LETÍCIA RODRIGUES DE ASSIS

REATOR DE BIOFILME COMO UNIDADE DE PRODUÇÃO E COLHEITA DE BIOMASSA ALGAL: APRIMORAMENTO DE UM SISTEMA DE TRATAMENTO DE ESGOTO VIA LAGOAS DE ALTA TAXA

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

Orientadora: Maria Lúcia Calijuri

Coorientadora: Paula Peixoto Assemany

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APROVADA: 05 de março de 2020.

Assentimento:

Letícia Rodrigues de Assis

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Autora

Maria L Caly

Maria Lúcia Calijuri
Orientadora

A Deus.
Aos meus pais Márcio e Maria.
À minha irmã Silmara.
Ao meu anjinho Miguel.
Dedico.

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Agradeço a Deus que me ampara, cuida e ama!

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“Tudo posso naquele que me fortalece”.

(Filipenses 4:13)

BIOGRAFIA

Letícia Rodrigues de Assis, filha de Márcio José Gonçalves de Assis e Maria Aparecida Rodrigues de Assis, nasceu em Viçosa-MG, em 23 de outubro de 1989. Cursou ensino fundamental e médio na “Escola Estadual Cônego José Ermelindo de Souza”, entre 1997 e 2007, em Araponga-MG. Em 2009 iniciou o curso de Engenharia Ambiental e Sanitária na Universidade Federal de Juiz de Fora (UFJF), onde formou-se em janeiro de 2014. Em março de 2014, ingressou no Programa de Pós-Graduação em Engenharia Civil da Universidade Federal de Viçosa (UFV), quando iniciou suas atividades do mestrado, submetendo-se à defesa da dissertação em fevereiro de 2016. Iniciou as atividades de doutorado em março de 2016. Em março de 2020 defendeu sua tese perante o Programa de Pós-Graduação em Engenharia Civil da UFV.

RESUMO

ASSIS, Letícia Rodrigues de, D.Sc., Universidade Federal de Viçosa, março de 2020. **Reator de biofilme como unidade de produção e colheita de biomassa algal: aprimoramento de um sistema de tratamento de esgoto via lagoas de alta taxa.** Orientadora: Maria Lúcia Calijuri. Coorientadora: Paula Peixoto Assemany.

A produção aderida de biomassa algal em águas residuárias é promissora para o desenvolvimento de reatores de biofilme (RBs) destinados à separação e colheita da biomassa de forma econômica. No entanto, ainda não existe um RB consolidado no âmbito de produção e separação de biomassa e, por isso, diversos projetos de reatores são encontrados na literatura. Consequentemente, diversas configurações desses reatores também são reportadas como: material suporte para a aderência da biomassa, inclinações dos reatores, posicionamento integrado ao sistema de cultivo suspenso, entre outros. De forma a propor estratégias de superação de algumas dessas limitações, este estudo teve como objetivo otimizar as características de um RB para a sua integração a uma lagoa de alta taxa (LAT), formando um sistema hibrido de produção e separação de biomassa. Experimentos preliminares de avaliação dos materiais suportes para o crescimento da biomassa e de inclinação dos reatores foram realizados. O poliéster demonstrou ser o material suporte com melhores resultados de durabilidade, de produção de biomassa e de menores impactos ambientais negativos. Enquanto os reatores inclinados a 15º (500,9 $g.m^{-2}$) e a 90º (528,1 $g.m^{-2}$) em relação ao plano horizontal, apresentaram melhores resultados de produção de biomassa, sob condições climáticas, em estações climáticas secas e chuvosas, respectivamente. Portanto, esses resultados preliminares foram considerados para a otimização e implementação de um RB a uma LAT, em escala piloto. Os resultados dessa integração demostraram que a produção de biomassa aderida ao RB (69,8 $g.m^{-2}$) foi, aproximadamente, o dobro do que a biomassa suspensa da lagoa de alta taxa (36,8 $g.m^{-2}$) e a colheita diária de biomassa no RB (13,3 $g.dia^{-1}$) foi cerca de cinco vezes maior em relação a biomassa sedimentada no decantador (2,6 $g.dia^{-1}$). Além disso, esse reator contribuiu substancialmente com a eficiência de separação de biomassa no sistema híbrido, que apresentou uma retenção total de 64% de biomassa contra apenas 22% no sistema composto por LAT e decantador. O RB apresentou maior densidade e diversidade de organismos fotossintéticos durante a maior parte do período experimental. Além disso, os resultados da avaliação de ciclo de vida (ACV) de 1 Kg de biomassa separada no sistema híbrido mostrou uma redução de impactos ambientais negativos de, aproximadamente, 19% em todas as categorias, quando comparado ao sistema de tratamento de esgotos e separação de biomassa convencional, composto por LAT e decantador. O RB contribuiu positivamente com as demandas energéticas, de água e nutrientes do sistema em que estava integrado. Portanto, o aprimoramento de sistemas de tratamento de esgotos com os RBs é promissor para a produção, separação e colheita de biomassa algal. Este estudo contribuiu com algumas características de projetos de RBs, visando a sua aplicação em larga escala de estações de tratamento de águas residuárias, tornando o saneamento ambiental mais atrativo do ponto de vista de sustentabilidade.

Palavras-chave: Águas residuárias. Biofilme algal. Crescimento aderido. Microalgas.

ABSTRACT

ASSIS, Letícia Rodrigues de, D.Sc., Universidade Federal de Viçosa, March 2020. **Biofilm reactor as an algal biomass production and harvesting unit: improvement of a sewage treatment system via high-rate algal ponds.** Adviser: Maria Lúcia Calijuri. Co-adviser: Paula Peixoto Assemany.

Attached algal biomass production in wastewater is promising for biofilm reactors (BRs) development intended to biomass separation and harvesting in an economic way. However, there is still no consolidated BR in the scope of biomass production and harvesting and, therefore, several reactor projects are found in the literature. Consequently, several configurations of these reactors are also reported as: support material for the biomass attachment, reactors slopes, positioning integrated to the suspended cultivation system, among others. In order to propose strategies to overcome some of these limitations, this study aimed to optimize the characteristics of a BR for its integration into a high-rate algal pond (HRAP), forming a hybrid system for biomass production and separation. Preliminary experiments to evaluate the supporting materials for biomass growth and reactors slope were carried out. Polyester proved to be the supporting material with the best results in terms of durability, biomass production and less negative environmental impacts. While the reactors inclined at 15º (500.9 g.m^{-2}) and 90º (528.1 g.m^{-2}) in relation to the horizontal plane, presented better results of biomass production, under climatic conditions, in dry and rainy seasons, respectively. Therefore, these preliminary results were considered for the optimization and implementation of a BR to a HRAP, on pilot scale. The results of this integration showed that the biomass production attached to the BR (69.8 g.m^{-2}) was approximately twice the biomass suspended in the HRAP (36.8 g.m^{-2}) and the daily biomass harvest in the BR (13.3 g.day^{-1}) was about five times higher in relation to the sedimented biomass in the settling tank (2.6 g.day^{-1}). In addition, this reactor contributed substantially to the efficiency of biomass separation in the hybrid system, which presented a total retention of 64% of biomass against only 22% in the system composed of HRAP and settling tank. The BR showed higher photosynthetic organisms density and diversity during most of the experimental period. In addition, the life cycle assessment (LCA) results of 1 kg of biomass separated in the hybrid system showed a reduction of negative environmental impacts of approximately 19% in all categories, when compared to the conventional wastewater treatment and biomass separation system, composed of HRAP and settling tank. BR contributed positively to the system energy, water and nutrient demands in which it was integrated. Therefore, wastewater treatment systems improvement with BRs is promising for algal biomass production, separation and harvesting. This study contributed to some characteristics of BR projects, aiming their large-scale application in wastewater treatment plants, making environmental sanitation more attractive from the sustainability point of view.

Keywords: Wastewater. Algal biofilm. Attached growth. Microalgae.

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ABREVIATURAS, SIGLAS E SÍMBOLOS

APHA – American Public Health Association
ACV – Avaliação do ciclo de vida
BR – Biofilm reactor
CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico
COD – Chemical oxygen demand
ETAR – Estação de tratamento de águas residuárias
FAPEMIG – Fundação de Amparo à Pesquisa do Estado de Minas Gerais
HRT – Hydraulic retention time
HRAP – High-rate algal pond
HRP – High-rate pond
ISO – International Organization for Standardization
LAT – Lagoa de alta taxa
LCA – Life cycle assessment
NH_4^+-N – Ammonia nitrogen
NO_3^--N – Nitrate nitrogen
OD – Dissolved oxygen
PAR – Photosynthetically active radiation
Ps – Soluble phosphorus
PVC – Polyvinyl chloride
RB – Reator de biofilme
ST – Settling tank
SVI – Sludge volume index
TKN –Total Kjeldahl Nitrogen
TP – Total phosphorus
TVS – Total volatile solids
UASB – Upflow anaerobic sludge blanket reactor
UFV – Universidade Federal de Viçosa
VSS – Volatile suspended solids
WWTP – Wastewater treatment plants
μ – Specific growth rate

SUMÁRIO

1. APRESENTAÇÃO

A presente pesquisa foi desenvolvida como continuidade dos estudos realizados no Núcleo de Pesquisas Ambientais Avançadas (nPA) do Departamento de Engenharia Civil da Universidade Federal de Viçosa (UFV), que abrangem a produção e separação de biomassa algal e tratamento de efluentes, além dos benefícios oriundos dessa integração. A origem da pesquisa foi no projeto “Biotecnologia de microalgas para valorização de nutrientes e conservação da água e do solo”, aprovado junto ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) no edital n°36/2013, Processo n° 403013/2013-5, que abordava o cultivo híbrido de microalgas. Essa forma de cultivo se desenvolve de forma suspensa em lagoas de alta taxa (LATs) e aderida a reatores de biofilmes (RBs), simultaneamente. Os resultados obtidos até o momento acerca dos reatores híbridos foram divulgados em uma dissertação de mestrado (Assis, 2016) e um artigo científico publicado em periódico internacional (Assis et al., 2017) e demonstraram que o uso de RBs integrados a uma lagoa de alta taxa foi eficiente tanto para o tratamento de esgoto doméstico quanto para produção de biomassa. Além disso, como os reatores de biofilme estavam em contato direto com o ar atmosférico e a radiação solar, os requisitos de carbono da biomassa das algas foram atendidos e não foi necessário suplementar carbono adicional no meio de cultivo.

Sendo assim, essa pesquisa representa a expansão dos conhecimentos acerca da produção e separação da biomassa cultivada em esgoto doméstico com a integração de reatores de biofilme (RBs) às lagoas de alta taxa (LATs). As LATs são reatores eficientes para o polimento das águas residuárias e produção de microalgas. Enquanto os RBs surgem como uma inovação tecnológica que consiste em uma matriz de microalgas e bactérias crescida de forma aderida a um suporte sólido. Esses reatores possuem menor demanda de área superficial, apresentam maior produtividade em relação às LATs e a concentração da sua biomassa dispensa a etapa de desidratação da biomassa. Nesse sentido, estratégias de melhoria do RB foram abordadas, dando ênfase ao material suporte utilizado para aderência da biomassa, à inclinação desse reator perante a LAT e a sua exposição às condições ambientais, para que um RB otimizado fosse integrado a uma LAT.

Este documento foi organizado em uma introdução geral, hipóteses, objetivos, quatro artigos, conclusão geral e sugestões para pesquisas futuras. O primeiro artigo diz respeito a avaliação de diferentes materiais suportes para o crescimento aderido da biomassa algal em relação a produção de biomassa e a durabilidade dos materiais. O mesmo já se encontra publicado no periódico Algal Research (ISSN: 2211-9264). O segundo artigo consistiu em um pré-teste para avaliação das inclinações dos reatores de biofilme e foi publicado como resumo expandido nos anais do evento *"2nd IWA Conference on Algal Technologies for Wastewater* Treatment and Resource Recovery and 12th IWA Specialist Group Conference in Wastewater Pond Technology - *IWAlgae2019",* realizado em Valladolid., na Espanha, em 2019. Os resultados obtidos durante esta etapa foram importantes para otimizar a operação do reator de biofilme ao longo das estações do ano, conforme descrito no terceiro artigo. Um RB otimizado (pelos resultados obtidos nos artigos 1 e 2) integrado a uma LAT e a um decantador, constituiu-se em um sistema híbrido de produção, separação e colheita de biomassa. A avaliação técnica deste sistema híbrido foi o principal objetivo do terceiro artigo, que se encontra publicado no periódico Journal of Environmental Management (ISSN: 0301-4797). Por fim, o quarto artigo apresenta a avaliação dos impactos ambientais dos materiais suportes avaliados no primeiro artigo 1 e a avaliação da sustentabilidade da integração de um RB a uma LAT (terceiro artigo). Ambas avaliações foram realizadas por meio da metodologia de avaliação do ciclo de vida (ACV). Com essa organização foi possível avaliar o RB como uma unidade promissora para produção e separação de biomassa a partir do tratamento de esgoto, visando a ampliação dessa tecnologia em larga escala como aprimoramento das estações de tratamento de águas residuárias (ETAR).

2. INTRODUÇÃO GERAL

A utilização de microalgas é uma estratégia na mitigação dos problemas relacionados à falta de saneamento. Do ponto de vista ecológico e econômico, as águas residuárias são fontes de água e nutrientes para o crescimento das células algais. A assimilação desses nutrientes pelas microalgas minimiza os impactos negativos causados pelo lançamento dos esgotos in natura nos corpos d'água, ao passo que contribui com o reuso e tratamento das águas residuárias. Após a etapa de separação e colheita da biomassa algal, diversos bioprodutos podem ser obtidos como biofertilizantes, bioenergia ou ração animal.

Apesar do potencial cultivo de microalgas em águas residuárias para a geração de diversos bioprodutos, o aproveitamento de sua biomassa ainda enfrenta os desafios associados às etapas de separação e colheita. A eficiência da separação e colheita é influenciada por diversos fatores como a morfologia das microalgas, a densidade e tamanho das células, o bioproduto final a ser obtido e o meio de cultivo utilizado (Singh e Patidar, 2018). Independentemente do método de separação adotado, ele deve superar as principais dificuldades associadas à separação das microalgas. Dessa forma, deve prever o pequeno tamanho das células (<20 µm), a proximidade da densidade das microalgas com a água, superfície com alta carga negativa (potencial zeta) e elevadas taxas de crescimento, que faz com que a colheita seja frequente. Nesse sentido, os desafios quanto a viabilidade econômica dos processos de recuperação e valorização de bioprodutos ainda persistem (Singh e Patidar, 2018; Menegazzo e Fonseca, 2019).

Diante das diversas técnicas de separação e colheita ainda não consolidadas no âmbito de recuperação da biomassa, novos reatores estão sendo projetados em menores escalas e em busca da minimização das desvantagens acima citadas (Foladori et al., 2018). A utilização de reatores de biofilmes (RBs), também conhecidos como reatores de perifíton, é uma inovação tecnológica que consiste em uma matriz de microalgas, bactérias e fungos crescida aderida a um suporte sólido (Sutherland e Craggs, 2017; Mantzorou e Ververidis, 2019). Nesses reatores, a luz, a temperatura e as concentrações de nitrogênio e fósforo são os principais fatores que determinam a taxa de crescimento da biomassa de microalgas, que são os organismos dominantes na matriz perifítica. A colheita periódica da biomassa remove os nutrientes assimilados pelas microalgas e o reator pode ser considerado um sistema de tratamento biológico para mititgar a poluição das águas residuarias (Sutherland e Craggs, 2017).

Sendo assim, a principal vantagem dos RBs quando comparado às formas convencionais de separação, é a facilidade de remoção das células de microalgas da superfície sólida por raspagem (Zhang et al., 2018a). Após a raspagem, as colônias que ainda permanecem no material aderente são utilizadas como inoculo para o próximo ciclo de crescimento (Assis et al., 2017). Esse reator apresenta maior produtividade, além de possuir menor demanda de área superficial, que os sistemas de cultivo suspensos (Caporgno et al., 2016; Rawat et al., 2013; Mantzorou e Ververidis, 2019). Ademais, o conteúdo úmido da biomassa é baixo, dispensando o processo de desaguamento (Liu et al., 2013) e a biomassa de microalgas aderida à superfície tem maior disponibilidade de luz quando comparado com à biomassa suspensa em meio líquido (Zhang et al., 2018a).

Apesar de todas as vantagens reportadas, há uma diversidade de projetos de RBs na literatura. Algumas características destes projetos incluem o material suporte ao qual a biomassa cresce aderida. A escolha desse material é de grande relevância para o desempenho de todo o sistema de produção e separação, e até o momento, não há material suporte considerado como padrão para o desenvolvimento de biofilme. Diferentes ângulos de inclinações do RBs, que variaram de 0,2º a 90º em relação ao plano horizontal, também já foram avaliados e os resultados de produtividades variaram entre 0,71 $g.m^{-2}.dia^{-1}$ e 9,1 $g.m^{-2}.dia^{-1}$ (Ozkan et al. 2012, Chen et al. 2014, Lee et al. 2014). Portanto, até o momento, a maioria dos projetos de RBs foram avaliados de acordo com sua finalidade e maioria dos estudos foram realizados em escala de laboratório (Choudhary et al., 2017). Estes estudos estão concentrados na comparação da produtividade das microalgas aderidas em relação aos sistemas de cultivo em suspensão. Ou simplesmente dizem respeito à produção de microalgas aderidas com o objetivo de tratar águas residuárias (Mantzorou e Ververidis, 2019). Sendo assim, a literatura ainda carece de mais pesquisas que otimizem o projeto e a operação desses reatores, procurando atender as adversidades das condições climáticas e visando a sua implantação em larga escala (Mantzorou e Ververidis, 2019).

Do ponto de vista ambiental, também são poucos os estudos avaliando RBs para o tratamento de efluentes e produção e colheita de biomassa de algas. Barlow et al. (2016) avaliaram a sustentabilidade da geração de diesel renovável por meio da liquefação hidrotérmica de biomassa anexada a uma algal RB em rotação por meio da avaliação do ciclo de vida (ACV). Morales et al. (2020) estudou a concentração de proteínas de uma biomassa cultivada em um sistema de biofilme de algas rotativo integrado com uma LAT. O biofilme de algas rotativas contribuiu para a redução dos impactos ambientais (20%) e do consumo de água (30%) por quilograma de biomassa de microalgas e por quilograma de concentrado protéico, sendo considerado pelos autores uma alternativa promissora em comparação à produção convencional de biomassa em LATs. No entanto, ainda não foram realizados estudos que avaliem os impactos relacionados aos materiais de suporte utilizados, ao funcionamento do reator e à energia necessária na separação da biomassa.

A identificação de rotas mais sustentáveis de aproveitamento da biomassa de microalgas durante o tratamento de água residuárias é uma ferramenta valiosa que pode ser obtida por meio da ACV. Essa metodologia permite a avaliação de impactos ambientais de cada estágio em um sistema, como em estações de tratamento de águas residuais, uma vez que leva em consideração e quantifica o uso de recursos naturais, energia, emissões e resíduos gerados (Parra-Saldivar et al., 2019).

Além disso, atualmente, existe a necessidade urgente de reforçar as concepções de tratamento de águas residuárias para a recuperação de recursos e minimização dos impactos relacionados com as emissões para a água e para o ar e com a escassez de recursos naturais (Arashiro et al., 2018). Diante cenário e dos benefícios dos RBs, a utilização desses reatores associados ao tratamento das águas residuárias com microalgas é promissor diante da produção de uma biomassa valorada, ao passo que ocorre a remoção dos poluentes simultaneamente. Sabe-se, porém, que nas estações de tratamento de águas residuárias (ETAR) em que já se encontram instaladas as tradicionais lagoas de alta taxas (LATs) não é benéfico economicamente recorrer a novas tecnologias de reatores para a otimização da produção e separação da biomassa. As LATs são reatores eficientes para o polimento das águas residuárias e produção de microalgas (Craggs et al., 2012). Nesse sentido, os RBs podem ser adaptados às LATs e a integração dessas duas tecnologias surge como aprimoramento das ETAR já existentes (Zhang et al., 2018b).

3. HIPÓTESES

O material suporte influencia na produção de biomassa algal de um reator de biofilme.

O material suporte interfere nos impactos ambientais negativos associados a produção de biomassa em um reator de biofilme.

A inclinação do reator de biofilme interfere na produção de biomassa algal.

A separação de biomassa em reator de biofilme é maior do que em um decantador secundário.

Os impactos ambientais negativos associados a um sistema híbrido, composto por um reator de biofilme e uma lagoa de alta taxa, são menores do que um sistema convencional de tratamento de esgoto e produção de biomassa via lagoas de alta taxa e decantador.

4. OBJETIVOS

4.1. Objetivo Geral

Avaliar o desempenho de um reator de biofilme como unidade de produção e colheita de biomassa algal em um sistema de tratamento de esgoto via lagoa de alta taxa.

4.2. Objetivos Específicos

- Avaliar diferentes materiais suportes quanto a produção da biomassa e a durabilidade ao contato com o esgoto doméstico;
- Avaliar a influência da inclinação dos reatores de biofilme na produção de biomassa cultivada no esgoto doméstico;
- Avaliar a separação e colheita de biomassa em reator de biofilme integrado a uma lagoa de alta taxa, em escala piloto.
- Avaliar os impactos ambientais gerados para a produção de biomassa em diferentes materiais suportes.
- Avaliar os impactos ambientais gerados para a separação de biomassa em um reator de biofilme integrado a uma lagoa de alta taxa.

5. ARTIGO 1. EVALUATION OF THE PERFORMANCE OF DIFFERENT MATERIALS TO SUPPORT THE ATTACHED GROWTH OF ALGAL BIOMASS[i]

Abstract

The attached microalgal biomass production in wastewater is promising for the development of biofilm reactors aimed at the economic separation and harvesting of biomass. However, the current impasse in the attached algal biomass production relies on the ability of materials to support such adherence. This study evaluated the effects of different support materials on the production and composition of algal biomass cultivated in domestic sewage. Durability and adherence of algal biomass to the threads of the support material were the most important criteria for choosing the material with the best performance. Three support materials were evaluated: cotton, nylon, and polyester. Polyester presented the best results in terms of durability; its resistance to friction tests was the highest, and even increased after its use in the experiment; this was associated with the high biomass production, mostly after the biomass inoculum (50.1 $g{\cdot}m^{-2}$). This support also demonstrated greater development of nitrifying bacteria, which are essential for biofilm formation due to the presence of filaments in their cells. As for biomass characterization, it was observed that the different support materials did not interfere in the composition of the cells present in the attached biomass.

Keywords: Biomass adherence, Wastewater, Algal biofilm, Materials durability, Microalgae, Biofilm reactors

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5.1.Introduction

The search for technologies that are less damaging to the environment, associated with the need to generate energy to supply the population demand, has stimulated research aimed at using renewable energy sources. The production of microalgae is within this context as a promising source of renewable energy. Among the applications of algal biomass, the following biofuels can be highlighted: the production of ethanol through sugar fermentation; bio-oil from thermal–chemical processing; and methane through anaerobic digestion. Other applications include the production of fertilizers, and aquaculture (Chisti, 2013).

Despite the great versatility of microalgae biomass use, it has not yet been commercialized due to its high production cost. Currently, its main production process is through high rate ponds, where the extraction of algal biomass is expensive. Grimma et al. (2003) estimated that the costs of harvesting and separating biomass represent nearly 20 to 30% of the costs for producing microalgae biofuels. In order to harvest algal biomass from a diluted sample, the algal cells in solution are usually concentrated through sedimentation, flotation, flocculation or centrifugation (Garbowski et al., 2017). In order to harvest algal biomass from a diluted sample, the algal cells in solution are usually concentrated through sedimentation, flotation, flocculation and centrifugation (Garbowski et al., 2017). These process, if used on large scale, have a time-consuming operation and are not considered as economically feasible (Gao et al., 2015). Newer technologies are available for algal biomass separation and for the production of high density biomass, such as membrane bioreactors. These bioreactors act efficiently in these requirements and also in the removal of nutrients when used for wastewater treatment (Xu et al., 2016).

In this context, the recent search for innovative strategies to optimize the production and separation of algal biomass can be highlighted. Nowadays, the method of the algal biofilm growth system has been widespread, in which the cells are fixed to the surface of a solid support material (Wang et al., 2017). The advantages of this new way of production are the low water demand and the ease of biomass harvesting, due to its greater concentration (Wang et al., 2017). Consequently, these systems have a low cost of biomass harvesting and separation when compared to the suspended growth system.

Adhered growth systems when used in wastewater treatment allow the formation of a consortium of microorganisms including microalgae and bacteria (Mantzorou and Ververidis, 2019). The interaction of these microorganisms and the association of several parameters interfere in the formation and structure of the formed biofilm (Bott, 2011). However, there is not much information about the formation of biofilms in these adhered growth systems, making the studies to deepen the optimization of the various parameters that involve flow velocity, support material, light availability, nutrients, among others (Mantzorou and Ververidis, 2019).

The support material to which microalgae adhere is of great relevance to the performance of the entire production system, and, to date, there is no support material considered as the standard for biofilm development (Kesaano and Sims, 2014).

In the literature, several materials for adherence growth study have already been tested in terms of cell binding, durability and cost of the support material. The materials with the best performers were cotton (Christenson and Sims, 2012; Gross et al., 2013), polystyrene foam (Johnson and Wen, 2010) and nylon mesh (Lee et al., 2014). However, with the exception of Lee et al. (2014), the tests for support materials choice were carried out on bench scale and under controlled environmental conditions. In addition, very few of them (Johnson and Wen, 2010; Lee et al., 2014), used real effluents during tests.

Furthermore, the different choices of materials reported in the literature were associated to the different culture media, microalgae species and environmental conditions to which they were submitted. Impacts related to real environmental conditions (radiation, precipitation, wind, etc.) and cultivation in real wastewater still require further investigations. Therefore, this study is aimed at assessing the performance of support materials (cotton, nylon and polyester) on microalgae production cultivated in domestic sewage, with respect to the adherence of the cells, durability of the material, and biomass composition. The research is also expected to contribute to the production of microalgae biomass and to separation and harvesting techniques through scraping, in real scale, to be simultaneously applied to wastewater treatment and biomass use.

5.2.Materials and Methods

5.2.1. Cultivation medium

The cultivation medium used for biomass growth was domestic sewage, previously treated in an upflow anaerobic sludge blanket reactor (UASB), located in a wastewater treatment plant in Viçosa, Brazil (20°45′14″S, 42°52′54″W). No inoculum addition was performed, therefore, autochthonous species have developed in the culture medium constituting a consortium between bacteria and microalgae. The monitoring was carried out between July and October 2016, which comprised winter and spring. The average temperature and mean relative air humidity during the experiment were, respectively, 19 °C and 73% (UFV, 2017).

5.2.2. Biofilm reactors design

The experiment was carried out using four reactors; three of them used adherent support material for microalgae growth: the first was cotton (Karsten, vagonite 1560, 100% cotton); the second was polyester (Sk Textil, oxford, 100% polyester); and the third was nylon (Tegape, ASTM-35, 100% polyamide). The choice of materials was based on the literature reports taking into consideration the materials that had best performance during biomass adhered production and their availability in the local market. Table 5.1 shows the characteristics of the yarn density of the support materials used in the study, as well as their source. The fourth reactor was a control reactor in which no adherent support was used. The control reactor was used to compare biomass suspended production and the removal of nutrients from domestic sewage to reactors that contained adherent support materials.

Table 5.1. Density of the threads of the support materials.

	Nylon	Polyester	Cotton
Source of the Fibers	Synthetic	Synthetic	Natural
Direction of the Weft (threads along the width of the material, per cm²)	5	21	24
Direction of the Warp (threads along the length of the material, per cm²)	7	19	21

Figure 5.1 presents the system of reactors used for the biomass attached growth. In each reactor there was an acrylic plate (51 x 33.5 cm) (2 mm, Plasttotal) coated with the adherent support material (51 x 33.5 cm), which was supported by a Styrofoam plate of equal dimensions (2 mm, Pluma). The recipient (55 x 35 x 9 cm; Plasútil), used to support the Styrofoam and allow recirculation of the sewage to the adherent support materials, presented a longitudinal

opening of 17.6 cm² at the bottom center, where the effluent was drained by gravity. This recipient was placed on a counter, 45.5 cm of the ground, with an inclination of 7.6° in relation to the horizontal.

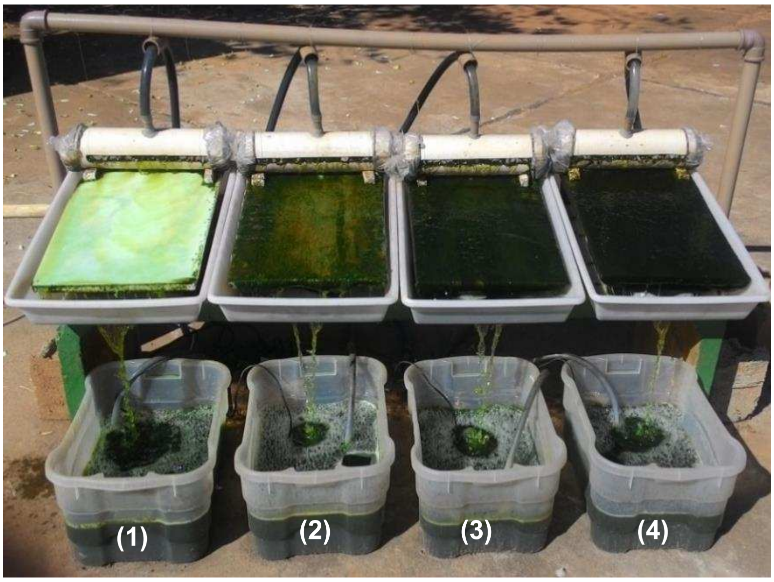

Figure 5.1. System of reactors for the attached biomass growth in different support materials: (1) control reactor; (2) nylon; (3) polyester; (4) cotton.

The application of domestic sewage to the adherent support materials was implemented through a longitudinal opening (30.5 x 1.5 cm) in a PVC pipe (diameter = 10 cm, length = 35 cm), which was filled with number 1 gravel and assembled above the adherent support materials. A submerged pump (Litwin 700/550) operating with a flow of 550 $L \cdot h^{-1}$ recirculated the sewage from the recipient on the soil (plastic box 33 x 27 x 46 cm, 15 L) to be distributed to the adherent support materials.

5.2.3. Biofilm reactors operation

In the first days of operation, samplings were not performed. It was a period of observation of the growth of the biomass adhered on the support materials. The criterion adopted for the scraping of the biomass was the occurrence of cell loss. On the 57th day of operation, it was observed that the adhered biomass was already formed in all the reactors and that there was also the detachment of the same. Then all the biomass was scraped and collected, characterizing itself as the first cycle of growth. The remaining cells were maintained in the adherent materials as inoculum for the next growth cycle, which lasted 32 days.

The reactors were operated in semi-continuous flow of 3 $L.d^{-1}$, so that the sewage input and output took place manually. All reactors were set in the East–West direction to guarantee the incidence of direct solar radiation, using a solar chart (Vianello and Alves, 2013) for the municipality of Viçosa as reference.

5.2.4. Biomass production and nutrients removal

The monitoring of the attached biomass growth was carried out directly on the support materials, and the monitoring of nutrients removal was performed on the suspended biomass in each reactor. Both monitoring was carried out weekly. The samples obtained from the support materials consisted of three scrapings of 1.0 cm^2 in three different positions (samples from the right side, left side and from the middle). Scrapings were alternated at each collection to guarantee that the previous collection did not compromise the following one, as the attached biomass accumulated. In the control reactor, monitoring was carried out with the harvesting of suspended biomass, which was concentrated using a centrifuge at 10,000 rpm for 10 min (HITACHI, High-Speed Refrigerated Centrifuge, CR 21GIII). The monitoring of the attached biomass occurred through the determination of the total volatile solids (TVS) (APHA, 2012) for the quantification of the total biomass, and of chlorophyll-a for the indirect quantification of algal biomass. Chlorophyll-a was hot extracted with 80% ethanol (Nush, 1980); the reading was carried out by spectrophotometry (APHA, 2012), and calculations were performed using the equations described by Schwarzbold et al. (2013), adapted from Marker et al. (1980) and Sartory and Grobbelaar (1984). Total biomass production curves were evaluated by linear regression over the entire period of second growth cycle.

For the suspended biomass of all the reactors, including the control, the parameters measured were dissolved oxygen (DO) concentration, temperature and pH through the Hach HQ40d which measured DO with luminescence-based optical sensors (luminescent dissolved oxygen, LDO). In addition, the concentrations of volatile suspended solids (VSS; 2540E), ammonia nitrogen (NH_3-N; 4500-NH_3C), nitrate (NO^-_3-N, 4500-NO_3A), and soluble phosphorus (P_s; 4500-P C; samples filtered at 0.45 µm), were measured according to APHA (2012). For the determination of chlorophyll-a, an 80% hot ethanol extraction (Nush, 1980) was used. The reading was performed by spectrophotometry (APHA, 2012) and the calculations were carried out using the equations described by the Dutch norm (NEN, 1981). The incident photosynthetically active radiations (PAR) were also measured, using the radiometer LI-COR LI-193 Underwater Spherical Quantum Sensor, between 12 and 1 pm.

The specific growth rate (μ, d^{-1}) of the microalgae was calculated using Equation (5.1) (Chiu et al., 2008).

$$\mu = \frac{(\ln Xi - \ln Xo)}{ti - to}$$

Equation 5.1.

Where Xo is the initial concentration of the biomass (dry weight) at time t and Xi is the concentration of biomass at time ti.

5.2.5. Characterization of biomass

The attached biomass was dried in an oven at 50°C for 24h and then macerated. Subsequently, contents of humidity, ash, and nitrogen were determined (adapted from APHA) (APHA, 2012). The protein content was determined using the conversion factor of nitrogen to protein of 6.25 (Zhong et al., 2012), and the content of neutral lipids was defined through the methodology described by Assis et al. (2017). The carbohydrate content was determined from the quantitative acid hydrolysis of the biomass (Hoebler et al, 1989) followed by the phenol-sulfuric method (Dubois et al., 1956); the reading was quantified by spectrophotometry (490 nm) using the glucose standard curve.

5.2.6. Biomass adherence and durability of the support materials

At the end of the experiment, the fibers of each support material were extracted and analyzed under an optical microscope (Olympus, model IX51) 40x magnification was used to observe the differences between the fibers prior to and after contact with the domestic sewage, as well as during the experiment to assess the adherence of the biomass to the support materials studied.

The durability of the adherent support materials was also assessed prior to and after the experiment through friction tests carried out in a torsiometer (Mathis CRO). In this equipment, a 4 cm² sample of each adherent support material was submitted to 1,000 frictions over 15 minutes. The procedure was repeated until the rupture of the fibers. When the fibers of the support materials showed no signs of rupture, the test after the experiment was interrupted with the same number of frictions reported in the test prior to the experiment. It should be noted that this friction test concerns only the mechanical durability of the support material and not the chemical durability of the contact and exposure to factors such as sewage, solar radiation, among others.

5.2.7. Statistical analysis

The Minitab 17 ® program was used to evaluate the differences between the mean values of the variables measured in biofilm reactors. Analysis of variance was also performed. The experiment used was a completely randomized design for Tukey test with 5% probability.

5.3. Results and Discussion

5.3.1. Biomass production

Figure 5.2 shows the growth curves of total biomass in the biofilms formed in each support material. Only in the polyester support (Figure 5.2b) the biomass growth curve was adjusted to a first order kinetics ($R^2 = 0{,}9369$). Knowledge about microalgae growth kinetics is still scarce in the literature and presents controversies (Wang et al., 2018). In some studies, linear kinetics were observed (Gross et al., 2013; Schnurr et al., 2013; Genin et al., 2014; Schnurr et al., 2016). Other authors believe in the existence of an exponential phase of growth until the biofilm presents maximum thickness, later, this biofilm suffers desquamation (Katarzyna et al., 2015). The nylon and cotton supports had low exponential adjustment and, therefore, other kinetics were tested. For these materials, second-order polynomial kinetics were the best fit, with $R^2 = 0.7084$ for nylon (Figure 5.2a) and $R^2 = 0.9808$ for cotton (Figure 5.2c).

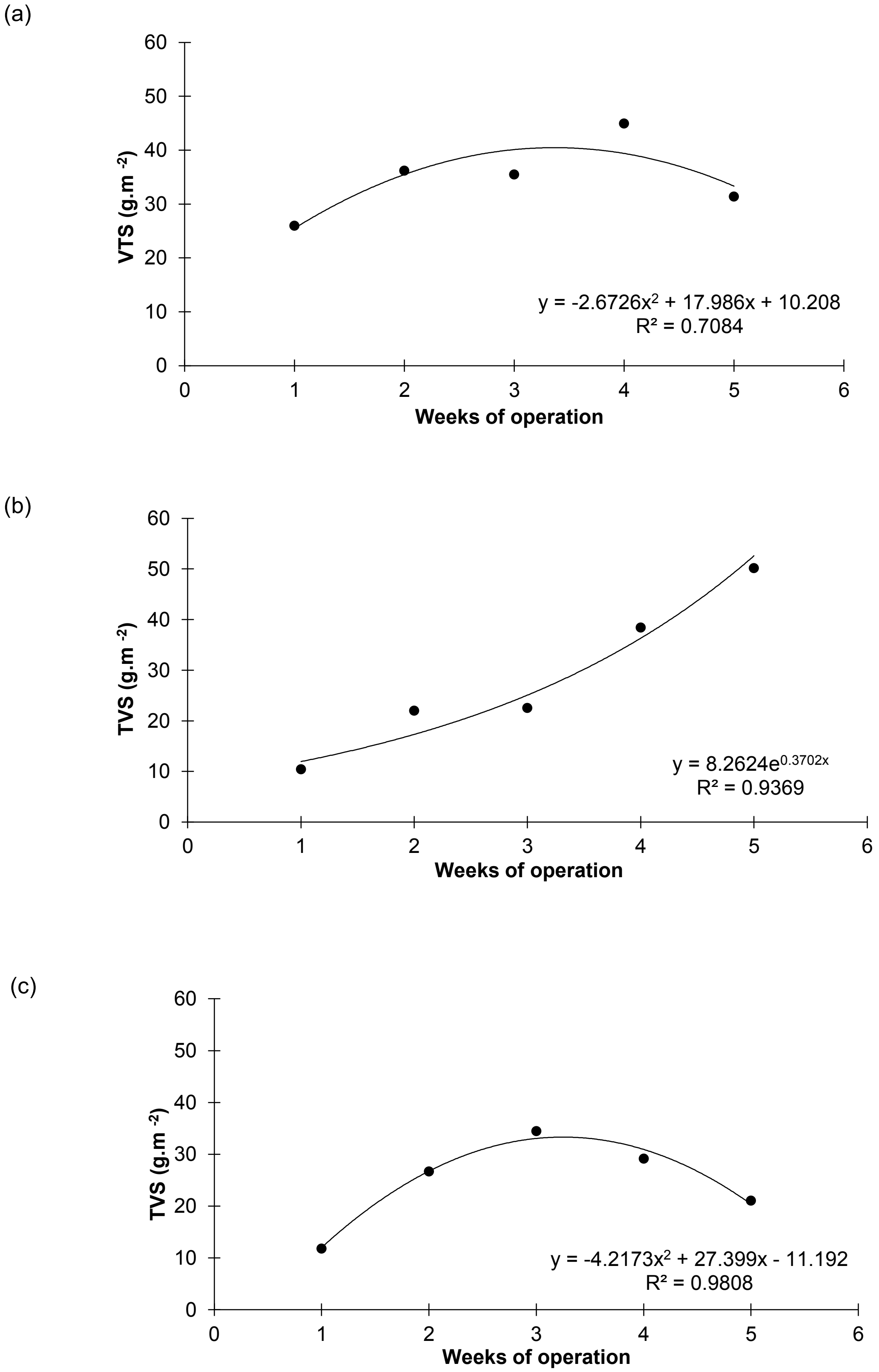

Figure 5.2. Growth kinetics curves for algal biofilms grown on three different materials: nylon (a), polyester (b) and cotton (c).

These results can be better interpreted through Table 5.2, which presents the production and productivities of total and algal biomass in each reactor. The total and algal biomass production in the control reactor were quantified with the purpose of verifying the growth of the biomass independent of the support materials, under the same environmental conditions. There was no statistical difference in total biomass production between the reactors ($p > 0.05$) and, for the algal biomass production, the averages were higher for the polyester support ($p = 0.018$).

The cotton support presented a specific growth rate of 0.12 d^{-1} and a production peak of 34.5 $g.m^{-2}$, which occurred on the 18th day of cultivation or in the 3rd week of operation (Figure 5.2c). This peak reached a productivity of 1.91 $g.m^{-2}.d^{-1}$. At the same time, the curves of the other support materials were still growing, showing that the stationary phases of the cell growth curves in the nylon and polyester supports occurred in higher yields of volatile solids. The nylon support had a maximum peak of 44.92 $g.m^{-2}$ of total biomass during the period of 25 days (4th week of operation, Figure 5.2a), yielding a productivity of 180 $g.m^{-2}.d^{-1}$ and a specific growth rate of 0.05 d^{-1}. The polyester support achieved a maximum total biomass production of 50.12 $g.m^{-2}$ in 32 days (5th week of operation, Figure 5.2b), which corresponds to a daily productivity of 1.57 $g.m.^{-2}.d^{-1}$ and a specific growth rate of 0.11 d^{-1}. As shown in Figure 5.2b, polyester was the only material that kept exponentially increasing its production throughout the growing period of the biomass.

Table 5.2. Total and algal biomass in the supports materials.

Support material	Total volatile solids			Chlorophyll-a		
	Maximum production ($g.m^{-2}$)	Maximum productivity ($g.m^{-2}.d^{-1}$)	$\mu_{maximum}$ (d^{-1})	Maximum production ($g.m^{-2}$)	Maximum productivity ($g.m^{-2}.d^{-1}$)	$\mu_{maximum}$ (d^{-1})
Nylon	44.92	1.80	0.05	0.59	0.02	0.14
Polyester	50.12	1.57	0.11	0.90	0.03	0.26
Cotton	34.46	1.91	0.12	0.42	0.02	0.28

The different properties of the substrates tested, such as hydrophobicity and surface roughness, may have influence the fixation of algal cells (Wang et al., 2018). Among the materials tested in this study, cotton was the most hydrophilic and also the rougher material and, therefore, showed a higher adhesion of the cells in a shorter culture time when compared to other materials. This can also be explained by the use of meshed substratum material, which is considered an alternative to increase the roughness of the surface of the support to be used for the adhesion of microalgae (Wang et al., 2018). As shown in Table 5.1, the opening of the cotton mesh used in this study was smaller than the openings of the nylon and polyester mesh, justifying once again the greater adhesion of the cotton.

The productivity results in this research were lower than the productivity found in other studies that evaluated the support materials for adhered growth of algal biomass. For cotton support, in the literature were found productivities between 1 and 6 $g.m^{-2}.d^{-1}$ (Christenson and Sims, 2012; Gross et al., 2013; Gross et al., 2016). For nylon and polyester, productivities of 13 $g.m^{-2}.d^{-1}$ (Gross et al., 2016) and 11.79 $g.m^{-2}.d^{-1}$ (Gross et al., 2016), respectively. It should be noted that all test results cited above were obtained in laboratory scale experiments under controlled environmental conditions.

When compared to other microalgae production and effluent treatment systems, the productivity of this study was also lower. Xu et al. (2014) evaluated a membrane bioreactor for high-density Chlorella emersoniie cultivationn with a hydraulic retention time of 1 day and obtained an average productivity of 6.2 $g.m^{-2}.d^{-1}$. Buchanan et al. [34] evaluated the treatment of domestic sewage from a septic tank in high-rate algal pond with a flow rate of 12 $m^3.d^{-1}$ and found an average productivity of 31.7 $g.m^{-2}.d^{-1}$.

The low biomass production was mainly related to the consortium of microorganisms, with the conditions of the experiment (larger scale, without control of the external environment interference), and also with the low frequency of scraping. In this study, the scraping of the total biomass was performed only twice, with a period of approximately 40 days between scraping. Some authors have pointed out that very long scaling intervals impair the distribution of nutrients and light in the algal cells located in the lower layers of the biofilm (Gross et al., 2015). Among the scraping frequency already studied, the once-weekly interval was more appropriate for the production of adhered biomass (Gross et al., 2013; Boelee et al., 2014; Choudhary et al., 2017).

In addition, in the mixotrophic environment as in this study, the development of microalgae may be inhibited by the extracellular metabolites produced by bacteria (Fukami et al., 1997). Also, low productivity can be justified for high cell density cultures. The presence of bacteria can reduce light availability to microalgae cells and, consequently, lower production of high algal biomass molecules, such as carotenoids and fatty acids (Fuentes et al., 2016).

5.3.2. Characterization of biomass

The total and algal biomass productions in the control reactor were quantified in order to verify the biomass growth independently of the support material, but under the same environmental conditions. Although the biomass production in the reactors with support material and the control reactor was assessed based on different growth conditions (adhered and suspended), some similarities were noticed and, in general, the different support materials did not interfere in the composition of the cells present in the attached biomass (Table 5.3).

Table 5.3. Mean characterization of the biomass in each reactor.

	Control	Nylon	Polyester	Cotton
Humidity (%)	82.9 (8.3)[a]	92.0 (1.1)[b]	89.0 (1.6)[b]	89.5 (2.4)[b]
Ash (%)	26.1 (15.8)	23.6 (7.4)	20.9 (6.5)	20.4 (8.1)
Proteins (%)	24.6 (5.4)	23.8 (1.5)	24.7 (0.5)	25.6 (2.4)
Carbohydrates (%)	20.8 (6.4)	21.2 (5.0)	20.7 (5.0)	21.1 (5.2)
Neutral lipids (%)	5.0 (2.3)	3.7 (0.04)	7.7 (1.1)	10.6 (3.9)

[a] Biomass humidity after centrifugation.
[b] Harvested biomass humidity. Mean values, standard deviation in parenthesis.

In this context, it is essential to understand the characterization of the cultivated algal biomass in order to apply its proper destination for better utilization and efficiency of the process. The biomass characteristics of all reactors remained similar, and was mostly composed by proteins (24 to 26%), followed by carbohydrates (20 to 22%) and ash (20 to 26%), and the lowest portion, the lipid content (3 to 7%). Moisture content of the collected biomass varied between 82 to 92%.

Despite the similarity in the moisture content of the collected biomass, for the control reactor, a centrifugation stage was necessary in other to harvest the suspended biomass, a step that demands energy and costs when it comes to large-scale production, reducing the economic viability of the whole process (Richardson et al., 2014). In the reactors with support materials, biomass was collected by scraping, a stage that require low energy, simpler and more profitable, when comparing to centrifugation. In addition, all tested reactors presented an acceptable moisture content for anaerobic digestion of the biomass (McKendry, 2002) without need for any extra concentration stage.

Also in terms of energy production, the ash content was high and was directly associated to the culture medium, which was the same for all reactors, being the amount related to the fixed solids content presented in domestic sewage. The high proportion of ash in biomass may impairs the production of biofuels and contributes negatively to the quality of biofuels in several ways, for example, increases the contamination of the fuel, increases the operational costs related to the harvesting and separation of biomass, makes more complicate thermochemical and biochemical energy conversions process, among others (Vassilev et al., 2015).

Neutral lipid content was generally considered to be very low in all reactors when compared to values reported for green algae (13 to 31%) and cyanobacteria (5 to 13%) (Griffiths e Harrison, 2009). The presence of bacteria in the domestic sewage causes a reduction in the energy content of the total biomass cultured, since they present lipid content typically lower than 10% (Brown et al., 1996; Mehrabadi et al., 2015).

Carbohydrate levels were close to the results found by Choudhary et al. (2017) for the biofilm growth reactor using domestic wastewater as the culture medium (24%). These authors stated that the range between 13 and 22% of carbohydrates is adequate for supplementation of bovine ration (20 to 25%). However, prior to this biomass processing for animal feed it is highly recommended the elimination of pathogens or any other toxic compounds that the biomass may presents (Choudhary et al., 2017)

Protein contents were below the range (32-38%) found by Gross et al. (2013) in adhered seaweed biomass. The authors considered that this range of protein content was promising to produce feed and fish farming. However, Cole et al. (2015) evaluated the protein content in the production of the Oedogonium macroalgae as an alternative feed production. The protein maximum value of 18% was found in dry biomass. The authors reported that this amount is equivalent or greater than many terrestrial cultures used as a source of protein for animal feeding. In addition, biomass could be used as a supplementary protein in animal feed.

5.3.3. Nutrient removal

The mean value of PAR during the whole experiment was 1,904.07 $\mu E.m^{-2}.s^{-1}$. This extra light was not a limiting factor for biomass production, since the culture medium presented a consortium of heterotrophic and autotrophic microorganisms. When the culture medium is composed of great variety of organisms, there is a reduction in the effect of photoinhibition (Brennan and Owende, 2010). PAR variable is important because it can directly influence the growth of biomass, favoring the photosynthetic activity, which, in turn, provides oxygen for the growth of heterotrophic bacteria, thus realizing the degradation of the organic matter present in the domestic sewage.

In Figure 5.3 are presented the concentrations of nutrients in the influent (reactor inlet) and effluents (outlet) of reactors. The ammonia nitrogen concentrations (Figure 5.3(a)) were almost completely removed, with rates higher than 90%.

The symbiotic relationship between microalgae and bacteria can lead to competition for the transformation of this nutrient. While microalgae incorporate this nutrient into their biomass, the bacteria oxidize ammonia to nitrite and then to nitrate (Daims et al., 2006; Muñoz and Guieysse, 2006). The increase in the nitrate concentration together with the high removals of ammonia nitrogen indicate the occurrence of nitrification. In the biofilm reactors there was an increase of approximately 12%, 41% and 9%, in the cotton, polyester and nylon support materials, respectively, while their removal in the control reactor was 32% (Figure 5.3(b)). Despite the preference of microalgae for ammonia nitrogen, nitrate can represent an important source of nitrogen for theier growth. However, as in all reactors pH values ranged from 10 to 11, the development of nitrifying bacteria may have been impaired (Daims et al., 2006) and another possible conversion route of NH_3-N may have been the volatilization (Sutherland et al., 2015).

All the reactors presented similar behavior curves in the removal of Ps (Figure 5.3(c)). However, the concentrations of P_s were higher in the control reactor, indicating a better performance of the biofilm in the removal of this nutrient. The P_s removals remained between 70 to 76% in the biofilm reactors and 46% in the control reactor. As in this study, pH values were relatively high (between 10 and 11) and as a culture medium of high algal density, it is possible that besides the assimilation by the microalgae, phosphorus precipitation occurred (Xu et al., 2014). These removal percentages can be considered larger than the conventional wastewater treatment technologies and microalgae production processes (Sutherland et al., 2015; Craggs et al., 2012).

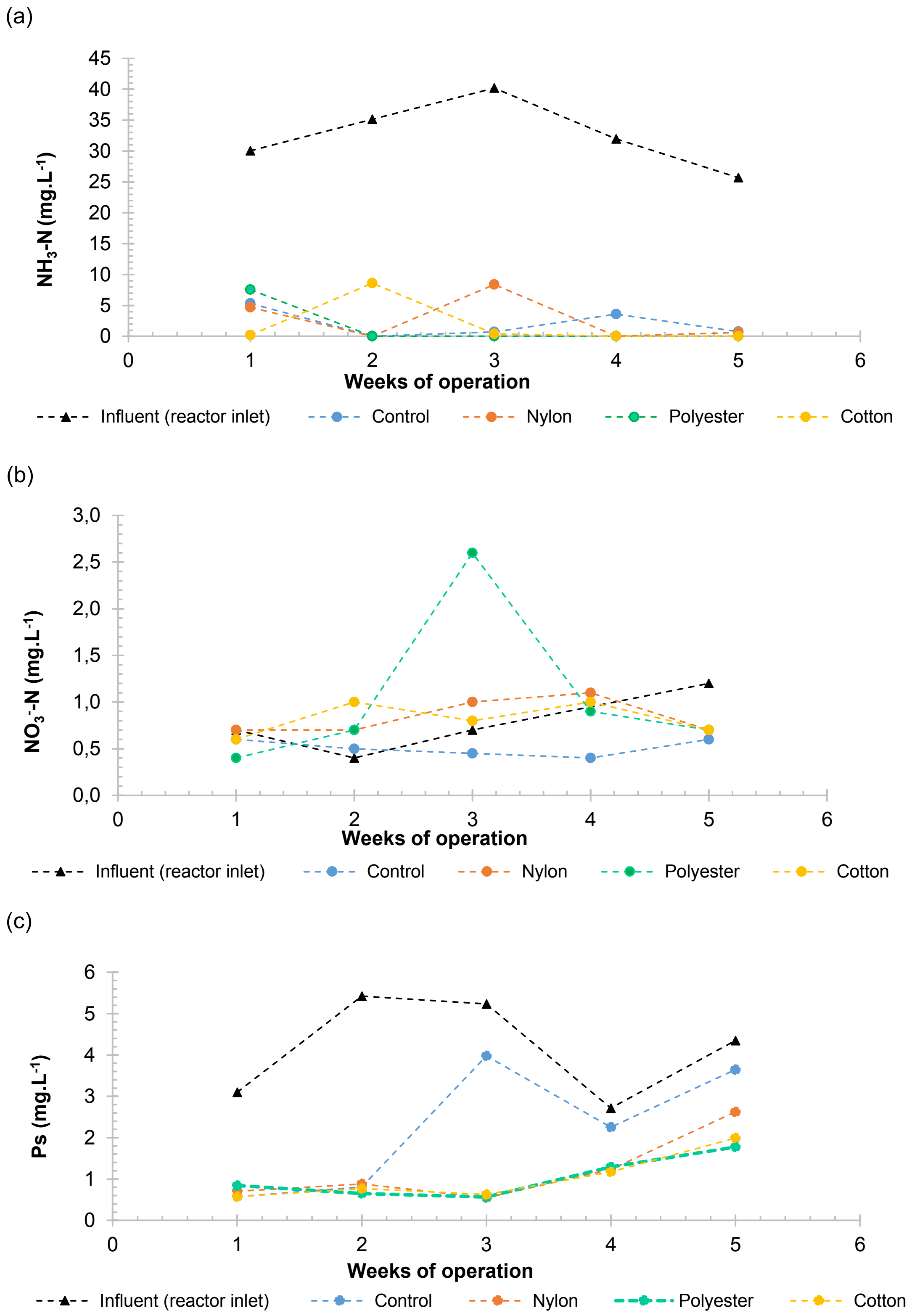

Figure 5.3. Nutrients concentration in domestic sewage, in control and biofilm reactors: (a) ammonia nitrogen (NH_3-N, mg.L^{-1}), (b) nitrate (NO_3^--N, mg.L^{-1}) and (c) soluble phosphorus (Ps, mg.L^{-1}).

5.3.4. Durability of the support materials and biomass adherence

The results of durability tests of the adherent support materials are shown in Table 5.4. After the experiment, the cotton was the most fragile material and presented a rupture of its fibers with only 1,102 frictions. Polyester's resistance to friction increased from 7,000 to 46,563 frictions. After the experiment, the polyester showed signs of breaking of its fibers, justifying the continuity of the test up to 46,563 frictions. While the nylon fibers did not rupture at all during evaluation, either at the time before or at the time after the experiment. For this reason, the test was interrupted at 20,000 frictions, without a sign of rupture. These durability results are largely due to the characteristics of these materials. According to Erhardt et al. (1976), polyester and nylon fibers are resistant to friction, climate conditions, light, and harmful insects.

Table 5.4. Friction test results for the adherent support materials.

	Cotton		Polyester		Nylon	
	Before contact with sewage	After contact with sewage	Before contact with sewage	After contact with sewage	Before contact with sewage	After contact with sewage
Number of frictions	1,518	1,102	7,000	46,563	20,000[a]	20,000[a]

[a] The test was carried out up to 20,000 frictions. However, the material did not present any sign of rupture and the test was interrupted.

Previous studies have reported different support materials with good adherence for biomass growth. Johnson and Wen (2010) assessed four support materials using dairy effluent on a laboratory scale, and concluded that the polystyrene foam presented the best results with respect to durability and reuse. Among the 16 different materials assessed by Gross et al. (2013) on a laboratory scale, the cotton support presented the highest durability and biomass productivity. In another laboratory study, Gross et al. (2016) used pure culture of microalgae and found that the nylon and polypropylene supports provide better adhesion to the initial fixation of the biomass and are also more resistant in the long term. Genin et al. (2014) used pure culture medium inoculated with wastewater for adhered production of algal biomass in a laboratory scale. Four types of support materials were tested and cellulose acetate presented the highest productivity. It is observed, however, that the different media considered by the authors as the most important in the development of the biofilm were associated with the different media of cultures and environmental conditions. Therefore, the choice of supporting material from one study will not always satisfy the demand for other work that considers different cultivation media and environmental conditions.

Figure 5.4 shows the fibers of the support materials prior to and subsequent the experiment. In this study, the cotton support presented higher biomass production and adhesion results (Figure 5.4(a)), however, as previously discussed, its durability was low compared to the other tested materials. During the experiment, cotton fibers were disrupted even before scraping was performed in the biofilm reactor.

The cotton fragility was also reported in the work of Gross and Wen (2014), where cotton was used as a support in a pilot scale reactor under environmental conditions of a greenhouse made from a transparent double polycarbonate wall. Within six months, the support material was totally damaged. Despite the high durability and biomass production, in the nylon support it was noticed that the biomass was more adhered to the acrylic plate that supported the nylon support than in the support itself. In Figure 5.4(c) it is possible to note how the biomass was little evidenced in nylon fibers. The greater aperture between the weft and warp yarns (Table 5.1) may also have favored the non-fixation of the biomass on this material.

Unlike the support made of nylon, in which the number of these yarns was smaller, the cotton and polyester supports had a greater number of wefts and warps, per cm², facilitating the adhesion of biomass. For this reason, the present study disregarded the possibility of nylon application to the adhered cultivation of algal biomass in domestic sewage.

The polyester support was the material that best satisfied the conditions of adhesion and durability taking into account the experiment conditions, i.e., contact with domestic sewage and solar radiation during 32 days of algal biofilm culture. Similarly, under effect evaluation of physicochemical surface of the support materials in the colonization and the adhered growth of the algal biofilm, Gross et al. (2016) found the polyester as one of the six materials that most fixed algal cells.

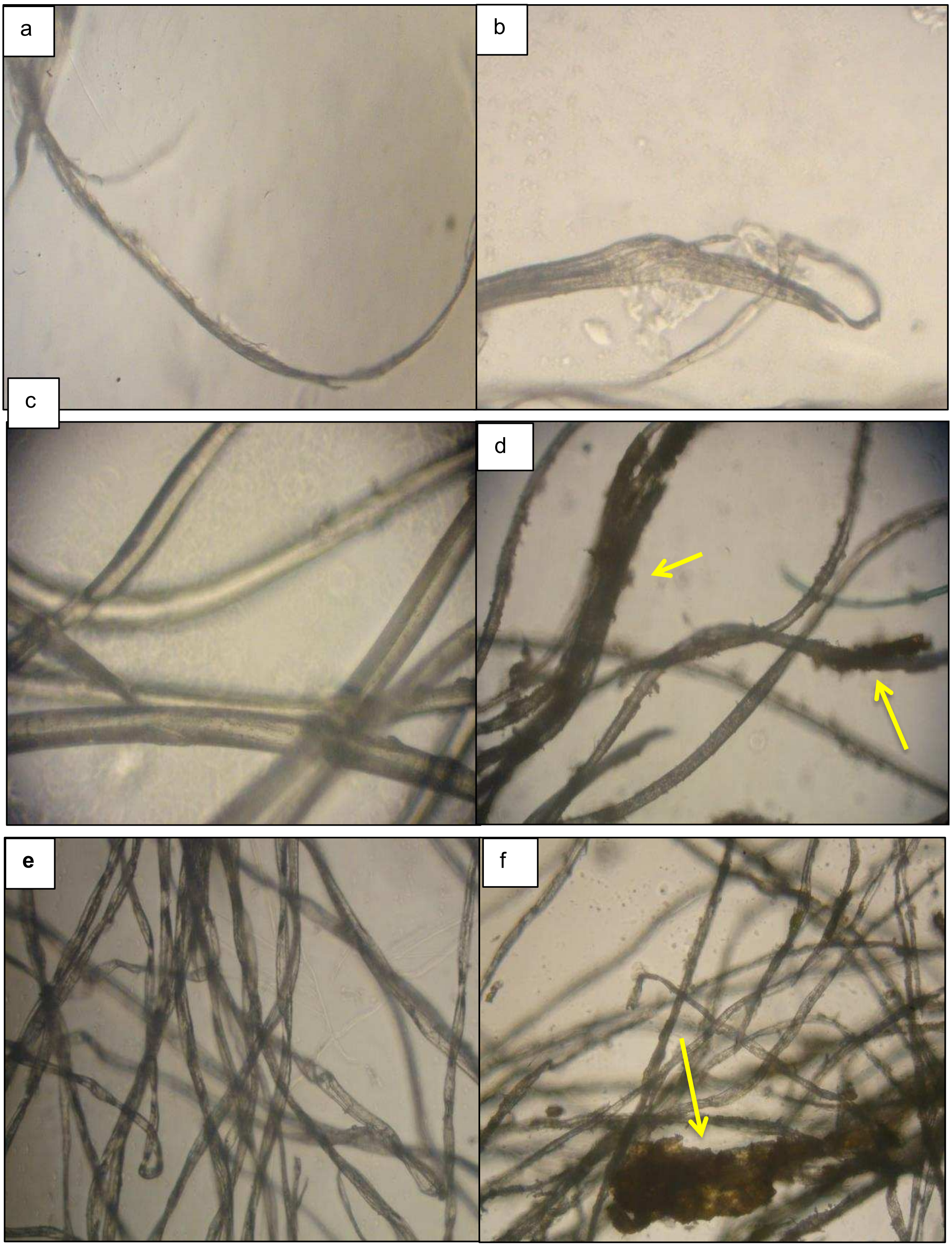

Figure 5.4. Fibers of the support materials: nylon (polyamide) prior to (a) and after (b) contact with cultivation medium; polyester prior to (c) and after (d) contact with cultivation medium; cotton prior to (e) and after (f) contact with cultivation medium.

5.4.Conclusion

The polyester support was the material that presented the best results in terms of durability and the highest biomass production during biomass regrowth (50.1 g.m^{-2}). As for biomass characterization, the different support materials did not interfere in the composition of the cells present in the attached biomass. In relation to the sewage treatment, the biofilm reactor with polyester support presented greater nitrate increase, being indirectly associated to the greater development of filamentous microorganisms. The removal of ammonia nitrogen was greater than 90% and the soluble phosphorus removals were between 70 and 76%.

The selection of the polyester support as the material that best performance to biofilm growth will contribute to future studies that consider the production of microalgae biomass and their separation and harvesting through scraping, in real scale, to be applied for wastewater treatment as well as for biomass reuse. It should be noted that in other cultivation medium and environmental conditions, other media may be considered.

5.5.Acknowledgements

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6. ARTIGO 2. EVALUATION OF THE PERFORMANCE OF REACTORS WITH DIFFERENT SLOPES FOR MICROALGAE PRODUCTION[ii]

6.1. Introduction

Attached growth reactors are new technologies within the algal biomass culture scenario and have been considered an alternative for adaptation and optimization of conventional systems of suspended biomass culture, such as high rate ponds. These reactors are capable of optimizing the production and separation of biomass due to its advantages, which are summarized in water economy, high biomass productivity and harvest efficiency (Wang et al. 2017). When used in wastewater treatment, attached growth reactors supply favorable growth conditions for a consortium of microorganisms that have the capacity to metabolize pollutants. In addition to the microalgae and bacteria organic matter degradation, the development of nitrifying bacteria, which have filaments in their cells and have a preference for static environments, also occurs (Babu et al. 2007).

In the attempt to increase biomass production and wastewater treatment efficiency, different types of algal biomass attached growth reactors have already been developed according to different slope angles, ranging from 0.2º a 90º in relation to plane horizontal, and reached yields between 0.7 $g.m^{-2}.dia^{-1}$ and 9.1 $g.m^{-2}.dia^{-1}$ (Ozkan et al. 2012, Chen et al. 2014, Lee et al. 2014). In this study, the production of algal biomass was evaluated in attached growth reactors with different slope angles (15º, 45º e 90º). The reactors were exposed directly to solar radiation and had as their culture medium the domestic sewage, pretreated in septic tank. The results of nutrient removal from domestic sewage and biomass production were compared in each reactor.

[ii] Resumo expandido publicado:
Assis, L.R., Calijuri, M.L., Assemany, P.P., Nascimento, L.A., Febroni, L.V. Evaluation of the performance of reactors with different slopes for microalgae production. In: 2nd IWA Conference on Algal Technologies for Wastewater Treatment and Resource Recovery and 12th IWA Specialist Group Conference in Wastewater Pond Technology - IWAlgae2019, 2019, Valladolid. IWA Specialist Group, Valladolid, 2019.

6.2. Materials and Methods

The experimental configuration consisted of three reactors of algal biomass adhered growth, which had different slopes in relation to the horizontal plane: 15º, 45º and 90º. In each reactor there was a container (plastic box 33 x 27 x 46 cm) with a useful volume of 15 L, where domestic sewage (pretreated in a real scale septic tank) was stored and recirculated by a submerged pump (Litwin 700/550, flow rate 550 L.h-1) up to an acrylic plate (51 x 33.5 cm) (2mm, Plasttotal) coated by polyester support material (Sk Textil, oxford, 100% polyester) (51 x 33,5 cm), in which the biomass grew attached. Every day a volume of 5.5L of sewage was replaced in the reactors, characterizing a semi-continuous operation. The reactors were operated for a period of 42 days, until the attached biomass layer was formed. From the 43rd day, the biomass growth was followed by weekly collections of attached biomass and analyzes of total volatile solids (TVS, APHA 2012) and chlorophyll-a (Nush 1980, NEN 1981). The nutrient removal was monitored through ammonia nitrogen, nitrate and soluble phosphorus analyzes, following the methodology APHA (2012). The biomass drag determined the biofilm growth cycle on each reactor and the collection by scraping, of the all the biomass. Precipitation data were obtained at the Main Climatological Station of the Federal University of Viçosa, where the experiment was carried out (UFV 2018). The software Minitab 17® was used to evaluate the differences between the mean values of the variables measured in the reactors, through the Tukey test, with a 5% probability.

6.3.Results and Discussion

Figure 6.1 shows the total biomass (TVS) production and precipitation throughout the experimental period. In all reactors, the curves showed a similar behavior and the TVS concentrations were statistically the same ($p>0.05$) during the period when there was no precipitation. From the 113th day of cultivation, the precipitation became frequent with values between 11 mm and 25 mm. In this period, the biomass of the 90º reactor remained attached (no losses were observed), and the mean TVS of this reactor was about 3 times higher than the other reactors ($p=0.00$). Slopes interfered in these results, since the reactors were exposed to environmental conditions, and frequent precipitation removed most of the attached biomass from the 15° and 45° reactors. The raindrops in direct contact with these reactors carried all the biomass already attached, whereas in the 90º reactor, the raindropsflowed parallel to the biomass, keeping it adhered to the reactor. Similarly, to the TVS results, chlorophyll-a concentrations showed statistically equal values during the dry season ($p>0.05$) and higher values for the 90° reactor during the rainy season ($p=0.005$). The concentrations of ammonia nitrogen, nitrate and phosphorus were statistically the same in all reactors ($p>0.05$).

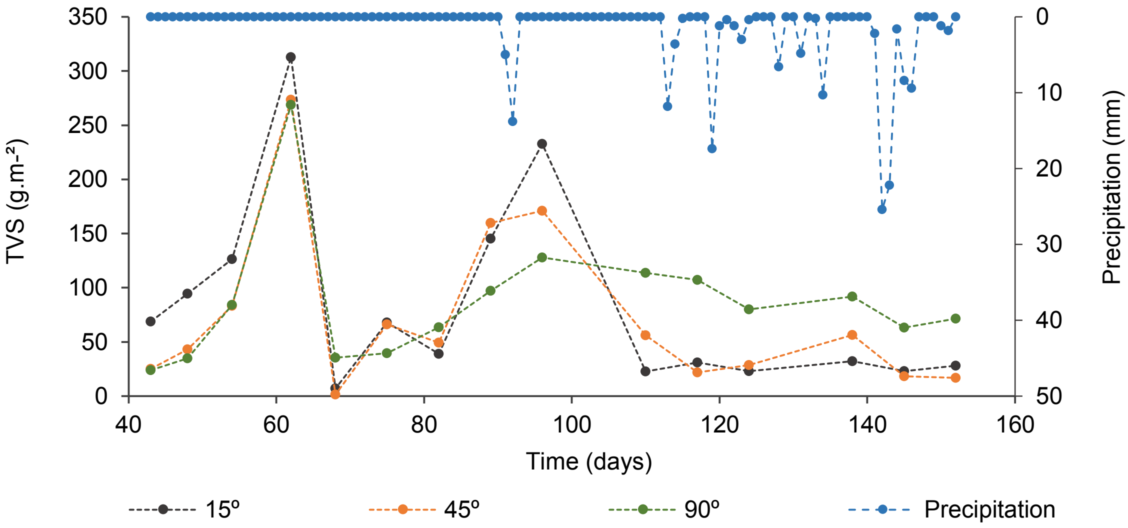

Figure 6.1. Time course of TVS (g.m^{-2}) in attached growth reactors and precipitation (mm) of the experimental period

The concentrations of ammonia nitrogen in all reactors were removed with efficiencies higher than 90% due to algal assimilation and nitrification. All reactors showed an increase of nitrate concentrations by approximately 260%. The presence of biofilm could have favored the development of nitrifying bacteria, as these have filaments, prefer static environments and rarely live as suspended and free bacteria (Hammer and Knight 1994). Phosphorus removals were between 60 and 75%, considered high when compared to conventional sewage treatment processes.

6.4.Acknowledgments

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Climatológica Principal de Viçosa. Boletim meteorológico 2018. Viçosa, 2018. http://www.posmet.ufv.br/?page_id=1253 (acessed 24 January 2018).

7. ARTIGO 3. INNOVATIVE HYBRID SYSTEM FOR WASTEWATER TREATMENT: HIGH-RATE ALGAL PONDS FOR EFFLUENT TREATMENT AND BIOFILM REACTOR FOR BIOMASS PRODUCTION AND HARVESTING[iii]

Abstract

The use of algal biomass still faces challenges associated with the harvesting stages. To address this issue, we propose an innovative hybrid system, in which a biofilm reactor (BR) operates as an algal biomass production and harvesting unit connected to a high-rate algal pond (HRAP), a wastewater treatment unit. BR did not interfered with the biomass chemical composition (protein = 32%, carbohydrates = 11% and total lipids = 18%), with the wastewater treatment (removals efficiency: chemical oxygen demand = 59%, ammonia nitrogen = 78%, total phosphorus = 16% and Escherichia coli = 1 log unit), and did not alter the sedimentation characteristics of the biomass (sludge volume index = 29 mg/L and humidity content = 92%) in the secondary settling tank of the hybrid system. On the other hand, the results showed that this technology achieved a biomass production about 2.6x greater than the conventional system without a BR, and the efficiency of harvesting of the hybrid system was 61%, against 22% obtained with the conventional system. In addition, the BR promoted an increase in the density (~ 1011 org/m²) and diversity of microalgae in the hybrid system. Chlorella vulgaris was the most abundant species (> 60%) from the 4th week of operation until the end of the experiment. Hence, results confirm that the integration of BR into a wastewater treatment plant optimised the production and harvesting of biomass of the hybrid system, making it a promising technology. The importance of economic and environmental analysis studies of BR is highlighted in order to enable its implementation on a large scale.

Keywords: algal biofilm, attached growth, domestic sewage, microalgae, secondary settling tank.

[iii] Artigo publicado:

Assis, L.R., Calijuri, M.L., Assemany, P.P., Silva, T.A., Teixeira, J.S. Innovative hybrid system for wastewater treatment: High-rate algal ponds for effluent treatment and biofilm reactor for biomass production and harvesting. Journal of Environmetal Management, 274, 111183, 2020. https://doi.org/10.1016/j.jenvman.2020.111183

7.1.Introduction

The cultivation of microalgae in wastewater is considered an economical approach to provide water and nutrients for algal growth, especially given the current need to change the wastewater treatment paradigm for resource recovery (Zhang et al., 2018a). After its separation and harvesting, microalgae biomass can then be used as an alternative source for energy, animal feed and fertiliser production (Choudhary et al., 2020).

Despite our comprehensive knowledge about the potential of microalgae in wastewater for the generation of several bioproducts, the use of algal biomass still faces challenges associated with the separation and harvesting stages. The efficiency of separation and harvesting can be influenced by several factors such as the morphology of microalgae, the density and size of cells, the final bioproduct to be obtained and the culture medium (Singh and Patidar, 2018). Regardless of the separation method, it must overcome the main difficulties associated with the separation of microalgae. Thus, it must consider the small size of the cells (< 20 μm), the similarity of the microalgae density to water, a surface with a high negative charge (zeta potential) and high growth rates, resulting in more frequent harvests. In this sense, the challenges regarding the economic viability of the recovery and valorisation of microalgae biomass still persist (Singh and Patidar, 2018).

In view of the various separation and harvesting techniques not yet consolidated in the scope of biomass recovery, new reactors are being designed on smaller scales with the objective of minimising the above-mentioned disadvantages (Foladori et al., 2018). The use of biofilm reactors (BRs) is a technological innovation that consists of a matrix of microalgae, bacteria and fungi grown attached to a solid support (Sutherland and Craggs, 2017; Mantzorou and Ververidis, 2019). In these reactors, light, temperature, as well as nutrients concentrations are the main factors that determine the growth rate of microalgae biomass, which are dominant in the microorganism consortium matrix. Periodic harvesting of biomass removes nutrients assimilated by microalgae and the reactor can be considered a biological treatment system used to mitigate wastewater pollution (Sutherland and Craggs, 2017).

Therefore, the main advantage of BRs when compared to conventional forms of separation is the ease of removing microalgal cells from the solid surface by scraping (Zhang et al., 2018b). After scraping, the organisms that remain on the material are used as inoculum for the next growth cycle (Assis et al., 2017). This reactor type has a higher productivity, in addition to a lower surface area demand, than suspended cultivation systems (Mantzorou and

Ververidis, 2019). Furthermore, the amount of water in the biomass is low, eliminating the dewatering process, and microalgal biofilm receives more light when compared to biomass suspended in the water column (Zhang et al., 2018b). High-rate algal ponds (HRAPs) are efficient reactors for the treatment of wastewater and the production of microalgae. In this sense, BRs can be connected to these units, forming a hybrid system for biomass production and wastewater treatment. The integration of these two technologies emerges as an improvement of the existing wastewater treatment plants (WWTP) (Zhang et al., 2018a).

In the literature it is possible to find positive results from the implementation of hybrid systems. Zhang et al. (2018a) cultivated microalgae in synthetic wastewater with different nutrient loading rates and reported biomass productivity values in the range of 10.54 – 14.68 g/m^2.day. Based on laboratory scale studies, a methane production potential of 21.471 - 29.136 m^3/ha.year and a biodiesel productivity of 0.57 - 1.15 ton/ha.year were estimated. The authors reinforce the need for studies on a pilot scale, outdoors, to assess the reality of nutrient removal and biomass production, as well as its by-products. In the study of Zhang et al. (2018b), also on a laboratory scale, the distances between the BRs submerged in HRAPs were evaluated with different synthetic wastewater. Productivity decreased with increasing distance, with a distance of 2 cm showing the best result (18.51 g/m^2.day).

Among the pilot scale studies, Assis et al. (2017) reported that the presence of BR in an HRAP was able to supply the demand for carbon dioxide, avoiding the additional supplementation of this gas in optimizing the growth of microalgae in wastewater. Lee et al. (2014) evaluated nylon meshes submerged in a HRAP for 18 days and reported a maximum biomass productivity of 13.5 g/m^2.day, about 4x higher than a conventional HRAP. Gross et al. (2013; 2015) evaluated a hybrid system composed of revolving algal biofilm (RAB) adapted in HRAPs. The hybrid systems showed greater evaporation of water, due to the greater contact of the RAB with the air (Gross et al., 2015) and authors emphasized that the RABs systems have the potential to commercially produce microalgae with high productivity and efficient use from water.

In view of the need for new reactors that improve the separation and harvesting of suspended grown microalgae, this study presents, as an innovation, the pilot-scale application of a BR connected to a wastewater treatment system, composed of HRAP and secondary settling tank. The main objective was to evaluate the interference of this reactor in the production, separation and harvesting of algal biomass.

7.2.Materials and Methods

7.2.1. Culture medium

The experiments were carried out in the experimental area of the Laboratory of Sanitary and Environmental Engineering at the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (20º45’14”S, 42º52’54”W). The domestic sewage used as a culture medium for biomass production was originated from a septic tank at a real-scale WWTP. No inoculum was added, and therefore, native species developed in the culture medium, with a consortium of bacteria, microalgae and other microorganisms.

7.2.2. Wastewater treatment systems and biomass production

Two systems of domestic sewage treatment, biomass production and harvesting, were evaluated on a pilot scale. System 1 was comprised of a conventional system consisting of a high-rate algal pond (HRAP 1) and a settling tank (ST 1). System 2 was comprised of a hybrid system of biomass production (suspended and biofilm), in which the suspended biomass grew in a HRAP (HRAP 2) and the biofilm was contained in a biofilm reactor (BR), followed by a settling tank (ST 2). Figure 7.1 shows the scheme of the two systems used in this study. Monitoring was carried out between February and August 2019, a period that included the end of summer, autumn and the beginning of winter. Photosynthetically active radiation (PAR) was measured weekly (always between 12 and 2 pm) using a LI-COR LI-1500 Light Sensor Logger radiometer. The daily precipitation (data obtained from climatic station and available at the UFV (2019)) and the PAR throughout the experimental period are shown in Figure 7.2.

The HRAPs had the following characteristics: width, 1.28 m; length, 2.86 m; total depth, 0.5 m; surface area, 3.3 m^2. They were made of fiberglass and steel paddlewheels, with six blades; paddlewheels were driven by a 0.5-hp electric motor. The speed was reduced by a speed reducer coupled to the motor and controlled by a frequency inverter (WEG CFW-10 series). The HRAPs were operated in continuous flow, with a useful depth of 0.3 m, a flow rate of 0.1 m^3/day and a hydraulic retention time (HRT) of 10 days. Settling tanks were positioned downstream of each HRAP and had a surface area of 0.16 m^2, a useful volume of 0.037 m^3, an HRT of 8.9 hours (8 hours and 54 minutes) and a surface loading rate of 0.625 m^3/m^2/day. The effluent from each HRAP was sent by gravity to each settling tank, and the concentrated biomass was collected weekly.

Figure 7.1. Scheme of wastewater treatment and biomass production systems: (a) System 1, composed of HRAP and settling tank (ST1); (b) System 2, composed of HRAP, BR (in the photo BR with α = 90º) and settling tank (ST2). The angle α in the BR indicates the variation in panel inclination during the experiment.

The BR was made of a flat acrylic panel and had the following characteristics: total surface area of 0.5 m^2, measuring 1.0 m in width and 0.5 m in length. The panel was kept in direct contact with atmospheric air and solar radiation; it was installed next to HRAP 2 and supported by PVC pipes at 0.85 m from the ground. To allow the growth of biofilm, the panel was coated with polyester (SkTextil, oxford, 100% polyester), as previously studied in Assis et al. (2019). The HRAP effluent was recirculated to the BR during 10 h per day, i.e. the useful volume of the pond was recirculated 10 times in a day, using an underwater pump (Sarlobetter SB 1000A) at a 1 m^3/h flow rate. After being pumped, effluent was percolated through the panel surface by dripping, collected in a gutter and returned to the HRAP by gravity.

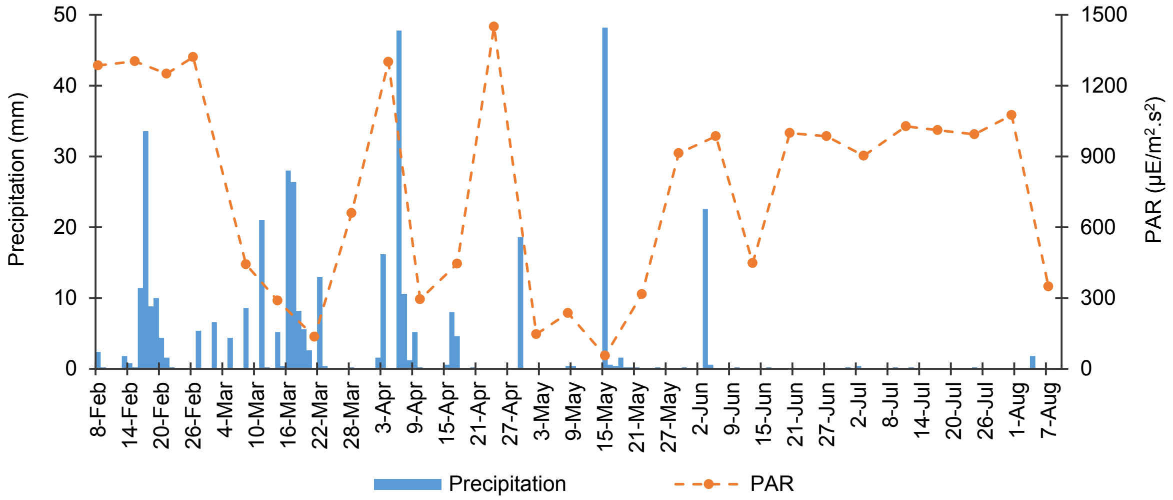

Figure 7.2. Daily precipitation (mm) and photosynthetically active radiation (PAR, μE/m^2. s^2) throughout the experimental period.

The inclination of the BR panel varied according to the season, due to the interference of precipitations in the growth of the biofilm. The inclination angles were defined in preliminary tests (data not shown); heavy rain events can compromise the performance of BRs, causing detachment of biomass, interruption in the capacity to remove nutrients and difficulty in colonising new algal cells, as reported by Sutherland and Craggs (2017). Therefore, in the rainy season (summer), the BR panel was maintained at 90° in relation to the horizontal plane, while in the dry seasons (autumn and winter), the BR panel was maintained at 15° in relation to the horizontal plane.

7.2.3. Scraping frequency of the biofilm reactor

For the determination of the total scraping frequency of the system 2 BR, a preliminary test with two growth periods was performed. The initial period of biofilm growth in the BR required one 1 week for the adaptation and attachment of the cells to the support material. From the second week of operation, sampling started to monitor biofilm development. The progressive accumulation of biofilm on the reactor was observed over 4 weeks of biomass monitoring. After the production peak, in the 5th week of monitoring, detachment of cells in the upper layers of the formed biofilm was noted, and the biomass was completely scraped off. The first 4 weeks of monitoring determined the first growth cycle of biomass. However, as stated by literature (Johnson and Wen, 2010; Christenson and Sims, 2012; Gross et al., 2013; Assis et al., 2017; Assis et al., 2019), after the first scraping of the biofilm, the new biofilm growth cycle has a higher yield compared to the initial growth, and the permanence of the inoculum saves initial downtime of the cells to the support materials (Gross et al., 2013). Therefore, it is not necessary to maintain the same period as for the first growth cycle and, in this study, the scraping frequency was determined every 3 weeks to ensure that no subsequent biomass loss occurred. The remaining biomass was kept on the support material and used as inoculum for the subsequent five biomass growth cycles.

7.2.4. Analytical measurements

The production of total biomass (g/m^2 of volatile solids) and the production of chlorophyll-a (g/m^2), an indirect measure of the algal biomass fraction, were monitored in each reactor of the systems on a pilot scale: in the effluents of the HRAPs, in the effluents from the settling tanks and, for system 2, both were monitored in the BR.

In the biofilm of the BR, samples were collected weekly by scraping with the aid of a spatula on the panel of the BR. Samples from three positions of the surface area of the panel were scraped (sample on the right, left and middle). To quantify the total biomass, an area of 6.25 cm^2 was sampled from each position of the panel, and total volatile solids (TVS) were analysed (APHA, 2012). In chlorophyll-a analysis, which 1.0 cm^2 of each panel position was sampled and subsequently diluted in 20 ml of distilled water. The 80% ethanol extraction technique (Nush, 1980) was used for the analysis, the reading was obtained by spectrophotometry (APHA, 2012), and the calculations were performed using the equations described by Schwarzbold et al. (2013), adapted from Marker et al. (1980) and Sartory and Grobbelaar (1984).

Suspended biomass was also analysed once per week. Volatile suspended solids (VSS) analysis was performed in the HRAPs effluent to determine the total biomass, according to the methodology described in APHA (2012); for determination of chlorophyll-a, the 80% ethanol extraction technique was used, the reading was performed by spectrophotometry (APHA, 2012), and the calculations were performed using the equations provided in the Dutch norm (NEN, 1981). In the effluent from the settling tanks, in addition to the same VSS and chlorophyll-a analyses described for the HRAPs samples, the following variables were also determined: ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP) and total chemical oxygen demand (COD), following the methodology described by APHA (2012). The chromogenic-fluorogenic method (Colilert®) was used to determine Escherichia coli (E.

coli). Dissolved oxygen (DO), temperature and pH of the culture medium were measured using the Hach HQ40d probe (Luminescent Dissolved Oxygen - LDO - for DO). In the raw domestic sewage, with the exception of chlorophyll-a, analyses of the same variables mentioned for the samples from the HRAPs and settling tanks were carried out.

7.2.4.1. Biomass separation and harvesting

The separation of biomass in the settling tanks was evaluated for humidity content, sedimentation and daily harvesting. The humidity content on a wet basis and the sedimentation of the sludge were evaluated using the sludge volume index (SVI) (APHA, 2012). The amount of harvested biomass (by mass) per unit of time was obtained according to Equation (7.1). Weekly, sludge was collected manually, and its volume was measured in a graduated cylinder.

$$\text{Harvesting}_{\text{ST}}\ (\text{g}\,.\,\text{day}^{-1}) = \left(\frac{\text{TVS}_{\text{ST}}\,.\text{V}_{\text{sludge}}}{\text{T}}\right) \qquad \text{Equation 7.1.}$$

where:

TVS_{ST}: TVS concentration in the sludge collected in each settling tank (g/L);

V_{sludge}: volume of collected sludge (L);

T: sludge accumulation time in the settling tanks (7 days).

Biomass separation in the BR was evaluated for humidity content and daily harvesting. The amount of harvested biomass (in mass) per unit of time in the BR was obtained according to Equation (7.2):

$$\text{Harvesting}_{\text{BR}}\ (\text{g}\,.\,\text{day}^{-1}) = \left(\frac{\text{TVS}_{\text{BR}}\,.\text{A}_{\text{system}}}{\text{T}}\right) \qquad \text{Equation 7.2.}$$

where:

TVS_{ST}: TVS concentration of biomass concentrated in the BR (g/m^2);

A_{system}: area of biomass production in the system. For system 2, the area was equivalent to the sum of the surface area of HRAP 2 (3.3 m^2) and the transversal area of the BR (0.5 m^2), resulting in an area of 3.8 m^2. For system 1, this equation was not used because this system did not have a BR.

T: growth period of the biofilm in the BR (21 days).

7.2.4.2. Calculation of biomass productivity

In the BR, the biofilm showed a cumulative growth, and in the HRAPs, due to the culture medium flow, the suspended biomass was in continuous growth and retention by the settling tank. Therefore, to compare the biomass productivity between the two systems, the average production of HRAP 1 was considered in system 1. For system 2, the average production of HRAP 2 and the average peak production of each growth cycle of the BR were considered. The area used to calculate productivity in system 2 was the sum of the area of HRAP 2 and the BR, as shown in Equation (7.3).

$$\mathrm{TPr} = \frac{\mathrm{P_{HRAP}} \cdot \mathrm{V_{HRAP}}}{\mathrm{A_{system}} \cdot \mathrm{HRT}} + \frac{\mathrm{P_{BR}}}{\mathrm{T}} \qquad \text{Equation 7.3.}$$

where:

TPr: total system productivity ($g/m^2/day$);

P_{HRAP}: VSS average production of HRAP (g/m^3);

V_{HRAP}: HRAP volume ($1\ m^3$);

A_{system}: area of biomass production in the system. For system 1, the area was equal to the surface area of HRAP 1 ($3.3\ m^2$). For system 2, the area was equivalent to the sum of the surface area of HRAP 2 ($3.3\ m^2$) and the transversal area of the BR ($0.5\ m^2$), resulting in an area of $3.8\ m^2$.

HRT: HRAPs hydraulic retention time (10 days);

P_{BR}: average peak production of TVS in each BR growth cycle (g/m^2). In system 1, P_{BR} was zero.

T: growth period of biofilm in BR (21 days).

7.2.5. Biomass characterisation

The biofilm of the BR and the biomass concentrated in each settling tank were lyophilised, followed by the determination of ash and Total Kjeldahl Nitrogen contents (adapted from APHA, 2012). The protein content was defined by the nitrogen-to-protein conversion factor of 6.25 (Zhong et al., 2012). The carbohydrate content was determined via quantitative acid hydrolysis of the biomass (Hoebler et al., 1989), followed by the phenol-sulfuric method (Dubois et al., 1956) and spectrophotometry (490 nm) using the standard glucose curve. The lipid content was determined using the Soxhlet extraction method (AOAC, 2000). After macerating the biomass, neutral lipids were extracted in the fat determiner (Tecnal TE-044-8 / 50) for 6 hours, with 99% hexane as solvent. Subsequently, in the same equipment, membrane lipids were extracted with 96% ethanol for 3 hours and quantified via gravimetry.

Characterisation of the microalgae community was carried out with the samples biofilm of the BR, collected weekly, during the first 6 weeks of operation to follow the initial development of the species. Subsequently, the samples biofilm of the BR, together with the suspended samples from both HRAPs, were collected monthly. For characterisation, 50 ml of suspended samples were collected on the HRAPs surfaces, and samples of 18.75 cm² were scraped form the BR and diluted in 50 ml of distilled water. All samples were kept in a 4% formaldehyde solution. Characterisation was performed at the genus level, and for the dominant genera, the species present were identified. For quantitative analysis, individuals were counted in a sedimentation chamber under an inverted microscope, using the method of Uthermöhl (1958). The density of the organisms was determined using the criteria described in APHA (2012). For qualitative analysis, identification was performed using an inverted microscope, according to Parra et al. (1982) and Komarek and Fott (1983).

7.2.6. Statistical analysis

All statistical analyses were performed using the Minitab®18 software. To compare the production, separation and characterisation of biomass between the two systems, analysis of variance (ANOVA) was performed, followed by the Tukey means test at 5% probability of error. Principal components analysis (PCA) was performed to assess the real contribution of each variable to the total variability of the quality data of the treated effluent, nutrients and organic matter, at the outlet of the settling tanks. An individual analysis was carried out to determine the main parameters influencing the dynamics of the conventional and hybrid systems.

7.3. Results and Discussion

7.3.1. Microalgae community

Figure 7.3 shows the density of organisms in the biofilm of the BR (Figure 7.3a) and in the suspended medium of the HRAPs (Figure 7.3b). Both HRAPs had similar results, with logarithmic units between 10^7 and 10^8 org/m^2 throughout the experimental period. The density of organisms in the BR was, during the entire experimental period, higher than that of HRAP, with a peak in late summer of 5 x 10^{11} org/m^2, while in the other months, it always remained around 10^{11} org/m^2. According to Sutherland and Craggs (2017), the development of the microalgae community in a biofilm system is strongly influenced by radiation and the concentration of nutrients in the culture medium. The amount of light available for photosynthesis by microalgae depends on the thickness of the biomass for BRs and on the depth of the water column for suspended systems. In the latter, the light that passes through the water column decreases exponentially with depth, as the particles absorb or disperse the light. In BRs, photons are instantly transformed into chemical energy by microalgae. In addition, productivity and nutrient assimilation are dependent on the availability of light, the absorption efficiency and the use of light by algae (Sutherland and Craggs, 2017).

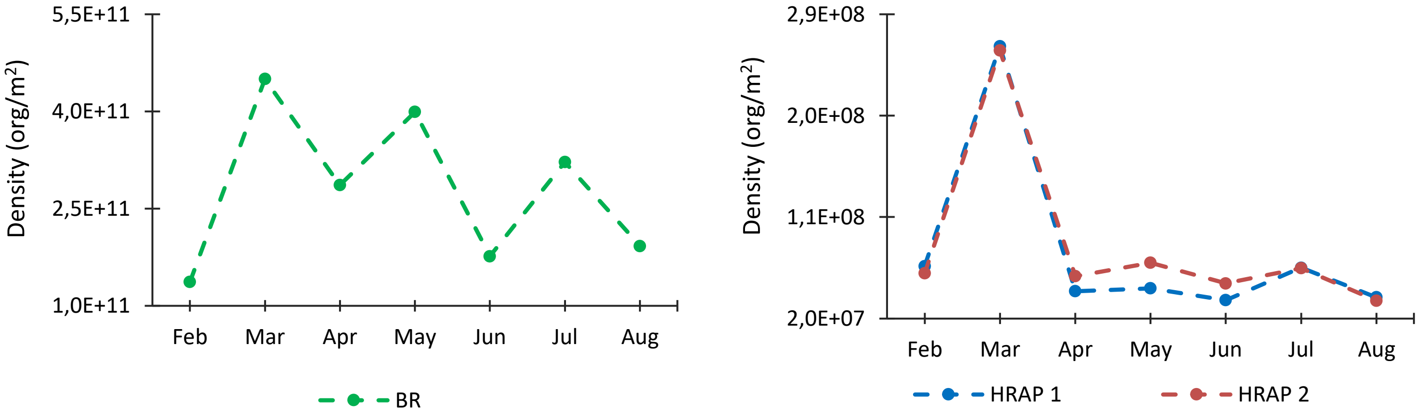

Figure 7.3. Density of organisms (org/m^2): (a) BR; (b) HRAPs.

This explains why the variability of organisms in the BR, throughout the experimental period, was less than that in the HRAPs. The coefficients of variation of the organisms over the experimental period were 94, 84 and 39%, respectively, for HRAP 1, HRAP 2 and BR. It is believed that seasonal changes did not interfere with the density of organisms, since the experiment was performed in a country with a hot subtropical climate, with high temperatures and incidence of radiation throughout the year, with little difference between seasons (Assemany et al., 2015).

Although BR had a higher organism density throughout the experimental period, this reactor did not influence the density of organisms in HRAP 2, when compared to HRAP 1.

However, as seen in Figure 7.4, the BR interfered with the abundance of HRAP 2 species and genera. During the first 6 weeks of operation (Figure 7.4a), the abundance of individuals in the BR was monitored weekly and, from the 3rd to the 7th month of operation (Figure 7.4b), the abundance of individuals in the BR was monitored monthly. This monitoring was important to record the ecological succession of the biofilm, in order to monitor the alternation of the pioneer community until the stabilization of the biofilm. In the first week, the species Tetradesmus obliquus (73%) was predominant in the BR. In the following weeks, Chlorella vulgaris became more abundant and, from the 4th week of operation, this species became predominant in the BR (> 60%) until the end of the experiment. The monthly samples, starting in April, showed the appearance of the species Eutetramorus fottie and the genus Desmodemus throughout the BR operation.

However, in both systems, other species and genera appeared throughout the experimental period. And, although the culture medium of both HRAPs remained within the optimum temperature range (between 15 to 30ºC) for most microalgae species to perform photosynthesis and cell division (Assis et al., 2017), the temperature decreased by approximately 8ºC throughout the experimental period, changing the species of the microalgae community of the suspended and biofilm systems.

In system 2, the abundances of the species Didymocystis inermis, Monoraphidium contortum and Tetradesmus obliquus and of the genera Navicula and Desmodemus were greater than in system 1 (HRAP 1, Figure 7.4c), both in the BR and the HRAP 2 (Figure 7.4d). In the last month of operation (August), the increase in the abundances of Eutetramorus fotti, Tetradesmus obliquus and Navicula altered the predominance of the HRAP 2 community. In the same period, Eutetramorus fotti and Navicula were in the BR at higher densities. The direct contact of the BR with solar radiation resulted in a greater species and genera heterogeneity of system 2 in relation to system 1. Similar results were found by Assemany et al. (2015), who evaluated the phytoplankton composition of three HRAPs submitted to different levels of solar radiation. The authors concluded that the HRAP with a 30% block of solar radiation allowed greater homogeneity in the phytoplankton community and less variability over the course of 1 year.

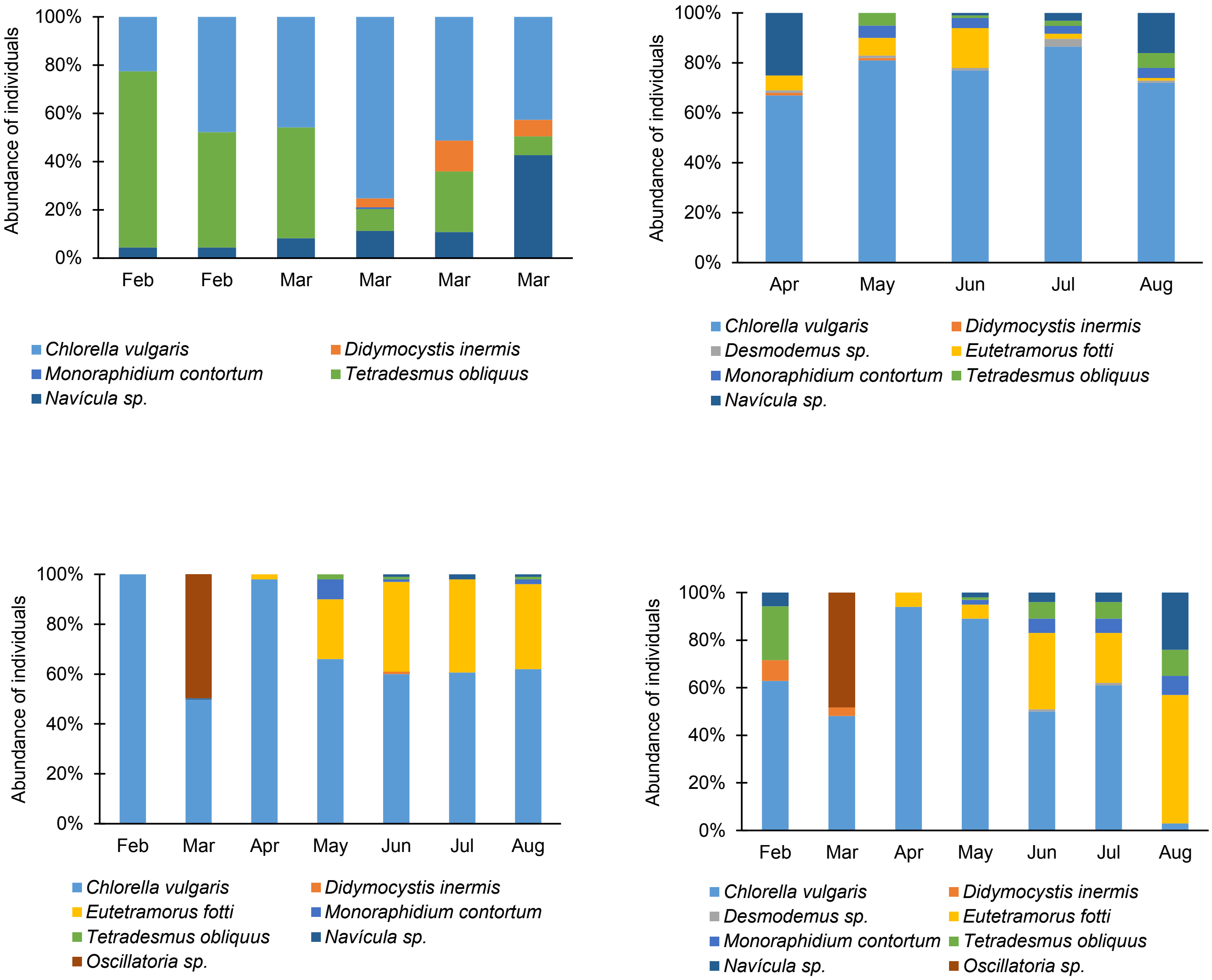

Figure 7.4. Abundance of individuals (%): (a) during the first six weeks of BR operation; (b) during the last months of BR's operation; (c) HRAP 1; (d) HRAP 2.

7.3.2. Biomass separation, harvesting and production

Table 7.1 shows the characteristics of separation and harvesting of the concentrated biomass in the settling tanks and in the BR. Biomass separation and harvesting were evaluated according to the values of SVI, humidity and daily harvesting. Regarding the SVI results, there was no difference between the settling tanks ($p > 0.05$), indicating that the presence of BR in system 2 did not change the quality of sludge sedimentation in ST 2. According to

Janczukowicz et al. (2001), both systems can have suitable properties for sedimentation of wastewater sludge (SVI < 150 mL/g), explained by the SVI optimisation of the sedimentation of wastewater sludge combined with microalgae (Wang et al., 2016).

Table 7.1. Characteristics of separation and harvesting of the concentrated biomass in the settling tanks and in the BR (standard deviations are shown in parentheses).

		SVI (ml/g)	Humidity* (%)	Harvesting (g/day)
System 1	ST 1	21.12^{a} (7.71)	91.84^{a} (1.79)	2.64^{b} (1.04)
System 2	ST 2	29.65^{a} (15.25)	91.67^{a} (2.01)	2.23^{b} (1.36)
	BR	-	93.89^{a} (4.99)	11.45^{a} (7.98)
	Total (ST 2 + BR)	-	-	13.68

Note: * Humidity relative to the harvested sludge in the settling tanks and the scraped biomass from BR. Significant at the 5% probability of error level by the Tukey test; the numbers followed by the same letter in the column did not differ statistically.

Wang et al. (2016), when evaluating the sedimentation of Chlorella sp. grown in contact with solar radiation and wastewater from activated sludge, found that the alga-bacteria combination obtained the best sedimentation efficiency, with a SVI of 42 mL/g, which represented a 44% reduction in SVI of sedimented sludge without the combination with microalgae. The alga-bacteria combination is formed through connections made via bioflocculation, in which the cell surface properties of the microalgae, combined with the extracellular polymeric substances and the cation content of the bacteria, influence the formation and stability of the biomass (Su et al., 2011). Therefore, due to the formation of denser and more compact flakes, the sedimentability of the sludge composed of algae and bacteria is improved, indicating the maturation of wastewater (Zhang et al., 2019).

The humidity content varied from 91 to 94%, and there was no statistical difference between the settling tanks of the two systems and the BR ($p > 0.05$). The humidity content and the biomass concentration are related and are important characteristics in determining the method for biomass harvesting. The equality in humidity content of the biomass harvested from the settling tanks and from the BR demonstrated the potential of the BR as a biomass separation unit, since the settling tanks are considered traditional systems of biomass separation in a WWTP. In addition to the secondary settling tank, the BR biomass concentration efficiency was comparable to those of other algae biomass harvesting technologies, such as dissolved air flotation (1 to 8%), centrifugation (10 to 22%), flocculation (1 to 15%) and filtration membranes (2 to 27%) (Deconinck et al., 2018). In a survey of microalgae harvesting methods carried out by Deconinck et al. (2018), these technologies demanded more energy for operation than the BR. In the same study, BRs required an energy of up to 0.2 KWh/m³, while other technologies such as filtration membranes, secondary settling tank and dissolved air flotation demanded up to 10, 10.8 and 20 kWh/m³, respectively. These results represent about 50 to 100 times more energy needed than for the functioning of the BR.

In relation to the daily biomass harvesting, the value for the BR (11.45 g/day) was about 5 times higher in relation to those for the settling tanks ($p > 0.05$). Although the BR is a simultaneous production and separation reactor, it was not operated in isolation, without a HRAP unit. Therefore, in this system, biomass was produced in HRAP 2 and BR and separated in ST 2 and BR. In system 1, biomass was produced in HRAP 1 and was only separated in ST 1. In view of these results, the BR showed potential as a biomass separation unit, and its large-scale application should be further investigated, aiming at its implantation in a WWTP to minimise the difficulties associated with biomass separation and harvesting.

The production of chlorophyll-a and volatile solids in HRAPs and BR is shown in Table 7.2. Chlorophyll-a production was approximately three times ($p = 0.00$) higher in the BR than in thee HRAPs, which is in agreement with the results previously discussed in 3.1 (Figure 7.4), in which the highest density of photosynthetic organisms occurred in the BR throughout the experimental period. The higher algal production in the BR was also confirmed by the ratio of chlorophyll-a to total biomass. The BR and both HRAPs showed good proportions of microalgae cultures in relation to total biomass, indicated by values from 1 to 1.5%, according to Veloso et al. (1991), showing healthy microalgae cultures. It is noteworthy, however, that in the BR, this ratio was 2.55, about 1.7 times greater than in HRAP 1 (1.49%) and 1.5 times greater than in HRAP 2 (1.68%).

Thus, the integration of BR in system 2 promoted the production of a biomass richer in photosynthetic organisms than system 1. The higher concentration of chlorophyll-a in the BR was associated with the intensification of the photosynthesis activity in this reactor. The interactions between microalgae and bacteria in biofilm systems allow a more stable symbiosis than in suspended systems (Liu et al., 2017). The binding of bacteria to the cell surfaces of microalgae provides a microenvironment with higher concentrations of nutrients and lesser effects of microalgae self-shading when compared to suspended cultivation medium, in addition to a greater transport efficiency of CO_2/O_2 masses (Zhang et al., 2020). Similar to the production of chlorophyll-a, the higher production of total biomass in BR plus the production of total biomass in HRAP caused an increase of about 2.6 times in relation to system 1 ($p = 0.05$).

Table 7.2. Biomass production in HRAPs and BR (standard deviations are shown in parentheses).

		Production of chlorophyll-a		Total biomass production (volatile solids)	
		Production (g/m^2)	Productivity (g/m^2.day)	Production (g/m^2)	Productivity (g/m^2.day)
System 1	HRAP 1	0.55^b (0.38)	0.05^a (0.04)	36.75^b (18.05)	3.68^a (1.81)
System 2	HRAP 2	0.48^b (0.29)	0.05^a (0.03)	28.79^b (11.28)	2.88^a (1.13)
	BR	1.64^a (0.93)	0.08^a (0.04)	64.28^a (29.83)	3.25^a (1.10)
	Total (HRAP 2 + BR)	2.12	0.13	93.07	6.13

Note: Significant at the 5% probability of error level by the Tukey test; the numbers followed by the same letter in the column did not differ statistically.

Comparing the results of biomass production and harvesting in each system, the integration of the BR increased the harvesting efficiency of system 2. In this system, the amount of total biomass produced was 22.39 g/day, representing 10.94 g/day produced in HRAP1 and 11.45 g/day in the BR. Of the produced biomass, 61% were harvested, comprising 11.45 g/day of harvested biomass in the BR and 2.23 g/day in the ST 2. For system 2, the BR accounted for 84% of the total harvesting efficiency. In system 1, total biomass retention was only 22%, with total production being 12.13 g/day and harvesting in ST 1 2.64 g/day.

Contrary to this study, most studies of hybrid systems evaluated the system's harvesting and biomass production only in the BR (Table 7.3). Therefore, in order to compare the results of this study with the literature, the daily biomass productivity of the BR (11.45 g/day) was converted into daily biomass productivity per surface area of the support material (0.5 m^2), totalling 22.9 g/m^2.day. This result was close to the biofilm production values reported by Zhang et al. (2018a; 2018b) and greater than the results found by Gross et al. (2013), Lee et al (2014), Gross et al. (2015), Assis et al. (2017) and Zhao et al. (2018). It is important to highlight that the BRs used in these studies have different configurations (submerged, rotating, not submerged), in addition to different support materials and, in some cases, the use of synthetic cultivation medium was done, as can be observed in Table 7.3.

Both settling tanks and BRs are reactors that involve mechanical methods of separating and harvesting algal biomass. When it comes to the application of these separation units on a large scale, some considerations must be highlighted. For example, the settling tanks used in the present study were not operated in the same way as in other studies that used settling tanks to separate algal biomass (Park et al., 2015). In the present study, the sludge accumulated over 1 week was only collected and was not recirculated to the HRAP. Park et al. (2015) demonstrated that the recirculation of biomass formed by algae and bacteria, collected by gravitational sedimentation, improved biomass productivity, sedimentation efficiency and microalgae species control. In relation to sedimentation, the recirculation of the biomass promoted an increase of approximately 60 to 85% in the size of the colony of Pediastrum boryanum, and the flakes formed exceeded 500 µm in diameter. However, it is noteworthy that in order to treat domestic sewage and separate biomass rich in microalgae, the results of the sedimentation characteristics of the settling tanks in this study are in line with the findings of other researches (Wang et al., 2016; Muylaert et al., 2017). When it is desired to separate the microalgae for the purpose of harnessing low added value biomass, gravity sedimentation combined with wastewater treatment is an attractive option, as in this culture medium, the microalgae can be deposited within about 30 minutes via natural bioflocculation (Wang et al., 2016; Muylaert et al., 2017).

The expectations for BRs in the production and separation of biomass in relation to conventional systems are better than those for settling tanks; however, few studies address the feasibility of their application on a large scale, mainly because of the different configurations of BRs already developed on a laboratory or pilot scale. In this sense, some prospects can be raised in order to advance future research for the optimisation of BRs, such as: life cycle assessment, economic feasibility, feasibility of expanding the scale of BRs for implantation in a WWTP.

Some criteria related to the integration of these reactors can be discussed from an economic perspective. For example, the costs involved in the stages of growth, separation and harvesting of biomass in suspended systems, aiming at the production of biofuels, account for about 70% of the total production costs. In suspended systems connected to BRs, these costs would be reduced because the system is simultaneously a biomass-producing and separating unit.

Table 7.3. Comparison of different hybrid systems.

References	**Culture medium**	**Operation**	**Support material**	**Harvesting frequency (days)**	**Biofilm harvesting* (g/m².day)**
Gross et al. (2013)	Chlorella vulgaris	Continuous	Cotton	7	3.5
Lee et al. (2014)	Wastewater	Batch	Nylon	18	9.1
Gross et al. (2015)	Chlorella vulgaris	Semi-continuous	Cotton	7	5.5
Assis et al. (2017)	Wastewater	Continuous	Cotton	42	9.9
Zhao et al. (2018)	Wastewater	Semi-continuous	Cotton	7	7.0
Zhang et al. (2018a)	Synthetic wastewater	Batch	Velvet	8	14.7
Zhang et al. (2018b)	Synthetic wastewater	Batch	Velvet	8	18.5
This study	Wastewater	Continuous	Polyester	21	22.9

*Harvesting performed only in the BR. Harvesting based on total surface area of the attaching material.

Due to the greater production and harvesting capacity of immobilised biomass, mixing and agitation costs would be saved in the BR (Moreno-Garcia et al., 2017; Wang et al., 2017), while pumping the culture medium to the BR would be linked to a higher energy cost. In addition, the energy costs of mixing the effluents for wastewater treatment in the HRAP would be maintained. Also, harvesting in the BRs can be carried out using the simplified scraping method, reducing the costs related to the downstream processing of biomass separation from the final effluent disposal (Zhang et al., 2018b). However, with the expanded scale, scraping may no longer be advantageous due to the huge investment in human labour, and therefore, automated technologies should be considered, further increasing financial investment (Wang et al., 2017).

Zhang et al. (2018b) designed a mechanical harvester for potential scale-up. The project was based on the automated operation of the harvesting step, without any human effort. However, the equipment has not yet been tested and information regarding investments and energy demand has not been reported. Cultivation time and harvesting frequency are operational aspects that deserve researchers' attention. The greater the production of biofilm in less period of time would be the optimal condition for expanding the scale of the BRs. However, it can be observed from the literature that for microalgae cultivation in wastewater, there is still a wide range of harvesting frequency applied (Table 7.3).

Harvesting frequency is strongly influenced by the ability of cells to adhere to the support material on which the biofilm grows. This technical aspect of BRs has attracted the attention of researchers and many materials have already been tested, including cotton, polyester, nylon and velvet (Table 7.3).The attachment of cells to the support material depends on the properties of the microalgae (surface charge, hydrophile, geometric structure, cell wall composition, secretion), properties of the support material (surface charge, hydrophile, surface structure), medium composition and the hydrodynamic conditions (Wang et al., 2017). However, little is known about the interactions between the physicochemical properties of microalgae cells and the support materials and how it affects the growth of biomass. These scientific issues are fundamental and deserve further study (Wang et al., 2017).

Regarding the establishment of a BR on a large scale, the fact that it protects the biofilm against the influence of weather, especially heavy rains, which can negatively affect the growth of microalgae, must also be considered (Sutherland and Craggs, 2017; Garbowski et al., 2017). The solution adopted in the present study was to rotate the BR panel according to the season. Measures to protect biofilm should be further investigated. Covering, for example, would substantially increase capital costs, making it economically impossible to mitigate the loss of biomass (Sutherland and Craggs, 2017).

The answers to all the questions raised about the economic point of view of the implementation of hybrid systems as an innovative technology can be better interpreted through a joint analysis of the results of economic viability accompanied by the feasibility of expanding the scale of the BRs for implantation in a WWTP. The convergence of these studies, in parallel with life cycle assessment, will allow a better judgment of the economic and sustainable efficiency of the BRs.

7.3.3. Wastewater treatment

Table 7.4 shows the removal of wastewater treatment monitoring variables. All variables had similar concentrations in the effluents from HRAPs (exit of their respective settling tanks) and, consequently, similar removals in the two systems. It is important to note that the high deviations of the environmental variables found in this study were associated with the uncontrollable dynamics of the system exposed to climatic conditions (Assemany et al., 2015).

Table 7.4. Removal of wastewater treatment monitoring variables.

Variable	Raw domestic sewage	HRAP 1	HRAP 1 removal (%)	HRAP 2	HRAP 2 removal (%)
pH	7.5 (0.6)	6.8 (0.6)	-	6.8 (0.7)	-
DO (mg/L)	1.0 (1.3)	7.9 (1.7)	-	7.5 (2.1)	-
COD (mg/L)	329.2 (276.2)	138.3 (132.1)	57.9	135.5 (64.5)	58.8
TP (mg/L)	9.1 (1.8)	7.9 (1.8)	13.4	7.7 (1.8)	16.2
NH_4^+-N (mg/L)	87.4 (19.8)	21.6 (6.4)	75.3	19.9 (7.6)	77.3
NO_3^--N (mg/L)	1.1 (1.5)	41.7 (17.2)	-	40.3 (14.3)	-
E. coli (MPN/100ml)	5.5×10^3* (7.3×10^4)	3.6×10^2* (2.7×10^3)	1 log unit**	4.8×10^2* (6.6×10^4)	1 log unit**

Note: * Geometric mean. ** Removal in logarithmic units. Standard deviation values in parentheses.

The integration of BR into HRAP 2 did not interfere with the treatment of the effluent, and therefore, these results will be discussed together with the results of the principal components (PC) analysis presented in Table 7.5.

Table 7.5. Wastewater treatment principal component analysis: (a) system 1, (b) system 2.

(a)

Variables	Principal components of system 1			
	PC 1	PC 2	PC 3	PC 4
COD (mg/L)	0.182	0.081	0.565	-0.733
TP (mg/L)	0.421	-0.463	-0.214	0.135
NH_4^+-N (mg/L)	0.508	-0.248	-0.262	-0.102
NO_3^--N (mg/L)	-0.008	-0.317	0.708	0.550
pH	0.315	0.618	0.124	0.344
DO (mg/L)	0.468	-0.204	0.222	-0.053
E. coli (MPN/100ml)	-0.463	-0.441	0.038	-0.106
Eigenvalue	2.8374	1.3337	1.1131	0.9354
Proportion	0.405	0.191	0.159	0.134
Accumulated	0.405	0.596	0.755	0.889

(b)

Variables	Principal components of system 2			
	PC 1	PC 2	PC 3	PC 4
COD (mg/L)	0.382	0.312	-0.351	0.617
TP (mg/L)	0.581	-0.021	0.035	-0.032
NH_4^+-N (mg/L)	0.543	-0.110	-0.346	-0.302
NO_3^--N (mg/L)	-0.264	0.408	-0.553	0.121
pH	0.162	-0.480	0.274	0.592
DO (mg/L)	0.354	0.399	0.324	-0.326
E. coli (MPN/100ml)	0.006	-0.577	-0.520	-0.238
Eigenvalue	2.4159	1.9123	0.9396	0.6769
Proportion	0.345	0.273	0.134	0.097
Accumulated	0.345	0.618	0.753	0.849

The monitoring variables of the wastewater treatment were reduced to four components of each system, which explained 88.9 and 84.9% of the variability in data from systems 1 and 2, respectively. The variables TP, NH_4^+-N and DO were more important for both systems (PC 1 and 2). The concentration of nutrients is closely related to the growth of microalgae and, consequently, to their removal due to biomass assimilation. Considering that pH values did not reach a sufficient magnitude for the volatilisation of ammonia nitrogen and chemical phosphorus precipitation (Table 7.4), it can be said that the biomass assimilation route was one of the main pathways responsible for TP removal (13 to 17%) and NH_4^+-N removal (75 to 78%).

The parameter DO is important in the consortium formed by microalgae and bacteria during the treatment of domestic sewage. This variable was indirectly associated with the proliferation of microalgae, which, through photosynthetic activity, increased the oxygen concentration in the culture medium from 1 to approximately 8 mg/L.

The concentration of E. coli in the HRAP effluents was important to diagnose the efficiency of domestic sewage treatment in relation to pathogen inactivation. The E. coli removal efficiencies were 1 logarithmic unit in both systems and were associated with pH and DO values, which did not undergo significant variations for pathogen inactivation. This variable was more important in system 1 because it had a high value on PC 1, while in system 2, its highest value was on PC 2.

The pH showed higher values on the PC 2 of both systems. This variable is extremely important to guarantee the efficiency of wastewater treatment. Both systems had similar pH values, close to neutrality (between 6 and 7), indicating that the concentration of organic matter in the effluent used as a culture medium was sufficient to ensure that microalgae growth was not limited by a lack of CO_2. Its variation is able to influence the balance of chemical compounds and to affect growth rates of microorganisms involved in biological wastewater treatment.

The variable NO_3^--N was also important in both systems as it provides information about the nitrification process. Nitrogen in the NH_4^+-N form is the preferred assimilation source, and the NO_3^--N form is the second most important nitrogen form to be consumed by microalgae. Nitrification was abundant in both systems, with concentrations between 40-42 mg/L, which represented an increase of about 3,800% of NO_3^--N. In system 2 (PC 2), this variable was more important than in system 1 (PC 3).

The concentration of organic matter in the effluents of the systems was represented by COD. The removal of COD in both systems was in the range of 57-59%, indicating a direct relationship of between microalgae and heterotrophic bacteria present in the culture medium. The DO concentrations confirmed this conclusion, since both systems had an increase of about 6 mg/L of DO, indicating the contribution of microalgae in the oxygen supply for the improvement of the activity of heterotrophic bacteria in the degradation of organic matter present in wastewater, such as domestic sewage, which has an insufficient level of oxygen (Maza-Marquez et al., 2017). The COD was more important in system 2 (PC 3) than in system 1 (PC 4).

7.3.4. Biomass characterisation

Table 7.6 shows the average values of the biomass properties harvested in the BR and in the settling tank of each system. There was no statistical difference in the composition of the biofilm of the BR and/or concentrated in the settling tanks ($p > 0.05$). Therefore, the biomass production and separation methods have no impact on its chemical composition. When compared to system 1, the BR maintained the biomass composition compared to that grown in the HRAP and separated in the settling tank.

Table 7.6. Chemical characterization of biomass (standard deviations are shown in parentheses).

Composition (%)		Ashes	Proteins	Carbohydrates	Neutral lipids	Membrane lipids	Total lipids
System 1	ST 1	20.54 (5.48)	35.21 (4.38)	10.43 (2.53)	4.15 (0.45)	10.86 (3.17)	15.01 (3.62)
System 2	BR	19.82 (4.16)	32.21 (2.80)	10.87 (3.62)	6.59 (3.44)	11.76 (4.71)	18.35 (6.31)
	ST 2	20.59 (4.24)	35.46 (2.86)	10.19 (1.84)	4.85 (0.08)	12.10 (5.68)	16.95 (5.77)

It is essential to understand the characterisation of biomass in order to properly allocate it, aiming at the best valorisation route. The biomass separated in the BR and in the settling tanks was mainly composed of proteins (32 to 36%), followed by ash (19 to 21%) and total lipids (16 to 19%) and, albeit to a lesser extent, carbohydrates (about 10%).

It is important to emphasise that the use of wastewater for the cultivation of microalgae, whether in open suspended or biofilm systems, creates a competitive environment. There is the presence of suspended solids that prevent the penetration of solar radiation, as well as toxic substances and other microorganisms that require space and nutrients, hindering the development of algal biomass and compromising the accumulation of components of interest.

In this context, the composition of algal biomass depends on the culture conditions, such as the dominant and predominant species and the conditions of the culture medium (Chew et al., 2017). In this study, Chlorella vulgaris was the dominant and predominant species in both systems. According to Chia et al. (2013), membrane lipids (structural) are dominant in the lipid composition of this microalgae, regardless of the used culture medium. In agreement with these authors, the results of the present study showed higher membrane lipid contents (between 10 and 12%) and lower levels of neutral lipids (< 7%), which are used for the production of quality biofuels.

Ash contents were directly associated with the accumulation of fixed solids from domestic sewage used as culture medium. A high ash content is usually found in algal biomass grown in wastewater, negatively influencing the properties of solid fuels. Therefore, pre-treatment of biomass is recommended to remove these impurities (Choudhary et al., 2020).

Carbohydrates are important nutrients in the composition of microalgae, and their use can be incorporated into the animal diet, contributing as a source of energy for animals and microorganisms of the gastrointestinal tract (Madeira et al., 2017). The biomass of this study had a carbohydrate content close to 10% and was close to a range of 13 to 22%, considered adequate for bovine supplementation (Choudhary et al., 2017).

Protein is one of the most important products of the microalgae biorefinery due to its contribution to human or animal nutrition. In this study, the largest composition of biomass was protein, which accounted for 32 to 36% in both systems. Cole et al. (2015) reported that the 18% protein content found in the Oedogonium macroalgae was equivalent or superior to that of many terrestrial cultures, and the algal biomass could be used as a source of supplementary protein in animal feed. In another study, it was estimated that the protein content of the microalgae Chlorella vulgaris was in the range of 50 to 60%, and its quality as similar to that of yeast, soy flour and milk protein (Madeira et al., 2017).

Therefore, given the composition of the algal biomass obtained in this study, the indicated valorisation route is the supplementation of animal feed. It is noteworthy, however, that before processing wastewater-grown algal biomass for animal feed, the removal of pathogens or any other potentially toxic compounds is highly recommended (Choudhary et al., 2017).

7.4.Conclusions

The innovative hybrid system achieved production and harvesting efficiencies 2.6 x and

5 x, respectively, greater than the conventional system composed of a HRAP without a BR. Moreover, the new technology promoted a greater diversity of autotrophic organisms and did neither interfere negatively with the biochemical composition of biomass nor with wastewater treatment. The integration of a BR optimised the production and harvesting of algal biomass, making it a promising technology to be deployed on a large scale. However, interactions between the physicochemical properties of cells and the support materials, cultivation time and harvesting frequency are aspects that deserve researchers' attention. Further research regarding the economic and environmental feasibility of the applicability of this reactor should be considered in the future.

7.5.Acknowledgements

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8. ARTIGO 4. ENVIRONMENTAL BENEFITS OF A HYBRID SYSTEM FOR ALGAL BIOMASS PRODUCTION, HARVESTING AND NUTRIENT RECOVERY UNDER A LIFE-CYCLE ASSESSMENT

Abstract

In the present study, two life cycle assessments (LCAs) were performed, both from primary data, on an experimental pilot scale. First, a sustainable choice of support material was made for the attached growth of algal biomass, and polyester was the support material chosen from the environmental perspectives. Subsequently, this support material was used in a pilot-scale BR adapted to a high-rate pond (HRP) and to a secondary settling tank, forming a hybrid system for domestic sewage treatment and biomass production and harvesting. The LCAs were conducted by Simapro® software using the ReCiPe 2016 midpoint method. The functional units were 10 g and 1 kg of harvested biomass, respectively, for the LCAs of the support materials and the hybrid system. The limits included flows of inputs and outputs of material and energy resources for the operation of the systems. The LCA of the hybrid system demonstrated that the BR contributed to a reduction of the negative environmental impacts by approximately 19% in all categories when compared to a system composed only of a high-rate pond and a secondary settling tank. Consequently, the efficiency of biomass production and harvesting by the BR reduced the energy, water, and nutrient demands of the hybrid system, which integrated approximately 23, 28 and 28%, respectively. Through LCA, the hybrid system, composed of an HRP and a biofilm reactor, proved to be a promising alternative for use in wastewater treatment in the production and harvesting of algal biomass. In addition, the increase in the number or area of reactors has included promising scenarios to optimize the harvesting of biomass grown in domestic sewage using biofilms from an environmental perspective.

Keywords: Algal biofilm; Environmental impacts; Microalgae; Sustainability; Wastewater treatment.

8.1.Introduction

The growing population and industrialization is a concern worldwide because consequently, the uncontrolled discharge of wastewaters results in serious pollution problems into the environment (Ferreira et al., 2018). Microalgae cultivation in wastewater is an ecological post treatment approach in which microalgae assimilate nutrients in the environment, reducing the environmental impacts compared to synthetic culture medium, since there is no addition of inputs (Schneider et al., 2018).

In this context, microalgae growth contributes to changing the wastewater management paradigm for resource recovery (Zhang et al., 2018a). Microalgae provide the oxygen needed by bacteria for the oxidation of organic matter through photosynthesis. Because of their combined auto- and heterotrophic growth, nutrients are assimilated into algal bacterial biomass (Ferreira et al., 2018).

The integration of microalgae with wastewater represents an economical source of water and nutrients for biomass production to be reused as a raw material for useful bioproducts such as biofuels (Ferreira et al., 2018; Callegari et al., 2020), animal food supplements (Javed et al., 2019; Zhang et al., 2019) and biofertilizers (Castro et al. 2020), among others. Simultaneously, the negative effects related to human activities are minimized, such as pollution of water bodies, scarcity of natural resources, and emissions of greenhouse gases (GHGs) (Arashiro et al., 2018). However, this resource is not widely used due to the high cost of production, especially with regard to the algal biomass separation and harvesting processes (Assis et al., 2019).

In the search for sustainable strategic measures that improve the separation and harvesting of suspended microalgae, biofilm reactors (BRs) emerge as a technological innovation in which the attached growth of algal biomass occurs on a solid support material, forming a matrix of microalgae, bacteria, and fungi (Sutherland and Craggs, 2017; Mantzorou and Ververidis, 2019). These BRs can be connected to high-rate ponds (HRPs), forming a hybrid system to improve wastewater treatment, biomass harvesting and production (Assis et al., 2020).

Hybrid systems have emerged as a technological innovation to improve resource recovery through algal biomass. The biomass production efficiency in this system is up to approximately 2.8 times higher than that in a conventional HRP (Assis et al., 2020; Lee et al., 2014). At the same time, this system produces biomass, which also promotes the separation of up to 5 times greater than the capacity of a gravitational sedimentation tank (Assis et al., 2020).

In addition, it is noteworthy that the harvesting of attached biomass to BR does not require the addition of chemical products and has low energy demand (Ferreira et al., 2020).

In the literature, it is possible to find positive results from the implementation of hybrid systems (Assis et al., 2020; Zhang et al., 2018a; Zhang et al., 2018b; Assis et al., 2017; Gross et al., 2015). However, in addition to the innovation of this proposal from a technical applicability, the need to assess the economic and environmental viability of this technology is highlighted (Assis et al., 2020).

From an environmental point of view, there are also few studies evaluating BRs for the treatment of wastewater and algal biomass production and harvesting. Barlow et al. (2016) evaluated the sustainability of renewable diesel generation through hydrothermal liquefaction of attached biomass to a rotating algal BR via life-cycle assessment (LCA). Morales et al. (2020) studied the concentration of proteins from a biomass grown in a rotating algal biofilm system integrated with an HRAP. The rotating algal biofilm contributed to the reduction of environmental impacts (20%) and water consumption (30%) per kilogram of microalgae biomass and per kilogram of protein concentrate, and the authors considered it a promising alternative in comparison to conventional production of biomass in HRAPs. However, studies that assess the impacts related to the support materials used, the operation of the reactor, and the energy required in the separation of biomass have not yet been carried out.

LCA is a valuable tool for identifying the most sustainable routes for using microalgae biomass during wastewater treatment. This methodology allows assessment of the environmental impacts of each stage in a system, such as in wastewater treatment plants, since it takes into account and quantifies the use of natural resources and energy and the emissions and waste generated (Parra-Saldivar et al., 2020). In addition, there is currently a need to change the concepts of wastewater treatment to resource recovery and minimize impacts related to emissions to water and air and the scarcity of natural resources (Arashiro et al., 2018). In view of the scenario and benefits of BRs, the use of these reactors associated with the treatment of wastewater with microalgae is promising for the production and separation of a valued biomass, while the pollutants are removed simultaneously. In addition, the presence of a BR in a sewage treatment and microalgae production system is able to supply the demand for carbon dioxide, dispensing with the additional supplementation of this gas in optimizing the growth of microalgae in wastewater (Assis et al., 2017), emerging as an innovative technology for wastewater treatment and resource recovery.

In this sense, the objective of this study was to evaluate from an environmental perspective the applicability of a BR as a biomass production and harvesting unit in sewage treatment and resource recovery by a microalgae system on a pilot scale. The main innovations of this study are (i) environmental comparison of different suitable support materials to be installed in the BR on a smaller scale (analysis 1) and (ii) quantification of the environmental impacts of the hybrid system on a pilot scale composed of a BR integrated with an HRP (analysis 2). The support material used in the BR was chosen as the most sustainable by analysis 1; (iii) the indication of an optimal environmental technology for resource recovery through algal biomass grown in wastewater.

8.2.Materials and Methods

This paper used the life-cycle approach to perform two different analyses to obtain real information about the sustainability of using an attached cultivation system for wastewater treatment and biomass production. The first (analysis 1) aims to investigate the environmental performance of different support materials, and the second (analysis 2) aims to quantify and compare the environmental impacts of two biomass harvesting and cultivation systems. For that, experimental data obtained during the study of Assis et al. (2019) and Assis et al. (2020) were used. These experiments were conducted at scales larger than most studies that have been reported in the literature and under real climatic conditions. Therefore, the materials and methods used in this study were divided into descriptions of the experimental configurations and LCA methodology.

8.2.1. Experimental configurations and primary data obtention

8.2.1.1. Description of biofilm reactor operation with different support materials

Three reactors were operated in a semi-continuous flow (3 L/day) of domestic sewage previously treated in an Upflow Anaerobic Sludge Blanket (UASB) reactor located in Viçosa, Minas Gerais, Brazil. The domestic sewage used had 40.6 mg/L of total nitrogen and 4.1 mg/L of phosphorus. (Assis et al., 2019). These reactors had a support material for the attached growth of biomass and were characterized as BRs, namely, a cotton BR, a nylon BR, and a polyester BR. Each reactor had a container with a useful volume of 15 L, where the domestic sewage was stored and recirculated by a submerged pump (Litwin 700/550, flow = 550 L/h, power = 12 W) until the support material (surface area of 0.12 m²) in which the algal biofilm grew. Biomass was collected by manual scraping with the aid of a spatula. Biomass was measured by the concentration of total volatile solids according to APHA (2012). Table 8.1 presents the main operating characteristics and the results of the biofilm reactors with different support materials. More details on the experimental methodology for producing biofilms on different support materials can be found in Assis et al. (2019).

Table 8.1. Primary data used as input to the life-cycle assessment (Analysis 1).

	Nylon BR	Polyester BR	Cotton BR
Biomass production (g/m²)	44.92	50.12	34.46
Harvesting efficiency (%)	100	100	100
Daily operation (h/day)	10	10	10
Total operation period (days)	32	32	32
Culture medium recirculation pump (kW)	0.012	0.012	0.012

8.2.1.2. Description of hybrid system operation

Two biomass cultivation and harvesting systems were operated on a pilot scale: first, HRP followed by a secondary settling tank (ST), and second, the hybrid system formed by an HRAP, an ST, and a BR. In both systems, HRAPs acted as a polishing unit for domestic sewage and as a producer of suspended biomass, while the STs were units for separating and harvesting biomass. In the hybrid system, both the production stage and the separation and harvesting stage occurred in two different ways: production was suspended at the HRP and attached to the BR; separation and harvesting were performed by manual scraping in the BR and ST sludge collection.

The HRAPs used for the production of biomass and ST used for harvest had the same dimensions. Both HRAPs were operated in continuous flow at a flow rate of 100 L/day and hydraulic retention time (HRT) of 10 days. The domestic sewage used as the culture medium was previously treated in a septic tank at a wastewater treatment plant located in the city of Viçosa. The domestic sewage used had 88.5 mg/L of total nitrogen and 9.1 mg/L of phosphorus (Assis et al., 2020). During the operation, the blades were driven by a 1 hp eletric motor. The speed was reduced by a reducer coupled to the motor and controlled by a frequency inverter (WEG brand, CFW-08 series), which guaranteed a speed between 0.10 and 0.15 m/s.

The BR (surface area of 0.5 m²) was made of a flat acrylic panel and was kept in direct contact with atmospheric air and solar radiation. It was installed next to HRP (of the hybrid system) and supported by polyvinyl chloride (PVC) pipes 0.85 m from the ground. To allow the attached growth of the biomass, the panel was coated with polyester (SkTextil, Oxford, 100% polyester). The HRP effluent was recirculated to the BR for 10 h/day; that is, the useful volume of the pond was recirculated 10 times a day using an underwater pump (Sarlobetter SB 1000A) at a flow rate of 1000 L/h. After pumping, the effluent was dripped onto the surface of the panel by dripping, collected in a gutter, and returned to the HRP by gravity.

The effluent from each HRP passed through gravity to each settling tank, and the sedimented sludge was collected weekly. On the other hand, the operation of the BRs was based on the progressive accumulation of biofilm on the BR panel. The period necessary for the attached growth of the microalgae was 21 days, when the peak of biomass production occurred. Therefore, the frequency of total BR scraping was performed manually every three weeks with the aid of a spatula. In both systems, the quantification of biomass was measured by the concentration of volatile suspended solids for HRAPs and STs and by the concentration of total volatile solids for the BR, both according to APHA (2012).

Table 8.2 presents the main operating characteristics and the results of the production and harvesting systems of algal biomass on a pilot scale. More details on the experimental methodology and the comparison between the technical performance of the two systems described can be found in Assis et al. (2020).

Table 8.2. Primary data used as input to the life-cycle assessment (Analysis 2).

	Scenario 4 (HRP with ST)		Scenario 5 (Hybrid System)		
	HRP	ST	HRP	ST	BR
Biomass production (g/m²)	36.75	-	28.79	-	64.28
Biomass harvesting (g/m²)	-	8.09	-	6.62	64.28
Harvesting efficiency (%)	-	22	-	23	100
Daily operation (h/day)	24	24	24	24	10
Total operation period (days)	21	21	21	21	21
Paddlewheels motor (kW)	0.0833	-	0.0833	-	-
HRP effluent recirculation pump to BR (kWh)	-	-	-	-	0.13

8.2.2. Life-cycle assessment

The LCA followed the procedures standardized by the International Organization for Standardization, ISO 14042 (2000), ISO 14040 (2006a), and ISO 14044 (2006b) and was carried out with the aid of SimaPro® software (PRé Consultants BV, Amersfoort, the Netherlands, PRé 2019, www.pre-sustentabilidade.com).

8.2.2.1. Goal, scope and functional unit

Analysis 1 aims to identify the least impactful support material. Three scenarios were modelled, in which cotton, nylon and polyester entered as inputs in scenarios 1, 2 and 3, respectively. On the other hand, analysis 2 aims to compare the environmental impacts of the reference system cultivation in HRP and harvesting through ST (scenario 4) with the hybrid system (scenario 5), an innovation technology that combines attached and suspended cultivation. Figure 8.1 shows the flowcharts of inputs and outputs, as well as the boundaries of the systems used in analyses 1 and 2. The functional units were fixed at 10 g and 1 kg of harvested algal biomass for analyses 1 and 2, respectively. Although the two modelled experiments were carried out on a pilot scale and in real climatic conditions, the experiment modelled in analysis 1 was performed on a smaller scale than that mentioned in the modelled in analysis 2. Thus, the functional units were adopted to avoid large extrapolations. Thus, the experimental data were normalised to compose the inventories.

The system boundaries of all scenarios include the stages of the cultivation and harvesting of biomass (Figure 8.1). The inputs considered in all scenarios were the support materials and electric energy used for the operation of submerged water pumps that revolved and recirculated the culture medium. Additionally, domestic sewage replaced the entry of sources of nitrogen, phosphorus, and water for the production of algal biomass; therefore, they were considered an avoided product in relation to those conventional sources in the cultivation stage. Some studies have reported that the use of nitrogen (fertilizer) is one of the main environmental impacts on microalgae production (D’Imporzano et al., 2018; Morales et al., 2020). Schneider et al. (2018) evaluated different microalgae biomass production scenarios to compare synthetic (NPK medium) and wastewater cultivation. Although electricity is the main input responsible for the cultivation impacts in an NPK medium, the authors reported that the use of wastewater collaborated to reduce the impacts of 11 evaluated categories. Therefore, based on these results reported by the literature and other studies that used the same approach (Castro et al., 2020; Ferreira et al., 2020; Souza et al., 2019), it was also adopted in this paper. Tables 8.3 and 8.4 present a summary of the description of the life-cycle inventory (LCI) used and its units, respectively, for analyses 1 and 2.

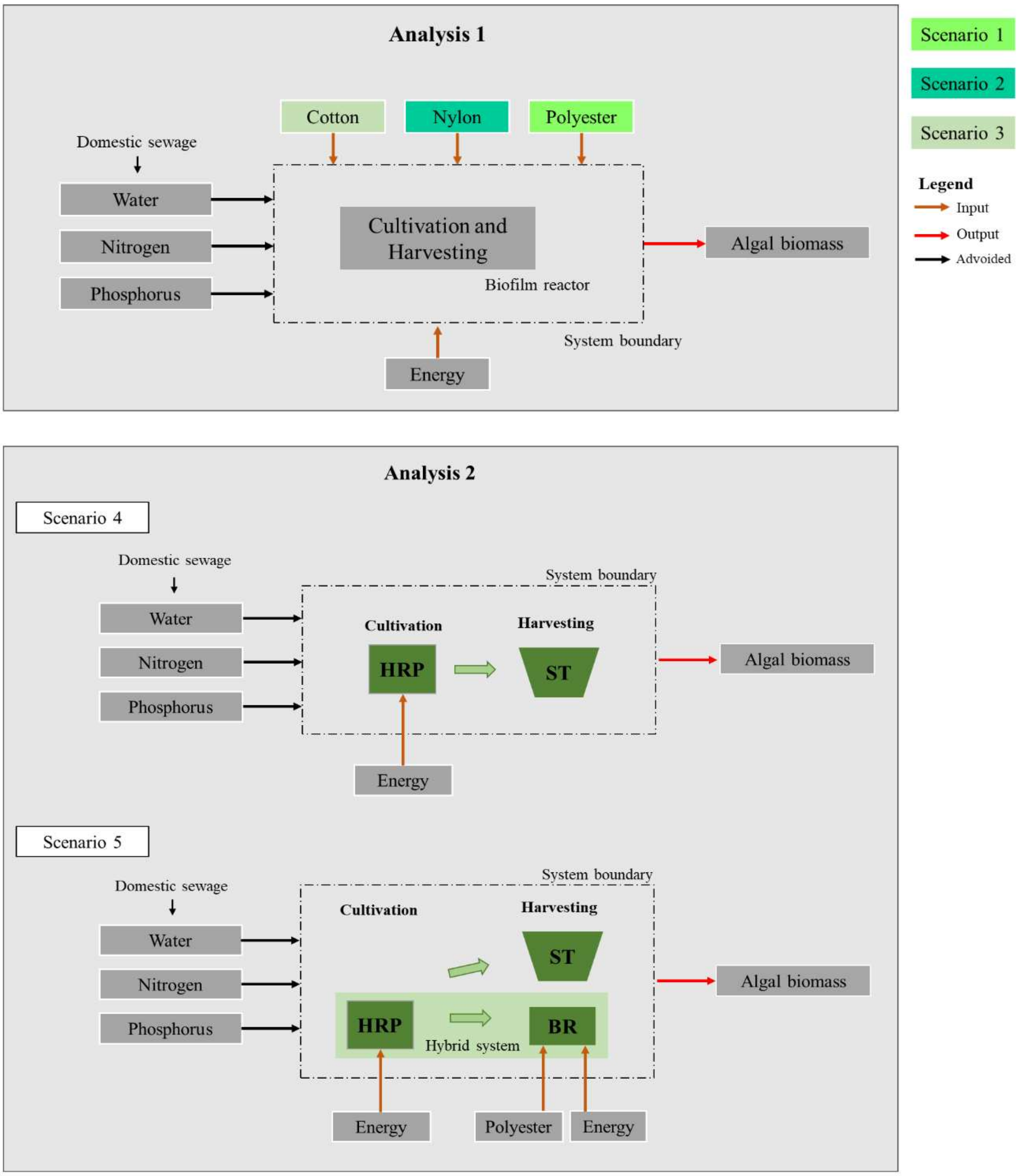

Figure 8.1. Product system for scenarios 1 to 3 (Analysis 1) and for scenarios 4 and 5 (Analysis 2).

Table 8.3. Description of the life-cycle inventory of the production of algal biomass on different support materials (Analysis 1).

	Parameters	Units	Descriptions
Avoided products	Nitrogen	mg	Use of nutrients and water from domestic sewage
	Phosphorus	mg	
	Water	kg	
Inputs	Cotton mesh	kg	Support material for the attached growth of biomass
	Nylon mesh	kg	
	Polyester mesh	kg	
	Electricity	kWh	Culture media recirculation
Outputs	Biomass	kg	Harvested biomass

Table 8.4. Description of the life-cycle inventory of the sewage treatment, production, separation, and harvesting systems for biomass on a pilot scale (Analysis 2).

Steps		Parameters	Units	Scenario 4 (HRP with ST)	Scenario 5 (Hybrid System)
Cultivation	Avoided products	Nitrogen	kg	Use of nutrients and water from domestic sewage	
		Phosphorus	kg		
		Water	kg		
	Inputs	Electricity	kWh	Culture media recirculation through the operation of the paddlewheels	
	Outputs	Biomass	kg	Biomass produced	
Harvesting	Inputs	Electricity	kWh	-	HRP culture media recirculation to BR
		Polyester	kg	-	Support material for the biomass attached growth
	Outputs	Biomass	kg	Harvested biomass	

Some assumptions were made during the modeling of the scenarios. The infrastructure of the project was also not taken into account, except for the BR polyester. The useful life of pilot-scale projects is approximately 25 years, and this support material will be changed several times during this period, whenever necessary (Souza et al., 2019). Moreover, the emissions to water were neglected because the concentrations of environmental parameters were in accordance with the Brazilian standard for sewage discharge at the state level (COPAM, 2008) and with international limits (European Commission, 1991). In addition, there was no difference in the wastewater treatment efficiency in both systems (Assis et al., 2020).

The biofilm showed cumulative growth and peak production at different times (Assis et al., 2019). After the production peak, detachment of cells occurs in the upper layers of the formed biofilm; therefore, the biomass needs to be completely scraped off (Assis et al., 2020). Thus, in analysis 1, it was considered that the scraping frequency of the BRs, i.e., the biomass harvest, occurs at peak production (Table 8.1).

In analysis 2, to quantify the harvested biomass in the hybrid system, the sum of the biomass harvested by gravitational sedimentation in ST and by manual scraping in BR was considered. Additionally, during the preparation of the inventories, the operating time was fixed at 21 days. It was considered to facilitate comparison between scenarios 4 and 5, since the HRP operating parameters (HRT = 10 days, harvest time = 7 days) were different from BR (HRT = 21 days, harvest time = 21 days). This is due to the differences between attached growth, which requires batch harvesting, and suspended cultivation, which allows continuous harvesting (daily). It is noteworthy that, in scenarios 4 and 5, the input of the energy is used to move the blades of the HRPs (Figure 8.1). More information on the systems operation can be found in the study of Assis et al. (2020).

8.2.2.2. LCA methodology

To quantify and evaluate life-cycle impacts, the ReCiPe midpoint methodology (hierarchist approach V1.12) was used. The ReCiPe methodology is commonly applied in LCA studies that include microalgae biomass production (Arashiro et al., 2018; Castro et al., 2020; D'Imporzano et al., 2018; Ferreira et al., 2020; Schneider et al., 2018; Souza et al., 2019, Yadav et al., 2020). ReCiPe is representative on a global scale (Schade and Meier, 2019) and is focused on environmental issues that consistently prioritize short- or medium-term impacts (Souza et al., 2019). The hierarchical approach was adopted because it is considered a standard model that represents a consensus between short and long term, since long-term emissions were not considered in this analysis (PRé, 2019).

The midpoint methodology was used and has 18 impact categories; however, in analysis 1, priority was given to some of the categories that suffered the most impacts during the biomass production and separation process, namely, climate change, human toxicity, terrestrial ecotoxicity, freshwater ecotoxicity, natural land transformation, and agricultural land occupation.

Seeking a more holistic approach to the scenarios of sewage treatment, production, separation, and harvesting of biomass, in analysis 2, the 18 impact categories of the midpoint methodology were considered. The use of all midpoint categories helps to ensure complete and robust comparisons of scenarios (D'Imporzano et al., 2018). Therefore, in addition to the categories mentioned above in analysis 1, the following categories were considered: ozone depletion, terrestrial acidification, freshwater eutrophication, marine eutrophication, photochemical oxidant formation, particulate matter formation, marine ecotoxicity, ionizing radiation, urban land occupation, water depletion, metal depletion, and fossil depletion. This choice was adopted to cover as much information as possible about the environmental impacts generated by pilot-scale systems and to enable the results to serve as providing support for large-scale deployment.

The database used was Ecoinvent - Allocation, Default (Ecoinvent, 2014). For energy, Brazilian data were adopted; however, because the database did not present Brazilian data for other processes (water, nitrogen, phosphorus, cotton, nylon and polyester), data from the 'rest of the world' (RoW), which represents all countries in the world except Europe, and 'Global' (GLO) were considered.

8.2.2.3. Sensitivity analysis

Based on analysis 2, sensitivity analysis was performed. According to Germec et al. (2020), increasing the surface area consists of the main purposes of BR design. In the literature, there are BRs with an amplitude of surface areas between 0.5 and 33.1 m² according to the scope of the study (Assis et al., 2020; Assis et al., 2017; Gross et al., 2015; Lee et al., 2014). In this sense, it is believed that the increase in the superficial area of the BR could contribute to the least impactful scenario due to higher biomass productivity. The present work investigated the increase in the BR surface area and the effects of the number of reactors in a hybrid system. Four different sensitivity analysis scenarios were created:

Hybrid system composed of two BRs (sensitivity i),

Hybrid system composed of three BRs (sensitivity ii),

Hybrid system composed of a BR with 1 m² surface area (Sensitivity iii).

It was assumed that the nutrients in domestic sewage were sufficient to allow the addition or increase the surface area of the BR. As attached growth is a research field at an early stage (Mantzorou and Ververidis, 2019), the proposed conditions have not yet been investigated and compared in technical terms, so this study has avoided further extrapolations. In addition, as the same pump was maintained for all modeled scenarios, it was considered that by doubling the BR surface area (Sensitivity iii) and, consequently, reducing the surface application rate by half, there is no great variation in the productivity found by Assis et al. (2020). Adding BRs in a hybrid system or a BR with a larger surface area could result in significant changes in productivity measured experimentally by Assis et al. (2020) and used in life cycle inventories. Thus, the considerations used increase the reliability of the results of the sensitivity analysis. The environmental impacts of these new scenarios were compared with scenario 5 (LCA of the hybrid system).

8.3.Results and Discussion

8.3.1. Analysis 1: Life-cycle assessment of different support materials

Table 8.5 shows the quantification of inputs and outputs of materials and energy from the algal biomass production and harvesting system calculated from the LCI for different support materials. It is observed that, except for cotton mesh, the input of energy and avoided products in scenario 1 (cotton BR) were smaller than in other scenarios. The reactor with cotton material has lower biomass productivity and, consequently, requires the operation of more reactors to produce 10 g of biomass. Additionally, as cotton has the highest fabric weight (137 g/m², Table 1), scenario 1 required the highest amount of support material. Despite this, as it requires less operating time to achieve peak production (18 days, Table 1), it has the lowest energy consumption (5.22 kWh) and the highest biomass production per volume of treated domestic sewage (159.61 kg of water, 2.68 g of nitrogen and 0.28 g of phosphorus). Unlike cotton BR, scenario 3 (polyester BR) had the highest values of energy (6.38 kWh), water (179.57 kg) and nutrient (4.38 g of nitrogen and 0.45 g of phosphorus) inputs and the lowest amount of material (16.36 g).

Table 8.5. Life-cycle inventory of different support materials (scenarios 1, 2 and 3) (Analysis 1).

	Parameters	Units	Scenario 1 (Nylon BR)	Scenario 2 (Polyester BR)	Scenario 3 (Cotton BR)
Avoided products	Nitrogen	mg	40.6	40.6	40.6
	Phosphorus	mg	4.17	4.17	4.17
	Water	kg	1	1	1
Inputs	Cotton mesh	kg	0.133	-	-
	Nylon mesh	kg	-	0.089	-
	Polyester mesh	kg	-	-	0.117
	Electricity	kWh	9.2	9.2	9.2
Outputs	Biomass	kg	1	1	1

Figure 8.2 shows the comparison between the environmental impacts modelled scenarios. Scenario 3 (polyester BR) and scenario 2 (nylon BR) had similar environmental performances and were the most ecological in all impact categories. Scenario 1 (cotton BR) had more negative impacts in all evaluated categories.

Despite the use of water as a product avoided, it is observed that in the water depletion category (Figure 8.2a), scenario 1 had a relevant impact (100%). This was because the use of water in cotton production and the spinning processes was so high that the use of wastewater failed to amortize to this impact, as in scenarios 2 (-22%) and 3 (-23%). Furthermore, the water depletion category is indirectly associated with the type of energy coming from hydroelectric energy. The main energy matrix in the country under study (Brazil) is hydroelectric plants, which have a high potential for transforming the soil due to the flooding of areas by damming water.

With respect to the treatment of land use and transformation, agricultural land occupation refers to the use of a type of soil cover for a specified period (Mattila et al., 2011). In this category, scenario 1 was the most impactful due to the use of cotton support material (Figure 8.2b). The environmental impacts associated with cotton production and the spinning process are heterogeneous and complex and include water consumption, soil occupation and the use of chemicals (Esteve-Turrillas and Guardia, 2017).

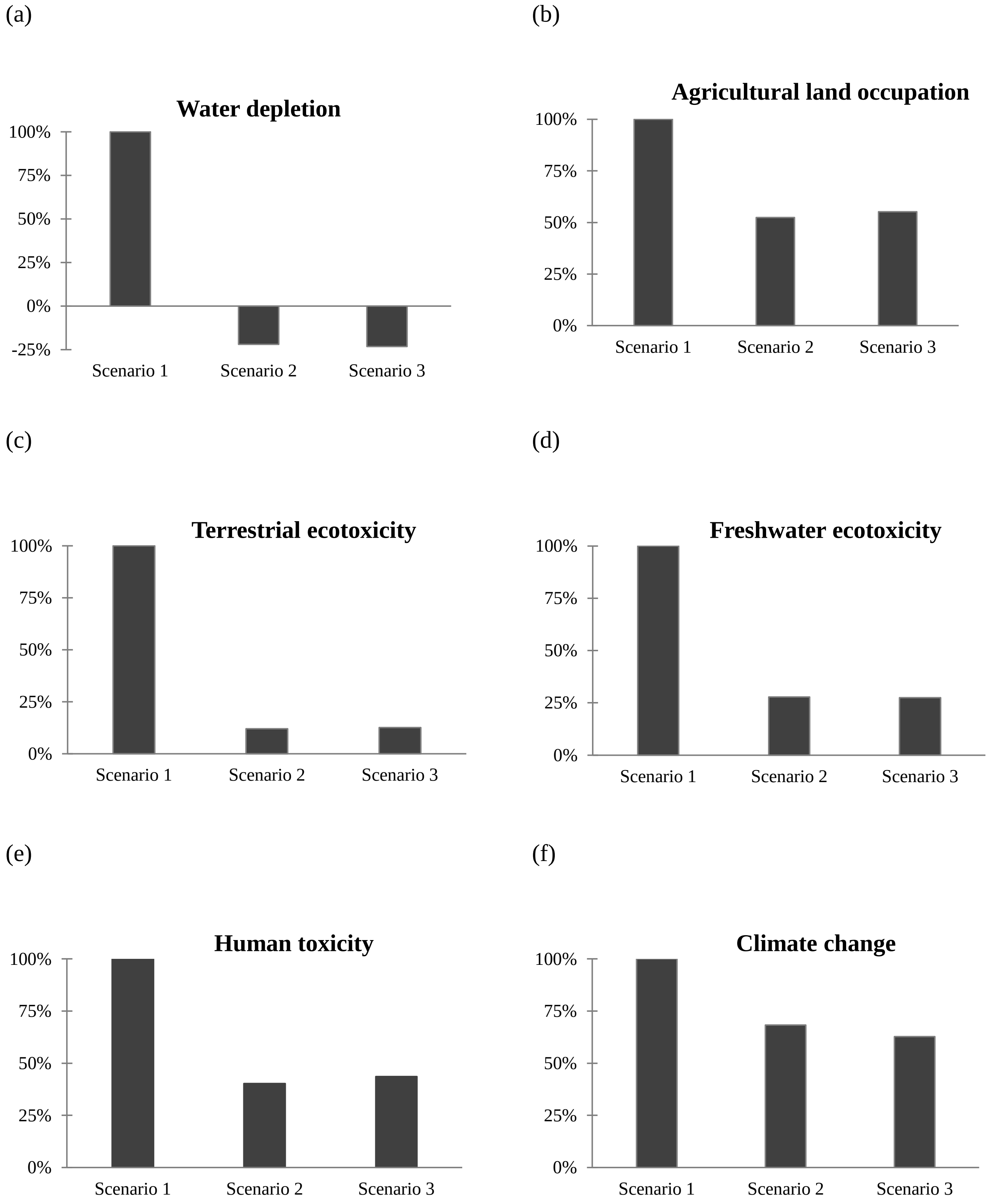

Figure 8.2. Potential environmental impacts of scenarios 1 (Cotton BR), 2 (Nylon BR) and 3 (Polyester BR) (Analysis 1) for categories: (a) water depletion, (b) agricultural land occupation, (c) terrestrial ecotoxicity, (d) freshwater ecotoxicity, (e) human toxicity, and (f) climate change.

According to a report released by the Pesticide Action Network (PAN) (2017), in Brazil, cotton is the crop with the highest use of pesticides, with a minimum of 17.5 L/ha and a maximum of 44.9 L/ha. Different chemical substances used in plantations can diffuse into the environment through runoff, leaching, and drainage, reaching water sources even though they are far from the point of application and groundwater (Gerónimo et al., 2014; Nayak et al., 2019; Wołejko et al., 2019). In this context, scenario 1 was, again, more impactful in terrestrial (Figure 8.2c) and freshwater (Figure 8.2d) ecotoxicity categories due to its cultivation. Occupational exposure to pesticides in cotton planting aggravates the impacts of human toxicity due to increased exposure when transporting, mixing, loading, and applying pesticides (Dhananjayan and Ravichandran, 2018). This category also had a greater impact on scenario 1 (Figure 8.2e). In addition, the use of any type of nitrogen-based fertilizer during the cultivation of cotton increases the nitrogen content in the soil and favours the emission of nitrous oxide, an important GHG that affects global warming (Souza et al., 2019). Therefore, for the climate change category, scenario 1 was the most impactful (Figure 8.2f).

In view of the above, the smallest impacts in all categories were caused by scenarios 2 and 3. Both are indicated, from an environmental point of view, for algal attached growth. However, based on the technical analysis of the three support materials developed by Assis et al. (2019), it was concluded that polyester was the material that presented the best results in terms of durability and higher biomass production (50.1 g/m^2). Therefore, this support material was chosen to be used in the pilot-scale hybrid system. Therefore, in analysis 2, by associating the BR with an HRP, only sustainability performance was evaluated, aiming at its large-scale application.

8.3.2. Analysis 2: Life-cycle assessment of the hybrid system

Table 8.6 shows the LCI for HRP with ST and the hybrid system scenarios. For the harvesting of 1 kg of biomass in the HRP with ST scenario, higher energy input and domestic sewage inputs (avoided products) were required than in the hybrid system scenario. The higher energy demand of HRP with ST (749.6 kWh) was related to the low efficiency of biomass harvesting in the ST (only 22%). It took 4.55 kg of biomass suspended in the HRP to separate 1 kg of sedimented biomass in the ST. On the other hand, BR integration increased the biomass harvest efficiency of the hybrid system by approximately nine times and, consequently, decreased the energy requirements (540.58 kWh).

Table 8.6. Life-cycle inventory of scenarios 4 and 5 (Analysis 2) and sensitivity analysis scenarios (Sensitivies i, ii and iii).

Stages		Parameters	Units	Scenario 4 (HRP with ST)	Scenario 5 (Hybrid System)	Sensitivity i (2 BRs)	Sensitivity ii (3 BRs)	Sensitivity iii (1 m^2 surface area)
Cultivation	Avoided products	Water	kg	37,480.56	27,029.05	19,131.17	14,798.27	19,131.17
		Nitrogen	g	3,317.40	2,392.34	1,693.30	1,309,79	1,693.30
		Phosphorus	g	342.57	247.05	174.86	135.26	174.86
	Inputs	Electricity	kWh	749.6	540.58	382.47	295.85	382.47
	Outputs	Biomass	kg	4.55	3.37	2.68	2.30	2.68
Harvesting	Inputs	Biomass	kg	4.55	3.37	2.68	2.30	2.68
		Electricity	kWh	-	35.14	49.74	57.71	24.87
		Polyester	g	-	528.16	747.03	866.76	747.03
	Outputs	Biomass	kg	1	1	1	1	1

Analysing only the BR as a biomass separation and harvesting unit, it represented 100% efficiency in harvesting the biomass that was produced in an attached form, and its energy demand was only 35.14 kWh. Therefore, the integration of BR into HRP (scenario 5) represented an energy reduction of approximately 23% for the hybrid system. Similarly, the demands for nutrients and water in HRP with ST (scenario 4) were approximately 28% higher than those in scenario 5. Therefore, to treat the same volume of domestic sewage, the hybrid system contributes a larger amount of harvested biomass. It should be noted that, in addition to the benefits in biomass production, separation, and harvesting, the integration of BR in the hybrid system contributed positively to the energy, water, and nutrient requirements, making BR promising for use in sewage treatment and biomass production.

The categories of environmental impacts referring to each scenario in Analysis 2 are shown in Figure 8.3. As scenario 5 presented a greater harvest efficiency of 1 kg of biomass, all categories of this system showed smaller impacts than scenario 4. It is observed that the terrestrial ecotoxicity category was the most impacted by scenario 4, with the equivalent of 0.038 kg 1.4-DB eq/kg of biomass, whereas in scenario 5, this category had an impact equivalent to 0.029 kg 1.4-DB eq/kg of biomass, which corresponds to a reduction of approximately 23%. The LCA of scenario 5 demonstrated that the BR contributed to a reduction of the environmental impacts by approximately 19% in all categories when compared to scenario 4.

In the hybrid system, the greatest impacts were observed in the categories of climate change, human toxicity, particulate matter formation, terrestrial ecotoxicity, freshwater ecotoxicity, marine ecotoxicity, agricultural land occupation, natural land occupation, and fossil depletion. Among these categories, Figure 8.4 identifies which inputs (polyester mesh or energy) were responsible for such impacts in the harvesting process via the BR, as well as the impacts avoided due to the use of wastewater as a culture medium for biomass growth.

The use of domestic sewage reduces the negative effects on the environment through avoided products. In the category with the greatest relative contribution of environmental impacts and marine ecotoxicity, the use of domestic sewage represented a reduction of approximately 29, 10 and 16%, respectively, for nitrogen, phosphorus and water. Among these avoided products, nitrogen was the one that most contributed to the reduction of impacts. These results reveal the environmental importance of reusing wastewater for the production of algal biomass and are in line with the literature (Schneider et al. 2018; D'Imporzano et al., 2018; Morales et al., 2020).

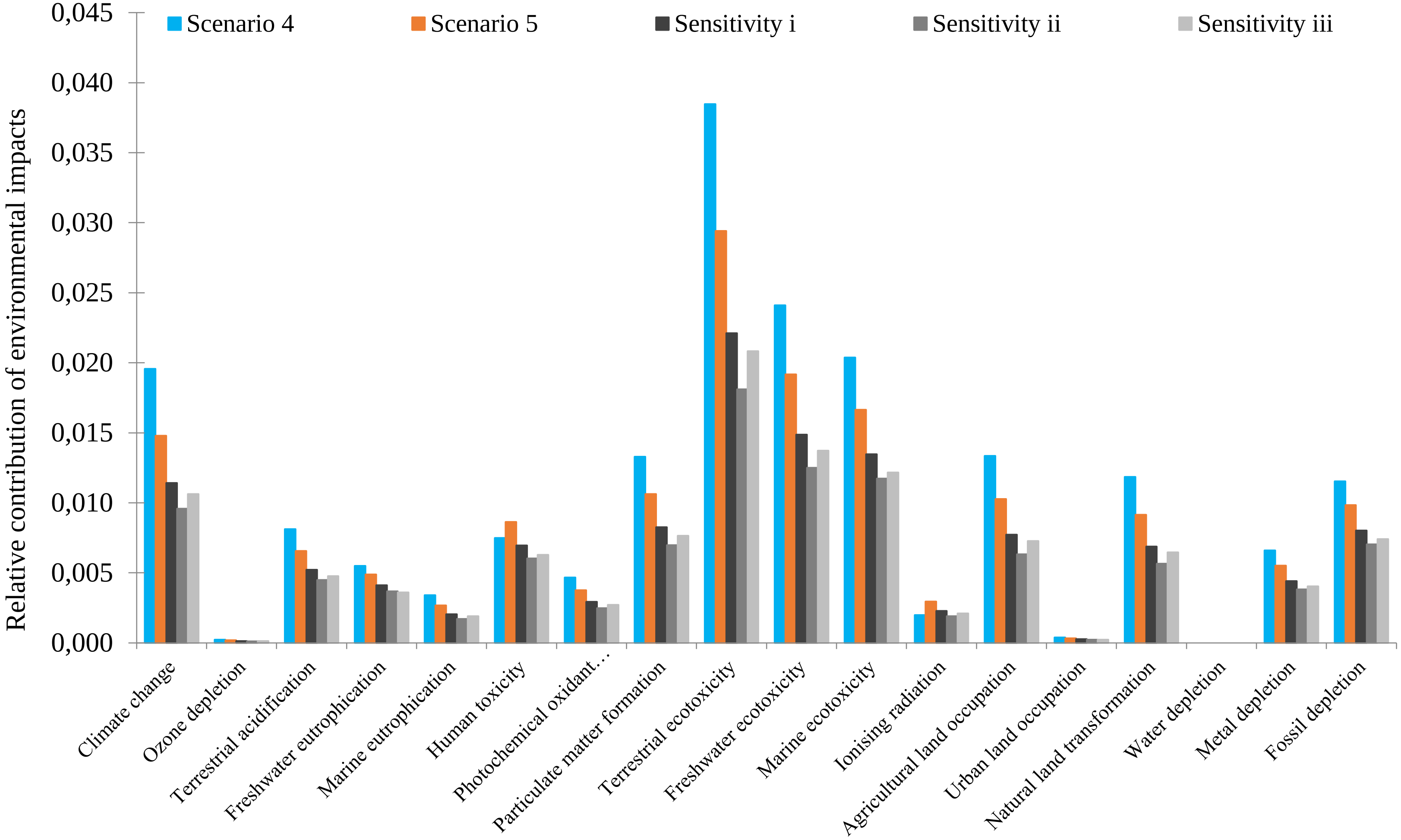

Figure 8.3. Relative contribution of environmental impacts of scenarios 4 (HRP with ST) and 5 (Hybrid System) (Analysis 2) and sensitivity analysis scenarios (Sensitivities i, ii and iii).

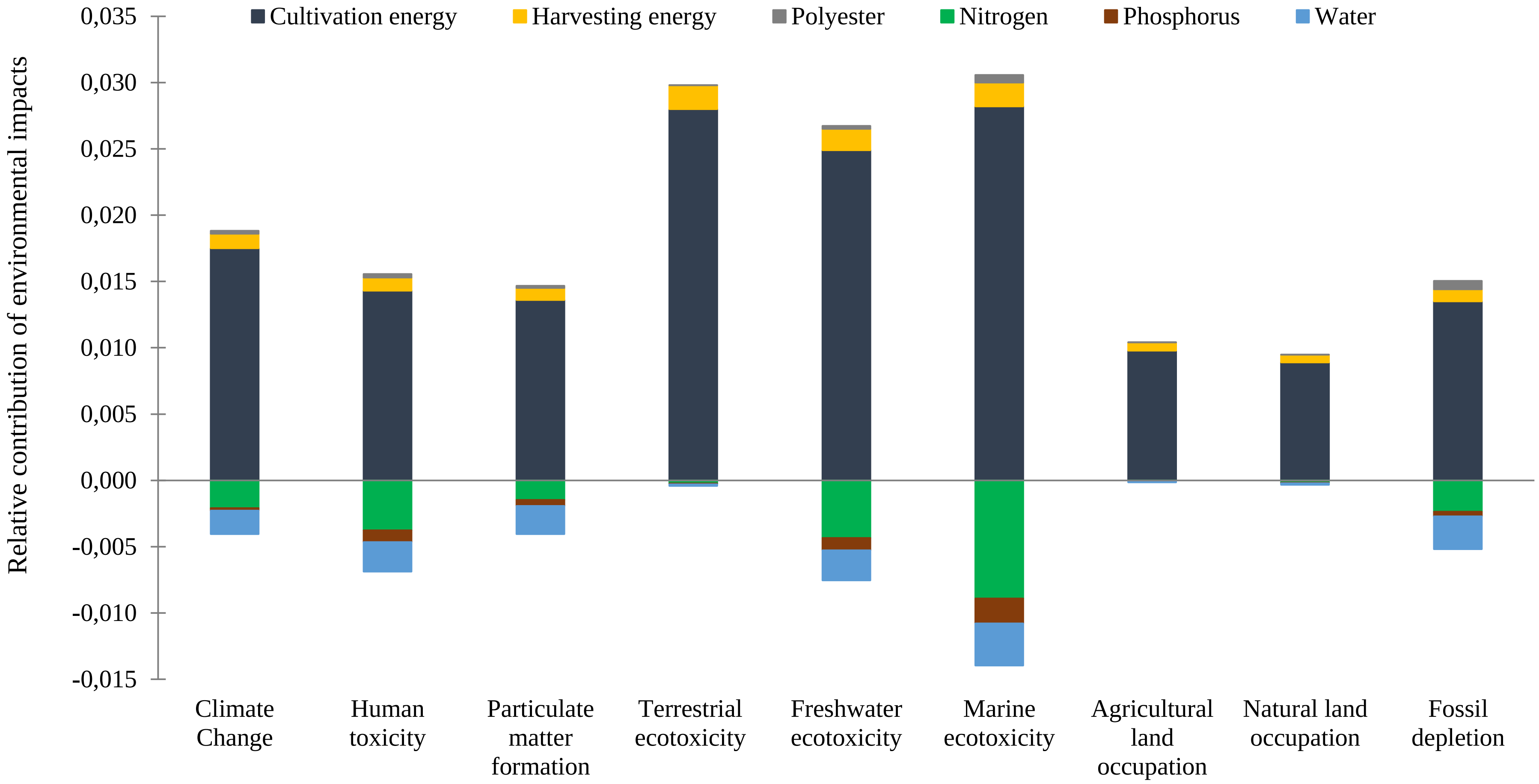

Figure 8.4. Relative contribution of environmental impacts of the energy, polyester, nitrogen, phosphorus and water for the most impactful categories in the hybrid system: climate change, human toxicity, particulate matter formation, terrestrial ecotoxicity, freshwater ecotoxicity, marine ecotoxicity, agricultural land occupation, natural land occupation, and fossil depletion (Analysis 2).

During the harvesting stage via the BR, energy was identified as having an impact, but in a proportion of approximately 15 times less than the demand of HRPs. As mentioned in the LCA of Analysis 1, the energy source from hydroelectric plants generates several environmental and social impacts, which affect many environmental categories, ranging from interference in the biodiversity of the native habitat and the production of GHGs to relocation of local communities due to the damming of water on natural, agricultural, or even urban land, transforming the type of cover (Mattilla et al., 2011; Pestana et al., 2019). Souza et al. (2019) and Castro et al. (2020) investigated the use of photovoltaic energy via LCA in the algal biomass production stage to obtain biofertilizers. Studies have considered this source of energy as more sustainable, with reduced impacts in most categories when compared to sources of energy from hydroelectric plants. However, this technology also deserves special attention regarding the proper final destination of its equipment at the end of its useful life, seeking to avoid the increase in other impacting categories, such as human toxicity caused by photovoltaic panels.

The polyester mesh was identified as having impacts mainly in the categories of marine ecotoxicity (0.001 kg 1.4-DB eq/kg of biomass) and fossil depletion (0.001 kg oil eq/kg of biomass). Polyester is formed from synthetic fibers made in the laboratory and synthesized from oil. The extraction of this non-renewable raw material for the manufacture of synthetic fibers favours fossil depletion. The oil extraction platforms are installed at sea or on land, with several implications for marine and terrestrial ecosystems and causing air contamination. According to Goedkoop et al. (2009), among the environmental mechanisms causing terrestrial and aquatic impacts is the damage caused to the diversity of ecosystems. In this context, the washing of synthetic fibers releases microplastics, which detach themselves from their fibers and reach aquatic sources, harming both microorganisms and larger animals.

The formation of particulate material by polyester (0.0001 kg MP10 eq/kg of biomass) was due to the release of chemicals into the air during the oil extraction process. In addition, during the manufacturing process, the various negative environmental effects of the textile industries include high air emissions (Toprak and Anis, 2017), such as nitric oxides and methanol, among other compounds that contribute to climate change (Stringfellow et al., 2017), corresponding to the impact of 0.0002 kg CO_2 eq/kg of biomass.

In soil, contamination occurs due to the spillage of fluids during transport by trucks or wastewater pipes, failure of well casings, or leaks from tank tubes (Pichtel, 2016). Indirectly, eating food grown on these soils or by contaminating surfaces and groundwater can cause damage to human health (Johnston et al., 2019). The human toxicity of the polyester mesh was 0.0002 kg 1.4-DB eq/kg of biomass). The contaminants most frequently detected in soil and water are total petroleum hydrocarbons, polycyclic aromatic hydrocarbons, arsenic, and manganese. Water pollution directly affects fishing, agriculture, and drinking water. This biological impact not only affects ecosystems and human health but also causes regional economic declines (Johnston et al., 2019).

Despite all the effects related to the energy and polyester mesh of the hybrid system, it is noteworthy that the BR contributed in terms of reducing environmental impacts and that the production and harvesting of biomass was positive in the search for solutions for building more sustainable reactors. It is known, however, that the literature still lacks studies that evaluate investments in the construction of these reactors. It is assumed that the infrastructure costs of these reactors must be higher than those of large-scale suspended systems, especially if harvesting via biomass scraping is done automatically. The manual scraping of a BR on a large scale becomes unviable due to the high demand for human labour (Wang et al., 2017), while automatic scraping demands more energy and raw material, increasing the associated impacts. Therefore, it is recommended that further studies evaluate the economic feasibility and implementation on a large scale so that BRs can provide an improvement compared to conventional wastewater treatment plants.

8.3.3. Sensitivity analysis

Table 8.6 presents the input data in the LCI of the sensitivity analysis scenarios. In the scenarios modeled in the sensitivity analysis, the values of energy used to paddlewheels decreased during the cultivation stage, while the energy used to recirculate the effluent and the amount of polyester increased when compared to scenario 5. This is because by increasing the number of reactors (sensitivity i and sensitivity iii) or the surface area of the reactor (sensitivity iii), attached growth is favored, which offers greater efficiency in harvesting biomass. Thus, fewer hybrid systems are needed to harvest 1 kg of biomass. In addition, it is noteworthy that the products avoided were also smaller, so to treat the same volume of domestic sewage, a greater amount of harvested biomass was obtained when compared to scenario 5.

Regarding the harvesting stage, it is observed that compared to scenario 5, the increase in energy and polyester demands were directly proportional to the increase in the number of reactors 2 and 3 (Table 8.6). However, environmental impacts followed an opposite trend, as shown in Figure 8.3. In all categories, for sensitivity ii (composed of three BRs), the reduction in environmental impacts was approximately 33%, followed by sensitivity iii (BR with 1 m² surface area) with a reduction of 28% and, finally, sensitivity ii (composed of two BRs), with a decrease of 21%, when compared to scenario 5.

The sensitivity analysis showed that despite the higher demand for energy and polyester inputs, the scenarios with two or three BRs and BR with 1 m² reduced the impact of biomass harvesting grown in domestic sewage in all the categories investigated. In this way, increasing the attached growth of biomass contributes to reducing the environmental impacts of the hybrid system. The energy required to paddlewheels in the HRP during the cultivation stage is the main impact observed (Figure 8.4). Thus, reducing the number of hybrid systems to obtain 1 kg of biomass reduces this energy demand and is more decisive in reducing environmental impacts, even though the inputs associated with BRs increase, whether in greater numbers or in a smaller area.

It is noteworthy that the sensitivity analysis was performed to values extrapolated from the experiment carried out on a pilot scale by Assis et al. (2020), and it is recommended that future research consider the technical feasibility of increasing the number of BRs, as well as the BR area, to validate the environmental assessment of these scenarios studied here.

8.4.Conclusions

Among the support materials advanced for the growth of microalgae, polyester was the material recommended because it presented smaller environmental impacts associated with greater biomass production and harvest, in addition to greater durability. The cotton BR was more impactful due to the effects of cotton planting, which caused high impacts in the categories of climate change (5.7 kg CO_2 eq/kg of biomass) and occupation of agricultural land (2.9 m². year/kg of biomass). The LCA of the hybrid system, on a pilot scale, showed a reduction in environmental impacts of approximately 19% in all categories compared to the system composed of HRP and ST. These results proved to be a promising alternative for use in wastewater treatment in the production and harvesting of algal biomass. Sensitivity analysis showed that the increase in the number or surface area of the BRs minimized the environmental impacts of algal harvesting grown in domestic sewage. The energy demand of HRPs causes the main environmental impact of the scenarios. Thus, it was found that by optimizing the harvesting through the BRs, there is a reduction in these impacts, since fewer hybrid systems are required and, consequently, fewer HRPs operate. It is recommended that future research consider the technical feasibility of increasing the number of BRs, as well as the BR area, to validate the environmental applicability of the biomass harvest found in this study.

8.5.Acknowledgements

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9. CONCLUSÃO GERAL

Os experimentos em escala reduzida foram realizados para promover a otimização do reator de biofilme aplicado em escala piloto. Dentre os materiais suportes avaliados (algodão, nylon e poliéster) para a produção aderida de biomassa algal, o poliéster foi o que apresentou os melhores resultados em termos de maior durabilidade, maior produção de biomassa e menores impactos ambientais negativos. As inclinações dos reatores avaliadas (15º, 45º e 90º), interferiram na produção de biomassa devido às condições ambientais a que estavam submetidos. Durante a estação seca, o reator de 15º foi o que apresentou maior produção de biomassa (500,9 $g.m^{-2}$), enquanto que o reator de 90º (528,1 $g.m^{-2}$) apresentou maior produção durante a estação chuvosa. Sendo assim, essa variação do ângulo de inclinação do painel, ao qual a biomassa cresceu aderida, foi mantida na operação do reator de biofilme otimizado, em escala piloto.

O reator de biofilme otimizado, em escala piloto, foi adaptado a uma LAT, escala piloto, formando um sistema híbrido de produção e colheita de biomassa. A sua integração proporcionou maior diversidade de organismos fotossintéticos no meio de cultivo do sistema híbrido e a sua produção foi o dobro do que uma LAT. A capacidade de separação e colheita de biomassa concentrada foi cerca de 5 vezes maior em relação ao decantador. Integrado ao sistema, o RB contribuiu com cerca de 84% de eficiência de colheita total do sistema híbrido. Além disso, o sistema híbrido obteve uma redução de impactos ambientais de, aproximadamente, 19% em todas as categorias, em relação ao sistema composto por uma LAT e um decantador convencionais. Os resultados da análise de sensibilidade ambiental revelaram que o aumento do número ou de área superficial dos RBs minimizou os impactos negativos relativos a colheita de biomassa.

Portanto, diante das vantagens apresentadas, conclui-se que a integração de reatores de biofilme em sistemas de tratamento de esgotos e produção de biomassa é promissora. Através do presente estudo foi possível contribuir com algumas características de projetos de reatores de biofilme, visando a sua utilização para produção e separação de biomassa algal a partir do tratamento de esgoto doméstico, tornando o saneamento ambiental mais atrativo do ponto de vista de sustentabilidade.

10. SUGESTÕES PARA PESQUISAS FUTURAS

Os resultados dessa pesquisa mostraram que a integração do reator de biofilme, como unidade de produção e separação de biomassa, a uma lagoa de alta taxa, aumentou a produção de biomassa, bem como a eficiência de separação e colheita de biomassa do sistema em escala piloto. Portanto, de forma a tornar a utilização dos reatores de biofilme em escala real, recomenda-se que estudos futuros avaliem parâmetros técnicos e operacionais e considerem a viabilidade econômica de implantação e operação destes reatores em uma estação de tratamento de esgotos.

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12. ANEXOS

Produtos originados dessa tese.

ANEXO 1

Assis, L.R., Calijuri, M.L., Assemany, P.P., Berg, E.C., Febroni, L.V., Bartolomeu, T.A. Evaluation of the performance of the different materials to support the attached growth of algal biomass. Algal Research, 39, 101440, 2019. https://doi.org/10.1016/j.algal.2019.101440

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Evaluation of the performance of different materials to support the attached growth of algal biomass

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ABSTRACT

The attached microalgal biomass production in wastewater is promising for the development of biofilm reactors aimed at the economic separation and harvesting of biomass. However, the current impasse in the attached algal biomass production relies on the ability of materials to support such adherence. This study evaluated the effects of different support materials on the production and composition of algal biomass cultivated in domestic sewage. Durability and adherence of algal biomass to the threads of the support material were the most important criteria for choosing the material with the best performance. Three support materials were evaluated: cotton, nylon, and polyester. Polyester presented the best results in terms of durability; its resistance to friction tests was the highest, and even increased after its use in the experiment; this was associated with the high biomass production, mostly after the biomass inoculum ($50.1\,g{\cdot}m^{-2}$). This support also demonstrated greater development of nitrifying bacteria, which are essential for biofilm formation due to the presence of filaments in their cells. As for biomass characterization, it was observed that the different support materials did not interfere in the composition of the cells present in the attached biomass.

1. Introduction

The search for technologies that are less damaging to the environment, associated with the need to generate energy to supply the population demand, has stimulated research aimed at using renewable energy sources. The production of microalgae is within this context as a promising source of renewable energy. Among the applications of algal biomass, the following biofuels can be highlighted: the production of ethanol through sugar fermentation; bio-oil from thermal–chemical processing; and methane through anaerobic digestion. Other applications include the production of fertilizers, and aquaculture [1].

Despite the great versatility of microalgae biomass use, it has not yet been commercialized due to its high production cost. Currently, its main production process is through high rate ponds, where the extraction of algal biomass is expensive. Grimma et al. [2] estimated that the costs of harvesting and separating biomass represent nearly 20 to 30% of the costs for producing microalgae biofuels. In order to harvest algal biomass from a diluted sample, the algal cells in solution are usually concentrated through sedimentation, flotation, flocculation or centrifugation [3]. These processes, if used on large scale, have a time-consuming operation and are not considered as economically feasible [4]. Newer technologies are available for algal biomass separation and for the production of high density biomass, such as membrane bioreactors. These bioreactors act efficiently in these requirements and also in the removal of nutrients when used for wastewater treatment [5].

In this context, the recent search for innovative strategies to optimize the production and separation of algal biomass can be highlighted. Nowadays, the method of the algal biofilm growth system has been widespread, in which the cells are fixed to the surface of a solid support material [6]. The advantages of this new way of production are the low water demand and the ease of biomass harvesting, due to its greater concentration [6]. Consequently, these systems have a low cost of biomass harvesting and separation when compared to the suspended growth system.

Adhered growth systems when used in wastewater treatment allow the formation of a consortium of microorganisms including microalgae and bacteria [7]. The interaction of these microorganisms and the association of several parameters interfere in the formation and structure of the formed biofilm [8]. However, there is not much information about the formation of biofilms in these adhered growth systems, making the studies to deepen the optimization of the various parameters that involve flow velocity, support material, light availability,

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nutrients, among others [7].

The support material to which microalgae adhere is of great relevance to the performance of the entire production system, and, to date, there is no support material considered as the standard for biofilm development [9].

In the literature, several materials for adherence growth study have already been tested in terms of cell binding, durability and cost of the support material. The materials with the best performers were cotton [10,11], polystyrene foam [12] and nylon mesh [13]. However, with the exception of Lee et al. [13], the tests for support materials choice were carried out on bench scale and under controlled environmental conditions. In addition, very few of them [12,13] used real effluents during tests.

Furthermore, the different choices of materials reported in the literature were associated to the different culture media, microalgae species and environmental conditions to which they were submitted. Impacts related to real environmental conditions (radiation, precipitation, wind, etc.) and cultivation in real wastewater still require further investigations. Therefore, this study is aimed at assessing the performance of support materials (cotton, nylon and polyester) on microalgae production cultivated in domestic sewage, with respect to the adherence of the cells, durability of the material, and biomass composition. The research is also expected to contribute to the production of microalgae biomass and to separation and harvesting techniques through scraping, in real scale, to be simultaneously applied to wastewater treatment and biomass use.

2. Materials and methods

2.1. Cultivation medium

The cultivation medium used for biomass growth was domestic sewage, previously treated in an upflow anaerobic sludge blanket reactor (UASB), located in a wastewater treatment plant in Viçosa, Brazil (20°45′14″S, 42°52′54″W). No inoculum addition was performed, therefore, autochthonous species have developed in the culture medium constituting a consortium between bacteria and microalgae. The monitoring was carried out between July and October 2016, which comprised winter and spring. The average temperature and mean relative air humidity during the experiment were, respectively, 19 °C and 73% [14].

2.2. Biofilm reactors design

The experiment was carried out using four reactors; three of them used adherent support material for microalgae growth: the first was cotton (Karsten, vagonite 1560, 100% cotton); the second was polyester (Sk Textil, oxford, 100% polyester); and the third was nylon (Tegape, ASTM-35, 100% polyamide). The choice of materials was based on the literature reports taking into consideration the materials that had best performance during biomass adhered production and their availability in the local market. Table 1 shows the characteristics of the yarn density of the support materials used in the study, as well as their source.

Table 1
Density of the threads of the support materials.

	Nylon	Polyester	Cotton
Source of the fibers	Synthetic	Synthetic	Natural
Direction of the weft (threads along the width of the material, per cm^2)	5	21	24
Direction of the warp (threads along the length of the material, per cm^2)	7	19	21

The fourth reactor was a control reactor in which no adherent support was used. The control reactor was used to compare biomass suspended production and the removal of nutrients from domestic sewage to reactors that contained adherent support materials.

Fig. 1 presents the system of reactors used for the biomass attached growth. In each reactor there was an acrylic plate (51 × 33.5 cm) (2 mm, Plasttotal) coated with the adherent support material (51 × 33.5 cm), which was supported by a Styrofoam plate of equal dimensions (2 mm, Pluma). The recipient (55 × 35 × 9 cm; Plasútil), used to support the Styrofoam and allow recirculation of the sewage to the adherent support materials, presented a longitudinal opening of 17.6 cm^2 at the bottom center, where the effluent was drained by gravity. This recipient was placed on a counter, 45.5 cm of the ground, with an inclination of 7.6° in relation to the horizontal.

The application of domestic sewage to the adherent support materials was implemented through a longitudinal opening (30.5 × 1.5 cm) in a PVC pipe (diameter = 10 cm, length = 35 cm), which was filled with number 1 gravel and assembled above the adherent support materials. A submerged pump (Litwin 700/550) operating with a flow of 550 $L{\cdot}h^{-1}$ recirculated the sewage from the recipient on the soil (plastic box 33 × 27 × 46 cm, 15 L) to be distributed to the adherent support materials.

2.3. Biofilm reactors operation

In the first days of operation, samplings were not performed. It was a period of observation of the growth of the biomass adhered on the support materials. The criterion adopted for the scraping of the biomass was the occurrence of cell loss. On the 57th day of operation, it was observed that the adhered biomass was already formed in all the reactors and that there was also the detachment of the same. Then all the biomass was scraped and collected, characterizing itself as the first cycle of growth. The remaining cells were maintained in the adherent materials as inoculum for the next growth cycle, which lasted 32 days.

The reactors were operated in semi-continuous flow of 3 $L{\cdot}d^{-1}$, so that the sewage input and output took place manually. All reactors were set in the East–West direction to guarantee the incidence of direct solar radiation, using a solar chart [15] for the municipality of Viçosa as reference.

2.4. Biomass production and nutrients removal

The monitoring of the attached biomass growth was carried out directly on the support materials, and the monitoring of nutrients removal was performed on the suspended biomass in each reactor. Both monitoring was carried out weekly. The samples obtained from the support materials consisted of three scrapings of 1.0 cm^2 in three different positions (samples from the right side, left side and from the middle). Scrapings were alternated at each collection to guarantee that the previous collection did not compromise the following one, as the attached biomass accumulated. In the control reactor, monitoring was carried out with the harvesting of suspended biomass, which was concentrated using a centrifuge at 10,000 rpm for 10 min (HITACHI, High-Speed Refrigerated Centrifuge, CR 21GIII). The monitoring of the attached biomass occurred through the determination of the total volatile solids (TVS) [16] for the quantification of the total biomass, and of chlorophyll-*a* for the indirect quantification of algal biomass. Chlorophyll-*a* was hot extracted with 80% ethanol [17]; the reading was carried out by spectrophotometry [16], and calculations were performed using the equations described by Schwarzbold et al. [18], adapted from Marker et al. [19] and Sartory and Grobbelaar [20]. Total biomass production curves were evaluated by linear regression over the entire period of second growth cycle.

For the suspended biomass of all the reactors, including the control, the parameters measured were dissolved oxygen (DO) concentration, temperature and pH through the Hach HQ40d which measured DO with

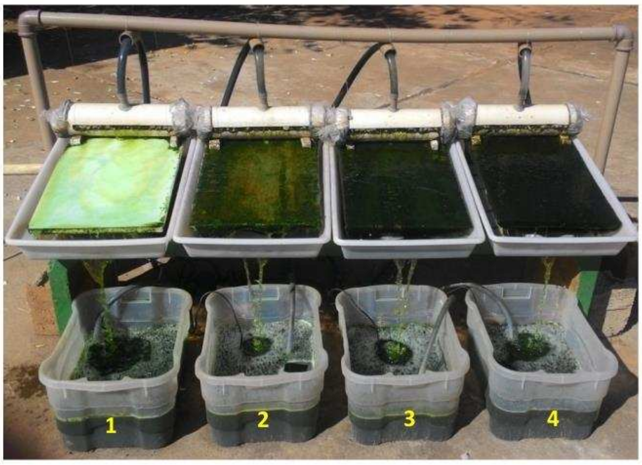

Fig. 1. System of reactors for the attached biomass growth in different support materials: (1) control reactor; (2) nylon; (3) polyester; (4) cotton.

luminescence-based optical sensors (luminescent dissolved oxygen, LDO). In addition, the concentrations of volatile suspended solids (VSS; 2540E), ammonia nitrogen (NH_3-N; 4500-NH_3C), nitrate (NO_3^--N, 4500-NO_3A), and soluble phosphorus (P_s; 4500-P C; samples filtered at 0.45 μm), were measured according to APHA [16]. For the determination of chlorophyll-*a*, an 80% hot ethanol extraction [17] was used. The reading was performed by spectrophotometry [16] and the calculations were carried out using the equations described by the Dutch norm [21]. The incident photosynthetically active radiations (PAR) were also measured, using the radiometer LI-COR LI-193 Underwater Spherical Quantum Sensor, between 12 and 1 pm.

The specific growth rate (μ, d^{-1}) of the microalgae was calculated using Eq. (1) [22].

$$\mu = \frac{(\ln Xi - \ln Xo)}{ti{-}to} \tag{1}$$

where Xo is the initial concentration of the biomass (dry weight) at time t and Xi is the concentration of biomass at time ti.

2.5. Characterization of biomass

The attached biomass was dried in an oven at 50 °C for 24 h and then macerated. Subsequently, contents of humidity, ash, and nitrogen were determined (adapted from APHA) [16]. The protein content was determined using the conversion factor of nitrogen to protein of 6.25 [23], and the content of neutral lipids was defined through the methodology described by Assis et al. [24]. The carbohydrate content was determined from the quantitative acid hydrolysis of the biomass [25] followed by the phenol-sulfuric method [26]; the reading was quantified by spectrophotometry (490 nm) using the glucose standard curve.

2.6. Biomass adherence and durability of the support materials

At the end of the experiment, the fibers of each support material were extracted and analyzed under an optical microscope (Olympus, model IX51) 40× magnification was used to observe the differences between the fibers prior to and after contact with the domestic sewage, as well as during the experiment to assess the adherence of the biomass to the support materials studied.

The durability of the adherent support materials was also assessed prior to and after the experiment through friction tests carried out in a torsiometer (Mathis CRO). In this equipment, a 4 cm^2 sample of each adherent support material was submitted to 1000 frictions over 15 min. The procedure was repeated until the rupture of the fibers. When the fibers of the support materials showed no signs of rupture, the test after the experiment was interrupted with the same number of frictions reported in the test prior to the experiment. It should be noted that this friction test concerns only the mechanical durability of the support material and not the chemical durability of the contact and exposure to factors such as sewage, solar radiation, among others.

2.7. Statistical analysis

The Minitab 17 ® program was used to evaluate the differences between the mean values of the variables measured in biofilm reactors. Analysis of variance was also performed. The experiment used was a completely randomized design for Tukey test with 5% probability.

3. Results and discussion

3.1. Biomass production

Fig. 2 shows the growth curves of total biomass in the biofilms formed in each support material. Only in the polyester support (Fig. 2b) the biomass growth curve was adjusted to a first order kinetics ($R^2 = 0{,}9369$). Knowledge about microalgae growth kinetics is still scarce in the literature and presents controversies [27]. In some studies linear kinetics were observed [11,28–30]. Other authors believe in the existence of an exponential phase of growth until the biofilm presents maximum thickness, later, this biofilm suffers desquamation [31]. The nylon and cotton supports had low exponential adjustment and, therefore, other kinetics were tested. For these materials, second-order polynomial kinetics were the best fit, with $R^2 = 0.7084$ for nylon (Fig. 2a) and $R^2 = 0.9808$ for cotton (Fig. 2c).

These results can be better interpreted through Table 2, which presents the production and productivities of total and algal biomass in each reactor. The total and algal biomass production in the control reactor were quantified with the purpose of verifying the growth of the biomass independent of the support materials, under the same environmental conditions. There was no statistical difference in total biomass production between the reactors ($p > 0.05$) and, for the algal biomass production, the averages were higher for the polyester support

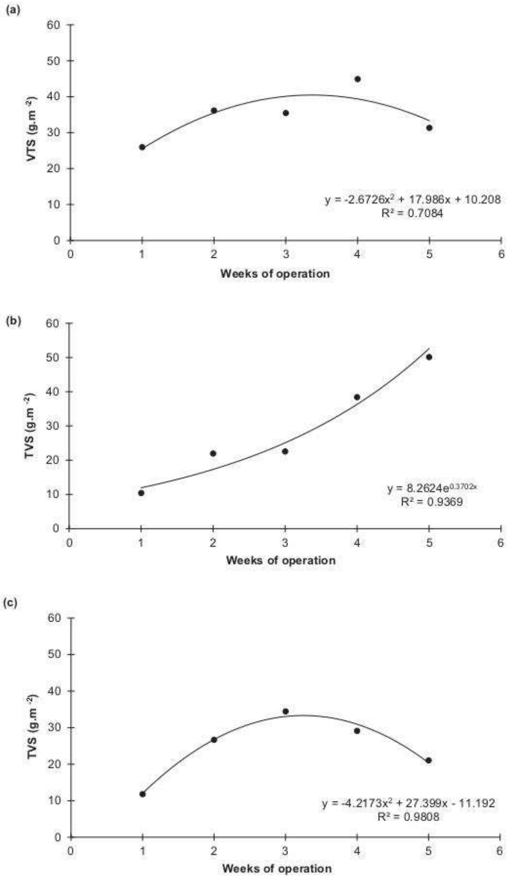

Fig. 2. Growth kinetics curves for algal biofilms grown on three different materials: nylon (a), polyester (b) and cotton (c).

(p = 0.018).

The cotton support presented a specific growth rate of 0.12 d^{-1} and a production peak of 34.5 $g{\cdot}m^{-2}$, which occurred on the 18th day of cultivation or in the 3rd week of operation (Fig. 2c). This peak reached a productivity of 1.91 $g.m^{-2}{\cdot}d^{-1}$. At the same time, the curves of the other support materials were still growing, showing that the stationary phases of the cell growth curves in the nylon and polyester supports occurred in higher yields of volatile solids. The nylon support had a maximum peak of 44.92 $g{\cdot}m^{-2}$ of total biomass during the period of 25 days (4th week of operation, Fig. 2a), yielding a productivity of 180 $g{\cdot}m^{-2}{\cdot}d^{-1}$ and a specific growth rate of 0.05 d^{-1}. The polyester support achieved a maximum total biomass production of 50.12 $g{\cdot}m^{-2}$ in 32 days (5th week of operation, Fig. 2b), which corresponds to a daily productivity of 1.57 $g{\cdot}m{\cdot}^{-2}{\cdot}d^{-1}$ and a specific growth rate of 0.11 d^{-1}. As shown in Fig. 2b, polyester was the only material that kept exponentially increasing its production throughout the growing period of the biomass.

The different properties of the substrates tested, such as hydrophobicity and surface roughness, may have influence the fixation of algal cells [27]. Among the materials tested in this study, cotton was the most hydrophilic and also the rougher material and, therefore, showed a higher adhesion of the cells in a shorter culture time when compared to other materials. This can also be explained by the use of meshed substratum material, which is considered an alternative to increase the roughness of the surface of the support to be used for the adhesion of microalgae [27]. As shown in Table 1, the opening of the cotton mesh used in this study was smaller than the openings of the nylon and polyester mesh, justifying once again the greater adhesion of the cotton.

The productivity results in this research were lower than the productivity found in other studies that evaluated the support materials for adhered growth of algal biomass. For cotton support, in the literature were found productivities between 1 and 6 $g{\cdot}m^{-2}{\cdot}d^{-1}$ [10,11,26]. For nylon and polyester, productivities of 13 $g{\cdot}m^{-2}{\cdot}d^{-1}$ [32] and 11.79 $g{\cdot}m^{-2}{\cdot}d^{-1}$ [32], respectively. It should be noted that all test results cited above were obtained in laboratory scale experiments under controlled environmental conditions.

When compared to other microalgae production and effluent treatment systems, the productivity of this study was also lower. Xu et al. [33] evaluated a membrane bioreactor for high-density *Chlorella emersoniie* cultivationn with a hydraulic retention time of 1 day and obtained na average productivity of 6.2 $g{\cdot}m^{-2}{\cdot}d^{-1}$. Buchanan et al. [34] evaluated the treatment of domestic sewage from a septic tank in high-rate algal pond with a flow rate of 12 $m^3{\cdot}d^{-1}$ and found na average productivity of 31.7 $g{\cdot}m^{-2}{\cdot}d^{-1}$.

The low biomass production was mainly related to the consortium of microorganisms, with the conditions of the experiment (larger scale, without control of the external environment interference), and also with the low frequency of scraping. In this study, the scraping of the total biomass was performed only twice, with a period of approximately 40 days between scraping. Some authors have pointed out that very long scaling intervals impair the distribution of nutrients and light in the algal cells located in the lower layers of the biofilm [35]. Among the scraping frequency already studied, the once-weekly interval was more appropriate for the production of adhered biomass [11,36,37].

In addition, in the mixotrophic environment as in this study, the development of microalgae may be inhibited by the extracellular metabolites produced by bacteria [38]. Also, low productivity can be justified for high cell density cultures. The presence of bacteria can reduce light availability to microalgae cells and, consequently, lower production of high algal biomass molecules, such as carotenoids and fatty acids [39].

3.2. Characterization of biomass

The total and algal biomass productions in the control reactor were

Table 2
Total and algal biomass in the supports materials.

Support material	Total volatile solids			Chlorophyll-*a*		
	Maximum production ($g{\cdot}m^{-2}$)	Maximum productivity ($g{\cdot}m^{-2}{\cdot}d^{-1}$)	$\mu_{maximum}$ (d^{-1})	Maximum production ($g{\cdot}m^{-2}$)	Maximum productivity ($g{\cdot}m^{-2}{\cdot}d^{-1}$)	$\mu_{maximum}$ (d^{-1})
Nylon	44.92	1.80	0.05	0.59	0.02	0.14
Polyester	50.12	1.57	0.11	0.90	0.03	0.26
Cotton	34.46	1.91	0.12	0.42	0.02	0.28

Table 3
Mean characterization of the biomass in each reactor.

	Control	Nylon	Polyester	Cotton
Humidity (%)	82.9 (8.3)[a]	92.0 (1.1)[b]	89.0 (1.6)[b]	89.5 (2.4)[b]
Ash (%)	26.1 (15.8)	23.6 (7.4)	20.9 (6.5)	20.4 (8.1)
Proteins (%)	24.6 (5.4)	23.8 (1.5)	24.7 (0.5)	25.6 (2.4)
Carbohydrates (%)	20.8 (6.4)	21.2 (5.0)	20.7 (5.0)	21.1 (5.2)
Neutral lipids (%)	5.0 (2.3)	3.7 (0.04)	7.7 (1.1)	10.6 (3.9)

[a] Biomass humidity after centrifugation.

[b] Harvested biomass humidity. Mean values, standard deviation in parenthesis.

quantified in order to verify the biomass growth independently of the support material, but under the same environmental conditions. Although the biomass production in the reactors with support material and the control reactor was assessed based on different growth conditions (adhered and suspended), some similarities were noticed and, in general, the different support materials did not interfere in the composition of the cells present in the attached biomass (Table 3).

In this context, it is essential to understand the characterization of the cultivated algal biomass in order to apply its proper destination for better utilization and efficiency of the process. The biomass characteristics of all reactors remained similar, and was mostly composed by proteins (24 to 26%), followed by carbohydrates (20 to 22%) and ash (20 to 26%), and the lowest portion, the lipid content (3 to 7%). Moisture content of the collected biomass varied between 82 and 92%.

Despite the similarity in the moisture content of the collected biomass, for the control reactor, a centrifugation stage was necessary in other to harvest the suspended biomass, a step that demands energy and costs when it comes to large-scale production, reducing the economic viability of the whole process [40]. In the reactors with support materials, biomass was collected by scraping, a stage that require low energy, simpler and more profitable, when comparing to centrifugation. In addition, all tested reactors presented an acceptable moisture content for anaerobic digestion of the biomass [41] without need for any extra concentration stage.

Also in terms of energy production, the ash content was high and was directly associated to the culture medium, which was the same for all reactors, being the amount related to the fixed solids content presented in domestic sewage. The high proportion of ash in biomass may impairs the production of biofuels and contributes negatively to the quality of biofuels in several ways, for example, increases the contamination of the fuel, increases the operational costs related to the harvesting and separation of biomass, makes more complicate thermochemical and biochemical energy conversions process, among others [42].

Neutral lipid content was generally considered to be very low in all reactors when compared to values reported for green algae (13 to 31%) and cyanobacteria (5 to 13%) [43]. The presence of bacteria in the domestic sewage causes a reduction in the energy content of the total biomass cultured, since they present lipid content typically lower than 10% [44,45].

Carbohydrate levels were close to the results found by Choudhary et al. [37] for the biofilm growth reactor using domestic wastewater as the culture medium (24%). These authors stated that the range between 13 and 22% of carbohydrates is adequate for supplementation of bovine ration (20 to 25%). However, prior to this biomass processing for animal feed it is highly recommended the elimination of pathogens or any other toxic compounds that the biomass may presents [37].

Protein contents were below the range (32–38%) found by Gross et al. [11] in adhered seaweed biomass. The authors considered that this range of protein content was promising to produce feed and fish farming. However, Cole et al. [46] evaluated the protein content in the production of the *Oedogonium* macroalgae as an alternative feed production. The protein maximum value of 18% was found in dry biomass. The authors reported that this amount is equivalent or greater than many terrestrial cultures used as a source of protein for animal feeding. In addition, biomass could be used as a supplementary protein in animal feed.

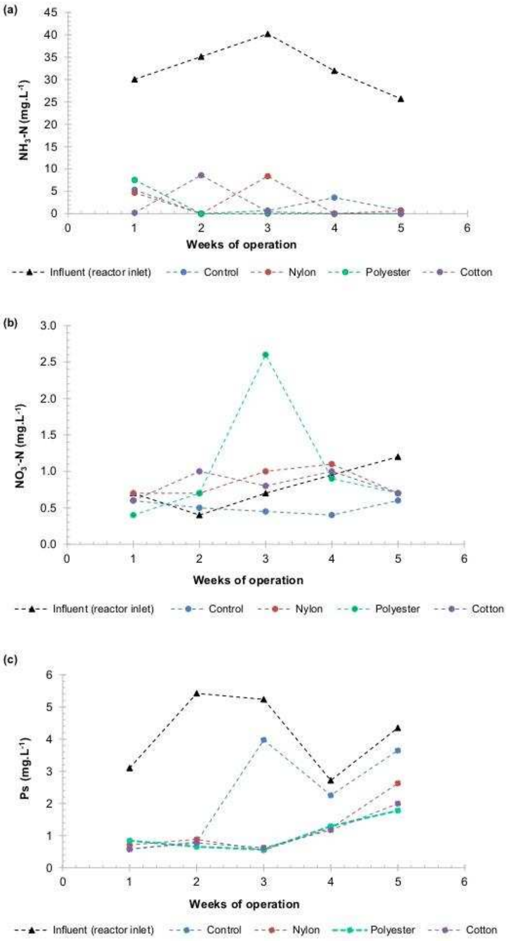

Fig. 3. Nutrients concentration in domestic sewage, in control and biofilm reactors: (a) ammonia nitrogen (NH_3-N, mg·L^{-1}), (b) nitrate (NO_3^--N, mg·L^{-1}) and (c) soluble phosphorus (Ps, mg·L^{-1}).

3.3. Nutrient removal

The mean value of PAR during the whole experiment was 1904.07 μE·m^{-2}·s^{-1}. This extra light was not a limiting factor for biomass production, since the culture medium presented a consortium of heterotrophic and autotrophic microorganisms. When the culture medium is composed of great variety of organisms, there is a reduction in the effect of photoinhibition [47]. PAR variable is important because it can directly influence the growth of biomass, favoring the photosynthetic activity, which, in turn, provides oxygen for the growth of heterotrophic bacteria, thus realizing the degradation of the organic matter present in the domestic sewage.

In Fig. 3 are presented the concentrations of nutrients in the influent (reactor inlet) and effluents (outlet) of reactors. The ammonia nitrogen concentrations (Fig. 3(a)) were almost completely removed, with rates higher than 90%. The symbiotic relationship between microalgae and bacteria can lead to competition for the transformation of this nutrient. While microalgae incorporate this nutrient into their biomass, the bacteria oxidize ammonia to nitrite and then to nitrate [48,49]. The increase in the nitrate concentration together with the high removals of ammonia nitrogen indicate the occurrence of nitrification. In the biofilm reactors there was an increase of approximately 12%, 41% and 9%, in the cotton, polyester and nylon support materials, respectively, while their removal in the control reactor was 32% (Fig. 3(b)). Despite the preference of microalgae for ammonia nitrogen, nitrate can represent an important source of nitrogen for their growth. However, as in all reactors pH values ranged from 10 to 11, the development of nitrifying bacteria may have been impaired [48] and another possible conversion route of NH_3-N may have been the volatilization [50].

All the reactors presented similar behavior curves in the removal of Ps (Fig. 3(c)). However, the concentrations of P_s were higher in the control reactor, indicating a better performance of the biofilm in the removal of this nutrient. The P_s removals remained between 70 and 76% in the biofilm reactors and 46% in the control reactor. As in this study, pH values were relatively high (between 10 and 11) and as a culture medium of high algal density, it is possible that besides the assimilation by the microalgae, phosphorus precipitation occurred [33]. These removal percentages can be considered larger than the conventional wastewater treatment technologies and microalgae production processes [50,51].

3.4. Durability of the support materials and biomass adherence

The results of durability tests of the adherent support materials are shown in Table 4. After the experiment, the cotton was the most fragile material and presented a rupture of its fibers with only 1102 frictions. Polyester's resistance to friction increased from 7000 to 46,563 frictions. After the experiment, the polyester showed signs of breaking of its fibers, justifying the continuity of the test up to 46,563 frictions. While the nylon fibers did not rupture at all during evaluation, either at the time before or at the time after the experiment. For this reason, the test was interrupted at 20,000 frictions, without a sign of rupture. These durability results are largely due to the characteristics of these materials. According to Erhardt et al. [52], polyester and nylon fibers are resistant to friction, climate conditions, light, and harmful insects.

Previous studies have reported different support materials with good adherence for biomass growth. Johnson and Wen [12] assessed four support materials using dairy effluent on a laboratory scale, and concluded that the polystyrene foam presented the best results with respect to durability and reuse. Among the 16 different materials assessed by Gross et al. [11] on a laboratory scale, the cotton support presented the highest durability and biomass productivity. In another laboratory study, Gross et al. [32] used pure culture of microalgae and found that the nylon and polypropylene supports provide better adhesion to the initial fixation of the biomass and are also more resistant in the long term. Genin et al. [29] used pure culture medium inoculated with wastewater for adhered production of algal biomass in a laboratory scale. Four types of support materials were tested and cellulose acetate presented the highest productivity. It is observed, however, that the different media considered by the authors as the most important in the development of the biofilm were associated with the different media of cultures and environmental conditions. Therefore, the choice of supporting material from one study will not always satisfy the demand for other work that considers different cultivation media and environmental conditions.

Fig. 4 shows the fibers of the support materials prior to and subsequent the experiment. In this study, the cotton support presented higher biomass production and adhesion results (Fig. 4(a)), however, as previously discussed, its durability was low compared to the other tested materials. During the experiment, cotton fibers were disrupted even before scraping was performed in the biofilm reactor. The cotton fragility was also reported in the work of Gross and Wen [53], where cotton was used as a support in a pilot scale reactor under environmental conditions of a greenhouse made from a transparent double polycarbonate wall. Within six months, the support material was totally damaged. Despite the high durability and biomass production, in the nylon support it was noticed that the biomass was more adhered to the acrylic plate that supported the nylon support than in the support itself. In Fig. 4(c) it is possible to note how the biomass was little evidenced in nylon fibers. The greater aperture between the weft and warp yarns (Table 1) may also have favored the non-fixation of the biomass on this material.

Unlike the support made of nylon, in which the number of these yarns was smaller, the cotton and polyester supports had a greater number of wefts and warps, per cm^2, facilitating the adhesion of biomass. For this reason, the present study disregarded the possibility of nylon application to the adhered cultivation of algal biomass in domestic sewage.

The polyester support was the material that best satisfied the conditions of adhesion and durability taking into account the experiment conditions, i.e., contact with domestic sewage and solar radiation during 32 days of algal biofilm culture. Similarly, under effect evaluation of physicochemical surface of the support materials in the colonization and the adhered growth of the algal biofilm, Gross et al. [32] found the polyester as one of the six materials that most fixed algal cells.

4. Conclusion

The polyester support was the material that presented the best results in terms of durability and the highest biomass production during biomass regrowth ($50.1\ g{\cdot}m^{-2}$). As for biomass characterization, the different support materials did not interfere in the composition of the cells present in the attached biomass. In relation to the sewage treatment, the biofilm reactor with polyester support presented greater nitrate increase, being indirectly associated to the greater development of filamentous microorganisms. The removal of ammonia nitrogen was $> 90\%$ and the soluble phosphorus removals were between 70 and 76%.

The selection of the polyester support as the material that best performance to biofilm growth will contribute to future studies that consider the production of microalgae biomass and their separation and

Table 4
Friction test results for the adherent support materials.

	Cotton		Polyester		Nylon	
	Before contact with sewage	After contact with sewage	Before contact with sewage	After contact with sewage	Before contact with sewage	After contact with sewage
Number of frictions	1518	1102	7000	46,563	20,000[a]	20,000[a]

[a] The test was carried out up to 20,000 frictions. However, the material did not present any sign of rupture and the test was interrupted.

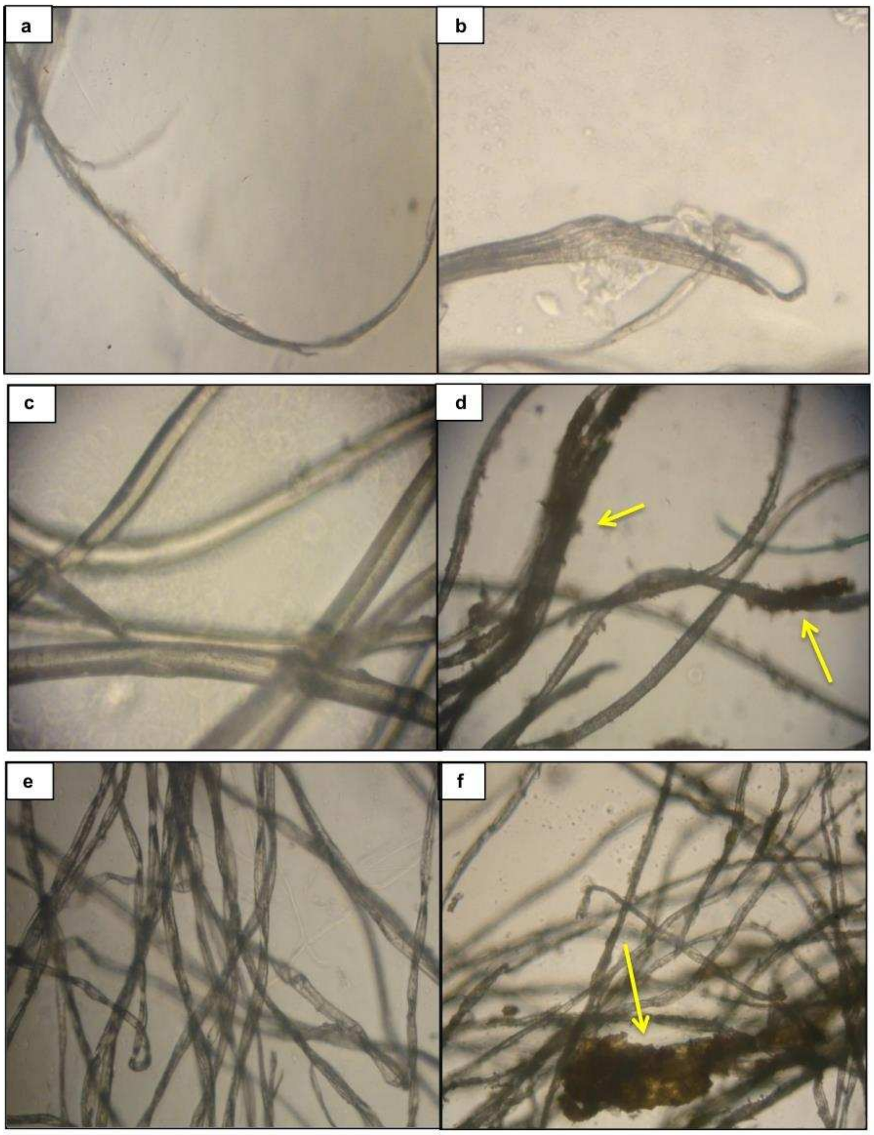

Fig. 4. Fibers of the support materials: nylon (polyamide) prior to (a) and after (b) contact with cultivation medium; polyester prior to (c) and after (d) contact with cultivation medium; cotton prior to (e) and after (f) contact with cultivation medium. The yellow arrows indicate the biomass attached to the fibers. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

harvesting through scraping, in real scale, to be applied for wastewater treatment as well as for biomass reuse. It should be noted that in other cultivation medium and environmental conditions, other media may be considered.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Declaration of authors' contribution

All authors were involved in the conception and design of the study, collection and assembly of data, analysis and interpretation of the data, and drafting of the paper. All authors agree to submission of the final version of the manuscript for peer review. L.R. Assis takes responsibility for the integrity of the entire work and can be contacted as leticia.assis@ufv.br.

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ANEXO 2

Assis, L.R., Calijuri, M.L., Assemany, P.P., Nascimento, L.A., Febroni, L.V. Evaluation of the performance of reactors with different slopes for microalgae production. In: 2nd IWA Conference on Algal Technologies for Wastewater Treatment and Resource Recovery and 12th IWA Specialist Group Conference in Wastewater Pond Technology - IWAlgae2019, 2019, Valladolid. IWA Specialist Group, Valladolid, 2019.

Evaluation of the performance of reactors with different slopes for microalgae production

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Introduction

Attached growth reactors are new technologies within the algal biomass culture scenario and have been considered an alternative for adaptation and optimization of conventional systems of suspended biomass culture, such as high-rate ponds. These reactors are capable of optimizing the production and separation of biomass due to its advantages, which are summarized in water economy, high biomass productivity and harvest efficiency (Wang et al. 2017). When used in wastewater treatment, attached growth reactors supply favorable growth conditions for a consortium of microorganisms that have the capacity to metabolize pollutants. In addition to the microalgae and bacteria organic matter degradation, the development of nitrifying bacteria, which have filaments in their cells and have a preference for static environments, also occurs (Babu et al. 2007).

In the attempt to increase biomass production and wastewater treatment efficiency, different types of algal biomass attached growth reactors have already been developed according to different slope angles, ranging from 0.2° a 90° in relation to plane horizontal, and reached yields between 0.7 $g.m^{-2}.dia^{-1}$ and 9.1 $g.m^{-2}.dia^{-1}$ (Ozkan et al. 2012, Chen et al. 2014, Lee et al. 2014). In this study, the production of algal biomass was evaluated in attached growth reactors with different slope angles (15°, 45° e 90°). The reactors were exposed directly to solar radiation and had as their culture medium the domestic sewage, pretreated in septic tank. The results of nutrient removal from domestic sewage and biomass production were compared in each reactor.

Materials and Methods

The experimental configuration consisted of three reactors of algal biomass adhered growth, which had different slopes in relation to the horizontal plane: 15°, 45° and 90°. In each reactor there was a container (plastic box 33 x 27 x 46 cm) with a useful volume of 15 L, where domestic sewage (pretreated in a real scale septic tank) was stored and recirculated by a submerged pump (Litwin 700/550, flow rate 550 $L.h^{-1}$) up to an acrylic plate (51 x 33.5 cm) (2 mm, Plasttotal) coated by polyester support material (Sk Textil, oxford, 100% polyester) (51 x 33,5 cm), in which the biomass grew attached. Every day a volume of 5.5L of sewage was replaced in the reactors, characterizing a semi-continuous operation. The reactors were operated for a period of 42 days, until the attached biomass layer was formed. From the 43rd day, the biomass growth was followed by weekly collections of attached biomass and analyzes of total volatile solids (TVS, APHA 2012) and chlorophyll-a (Nush 1980, NEN 1981). The nutrient removal was monitored through ammonia nitrogen, nitrate and soluble phosphorus analyzes, following the methodology APHA (2012). The biomass drag determined the biofilm growth cycle on each reactor and the collection by scraping, of the all the biomass. Precipitation data were obtained at the Main Climatological Station of the Federal University of Viçosa, where the experiment was carried out (UFV 2018). The software Minitab 17® was used to evaluate the differences between the mean values of the variables measured in the reactors, through the Tukey test, with a 5% probability.

Preliminary Results

Figure 1 shows the total biomass (TVS) production and precipitation throughout the experimental period. In all reactors, the curves showed a similar behavior and the TVS concentrations were statistically the same ($p>0.05$) during the period when there was no precipitation. From the 113th day of cultivation, the precipitation became frequent with values between 11 mm and 25 mm. In this period, the biomass of the 90° reactor remained attached (no losses were observed), and the mean TVS of this reactor was about 3 times higher than the other reactors (p=0.00). Slopes interfered in these results, since the reactors were exposed to environmental conditions, and frequent precipitation removed most of the attached biomass from the 15° and 45° reactors. The raindrops in direct contact with these reactors carried all the biomass already attached, whereas in the 90° reactor, the raindrops flowed parallel to the biomass, keeping it adhered to the reactor. Similarly, to the TVS results, chlorophyll-a concentrations showed statistically equal values during the dry season (p>0.05) and higher values for the 90° reactor during the rainy season (p=0.005).

The concentrations of ammonia nitrogen, nitrate and phosphorus were statistically the same in all reactors (p>0.05). The concentrations of ammonia nitrogen in all reactors were removed with efficiencies higher than 90% due to algal assimilation and nitrification. All reactors showed an increase of nitrate concentrations by approximately 260%. The presence of biofilm could have favored the development of nitrifying bacteria, as these have filaments, prefer static environments and rarely live as suspended and free bacteria (Hammer and Knight 1994). Phosphorus removals were between 60 and 75%, considered high when compared to conventional sewage treatment processes.

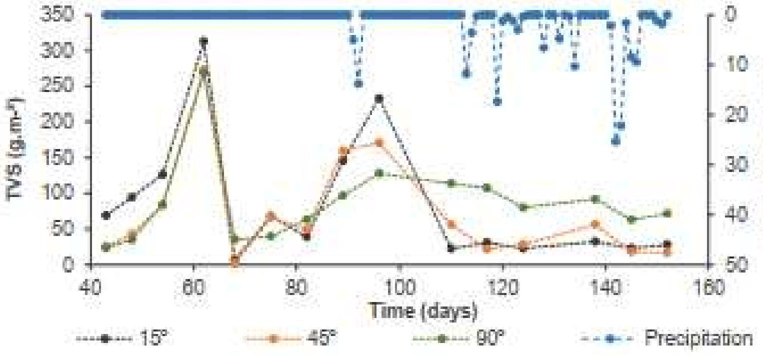

Fig.1. Time course of TVS ($g.m^{-2}$) in attached growth reactors and precipitation (mm) of the experimental period.

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ANEXO 3

Assis, L.R., Calijuri, M.L., Assemany, P.P., Silva, T.A., Teixeira, J.S. Innovative hybrid system for wastewater treatment: High-rate algal ponds for effluent treatment and biofilm reactor for biomass production and harvesting. Journal of Environmetal Management, 274, 111183, 2020. https://doi.org/10.1016/j.jenvman.2020.111183

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Research article

Innovative hybrid system for wastewater treatment: High-rate algal ponds for effluent treatment and biofilm reactor for biomass production and harvesting

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ABSTRACT

The use of algal biomass still faces challenges associated with the harvesting stages. To address this issue, we propose an innovative hybrid system, in which a biofilm reactor (BR) operates as an algal biomass production and harvesting unit connected to a high-rate algal pond (HRAP), a wastewater treatment unit. BR did not interfered with the biomass chemical composition (protein = 32%, carbohydrates = 11% and total lipids = 18%), with the wastewater treatment (removals efficiency: chemical oxygen demand = 59%, ammonia nitrogen = 78%, total phosphorus = 16% and *Escherichia coli* = 1 log unit), and did not alter the sedimentation characteristics of the biomass (sludge volume index = 29 mg/L and humidity content = 92%) in the secondary settling tank of the hybrid system. On the other hand, the results showed that this technology achieved a biomass production about 2.6x greater than the conventional system without a BR, and the efficiency of harvesting of the hybrid system was 61%, against 22% obtained with the conventional system. In addition, the BR promoted an increase in the density (~1011 org/m^2) and diversity of microalgae in the hybrid system. Chlorella vulgaris was the most abundant species (>60%) from the 4th week of operation until the end of the experiment. Hence, results confirm that the integration of BR into a wastewater treatment plant optimised the production and harvesting of biomass of the hybrid system, making it a promising technology. The importance of economic and environmental analysis studies of BR is highlighted in order to enable its implementation on a large scale.

1. Introduction

The cultivation of microalgae in wastewater is considered an economical approach to provide water and nutrients for algal growth, especially given the current need to change the wastewater treatment paradigm for resource recovery (Zhang et al., 2018a). After its separation and harvesting, microalgae biomass can then be used as an alternative source for energy, animal feed and fertiliser production (Choudhary et al., 2020).

Despite our comprehensive knowledge about the potential of microalgae in wastewater for the generation of several bioproducts, the use of algal biomass still faces challenges associated with the separation and harvesting stages. The efficiency of separation and harvesting can be influenced by several factors such as the morphology of microalgae, the density and size of cells, the final bioproduct to be obtained and the culture medium (Singh and Patidar, 2018). Regardless of the separation method, it must overcome the main difficulties associated with the separation of microalgae. Thus, it must consider the small size of the cells (<20 μm), the similarity of the microalgae density to water, a surface with a high negative charge (zeta potential) and high growth rates, resulting in more frequent harvests. In this sense, the challenges regarding the economic viability of the recovery and valorisation of microalgae biomass still persist (Singh and Patidar, 2018).

In view of the various separation and harvesting techniques not yet consolidated in the scope of biomass recovery, new reactors are being designed on smaller scales with the objective of minimising the above-

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mentioned disadvantages (Foladori et al., 2018). The use of biofilm reactors (BRs) is a technological innovation that consists of a matrix of microalgae, bacteria and fungi grown attached to a solid support (Sutherland and Craggs, 2017; Mantzorou and Ververidis, 2019). In these reactors, light, temperature, as well as nutrients concentrations are the main factors that determine the growth rate of microalgae biomass, which are dominant in the microorganism consortium matrix. Periodic harvesting of biomass removes nutrients assimilated by microalgae and the reactor can be considered a biological treatment system used to mitigate wastewater pollution (Sutherland and Craggs, 2017).

Therefore, the main advantage of BRs when compared to conventional forms of separation is the ease of removing microalgal cells from the solid surface by scraping (Zhang et al., 2018b). After scraping, the organisms that remain on the material are used as inoculum for the next growth cycle (Assis et al., 2017). This reactor type has a higher productivity, in addition to a lower surface area demand, than suspended cultivation systems (Mantzorou and Ververidis, 2019). Furthermore, the amount of water in the biomass is low, eliminating the dewatering process, and microalgal biofilm receives more light when compared to biomass suspended in the water column (Zhang et al., 2018b). High-rate algal ponds (HRAPs) are efficient reactors for the treatment of wastewater and the production of microalgae. In this sense, BRs can be connected to these units, forming a hybrid system for biomass production and wastewater treatment. The integration of these two technologies emerges as an improvement of the existing wastewater treatment plants (WWTP) (Zhang et al., 2018a).

In the literature it is possible to find positive results from the implementation of hybrid systems. Zhang et al. (2018a) cultivated microalgae in synthetic wastewater with different nutrient loading rates and reported biomass productivity values in the range of 10.54–14.68 g/m^2. day. Based on laboratory scale studies, a methane production potential of 21.471–29.136 m^3/ha.year and a biodiesel productivity of 0.57–1.15 ton/ha. year were estimated. The authors reinforce the need for studies on a pilot scale, outdoors, to assess the reality of nutrient removal and biomass production, as well as its by-products. In the study of Zhang et al. (2018b), also on a laboratory scale, the distances between the BRs submerged in HRAPs were evaluated with different synthetic wastewater. Productivity decreased with increasing distance, with a distance of 2 cm showing the best result (18.51 g/m^2. day).

Among the pilot scale studies, Assis et al. (2017) reported that the presence of BR in an HRAP was able to supply the demand for carbon dioxide, avoiding the additional supplementation of this gas in optimizing the growth of microalgae in wastewater. Lee et al. (2014) evaluated nylon meshes submerged in a HRAP for 18 days and reported a maximum biomass productivity of 13.5 g/m^2. day, about 4x higher than a conventional HRAP. Gross et al. (2013, 2015) evaluated a hybrid system composed of revolving algal biofilm (RAB) adapted in HRAPs. The hybrid systems showed greater evaporation of water, due to the greater contact of the RAB with the air (Gross et al., 2015) and authors emphasized that the RABs systems have the potential to commercially produce microalgae with high productivity and efficient use from water.

In view of the need for new reactors that improve the separation and harvesting of suspended grown microalgae, this study presents, as an innovation, the pilot-scale application of a BR connected to a wastewater treatment system, composed of HRAP and secondary settling tank. The main objective was to evaluate the interference of this reactor in the production, separation and harvesting of algal biomass.

2. Materials and methods

2.1. Culture medium

The experiments were carried out in the experimental area of the Laboratory of Sanitary and Environmental Engineering at the Federal University of Viçosa, Viçosa, Minas Gerais, Brazil (20°45′14″S, 42°52′54″W). The domestic sewage used as a culture medium for biomass production was originated from a septic tank at a real-scale WWTP. No inoculum was added, and therefore, native species developed in the culture medium, with a consortium of bacteria, microalgae and other microorganisms.

2.2. Wastewater treatment systems and biomass production

Two systems of domestic sewage treatment, biomass production and harvesting, were evaluated on a pilot scale. System 1 was comprised of a conventional system consisting of a high-rate algal pond (HRAP 1) and a settling tank (ST 1). System 2 was comprised of a hybrid system of biomass production (suspended and biofilm), in which the suspended biomass grew in a HRAP (HRAP 2) and the biofilm was contained in a biofilm reactor (BR), followed by a settling tank (ST 2). Fig. 1 shows the scheme of the two systems used in this study. Monitoring was carried out between February and August 2019, a period that included the end of summer, autumn and the beginning of winter. Photosynthetically active radiation (PAR) was measured weekly (always between 12 and 2 p.m.) using a LI-COR LI-1500 Light Sensor Logger radiometer. The daily precipitation (data obtained from climatic station and available at the UFV (2019)) and the PAR throughout the experimental period are shown in Fig. 2.

The HRAPs had the following characteristics: width, 1.28 m; length, 2.86 m; total depth, 0.5 m; surface area, 3.3 m^2. They were made of fiberglass and steel paddlewheels, with six blades; paddlewheels were driven by a 0.5-hp electric motor. The speed was reduced by a speed reducer coupled to the motor and controlled by a frequency inverter (WEG CFW-10 series). The HRAPs were operated in continuous flow, with a useful depth of 0.3 m, a flow rate of 0.1 m^3/day and a hydraulic retention time (HRT) of 10 days. Settling tanks were positioned downstream of each HRAP and had a surface area of 0.16 m^2, a useful volume of 0.037 m^3, an HRT of 8.9 h (8 h and 54 min) and a surface loading rate of 0.625 m^3/m^2/day. The effluent from each HRAP was sent by gravity to each settling tank, and the concentrated biomass was collected weekly.

The BR was made of a flat acrylic panel and had the following characteristics: total surface area of 0.5 m^2, measuring 1.0 m in width and 0.5 m in length. The panel was kept in direct contact with atmospheric air and solar radiation; it was installed next to HRAP 2 and supported by PVC pipes at 0.85 m from the ground. To allow the growth of biofilm, the panel was coated with polyester (SkTextil, oxford, 100% polyester), as previously studied in Assis et al. (2019). The HRAP effluent was recirculated to the BR during 10 h per day, i.e. the useful volume of the pond was recirculated 10 times in a day, using an underwater pump (Sarlobetter SB 1000 A) at a 1 m^3/h flow rate. After being pumped, effluent was percolated through the panel surface by dripping, collected in a gutter and returned to the HRAP by gravity.

The inclination of the BR panel varied according to the season, due to the interference of precipitations in the growth of the biofilm. The inclination angles were defined in preliminary tests (data not shown); heavy rain events can compromise the performance of BRs, causing detachment of biomass, interruption in the capacity to remove nutrients and difficulty in colonising new algal cells, as reported by Sutherland and Craggs (2017). Therefore, in the rainy season (summer), the BR panel was maintained at 90° in relation to the horizontal plane, while in the dry seasons (autumn and winter), the BR panel was maintained at 15° in relation to the horizontal plane.

2.3. Scraping frequency of the biofilm reactor

For the determination of the total scraping frequency of system 2 BR, a preliminary test with two growth periods was performed. The initial period of biofilm growth in the BR required one 1 week for the adaptation and attachment of the cells to the support material. From the second week of operation, sampling started to monitor biofilm development. The progressive accumulation of biofilm on the reactor was

Fig. 1. Scheme of wastewater treatment and biomass production systems: (a) System 1, composed of HRAP and settling tank (ST1); (b) System 2, composed of HRAP, BR (in the photo BR with $\alpha = 90°$) and settling tank (ST2). The angle α in the BR indicates the variation in panel inclination during the experiment.

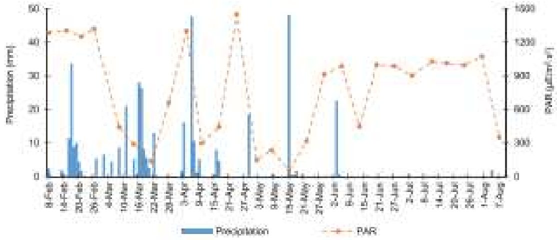

Fig. 2. Daily precipitation (mm) and photosynthetically active radiation (PAR, $\mu E/m^2 . s^2$) throughout the experimental period.

observed over 4 weeks of biomass monitoring. After the production peak, in the 5th week of monitoring, detachment of cells in the upper layers of the formed biofilm was noted, and the biomass was completely scraped off. The first 4 weeks of monitoring determined the first growth cycle of biomass. However, as stated by literature (Johnson and Wen, 2010; Christensen and Sims, 2012; Gross et al., 2013; Assis et al., 2017, 2019), after the first scraping of the biofilm, the new biofilm growth cycle has a higher yield compared to the initial growth, and the permanence of the inoculum saves initial downtime of the cells to the support materials (Gross et al., 2013). Therefore, it is not necessary to maintain the same period as for the first growth cycle and, in this study, the scraping frequency was determined every 3 weeks to ensure that no subsequent biomass loss occurred. The remaining biomass was kept on the support material and used as inoculum for the subsequent five biomass growth cycles.

2.4. Analytical measurements

The production of total biomass (g/m^2 of volatile solids) and the production of chlorophyll-*a* (g/m^2), an indirect measure of the algal biomass fraction, were monitored in each reactor of the systems on a pilot scale: in the effluents of the HRAPs, in the effluents from the settling tanks and, for system 2, both were monitored in the BR.

In the biofilm of the BR, samples were collected weekly by scraping with the aid of a spatula on the panel of the BR. Samples from three positions of the surface area of the panel were scraped (sample on the right, left and middle). To quantify the total biomass, an area of 6.25 cm^2 was sampled from each position of the panel, and total volatile solids (TVS) were analysed (APHA-AWWA-WEF, 2012). In chlorophyll-*a* analysis, which 1.0 cm^2 of each panel position was sampled and subsequently diluted in 20 ml of distilled water. The 80% ethanol extraction technique (Nush, 1980) was used for the analysis, the reading was obtained by spectrophotometry (APHA-AWWA-WEF, 2012), and the calculations were performed using the equations described by Schwarzbold et al. (2013), adapted from Marker et al. (1980) and Sartory and Grobbelaar (1984).

Suspended biomass was also analysed once per week. Volatile suspended solids (VSS) analysis was performed in the HRAPs effluent to determine the total biomass, according to the methodology described in APHA-AWWA-WEF (2012); for determination of chlorophyll-*a*, the 80% ethanol extraction technique was used, the reading was performed by spectrophotometry (APHA-AWWA-WEF, 2012), and the calculations were performed using the equations provided in the Dutch norm (NEN, 1981). In the effluent from the settling tanks, in addition to the same VSS and chlorophyll-*a* analyses described for the HRAPs samples, the following variables were also determined: ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total phosphorus (TP) and total chemical oxygen demand (COD), following the methodology described by APHA-AWWA-WEF (2012). The chromogenic-fluorogenic method (Colilert®) was used to determine *Escherichia coli* (*E. coli*). Dissolved oxygen (DO), temperature and pH of the culture medium were measured using the Hach HQ40d probe (Luminescent Dissolved Oxygen - LDO - for DO). In the raw domestic sewage, with the exception of chlorophyll-*a*, analyses of the same variables mentioned for the samples from the HRAPs and settling tanks were carried out.

2.4.1. Biomass separation and harvesting

The separation of biomass in the settling tanks was evaluated for humidity content, sedimentation and daily harvesting. The humidity content on a wet basis and the sedimentation of the sludge were evaluated using the sludge volume index (SVI) (APHA-AWWA-WEF, 2012). The amount of harvested biomass (by mass) per unit of time was obtained according to Equation (1). Weekly, sludge was collected manually, and its volume was measured in a graduated cylinder.

$$\text{Harvesting}_{ST}\ (g\ /\text{day}) = \left(\frac{TVS_{ST}\ .V_{sludge}}{T}\right) \tag{1}$$

where:

TVS_{ST}: TVS concentration in the sludge collected in each settling tank (g/L);
V_{sludge}: volume of collected sludge (L);
T: sludge accumulation time in the settling tanks (7 days).

Biomass separation in the BR was evaluated for humidity content and daily harvesting. The amount of harvested biomass (in mass) per unit of time in the BR was obtained according to Equation (2):

$$\text{Harvesting}_{BR}\ (g\ /\text{day}) = \left(\frac{TVS_{BR}\ .A_{system}}{T}\right) \tag{2}$$

where:

TVS_{ST}: TVS concentration of biomass concentrated in the BR (g/m^2);
A_{system}: area of biomass production in the system. For system 2, the area was equivalent to the sum of the surface area of HRAP 2 (3.3 m^2) and the transversal area of the BR (0.5 m^2), resulting in an area of 3.8 m^2. For system 1, this equation was not used because this system did not have a BR.
T: growth period of the biofilm in the BR (21 days).

2.4.2. Calculation of biomass productivity

In the BR, the biofilm showed a cumulative growth, and in the HRAPs, due to the culture medium flow, the suspended biomass was in continuous growth and retention by the settling tank. Therefore, to compare the biomass productivity between the two systems, the average production of HRAP 1 was considered in system 1. For system 2, the average production of HRAP 2 and the average peak production of each growth cycle of the BR were considered. The area used to calculate productivity in system 2 was the sum of the area of HRAP 2 and the BR, as shown in Equation (3):

$$TPr(g/m^2.day) = \frac{P_{HRAP}.V_{HRAP}}{A_{system}\ .HRT} + \frac{P_{BR}}{T} \tag{3}$$

where:

TPr: total system productivity (g/m^2.day);
P_{HRAP}: VSS average production of HRAP (g/m^3);
V_{HRAP}: HRAP volume (1 m^3);
A_{system}: area of biomass production in the system. For system 1, the area was equal to the surface area of HRAP 1 (3.3 m^2). For system 2, the area was equivalent to the sum of the surface area of HRAP 2 (3.3 m^2) and the transversal area of the BR (0.5 m^2), resulting in an area of 3.8 m^2.
HRT: HRAPs hydraulic retention time (10 days);
P_{BR}: average peak production of TVS in each BR growth cycle (g/m^2). In system 1, P_{BR} was zero.
T: growth period of biofilm in BR (21 days).

2.5. Biomass characterisation

The biofilm of the BR and the biomass concentrated in each settling tank were lyophilised, followed by the determination of ash and Total Kjeldahl Nitrogen contents (adapted from APHA-AWWA-WEF, 2012). The protein content was defined by the nitrogen-to-protein conversion factor of 6.25 (Zhong et al., 2012). The carbohydrate content was determined via quantitative acid hydrolysis of the biomass (Hoebler et al., 1989), followed by the phenol-sulfuric method (Dubois et al., 1956) and spectrophotometry (490 nm) using the standard glucose curve. The lipid content was determined using the Soxhlet extraction method (AOAC, 2000). After macerating the biomass, neutral lipids were extracted in the fat determiner (Tecnal TE-044-8/50) for 6 h, with 99% hexane as solvent. Subsequently, in the same equipment, membrane lipids were extracted with 96% ethanol for 3 h and quantified via gravimetry.

Characterisation of the microalgae community was carried out with the samples biofilm of the BR, collected weekly, during the first 6 weeks of operation to follow the initial development of the species. Subsequently, the samples biofilm of the BR, together with the suspended samples from both HRAPs, were collected monthly. For characterisation, 50 ml of suspended samples were collected on the HRAPs surfaces, and samples of 18.75 cm^2 were scraped form the BR and diluted in 50 ml of distilled water. All samples were kept in a 4% formaldehyde solution. Characterisation was performed at the genus level, and for the dominant genera, the species present were identified. For quantitative analysis, individuals were counted in a sedimentation chamber under an inverted microscope, using the method of Uthermöl (1958). The density of the organisms was determined using the criteria described in APHA-AWWA-WEF (2012). For qualitative analysis, identification was performed using an inverted microscope, according to Parra et al. (1982) and Komarek and Fott (1983).

2.6. Statistical analysis

All statistical analyses were performed using the Minitab®18 software. To compare the production, separation and characterisation of biomass between the two systems, analysis of variance (ANOVA) was performed, followed by the Tukey means test at 5% probability of error. Principal components analysis (PCA) was performed to assess the real contribution of each variable to the total variability of the quality data of the treated effluent, nutrients and organic matter, at the outlet of the settling tanks. An individual analysis was carried out to determine the main parameters influencing the dynamics of the conventional and hybrid systems.

3. Results and discussion

3.1. Microalgae community

Fig. 3 shows the density of organisms in the biofilm of the BR (Fig. 3a) and in the suspended medium of the HRAPs (Fig. 3b). Both HRAPs had similar results, with logarithmic units between 10^7 and 10^8 org/m^2 throughout the experimental period. The density of organisms in the BR was, during the entire experimental period, higher than that of HRAP, with a peak in late summer of 5×10^{11} org/m^2, while in the other months, it always remained around 10^{11} org/m^2. According to Sutherland and Craggs (2017), the development of the microalgae community in a biofilm system is strongly influenced by radiation and the concentration of nutrients in the culture medium. The amount of light available for photosynthesis by microalgae depends on the thickness of the biomass for BRs and on the depth of the water column for suspended systems. In the latter, the light that passes through the water column decreases exponentially with depth, as the particles absorb or disperse the light. In BRs, photons are instantly transformed into chemical energy by microalgae. In addition, productivity and nutrient assimilation are dependent on the availability of light, the absorption efficiency and the use of light by algae (Sutherland and Craggs, 2017).

This explains why the variability of organisms in the BR, throughout the experimental period, was less than that in the HRAPs. The coefficients of variation of the organisms over the experimental period

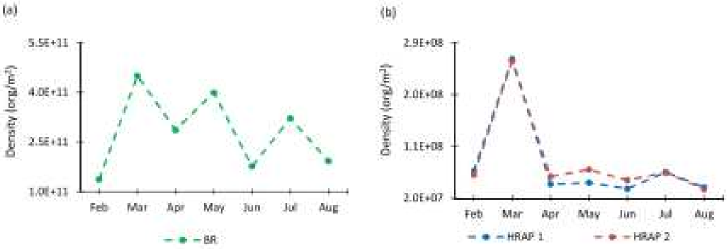

Fig. 3. Density of organisms (org/m^2): (a) BR; (b) HRAPs.

were 94, 84 and 39%, respectively, for HRAP 1, HRAP 2 and BR. It is believed that seasonal changes did not interfere with the density of organisms, since the experiment was performed in a country with a hot subtropical climate, with high temperatures and incidence of radiation throughout the year, with little difference between seasons (Assemany et al., 2015).

Although BR had a higher organism density throughout the experimental period, this reactor did not influence the density of organisms in HRAP 2, when compared to HRAP 1. However, as seen in Fig. 4, the BR interfered with the abundance of HRAP 2 species and genera. During the first 6 weeks of operation (Fig. 4a), the abundance of individuals in the BR was monitored weekly and, from the 3rd to the 7th month of operation (Fig. 4b), the abundance of individuals in the BR was monitored monthly. This monitoring was important to record the ecological succession of the biofilm, in order to monitor the alternation of the pioneer community until the stabilization of the biofilm. In the first week, the species *Tetradesmus obliquus* (73%) was predominant in the BR. In the following weeks, *Chlorella vulgaris* became more abundant and, from the 4th week of operation, this species became predominant in the BR (>60%) until the end of the experiment. The monthly samples, starting in April, showed the appearance of the species *Eutetramorus fottie* and the genus *Desmodemus* throughout the BR operation.

However, in both systems, other species and genera appeared throughout the experimental period. And, although the culture medium of both HRAPs remained within the optimum temperature range (between 15 and 30 °C) for most microalgae species to perform photosynthesis and cell division (Assis et al., 2017), the temperature decreased by approximately 8 °C throughout the experimental period, changing the species of the microalgae community of the suspended and biofilm systems.

In system 2, the abundances of the species *Didymocystis inermis*, *Monoraphidium contortum* and *Tetradesmus obliquus* and of the genera *Navicula* and *Desmodemus* were greater than in system 1 (HRAP 1, Fig. 4c), both in the BR and the HRAP 2 (Fig. 4d). In the last month of operation (August), the increase in the abundances of *Eutetramorus fotti*, *Tetradesmus obliquus* and *Navicula* altered the predominance of the HRAP 2 community. In the same period, *Eutetramorus fotti* and *Navicula* were in the BR at higher densities. The direct contact of the BR with solar radiation resulted in a greater species and genera heterogeneity of system 2 in relation to system 1. Similar results were found by Assemany et al. (2015), who evaluated the phytoplankton composition of three HRAPs submitted to different levels of solar radiation. The authors

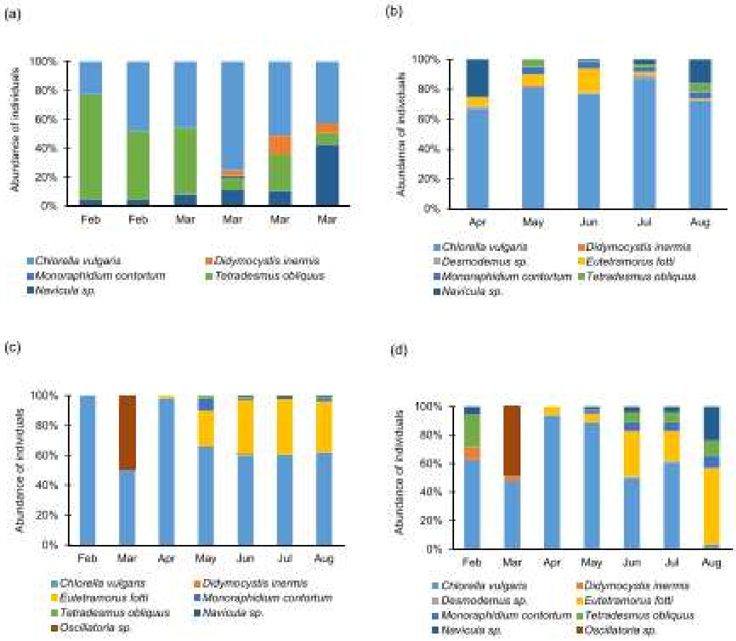

Fig. 4. Abundance of individuals (%): (a) during the first six weeks of BR operation; (b) during the last months of BR's operation; (c) HRAP 1; (d) HRAP 2.

concluded that the HRAP with a 30% block of solar radiation allowed greater homogeneity in the phytoplankton community and less variability over the course of 1 year.

3.2. Biomass separation, harvesting and production

Table 1 shows the characteristics of separation and harvesting of the concentrated biomass in the settling tanks and in the BR. Biomass separation and harvesting were evaluated according to the values of SVI, humidity and daily harvesting. Regarding the SVI results, there was no difference between the settling tanks ($p > 0.05$), indicating that the presence of BR in system 2 did not change the quality of sludge sedimentation in ST 2. According to Janczukowicz et al. (2001), both systems can have suitable properties for sedimentation of wastewater sludge (SVI < 150 mL/g), explained by the SVI optimisation of the sedimentation of wastewater sludge combined with microalgae (Wang et al., 2016).

Wang et al. (2016), when evaluating the sedimentation of *Chlorella* sp. grown in contact with solar radiation and wastewater from activated sludge, found that the alga-bacteria combination obtained the best sedimentation efficiency, with a SVI of 42 mL/g, which represented a 44% reduction in SVI of sedimented sludge without the combination with microalgae. The alga-bacteria combination is formed through connections made via bioflocculation, in which the cell surface properties of the microalgae, combined with the extracellular polymeric substances and the cation content of the bacteria, influence the formation and stability of the biomass (Su et al., 2011). Therefore, due to the formation of denser and more compact flakes, the sedimentability of the sludge composed of algae and bacteria is improved, indicating the maturation of wastewater (Zhang et al., 2019).

The humidity content varied from 91 to 94%, and there was no statistical difference between the settling tanks of the two systems and the BR ($p > 0.05$). The humidity content and the biomass concentration are related and are important characteristics in determining the method for biomass harvesting. The equality in humidity content of the biomass harvested from the settling tanks and from the BR demonstrated the potential of the BR as a biomass separation unit, since the settling tanks are considered traditional systems of biomass separation in a WWTP. In addition to the secondary settling tank, the BR biomass concentration efficiency was comparable to those of other algae biomass harvesting technologies, such as dissolved air flotation (1–8%), centrifugation (10–22%), flocculation (1–15%) and filtration membranes (2–27%) (Deconinck et al., 2018). In a survey of microalgae harvesting methods carried out by Deconinck et al. (2018), these technologies demanded more energy for operation than the BR. In the same study, BRs required an energy of up to 0.2 KWh/m^3, while other technologies such as filtration membranes, secondary settling tank and dissolved air flotation demanded up to 10, 10.8 and 20 kWh/m^3, respectively. These results represent about 50–100 times more energy needed than for the functioning of the BR.

Table 1
Characteristics of separation and harvesting of the concentrated biomass in the settling tanks and in the BR (standard deviations are shown in parentheses).

		SVI (ml/g)	Humidity* (%)	Harvesting (g/day)
System 1	ST 1	21.12^{a} (7.71)	91.84^{a} (1.79)	2.64^{b} (1.04)
System 2	ST 2	29.65^{a} (15.25)	91.67^{a} (2.01)	2.23^{b} (1.36)
	BR	–	93.89^{a} (4.99)	11.45^{a} (7.98)
	Total (ST 2 + BR)	–	–	13.68

Note: * Humidity relative to the harvested sludge in the settling tanks and the scraped biomass from BR. Significant at the 5% probability of error level by the Tukey test; the numbers followed by the same letter in the column did not differ statistically.

In relation to the daily biomass harvesting, the value for the BR (11.45 g/day) was about 5 times higher in relation to those for the settling tanks ($p > 0.05$). Although the BR is a simultaneous production and separation reactor, it was not operated in isolation, without a HRAP unit. Therefore, in this system, biomass was produced in HRAP 2 and BR and separated in ST 2 and BR. In system 1, biomass was produced in HRAP 1 and was only separated in ST 1. In view of these results, the BR showed potential as a biomass separation unit, and its large-scale application should be further investigated, aiming at its implantation in a WWTP to minimise the difficulties associated with biomass separation and harvesting.

The production of chlorophyll-*a* and volatile solids in HRAPs and BR is shown in Table 2. Chlorophyll-*a* production was approximately three times ($p = 0.00$) higher in the BR than in thee HRAPs, which is in agreement with the results previously discussed in 3.1 (Fig. 3), in which the highest density of photosynthetic organisms occurred in the BR throughout the experimental period. The higher algal production in the BR was also confirmed by the ratio of chlorophyll-*a* to total biomass. The BR and both HRAPs showed good proportions of microalgae cultures in relation to total biomass, indicated by values from 1 to 1.5%, according to Veloso et al. (1991), showing healthy microalgae cultures. It is noteworthy, however, that in the BR, this ratio was 2.55, about 1.7 times greater than in HRAP 1 (1.49%) and 1.5 times greater than in HRAP 2 (1.68%).

Thus, the integration of BR in system 2 promoted the production of a biomass richer in photosynthetic organisms than system 1. The higher concentration of chlorophyll-*a* in the BR was associated with the intensification of the photosynthesis activity in this reactor. The interactions between microalgae and bacteria in biofilm systems allow a more stable symbiosis than in suspended systems (Liu et al., 2017). The binding of bacteria to the cell surfaces of microalgae provides a microenvironment with higher concentrations of nutrients and lesser effects of microalgae self-shading when compared to suspended cultivation medium, in addition to a greater transport efficiency of CO_2/O_2 masses (Zhang et al., 2020). Similar to the production of chlorophyll-*a*, the higher production of total biomass in BR plus the production of total biomass in HRAP caused an increase of about 2.6 times in relation to system 1 ($p = 0.05$).

Comparing the results of biomass production and harvesting in each system, the integration of the BR increased the harvesting efficiency of system 2. In this system, the amount of total biomass produced was 22.39 g/day, representing 10.94 g/day produced in HRAP1 and 11.45 g/day in the BR. Of the produced biomass, 61% were harvested, comprising 11.45 g/day of harvested biomass in the BR and 2.23 g/day in the ST 2. For system 2, the BR accounted for 84% of the total harvesting efficiency. In system 1, total biomass retention was only 22%,

Table 2
Biomass production in HRAPs and BR (standard deviations are shown in parentheses).

		Production of chlorophyll-*a*		Total biomass production (volatile solids)	
		Production (g/m^2)	Productivity (g/m^2.day)	Production (g/m^2)	Productivity (g/m^2.day)
System 1	HRAP 1	0.55^{b} (0.38)	0.05^{a} (0.04)	36.75^{b} (18.05)	3.68^{a} (1.81)
System 2	HRAP 2	0.48^{b} (0.29)	0.05^{a} (0.03)	28.79^{b} (11.28)	2.88^{a} (1.13)
	BR	1.64^{a} (0.93)	0.08^{a} (0.04)	64.28^{a} (29.83)	3.25^{a} (1.10)
	Total (HRAP 2 + BR)	2.12	0.13	93.07	6.13

Note: Significant at the 5% probability of error level by the Tukey test; the numbers followed by the same letter in the column did not differ statistically.

with total production being 12.13 g/day and harvesting in ST 1 2.64 g/day.

Contrary to this study, most studies of hybrid systems evaluated the system's harvesting and biomass production only in the BR (Table S1). Therefore, in order to compare the results of this study with the literature, the daily biomass productivity of the BR (11.45 g/day) was converted into daily biomass productivity per surface area of the support material (0.5 m^2), totalling 22.9 g/m^2. day. This result was close to the biofilm production values reported by Zhang et al. (2018a, 2018b) and greater than the results found by Gross et al. (2013), Lee et al. (2014), Gross et al. (2015), Assis et al. (2017) and Zhao et al. (2018). It is important to highlight that the BRs used in these studies have different configurations (submerged, rotating, not submerged), in addition to different support materials and, in some cases, the use of synthetic cultivation medium was done, as can be observed in Table S1.

Both settling tanks and BRs are reactors that involve mechanical methods of separating and harvesting algal biomass. When it comes to the application of these separation units on a large scale, some considerations must be highlighted. For example, the settling tanks used in the present study were not operated in the same way as in other studies that used settling tanks to separate algal biomass (Park et al., 2015). In the present study, the sludge accumulated over 1 week was only collected and was not recirculated to the HRAP. Park et al. (2015) demonstrated that the recirculation of biomass formed by algae and bacteria, collected by gravitational sedimentation, improved biomass productivity, sedimentation efficiency and microalgae species control. In relation to sedimentation, the recirculation of the biomass promoted an increase of approximately 60–85% in the size of the colony of *Pediastrum boryanum*, and the flakes formed exceeded 500 μm in diameter. However, it is noteworthy that in order to treat domestic sewage and separate biomass rich in microalgae, the results of the sedimentation characteristics of the settling tanks in this study are in line with the findings of other researches (Wang et al., 2016; Muylaert et al., 2017). When it is desired to separate the microalgae for the purpose of harnessing low added value biomass, gravity sedimentation combined with wastewater treatment is an attractive option, as in this culture medium, the microalgae can be deposited within about 30 min via natural bioflocculation (Wang et al., 2016; Muylaert et al., 2017).

The expectations for BRs in the production and separation of biomass in relation to conventional systems are better than those for settling tanks; however, few studies address the feasibility of their application on a large scale, mainly because of the different configurations of BRs already developed on a laboratory or pilot scale. In this sense, some prospects can be raised in order to advance future research for the optimisation of BRs, such as: life cycle assessment, economic feasibility, feasibility of expanding the scale of BRs for implantation in a WWTP.

Some criteria related to the integration of these reactors can be discussed from an economic perspective. For example, the costs involved in the stages of growth, separation and harvesting of biomass in suspended systems, aiming at the production of biofuels, account for about 70% of the total production costs. In suspended systems connected to BRs, these costs would be reduced because the system is simultaneously a biomass-producing and separating unit. Due to the greater production and harvesting capacity of immobilised biomass, mixing and agitation costs would be saved in the BR (Moreno-Garcia et al., 2017; Wang et al., 2017), while pumping the culture medium to the BR would be linked to a higher energy cost. In addition, the energy costs of mixing the effluents for wastewater treatment in the HRAP would be maintained. Also, harvesting in the BRs can be carried out using the simplified scraping method, reducing the costs related to the downstream processing of biomass separation from the final effluent disposal (Zhang et al., 2018b). However, with the expanded scale, scraping may no longer be advantageous due to the huge investment in human labour, and therefore, automated technologies should be considered, further increasing financial investment (Wang et al., 2017).

Zhang et al. (2018b) designed a mechanical harvester for potential scale-up. The project was based on the automated operation of the harvesting step, without any human effort. However, the equipment has not yet been tested and information regarding investments and energy demand has not been reported. Cultivation time and harvesting frequency are operational aspects that deserve researchers' attention. The greater the production of biofilm in less period of time would be the optimal condition for expanding the scale of the BRs. However, it can be observed from the literature that for microalgae cultivation in wastewater, there is still a wide range of harvesting frequency applied (Table S1).

Harvesting frequency is strongly influenced by the ability of cells to adhere to the support material on which the biofilm grows. This technical aspect of BRs has attracted the attention of researchers and many materials have already been tested, including cotton, polyester, nylon and velvet (Table S1). The attachment of cells to the support material depends on the properties of the microalgae (surface charge, hydrophile, geometric structure, cell wall composition, secretion), properties of the support material (surface charge, hydrophile, surface structure), medium composition and the hydrodynamic conditions (Wang et al., 2017). However, little is known about the interactions between the physicochemical properties of microalgae cells and the support materials and how it affects the growth of biomass. These scientific issues are fundamental and deserve further study (Wang et al., 2017).

Regarding the establishment of a BR on a large scale, the fact that it protects the biofilm against the influence of weather, especially heavy rains, which can negatively affect the growth of microalgae, must also be considered (Sutherland and Craggs, 2017; Garbowski et al., 2017). The solution adopted in the present study was to rotate the BR panel according to the season. Measures to protect biofilm should be further investigated. Covering, for example, would substantially increase capital costs, making it economically impossible to mitigate the loss of biomass (Sutherland and Craggs, 2017).

The answers to all the questions raised about the economic point of view of the implementation of hybrid systems as an innovative technology can be better interpreted through a joint analysis of the results of economic viability accompanied by the feasibility of expanding the scale of the BRs for implantation in a WWTP. The convergence of these studies, in parallel with life cycle assessment, will allow a better judgment of the economic and sustainable efficiency of the BRs.

3.3. Wastewater treatment

Table 3 shows the removal of wastewater treatment monitoring variables. All variables had similar concentrations in the effluents from HRAPs (exit of their respective settling tanks) and, consequently, similar removals in the two systems. It is important to note that the high

Table 3
Removal of wastewater treatment monitoring variables.

Variable	Raw domestic sewage	HRAP 1	HRAP 1 removal (%)	HRAP 2	HRAP 2 removal (%)
pH	7.5 (0.6)	6.8 (0.6)	–	6.8 (0.7)	–
DO (mg/L)	1.0 (1.3)	7.9 (1.7)	–	7.5 (2.1)	–
COD (mg/L)	329.2 (276.2)	138.3 (132.1)	57.9	135.5 (64.5)	58.8
TP (mg/L)	9.1 (1.8)	7.9 (1.8)	13.4	7.7 (1.8)	16.2
NH_4^+-N (mg/L)	87.4 (19.8)	21.6 (6.4)	75.3	19.9 (7.6)	77.3
NO_3^--N (mg/L)	1.1 (1.5)	41.7 (17.2)	–	40.3 (14.3)	–
E. coli (MPN/100 ml)	5.5×10^3* (7.3×10^4)	3.6×10^2* (2.7×10^3)	1 log unit**	4.8×10^2* (6.6×10^4)	1 log unit**

Note: * Geometric mean. ** Removal in logarithmic units. Standard deviation values in parentheses.

deviations of the environmental variables found in this study were associated with the uncontrollable dynamics of the system exposed to climatic conditions (Assemany et al., 2015).

The integration of BR into HRAP 2 did not interfere with the treatment of the effluent, and therefore, these results will be discussed together with the results of the principal components (PC) analysis presented in Table S2. The monitoring variables of the wastewater treatment were reduced to four components of each system, which explained 88.9 and 84.9% of the variability in data from systems 1 and 2, respectively. The variables TP, NH_4^+-N and DO were more important for both systems (PC 1 and 2). The concentration of nutrients is closely related to the growth of microalgae and, consequently, to their removal due to biomass assimilation. Considering that pH values did not reach a sufficient magnitude for the volatilisation of ammonia nitrogen and chemical phosphorus precipitation (Table 3), it can be said that the biomass assimilation route was one of the main pathways responsible for TP removal (13–17%) and NH_4^+-N removal (75–78%).

The parameter DO is important in the consortium formed by microalgae and bacteria during the treatment of domestic sewage. This variable was indirectly associated with the proliferation of microalgae, which, through photosynthetic activity, increased the oxygen concentration in the culture medium from 1 to approximately 8 mg/L.

The concentration of *E. coli* in the HRAP effluents was important to diagnose the efficiency of domestic sewage treatment in relation to pathogen inactivation. The *E. coli* removal efficiencies were 1 logarithmic unit in both systems and were associated with pH and DO values, which did not undergo significant variations for pathogen inactivation. This variable was more important in system 1 because it had a high value on PC 1, while in system 2, its highest value was on PC 2.

The pH showed higher values on the PC 2 of both systems. This variable is extremely important to guarantee the efficiency of wastewater treatment. Both systems had similar pH values, close to neutrality (between 6 and 7), indicating that the concentration of organic matter in the effluent used as a culture medium was sufficient to ensure that microalgae growth was not limited by a lack of CO_2. Its variation is able to influence the balance of chemical compounds and to affect growth rates of microorganisms involved in biological wastewater treatment.

The variable NO_3^--N was also important in both systems as it provides information about the nitrification process. Nitrogen in the NH_4^+-N form is the preferred assimilation source, and the NO_3^--N form is the second most important nitrogen form to be consumed by microalgae. Nitrification was abundant in both systems, with concentrations between 40 and 42 mg/L, which represented an increase of about 3800% of NO_3^--N. In system 2 (PC 2), this variable was more important than in system 1 (PC 3).

The concentration of organic matter in the effluents of the systems was represented by COD. The removal of COD in both systems was in the range of 57–59%, indicating a direct relationship of between microalgae and heterotrophic bacteria present in the culture medium. The DO concentrations confirmed this conclusion, since both systems had an increase of about 6 mg/L of DO, indicating the contribution of microalgae in the oxygen supply for the improvement of the activity of heterotrophic bacteria in the degradation of organic matter present in wastewater, such as domestic sewage, which has an insufficient level of oxygen (Maza-Marquez et al., 2017). The COD was more important in system 2 (PC 3) than in system 1 (PC 4).

3.4. Biomass characterisation

Table 4 shows the average values of the biomass properties harvested in the BR and in the settling tank of each system. There was no statistical difference in the composition of the biofilm of the BR and/or concentrated in the settling tanks ($p > 0.05$). Therefore, the biomass production and separation methods have no impact on its chemical composition. When compared to system 1, the BR maintained the biomass composition compared to that grown in the HRAP and separated in the settling tank.

It is essential to understand the characterisation of biomass in order to properly allocate it, aiming at the best valorisation route. The biomass separated in the BR and in the settling tanks was mainly composed of proteins (32–36%), followed by ash (19–21%) and total lipids (16–19%) and, albeit to a lesser extent, carbohydrates (about 10%).

It is important to emphasise that the use of wastewater for the cultivation of microalgae, whether in open suspended or biofilm systems, creates a competitive environment. There is the presence of suspended solids that prevent the penetration of solar radiation, as well as toxic substances and other microorganisms that require space and nutrients, hindering the development of algal biomass and compromising the accumulation of components of interest.

In this context, the composition of algal biomass depends on the culture conditions, such as the dominant and predominant species and the conditions of the culture medium (Chew et al., 2017). In this study, *Chlorella vulgaris* was the dominant and predominant species in both systems. According to Chia et al. (2013), membrane lipids (structural) are dominant in the lipid composition of this microalgae, regardless of the used culture medium. In agreement with these authors, the results of the present study showed higher membrane lipid contents (between 10 and 12%) and lower levels of neutral lipids (<7%), which are used for the production of quality biofuels.

Ash contents were directly associated with the accumulation of fixed solids from domestic sewage used as culture medium. A high ash content is usually found in algal biomass grown in wastewater, negatively influencing the properties of solid fuels. Therefore, pre-treatment of biomass is recommended to remove these impurities (Choudhary et al., 2020).

Carbohydrates are important nutrients in the composition of microalgae, and their use can be incorporated into the animal diet, contributing as a source of energy for animals and microorganisms of the gastrointestinal tract (Madeira et al., 2017). The biomass of this study had a carbohydrate content close to 10% and was close to a range of 13–22%, considered adequate for bovine supplementation (Choudhary et al., 2017).

Protein is one of the most important products of the microalgae biorefinery due to its contribution to human or animal nutrition. In this study, the largest composition of biomass was protein, which accounted for 32–36% in both systems. Cole et al. (2015) reported that the 18% protein content found in the *Oedogonium* macroalgae was equivalent or superior to that of many terrestrial cultures, and the algal biomass could be used as a source of supplementary protein in animal feed. In another study, it was estimated that the protein content of the microalgae *Chlorella vulgaris* was in the range of 50–60%, and its quality as similar to that of yeast, soy flour and milk protein (Madeira et al., 2017).

Therefore, given the composition of the algal biomass obtained in this study, the indicated valorisation route is the supplementation of animal feed. It is noteworthy, however, that before processing

Table 4
Chemical characterisation of biomass (standard deviations are shown in parentheses).

Composition (%)		Ashes	Proteins	Carbohydrates	Neutral lipids	Membrane lipids	Total lipids
System 1	ST 1	20.54 (5.48)	35.21 (4.38)	10.43 (2.53)	4.15 (0.45)	10.86 (3.17)	15.01 (3.62)
System 2	BR	19.82 (4.16)	32.21 (2.80)	10.87 (3.62)	6.59 (3.44)	11.76 (4.71)	18.35 (6.31)
	ST 2	20.59 (4.24)	35.46 (2.86)	10.19 (1.84)	4.85 (0.08)	12.10 (5.68)	16.95 (5.77)

wastewater-grown algal biomass for animal feed, the removal of pathogens or any other potentially toxic compounds is highly recommended (Choudhary et al., 2017).

4. Conclusions

The innovative hybrid system achieved production and harvesting efficiencies 2.6 x and 5 x, respectively, greater than the conventional system composed of a HRAP without a BR. Moreover, the new technology promoted a greater diversity of autotrophic organisms and did neither interfere negatively with the biochemical composition of biomass nor with wastewater treatment. The integration of a BR optimised the production and harvesting of algal biomass, making it a promising technology to be deployed on a large scale. However, interactions between the physicochemical properties of cells and the support materials, cultivation time and harvesting frequency are aspects that deserve researchers' attention. Further research regarding the economic and environmental feasibility of the applicability of this reactor should be considered in the future.

CRediT authorship contribution statement

Letícia Rodrigues de Assis: Conceptualization, Data curation, Methodology, Writing - original draft, Formal analysis, Investigation, Visualization, Writing - review & editing. **Maria Lúcia Calijuri:** Supervision, Writing - review & editing, Resources. **Paula Peixoto Assemany:** Conceptualization, Writing - review & editing, Investigation, Visualization. **Thiago Abrantes Silva:** Data curation, Methodology, Writing - review & editing. **Jamily Santos Teixeira:** Data curation, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2020.111183.

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