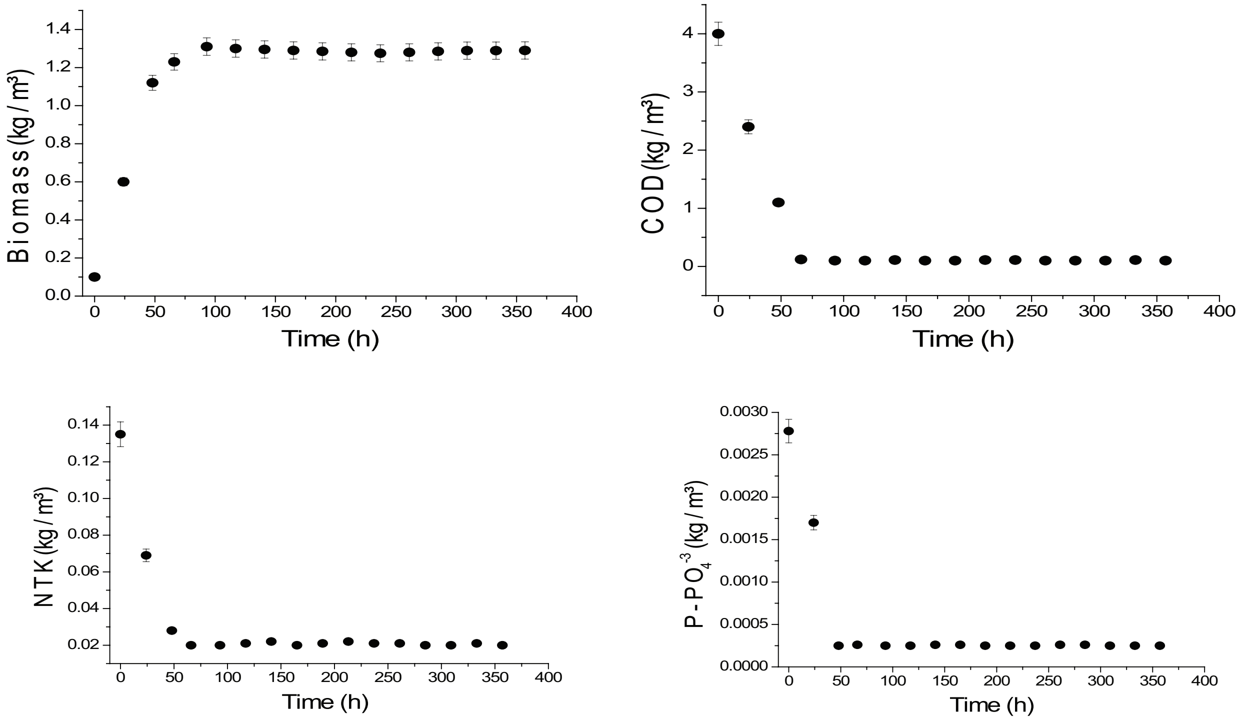

Fig. 3. Cellular concentration and substrate consumption dynamics in heterotrophic microalgal bioreactor.

Table 3
Major equipment costs used in the process

Item	Capacity	Cost (USD)	No. of units	Total cost (USD)
1. Fine screen	(0.70 m², carbon steel)	261,000.00	1	261,000.00
2. Rotary sieve	(1,036.20 m², stainless steel)	325,600.00	1	325,600.00
3. Equalization tank	(3,345.45 m³, carbon steel)	583,100.00	1	583,100.00
4. Parshall flume	(9’’, stainless steel)	19,000.00	1	19,000.00
5. Bioreactor	(30,666.7 m³, stainless steel)	12,944,200.00	1	12,944,200.00
6. Decanter	(11.29 m, carbon steel)	1,114,700.00	2	2,229,400.00
7. Centrifugal pump	(700.5 m³/h, stainless steel)	39,900.00	3	119,700.00
8. Drum dryer	(2,660 m², stainless steel)	5,258,400.00	1	5,258,400.00
9. Blowers	(360 m³/min, carbon steel)	133,400.00	5	667,000.00
10. Belt filter	(399.96 m², carbon steel)	3,561,400.00	1	3,561,400.00
Total MEC (USD)				25,968,800.00

Table 5 shows that the total amount of the raw materials was summarized as USD 1,017,676.80, wherein the consumption of caustic soda was the main cost. The costs of utilities, based only on power consumption, were estimated as USD 1,417,896.40. Finally, other costs (labor, supervision, payroll charges, maintenance, operating supplies, overheads, taxes, and contingencies) reached USD 3,773,008.70. In this sense, the total WC was estimated at USD 14,320,974.00/year.

Regarding the analysis of the major costs of the process, the major purchases of equipment showed that the bioreactor represents a cost close to 50% of the total facility, followed by the drum-dryer and the belt filter, showing the relationship of these pieces of equipment with their high power consumption. The FCI, depreciation over 10 years, contributed to approximately 56% to the cost of the process. The remaining 44% of the production cost originated in the direct production of the WC. Depreciation charges contributed an approximately 48% to the annual production cost while raw materials, utilities, and labor contributed 7%, 9%, and 5%, respectively, to the production cost.

Based on the determination of cost analysis and the calculation basis of the industry in analysis

(16,000 m^3/d), the wastewater treatment cost was estimated at USD 2.66/m^3 (USD 0.70/m^3 considering only operational costs). Additionally, through the microalgae sludge formation, one can predict a cost of USD 0.03 cent/kg of the dried biomass.

Comparatively, Fig. 4 shows the operational costs of conventional wastewater treatment processes and the costs of the main processes for microalgal biomass production.

Table 4
Fixed capital investment of the process

Item	Factor	Cost (USD)
1. Major purchased equipment (MEC)	1	25,968,800.00
2. Installations	0.2	5,193,760.00
3. Instrumentation and control	0.4	10,387,520.00
4. Piping	0.4	10,387,520.00
5. Electrical	0.09	2,337,192.00
6. Buildings	0.11	2,856,568.00
7. Services	0.14	3,635,632.00
8. Land	0.06	1,558,128.00
9. Engineering and supervision	0.13	3,375,944.00
10. Contractor's fee (0.05 Σ items 1–8)	0.05	3,116,256.00
11. Contingency	0.08	2,077,504.00
Total fixed capital, *A*		70,894,824.00
Depreciation (Σ items 1–7, 9–11)/10 years		6,933,669.60
Property tax (0.01 depreciation)	0.01	69,336.70
Purchase tax (0.16 items 1–10/10)	0.16	1,109,387.10
Total fixed capital per year, *B*		8,112,393.40

Table 5
Working capital of the process

Raw materials	Total quantity	Cost (USD)
1. Caustic soda (USD 0.348 kg)	0.464 kg/m^3	867,686.40
2. Flocculants (USD 2.79 kg)	160 kg/d	149,990.40
Total raw materials, *C*		1,017,676.80
Utilities		
3. Power consumption (0.02 FCI)	kWh	1,417,896.40
Total utilities, *D*		1,417,896.40
Others		
4. Labor (USD 8.50/h, 3 shifts)	5 workers	685,440.00
5. Supervision (0.2 labor)		137,088.00
6. Payroll charges (0.25 labor + supervision)		205,632.00
7. Maintenance (0.04 MEC)		1,038,752.00
8. Operating supplies (0.004 *C*)		4,070.70
9. General plant overheads		1,023,704.00
(0.55 labor + supervision + maintenance)		
10. Tax (0.16 items 1–3, 7 and 8)		556,543.34
11. Contingency (0.05 items 1–3)		121,778.66
Total others, *E*		3,773,008.70
Total working capital, *F* (*B* (Table 3) + *C*+ *D* + *E*) (USD)		14,320,974.00

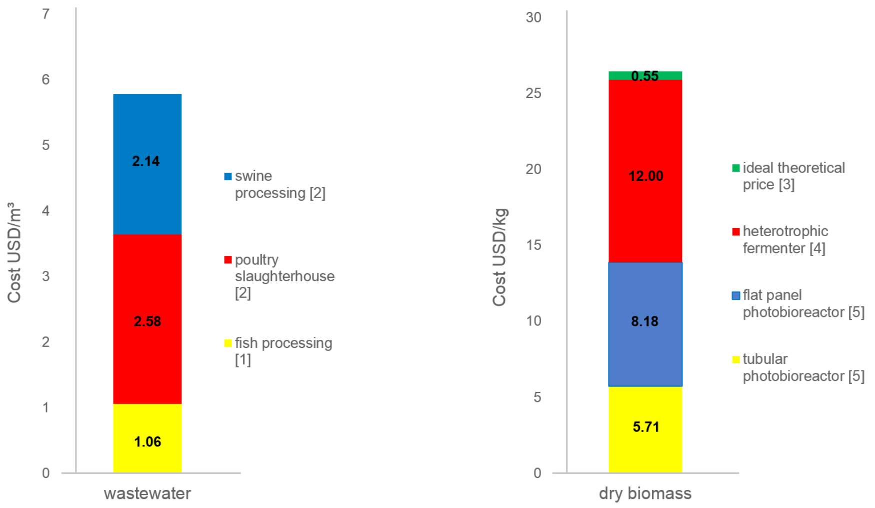

Fig. 4. Comparative costs of the wastewater treatment processes and dried microalgal biomass.
Note: [1] – Cristóvão et al. [29], [2] – Asselin et al. [30], [4] – Wijffels et al. [17], [5] – Lee [14], and [6] – Norske et al. [15].

The conventional technologies for wastewater treatment have operational costs estimated between USD 1.06/m³ to USD 2.58/m³ [29,30]. In particular, for meat processing wastewater, chemical treatment followed by activated sludge with extended aeration are the most usual treatments, with higher operational costs than those estimated in this study. The application of microalgal heterotrophic bioreactors could represent substantial savings per cubic meter of treated wastewater, and furthermore, it generates sludge with commercial value, viable to the exploitation of bioproducts. The low production cost of this biomass (USD 0.03/kg) makes it viable to exploit low added value products, which is currently an infeasible scenario. The production costs of the microalgal biomasses are estimated at USD 5.71/kg for the tubular photobioreactor and USD 8.18/kg for the flat panel photobioreactor [15]. Additionally, the production cost of the heterotrophic fermenters is close to USD 12.00/kg [14]. According to Wijffels et al. [17], the production cost of microalgae biomass may not be higher than USD 0.55 cent/kg (ideal theoretical price) for manufacture of bulk products such as biofuels, which makes this process highly attractive in a commercial point of view.

3.3. Applicability of the process

The feasibility of the process was determined based on the estimate of EE, profitability, rentability, and period of return on investment (Table 6).

The wastewater treatment generates a substantial amount of microalgal biomass of rich composition, similar to commodity products such as soybeans. Soybeans have an average international price in the market estimated at USD 0.48 cent/kg [31], and therefore, the commercial value of microalgal biomass was compared with the price of soybeans, resulting in USD 480/ton.

The net profit was estimated at USD 227,583,522.00 with a profit margin of 94%. The profitability of the process reports that, each year, the industry recovers approximately 321% of the amount invested, and when the revenue reaches the value of USD 71,610,933.30, the payment of the total costs is made. The time of return on investment was estimated at 0.29 years, which means when this period of operation is achieved, the industry recovers the invested capital. These values are highly attractive, since most companies use a value of 12% as minimum acceptable rate of return [32]. This rate is usually determined by evaluating existing opportunities in the expansion of operations, rate of return for investments, and other factors deemed relevant by management. However, companies operating in industries with more volatile markets might use a slightly higher rate in order to offset risk and attract investors [33]. In this sense, the feasibility analysis of the process showed that heterotrophic microalgal bioreactors applied to poultry and swine slaughterhouse wastewater treatments have a wide economic margin to be explored industrially and commercially.

Table 6
Economical feasibility indicators of process

Parameter	Value
Economic equilibrium (USD)	71,610,933.30
Profitability (%/year)	94.00
Rentability (%/year)	321.00
Period of return on investment (year)	0.29

Additionally, the feasibility of the process demonstrates that this microalgal biomass produced in the agroindustrial wastewater has an economic margin that allows for work with fine chemical products but also commodities from microalgae, clearly showing the benefit-cost relationship for both of them.

The heterotrophic microalgal bioreactor is associated with improvements in the productive process, since it complies with the general guidelines for intensive processes, combining more than one function. It requires lower power densities during operation, confirming the high performance of the bioreactor, snapping it into the category of multifunctional reactors [22]. The cultivation of microalgae in wastewater offers combined advantages for the wastewater treatment and simultaneously the production of a valuable biomass. This bioreactor serves as an alternative to reduce the high costs of conventional secondary and tertiary treatments. Inherent in the treatment process, microalgal sludge is generated with a minimum cost of production, since it is a resultant product of an intensive process based on inputs of negligible cost (agroindustrial wastewater).

The current agroindustrial wastewater treatment systems utilize processes operating in multiple unit operations, which require high energetic demand, thus impacting finances throughout the production chain. Furthermore, these systems are still linked to expensive processes, with high capital and operation costs, besides the massive generation of biological sludge, with a low potential of reuse. The microalgal heterotrophic bioreactor not only offers a low-cost alternative for conventional wastewater treatment processes, but also produces biomass with reuse potential; thus, bioproducts with commercial value can be marketed. The process conducted from the use of a heterotrophic microalgae bioreactor contributes to the maturation of the technology, in order to possibly explore these technological routes.

Finally, one should consider the scale limitations of these estimates, currently supported exclusively by lab-scale experiments. In any case, it is of paramount importance to base any performance and economic estimates on field data coming from pilot plants of suitable size, to finally reach an industrial scale. There is a clear need for further studies about this theme, integrating the biological aspects with engineering ones and producing field experimental data from pilot plants, in order to achieve a rapid development of the technology needed for application at the industrial level.

4. Conclusion

The emerging microalgae industry continues its march toward industrial application. The agroindustrial wastewater treatment with the parallel production of microalgal biomass could contribute to the consolidation of this technology.

The multifunctional heterotrophic microalgal bioreactor simultaneously converts the three main pollutants of the poultry and swine slaughterhouse wastewater, reaching removal efficiencies of 97.6%, 85.5%, and 92.4% for COD, N–TKN, and P–PO_4^{-3}, respectively. In addition, a microalgal sludge productivity of 0.27 kg/m^3/d is obtained, potentializing reuse in multiple production platforms.

The economic analysis showed a cost of USD 2.66/m^3 of treated industrial wastewater, and as a consequence of this process, the production cost of microalgal sludge was USD 0.03/kg of dehydrated biomass.

The feasibility analysis for the industrial applicability of the proposed technology shows that if the commercial value of microalgal biomass is estimated at USD 480/ton, a profit margin of 94% can be obtained.

Acknowledgments

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Symbols

μ_{max} — Maximum specific growth rate
C — Oxygen concentration at time $t = t_i$, mg/L
C^* — Oxygen concentration in saturation, mg/L
C/N — Carbon/nitrogen ratio
C_0 — Critical oxygen concentration, mg/L
COD — Chemical oxygen demand, mg/L
d_1 — Diameter of bench-scale reactor, m
d_2 — Diameter of full-scale reactor, m
EE — Economic equilibrium
FCI — Fixed capital investment
FS — Fixed solids, mg/L
H_1 — Height of bench-scale reactor, m
H_2 — Height of full-scale reactor, m
HDT — Hydraulic detention time
ICM — Index contribution margin
KL_a — Volumetric mass transfer coefficient, min^{-1}
MEC — Major equipment costs
N/P — Nitrogen/phosphorous ratio
P — Profitability
PO_4^{-3} — Total phosphorus, mg/L
PRI — Period of return on investment
P_X — Average cellular productivity
Q_1 — Air flow rate of bench-scale reactor, m^3/min
Q_2 — Air flow rate of full-scale reactor, m^3/min
R — Rentability
RE — Removal efficiency
r_S — Consumption rate
SS — Suspended solids, mg/L
t — Time, min
TCI — Total capital investment
TKN — Total nitrogen, mg/L
TS — Total solids, mg/L
V_1 — Reactor volume at bench-scale, m^3
V_2 — Reactor volume at full-scale, m^3
VS — Volatile solids, mg/L
WC — Working capital
$Y_{X/COD}$ — Biomass yield coefficient

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CAPÍTULO 5

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Nutrient cycling in meat processing industry by microalgae-based-processes

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Nutrient cycling in meat processing industry by microalgae-based processes

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ABSTRACT

The nutrient cycling from microalgae-based processes is a potential technological route to be applied in wastewater treatment plants. The pollutants conversion in parallel with microalgal sludge formation results in a renewable feedstock for bioproducts production that potentially reduces the cost of wastewater treatment. The experiments have been performed in a bubble column bioreactor, operating at 25ºC, pH 7.5, 100 mg/L of initial inoculum, absence of light and flow rate per unit volume (Q/V) of 1.0 VVM (volume of air per volume of culture per minute). Thereby, the kinetic parameters of cell growth, substrate consumption, analysis of microalgal sludge composition and the biodiesel quality properties have been realized. The nutrient cycling by *Phormidium autumnale* in wastewater has presented high removal efficiencies of pollutants. The generated microalgal sludge shows predominance of saturated fatty acids, indicates the potential of its use as a suitable lipid input for biodiesel synthesis. The lipid-extracted microalgae (LEM) showed high content of free amino acids, minerals and pigments. The heterotrophic microalgae cultivation in wastewater has demonstrated to be capable of removing pollutants and, simultaneously, producing biofuel and being a potential source for animal feeding from microalgal sludge, contributing, therefore, to the multi-purpose microalgal bioprocess development.

Keywords: Microalgae; Cyanobacteria; Wastewater; Waste valorization; Biodiesel; Animal feed

1. Introduction

Safeguard water resources policies have influenced the development of wastewater treatment systems and their management, focusing on energy consumption and sustainable performance of these industrial processes in recent decades. One direction towards renovating the wastewater treatment into a more sustainable one is to recover the resources that it holds, such as water, nutrients (e.g. C, N and P) and energy [1].

Nutrient cycling by microalgae emerges as a promising technology because it balances sustainable vectors by reused pollutants, like carbon, nitrogen and phosphorus, present in wastewater generated by the industry. Thus, they create a biomass that one can extract a great variety of bioproducts with substantial added value [2].

Together, wastewater treatment and valuable algal biomass production enhances environmental and economic benefits from this process [3,4]. According to Brennan and Owende [5], the combination of these processes will be the most conceivable commercial application in the short term; and it is probably one of the most sustainable ways to produce bioenergy and bio-products.

As an effort to reduce the cost of microalgal bioproducts, the biorefinery approaches have arise [6,7]. Biorefinery is a totally integrative and multifunctional process that uses raw material to generate a spectrum of different products in a sustainable way, such as biofuel and animal feeding. The objective of this study is to use comprehensively all the raw material components and to improve the resource flow in order to reduce the resource loss [8].

The fatty acids composition is crucial to the single-cell oil production, since it directly influences on the biodiesel quality. Some microalgae are acknowledged because they

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produce high amounts of lipids and they can be used as a mean of bioprocessing, in order to produce alternative oils for biofuel manufacturers [9,10]. The choice for oil accumulation through the manipulation of environmental culture conditions has a great potential in the single-cell oil production [11].

Furthermore, many non-lipid portions of microalgal biomass can be processed into wide ranges of bioproducts. Proteins and minerals in microalgal tissues can be used as food or feeding. Microalgae are the ideal source for the production of chemicals, human nutrition products, pharmaceuticals and cosmetics [8].

Phormidium is a genus of single-cell blue green algae that belongs to the cyanobacteria. Its filamentous can measure about 3–4 µm diameter. Several species live in limiting environments. These blue green algae show considerable potential as biocatalysts in environmental biotechnology processes because of their robustness and simple nutritional requirements [12,13].

Therefore, this study aims to develop a microalgae-based process through nutrient cycling of meat processing industry wastewater, with emphasis on biodiesel and animal feed manufacturing.

2. Material and methods

2.1. Microorganism and culture conditions

A monoculture of *Phormidium autumnale* has been originally isolated from the Cuatro Cienegas desert (26°59′N, 102°03′W-Mexico). Stock cultures have been propagated and remained in solidified agar-agar (20 g/L) containing synthetic BG11 medium [14] with the following composition (mg/L): K_2HPO_4 (30.0), $MgSO_4$ (75.0), $CaCl_2{\cdot}2H_2O$ (36.0), ammonium citrate and iron (0.6), Na_2 EDTA (1.0), NaCl (0.72), $NaNO_3$ (15.0), citric acid (0.6), Na_2CO_3 (1500.0), trace metals [H_3BO_3 (2.8), $MnCl_2{\cdot}4H_2O$ (1.8), $ZnSO_4{\cdot}7H_2O$ (0.22), $Na_2MoO_4{\cdot}2H_2O$ (0.39), $CoSO_4{\cdot}6H_2O$ (0.04). The incubation conditions were the following: 20°C, photon flux density of 15 µmol/m^2/s and photo period of 12 h.

2.2. Wastewater

The poultry and swine slaughterhouse wastewater used in the experiments has been obtained from an industry located in the State of Santa Catarina, Brazil (27°14′02″S, 52°01′40″W). It has been collected from the discharge point of an equalization tank (after fine screen and rotary sieve) over the period of one year, and it has been analyzed according to its pH, COD, N-TKN, P-PO_4^{-3}, TS, SS, VS, and FS, following the Standard Methods for the Examination of Water and Wastewater [15]. Table 1 shows the average composition of the wastewater, in a one-year sampling. The C/N and N/P ratio has been calculated through COD, N-TKN, and P-PO_4^{-3}.

Table 1
Average composition of wastewater

Parameter	Value
pH	5.9 ± 0.05
COD (mg/L)	4100 ± 874
N-TKN (mg/L)	128.5 ±12.1
P-PO_4^{-3} (mg/L)	2.84 ± 0.2
TS (mg/L)	3.8 ± 2.7
FS (mg/L)	0.9 ± 0.3
VS (mg/L)	2.9 ± 1.4
SS (mg/L)	1.9 ± 0.8
C/N	31.9 ± 1.2
N/P	45.2 ± 2.6

$n = 54$

2.3. Bioreactor configuration

Measurements have been made in a bubble column bioreactor. The system has been built by using borosilicate glass. It has an external diameter of 12.5 cm and height of 16 cm, resulting in a height/diameter (h/D) ratio equals to 1.28 and a nominal working volume of 2.0 L. The reactor dispersion system has been composed of a 2.5 cm diameter air diffuser located inside the bioreactor. The air flow has been monitored by a flow meter (KI-Key Instruments®, Trevose-PA, USA) and the inlet and outlet of gases have worked through filtering units, which have been created by using a polypropylene membrane with a pore diameter 0.22 µm and total diameter 50 mm (Millex FG®, Billerica-MA, USA).

2.4. Obtaining the kinetic data

Experiments have been conducted in a batch bioreactor. The bioreactor has been fed with 2.0 L of previously sterilized wastewater (15 psi/121°C). The experimental conditions were initial cell concentration of 100 mg/L, pH adjusted to 7.6, temperature of 25°C, flow rate per unit volume of 1.0 VVM, C/N ratio of 31.9 and absence of light.

2.5. Sampling and analytical methods for wastewater

Samples have been collected at regular intermissions of 12 h and characterized by chemical oxygen demand (COD), total nitrogen (N-TKN), total phosphorus (P-PO_4^{-3}) and cell biomass.

The chemical oxygen demand, total nitrogen and total phosphorus have been determined according to the methodology described in Standard Methods for the Examination of Water and Wastewater [15]. Cell biomass has been gravimetrically evaluated by wastewater filtering through a 0.45 µm membrane filter (Millex FG®, Billerica-MA, USA), drying at 60°C until it reaches constant weight.

External contamination has been monitored by the heterotrophic plate count method, according to Maroneze et al. [12].

The experiments were performed in duplicate, and kinetic data refer to the average of four repetitions.

2.6. Kinetic parameters

The cell growth and substrate consumption data have been used to calculate the biomass productivity (P_X = dX/

dt, mg/L/h); maximum specific growth rate ($\ln(X/X_0) = \mu_{max}\cdot t$, 1/h); consumption rates of COD, N-TKN and $P\text{-}PO_4^{-3}$ ($r_S = dS/dt$, mg/L/h) and removal efficiencies of COD, N-TKN and $P\text{-}PO_4^{-3}$ ($RE = (S_0 - S)/(S_0)$, %), where X is the cell biomass at time t = t (mg/L), X_0 is the initial cell biomass (mg/L), t is time (h), S is the final concentration of COD, N-TKN and $P\text{-}PO_4^{-3}$ (mg/L) and S_0 is the initial concentration of COD, N-TKN and $P\text{-}PO_4^{-3}$ (mg/L).

2.7. Sampling and analytical methods for microalgal sludge

2.7.1. Centesimal composition

Microalgal sludge chemical composition has been characterized according to AOAC [16]. Carbohydrate concentration has been determined by differentiation. Total lipid has been extracted by Bligh and Dyer modified method [17].

2.7.2. Fatty acid profile

Hartman and Lago method [18] has been used to saponify and esterify (methylation reaction) the dried lipid extract in order to obtain the fatty acid methyl esters (biodiesel). Fatty acid composition has been determined using a VARIAN 3600 CX gas chromatograph (Varian, Palo Alto, CA, USA) equipped with FID and a fused silica capillary column (SP 2560 Supelco), 100 m × 0.25 mm id, film thickness 0.20 µm. The fatty acid methyl esters (FAMEs) have been identified by comparing the retention times with external patterns (Supelco, Bellefonte, PA, USA). They have also been quantified by their area normalization using Varian Star 4.51 software.

2.7.3. Free amino acids

Amino acids have been determined by a Beckman 7300 (Beckman Instruments, Palo Alto, CA, USA) auto analyzer from hydrolysates obtained by the hydrolysis of 15–25 mg sample with 2.0 ml 6.0 N HCl in an evacuated sealed tube at 110°C for 24 h. The identification and quantification of the amino acids have been analyzed by comparison, according to external patterns (Sigma–Aldrich, St. Louis, MO, USA).

2.7.4. Minerals

The minerals chemical composition has been determined as described by Mesko et al. [19]. The solid samples have been exposed firstly to an acidic digestion; then, they have been heated up to 6000–8000 K in order to vaporize and ionize metallic compounds to be quantified. The ions have been detected and analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

2.7.5. Pigments

The carotenoids and chlorophylls have been analyzed through a high performance liquid chromatography HPLC (Shimadzu, Kyoto, Japan) equipped with quaternary pumps (model LC-20AD), online degasser, and injection valve 20 µL loop (Rheodyne, Rohnert Park, CA, USA). The equipment has been connected in series to a photodiode array detector (model SPD-M20A) and a mass spectrometer with an ion-trap analyzer and an atmospheric pressure chemical ionization (APCI) source (model Esquire 4000, Bruker Daltonics, Bremen, Germany). The carotenoid separation has been performed on a C30 YMC column (5 µm, 250 × 4.6 mm) (Waters, Wilmington, DE, USA). The mobile phase consisted in a mixture of methanol and methyl tert-butyl ether. The chlorophyll separation has been performed on a C18 NST column (5 µm, 150 × 4.4 mm) (Nano Separation Technologies, São Paulo, Brazil). The quantification process of total carotenoids and total chlorophyll has been conducted according to Rodrigues et al. [20].

When it comes to phycobiliproteins, freeze-dried biomass (1.0 ± 0.2 g) has been extracted with 50 mM sodium phosphate buffer pH 6.8 in a mortar with a pestle followed by filtration. The filtrate containing phycocyanin (C-PC) has been kept on. The phycocyanin extract recovery (%) has been calculated according to Soni et al. [21], and the pigment purity has been assessed by calculating the ratio and the absorbance of total protein (280 nm), phycoerythrin (C-PE) (540 nm) and allophycocyanin (C-APC) (650 nm). The phycobiliproteins amount has been calculated according to Bennett and Bogorad [22]. The UV–Vis absorption of C-PC and fluorescence emission and excitation spectra have been obtained through a SpectraMax M5 (Molecular Devices Corp, Los Angeles, CA, USA).

2.7.6. Antioxidant capacity

The antioxidant capacity of lipophilic extracts (carotenoids and chlorophylls) has been carried out according to Rodrigues et al. [23]. The antioxidant capacity of analyzing the hydrophilic extracts (phycobiliproteins) have been carried out according to the oxygen radical absorbance capacity (ORAC) method [24].

2.7.7. Biodiesel quality

The biodiesel quality properties (ester content, EC; cetane number, CN; iodine value, II; degree of unsaturation, DU; saponification value, SV; long-chain saturated factor, LCSF; cold filter plugging point, CFPP; cloud point, CP; allylic position equivalents, APE; bisallylic position equivalents, BAPE; oxidation stability, OS; higher heating value, HVV; kinematic viscosity, µ and kinematic density, ρ) have been calculated by Biodiesel Analyzer© 1.1 software [25].

2.8. Statistical analysis

Analysis of variance (one-way ANOVA) and Tukey's test ($p < 0.05$) have been made in this study. The analyses have been performed using Statistica 10 software (StatSoft, Tulsa-OK, USA).

3. Results and discussion

3.1. Nutrient cycling by microalgae-based process

The heterotrophic microalgal metabolism can simultaneously convert the main pollutants (C, N and P) presented in wastewater in a single step. It's represents a considerable potential operational cost reduction [26]. Table 2 and Fig. 1 show the kinetic parameters of cell growth and substrate consumption for microalgal sludge grown in poultry and swine slaughterhouse wastewater. The microalgal heterotrophic culture has demonstrated high removal efficiencies of COD (97.5%), N-TKN (87.5%) and P-PO_4^{-3} (100%). Besides, it has presented maximum cellular concentration 1647 mg/L, average cellular productivity 9.0 mg/L. h and maximum specific growth rate 0.6 1/h. Thereby, the microalgal heterotrophic bioreactor has demonstrated to be able to treat this wastewater, not requiring additional unit operations to remove the pollutants, except the primary treatment. Additionally, substantial production of microalgal sludge occurs in this process.

Table 2
Kinetic parameters of cell growth and substrate consumption for microalgal sludge

Parameters	Value
X_{max} (mg/L)	1647 ± 120.2
P_X (mg/L·h)	9 ± 1.86
μ_{max} (1/h)	0.6 ± 0.1
r_{SCOD} (mg/L·h)	18.84 ± 4.54
$r_{SN\text{-}TKN}$ (mg/L·h)	0.96 ± 0.2
$r_{S\,P\text{-}PO4}{}^{-3}$ (mg/L·h)	0.072 ± 0.001
RE $_{COD}$ (%)	97.57 ± 6.83
RE $_{N\text{-}TKN}$ (%)	87.5 ± 3.2
RE $_{P\text{-}PO4}{}^{-3}$ (%)	100 ± 1.1
HRT (h)	120

The removal mechanisms that enables these nutrient cycling is the respiration for organic matter, assimilation for nitrogen and phosphorylation for phosphorus [2]. Conversely, it should be considered that other mechanisms capable of removing nitrogen and phosphorus in this system are stripping, volatilization, adsorption, and sedimentation [27].

The aseptic procedures adopted have been suitable for preventing microbial contamination of the cultures (data not shown), since null results have been observed through the heterotrophic plate count method.

3.2. Centesimal composition of microalgal sludge

The nutrient cycling is inherent to the production of microalgal sludge. Thus, the chemical composition analysis of this bioproduct (Table 3) has showed that the proteins are

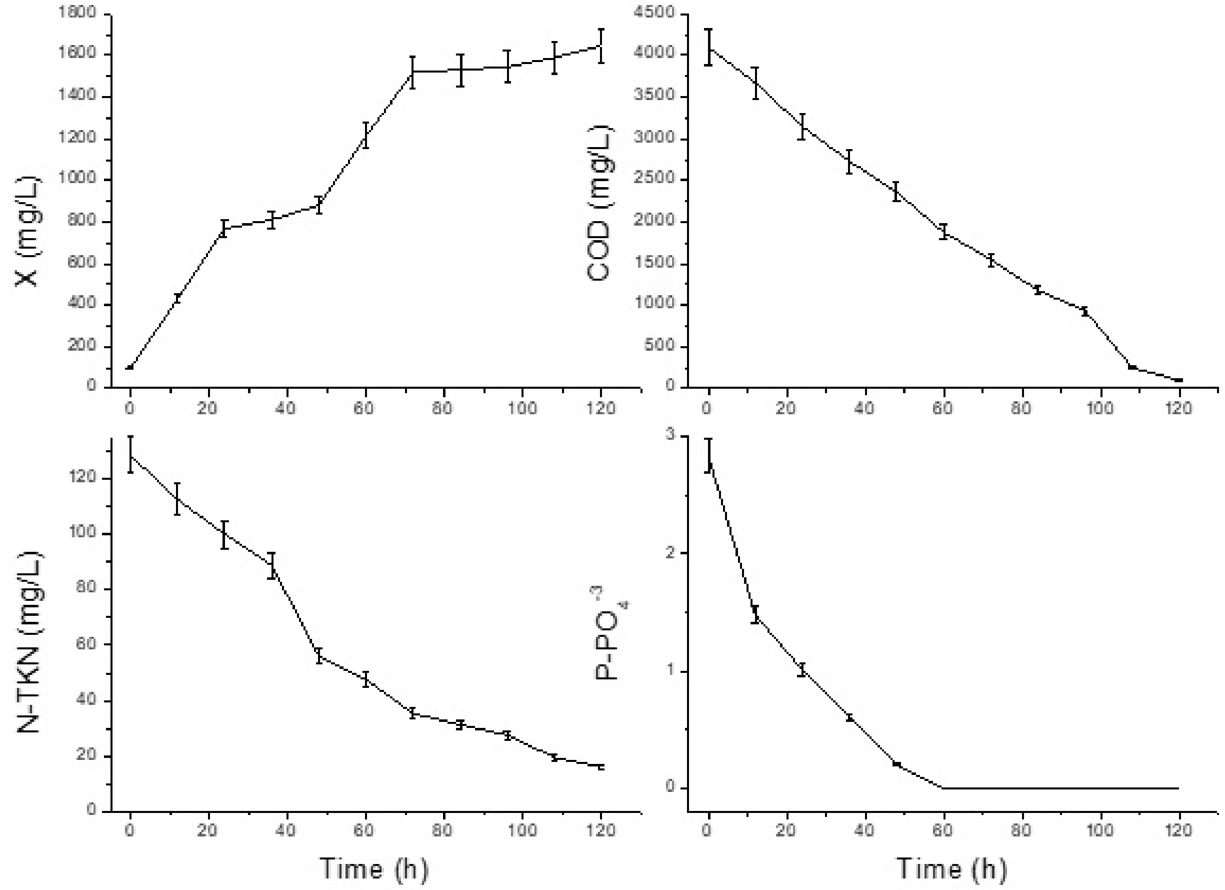

Fig. 1. Dynamics of cell biomass (X), organic carbon (COD), total nitrogen (N-TKN) and total phosphorus (P-PO_4^{-3}) in microalgal heterotrophic bioreactor.

Table 3
Centesimal composition of microalgal sludge produced in poultry and swine slaughterhouse wastewater

Constituent	Value (% dry weight)
Proteins	31.7 ± 0.9
Minerals	21.7 ± 0.2
Carbohydrates	15.9 ± 0.1
Lipids	15.4 ± 0.4
Moisture	15.3 ± 0.7

Table 4
Fatty acids profile of microalgal sludge

Fatty acid	Value (%)
C6:0	4.42 ± 0.2
C8:0	66.62 ± 0.4
C12:0	13.07 ± 0.2
C16:0	8.96 ± 0.5
C18:1n9c	6.91 ± 0.03
SFAs	93.08 ± 1.1
MUFAs	6.91 ± 0.6
PUFAs	ND

its major constituent (31.7%). On the other hand, concentrations of minerals (21.7%), carbohydrates (15.9%), lipids (15.4%) and moisture (15.1%) have also been observed in these conditions. These values show the potential for reusing microalgal sludge as a feedstock for several commercial products.

3.3. Single-cell oil as feedstock for biodiesel synthesis

3.3.1. Fatty acid profile of microalgal sludge

Table 4 shows the profile of fatty acids in the lipid fraction of microalgal sludge. There is a saturated fatty acid predominance (93.1%). The caprylic acid is the major fatty acid (66.6%), followed by lauric (13.1%), palmitic (8.9%) oleic acid (6.9%) and caproic acid (4.4%). It is normally expected that microalgae produce large amounts of polyunsaturated fatty acids (PUFAs) [28]. However, microalgae in heterotrophic cultivation tend to produce saturated and mono unsaturated fatty acids [29,30].

3.3.2. Biodiesel quality

Table 5 shows the quality parameters of biodiesel produced by microalgae *Phormidium autumnale*.

The biodiesel produced from single-cell oil contains: ester content of 99.8%, cetane number of 60.51, iodine value of 6.21 gl_2/100g, degree of unsaturation of 6.91%, saponification value of 349.81, long-chain saturated factor of 0.90%, cold filter plugging point at –13.65°C, cloud point at –0.28°C, allylic position equivalents of 6.91, bis-allylic position equivalents of 22.0, oxidation stability of 8.52 h, higher heating value of 34.96, kinematic viscosity of 0.22 mm^2/s, and kinematic density of 0.88 g/cm^3. All these parameters fulfill the limits established by U.S., European, and Brazilian patterns [32–34]. A high quality biodiesel derived from microalgal sludge has been verified and compared to soybean biodiesel [31]. These values are a direct consequence of the low rate of unsaturated fatty acids, present in single-cell oil, resulting in a high cetane number and a high oxidative stability [35]. These values indicate a potential use of microalgal sludge as a suitable lipid input for biodiesel manufacturing.

Table 5
Parameters for determining biodiesel quality

Properties	Microalgal sludge	Soybean [31]
EC (%)	99.8	96.9
CN	60.5	49.0
IV (gI_2/100 g)	6.2	128
DU (%)	6.9	143.8
SV	349.8	–
LCSF (%)	0.9	1.6
CFPP (°C)	–13.6	–5.0
CP (°C)	–0.28	–
APE	6.9	–
BAPE	–	–
OS (h)	–	1.3
HHV	34.9	–
μ (mm^2/s)	0.22	4.2
ρ (g/cm^3)	0.88	–

3.4. Lipid-extracted microalgae

3.4.1. Free amino acids content

The LEM microalgae are rich in protein and, therefore, in amino acids. Table 6 shows free amino acid content of microalgal sludge. Eighteen amino acids (tryptophan, lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine) have been detected and quantified.

In general, six amino acids out of the 18 ones have been responsible for more than 50% of the total concentration (Table 6): glutamic acid, aspartic acid, glycine, leucine, alanine and arginine. Although the major amino acids have been non-essential amino-acids as glutamic acid, aspartic acid and glycine, the product contains essential amino acids at percentages higher than 40% of the total content. Leucine has the highest concentration (5.16 g/16 gN). The microalgal sludge present some essential amino acids content (isoleucine, valine and threonine) higher than amino acid scoring patterns in FAO [36]. Therefore, they can be considered a potential protein source alternative.

3.4.2. Minerals

Table 7 shows the mineral element content in microalgal sludge. Twenty-nine different minerals have been

Table 6
Free amino acid content of microalgal sludge expressed as g/16 gN

Amino acids	Microalgal sludge (g/16gN)	FAO [36] (g/16gN)
Essential		
Lysine	3.96 ± 0.06	5.8
Methionine + Cystine	1.73 ± 0.00	2.5
Phenylalanine + Tyrosine	4.59 ± 0.01	6.3
Leucine	5.16 ± 0.03	6.6
Isoleucine	3.69 ± 0.03	2.8
Valine	3.94 ± 0.01	3.5
Threonine	3.75 ± 0.02	3.4
Tryptophan	0.92 ± 0.00	1.1
Non-essential		
Histidine	1.43 ± 0.01	–
Arginine	4.37 ± 0.03	–
Aspartic acid	7.00 ± 0.08	–
Serine	3.14 ± 0.03	–
Glutamic acid	7.54 ± 0.01	–
Proline	2.28 ± 0.06	–
Glycine	5.24 ± 0.02	–
Alanine	4.70 ± 0.01	–

found in microalgal sludge composition. Minerals analysis has showed that the main constituent of ash fraction was Na (103707 µg/g). Other minerals that showed high levels are the following: K (26042 µg/g), P (13876 µg/g), S (8629 µg/g), Ca (3302 µg/g), Mg (3302 µg/g) and Fe (1266 µg/g). One of the most important problems concerning microalgae for feeding is the elevated amounts of heavy metals contaminants (lead, cadmium, arsenic, mercury, chrome, manganese). However, toxic heavy metal values in microalgal sludge were lower than the recommended values, according to the Commission Regulation (EC) No 1881/2006, which sets maximum contaminant levels in foodstuffs [37]. The microalgal sludge also contains biologically important macro (Na, K, P, Ca, Mg) and micro minerals (Fe, Mn, Zn, Cu), indispensable for animal feeding [38].

3.4.3. Pigments content

The microalgae presents three natural pigments basic classes: carotenoids, chlorophylls and phycobiliproteins [39]. Table 8 shows the pigments characterization and scavenger capacity compared to peroxyl radicals, using hydrophilic and lipophilic extracts in microalgal sludge. The total carotenoid and total chlorophyll content was 714.3 ± 0.9 and 3400 ± 0.1 µg/g, respectively. In terms of phycobiliproteins, the results have showed 214000 ± 0.5µg/g. These natural pigments have an important role in the photosynthetic and pigmentation metabolism of microalgae, and they display several beneficial biological activities like antioxidant, anti-carcinogenic, anti-inflammatory and others [40–42].

Table 7
Mineral element content in microalgal sludge

Element	Microalgal sludge (µg/g)
Ag	6.40 ± 0,34
Al	111 ± 11
As	< 5.97
Ba	11 ± 1
Be	<0.014
Bi	<6.90
Ca	3302 ± 201
Cd	<0.889
Co	<0.909
Cr	3.29 ± 0.24
Cu	42.6 ± 0.7
Fe	1266 ± 51
Li	<0.508
K	26042 ± 2349
Mg	3302 ± 145
Mn	120 ± 9
Mo	15.8 ± 2.5
Na	103757 ± 667
Ni	<1.32
P	13876 ± 258
Pb	<2.72
S	8629 ± 217
Sb	<12.6
Se	<5.89
Sn	<0.974
Sr	20 ± 1.7
Ti	4.49 ± 0.35
V	<0.101
Zn	71.9 ± 3.9

Table 8
Characterization of pigments and scavenger capacity against peroxyl radicals by hydrophilic and lipophilic extracts in microalgal sludge

Extract	Concentration µg/g (dry weight)	Antioxidant capacity	
		Hydrophilic[a]	Lipophilic[b]
Total carotenoids	714.3 ± 0.9	–	28.1 ± 1.3
Total chlorophylls	3400 ± 0.1	–	84.9 ± 1.9
Total phycobiliproteins	214000 ± 0.5	237.4 ± 2.2	–

[a] micromoles of trolox equivalent per gram of microalgal sludge;
[b] α-tocopherol relative.

In terms of antioxidant capacity of pigments extracts, the carotenoid extract of microalgal sludge has been 28 times a more potent peroxyl radicals scavenger than α-tocopherol (Table 8). This value is higher than the one found on the expressive sources of bioactive compounds [24]. Another class pigment assessed by the peroxyl radicals scavenger capacity for lipophilic extracts was the chlorophyll. It is almost 85 times more potent than α-tocopherol. Finally, the in vitro scavenging capacity in contrast to peroxyl radicals of phycobiliproteins extract from microalgal sludge was 237.4 μmol_{trolox}/g, which is about 10 times higher than that the one found in commercial microalgae [43].

This pigments work as natural antioxidants to remove harmful free radicals and they are produced through normal cellular activity and environmental stressors. So, it maintains the immune cells structural integrity. Therefore, the antioxidant capacity presented in LEM microalgae (Table 8) might play an important role to animal health by increasing their immunity [39]. A compromised immune system will increase animal morbidity and mortality rates and, consequently, decrease animal production efficiency [44].

3.5. Multi-purpose microalgal bioprocess

Multi-purpose bioprocess systems that utilize microalgae for treating wastewater, producing biofuels and potential animal feed are an attractive alternative to microalgae-based systems aimed exclusively at biofuels production [45,46]. The remaining portions of microalgae not used in biofuel production (carbohydrates, protein, minerals and unextracted lipids) can be a prospective coproduct that may be available in excess of oil production wasting. The use of LEM microalgae as a coproduct requires the evaluation of industries to determine its suitability.

Fig. 2 shows a nutrient cycling strategy based in poultry and swine slaughterhouse wastewater. Taking as basis for calculation 1 m^3 wastewater, it is possible to obtain, in a batch process, 279 g [biomass]/d, 88.5 g [proteins]/d, 60.5 g [minerals]/day, 42.9 g [lipids]/d and 44.3 g [carbohydrates]/d.

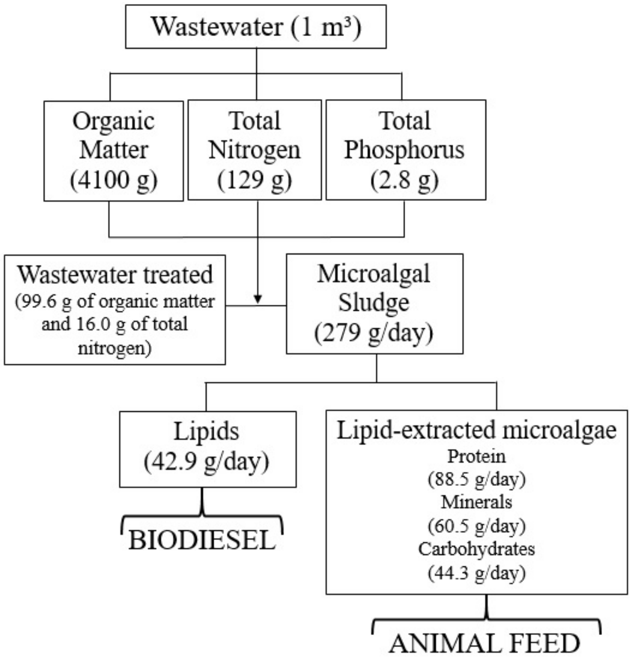

Fig. 2. Nutrient cycling by microalgae-based-processes.

Biorefining may be considered a good opportunity for industry, since microalgal biodiesel presents potential, as evidenced by its qualitative properties, and the remaining biomass fractions can be viable sources of protein, minerals and pigments supplement in animal formulations. Besides, in this calculation basis there are conversions of 4100 g into organic material, 129 g of total nitrogen and 2.8 g of total phosphorus, allowing proper issuance of wastewater to the receptor water bodies.

4. Conclusion

This study has demonstrated the feasibility of microalgae cultivation in wastewater for the sustainable production of microalgal sludge. Moreover, this opportunity to produce biofuel and potential animal feed from *Phormidium autummnale* biomass contributes to the development of a multi-purpose microalgal bioprocess concept, which enables the successful transition to a biobased economy.

Symbols

Q/V — Flow rate per unit volume
VVM — Volume of air per volume of wastewater per minute
LEM — Lipid-extracted microalgae
COD — Chemical oxygen demand (mg/L)
N-TKN — Total nitrogen (mg/L)
$P\text{-}PO_4^{-3}$ — Total phosphorus (mg/L)
TS — Total solids (mg/L)
SS — Suspended solids (mg/L)
VS — Volatile solids (mg/L)
FS — Fixed solids (mg/L)
C/N — Carbon/nitrogen ratio
N/P — Nitrogen/phosphorous ratio
h/D — Height/diameter ratio
Px — Biomass productivity (mg/L)
μmax — Maximum specific growth rate (1/h)
rs — Consumption rates (mg/L·h)
RE — Removal efficiency (%)
X — Cell biomass at time t = t (mg/L)
X_0 — Initial cell biomass (mg/L)
t — Time (h)
S — Final concentration (mg/L)
S_0 — Initial concentration (mg/L)
HRT — Hydraulic retention time (h)
ORAC — Oxygen radical absorbance capacity
EC — Ester content (%)
CN — Cetane number
II — Iodine value (gI_2/100g)
DU — Degree of unsaturation (%)
SV — Saponification value
LCSF — Long-chain saturated factor (%)
CFPP — Cold filter plugging point (°C)
CP — Cloud point (°C)
APE — Allylic position equivalents
BAPE — Bisallylic position equivalents
OS — Oxidation stability (h)
HVV — Higher heating value
m — Kinematic viscosity (mm^2/s)
ρ — Kinematic density (g/cm^3)

PUFAs	—	Polyunsaturated fatty acids
SFAs	—	Saturated fatty acids
MUFAs	—	Monounsaturated fatty acids
ND	—	Not detected

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CONCLUSÃO GERAL

Este estudo demonstrou a viabilidade do cultivo de microalgas em águas residuais para a produção sustentável de lamas de microalgal.

O cultivo heterotrófico de *Phormidium autummnale* converte simultaneamente os três principais poluentes das águas residuais proveniente da agroindústria do abate e processamento de aves e suínos, atingindo eficiências de remoção de 97,6%, 85,5% e 92,4% para DQO, N-TKN e $P\text{-}PO_4^{-3}$, respectivamente. Além disso, é obtida uma produtividade de lodo microalgal de 0,27 $kg/m^3/d$.

A análise econômica mostrou um custo de US\$ 2,66/m^3 de águas residuais agroindustriais tratadas e, como consequência desse processo, o custo de produção de lama microalgal foi de US\$ 0,03/kg de biomassa desidratada.

O lodo microalgal gerado mostra predominância de ácidos graxos saturados, indicando uma fonte potencial para produção de biodiesel. A biomassa microalgal extraída de lipídios apresentaram alto teor de aminoácidos, minerais e pigmentos livres, sendo uma potencial fonte para alimentação animal.

A análise de viabilidade para a aplicabilidade industrial da tecnologia proposta mostra que se o valor comercial da biomassa de microalgas é estimado em US\$ 480/tonelada, pode ser obtida uma margem de lucro de 94%.

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