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Bruna Porto

Microalgal biomass production and nutrients removal from industrial wastewater using different culture systems

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Thesis submitted to the Graduate Program in Chemical Engineering at the Federal University of Santa Catarina to obtain the title of Doctor in Chemical Engineering.
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RESUMO

As microalgas são vistas como uma matéria-prima flexível e muito promissora e, por isso, têm sido amplamente estudadas nos últimos anos em diferentes aplicações: captura de CO_2; produção de biomassa/biocombustível e absorção de nutrientes de águas residuárias. Porém, é preciso tornar o processo mais eficiente e barato. Nesse contexto, o crescimento de microalgas em águas residuárias, promovendo concomitantemente sua biorremediação, tem sido proposto como uma alternativa viável para redução de custos e da pegada ambiental. Assim, neste estudo, um lixiviado de aterro e um efluente da indústria de papel, concentrado e em diferentes diluições, foram avaliados como meio de cultura para *Chlorella vulgaris* e *Tetradesmus obliquus*. Como o fósforo e o nitrogênio são nutrientes essenciais para as microalgas, sua concentração foi monitorada ao longo dos cultivos em termos de fosfato, nitrato e nitrito. Nas condições estudadas, ambas as espécies foram capazes de crescer nesses meios e promover a remoção desses nutrientes. As eficiências máximas de remoção obtidas foram 83 ± 1% e 56 ± 1% para nitrogênio e fósforo, respectivamente. No entanto, observou-se que os efluentes não diluídos inibiram o crescimento da espécie. Assim, pode-se concluir que o processo de biorremediação é possível após uma adequada diluição do efluente. A diluição do efluente minimiza a toxicidade e reduz a cor/turbidez. No cultivo de *T. obliquus*, no entanto, foi observado um comportamento diferente em relação aos demais testes, pois as microalgas cresceram na forma de flocos. Este fato pode representar uma real vantagem econômica para a etapa de recuperação da biomassa (processo de colheita), embora, por outro lado, resulte em produtividades mais baixas. Em relação ao lixiviado de aterro, após a seleção de uma concentração adequada de efluente nos testes realizados em frascos de 1 L, também foram realizados testes em um fotobiorreator tubular (PBR) inovador. A configuração empregada foi composta por um tubo absorvedor de vidro de borossilicato e uma superfície refletora com geometria de dupla parábola (DP) feita de material de alumínio anodizado (R85). Este PBR é conhecido por sua capacidade de melhorar a absorção e distribuição da luz dentro do meio reacional, um parâmetro crucial para os processos fotossintéticos. Uma melhoria significativa nas taxas específicas de crescimento de *C. vulgaris* (de 0,15±0,04 d^{-1} a 0,61±0,04 d^{-1}) e eficiências de remoção de nitrogênio (um ganho de aproximadamente 26% e 33% para *C. vulgaris* e *T. obliquus*, respectivamente) foram alcançados neste teste preliminar, confirmando que o desenvolvimento de novos PBRs também é uma estratégia importante a ser explorada. Com base nisso, estudos foram realizados com diferentes configurações deste

PBR, onde foram avaliados os seguintes aspectos: (i) geometria da superfície refletora plana (F), parábola dupla simples (SP) e parábola dupla tradicional (DP)) e (ii) os materiais utilizados na sua fabricação (alumínio anodizado com revestimento protetor MIRO-SUN® (MS) e sem revestimento protetor (R85) e aço inoxidável (SS)). Para determinar o impacto desses parâmetros no aproveitamento da energia luminosa foram realizados testes actinométricos e determinado o crescimento de *C. vulgaris* em função do tempo de cultivo e da energia acumulada no sistema. Em função do tempo, os sistemas com refletores parabólicos (SP e DP) feitos de materiais com maior refletância especular (R85 e MS) alcançaram resultados superiores, o que está de acordo com uma maior potência radiante incidente (RP_i), obtida com os testes actinométricos. Assim, a taxa específica de crescimento máxima (μ, d^{-1}) obtida foi de 0,230±0,005 d^{-1} no ensaio com PBR R85-DP (RP_i de 0,167±0,005 J s^{-1}). Em termos de energia, houve uma pequena queda nas taxas específicas de crescimento, com o aumento do fluxo de fótons, o que pode estar associado à baixa transmissibilidade das suspensões de microalgas, principalmente quando se atinge maior densidade celular. Os testes utilizando dois tubos absorvedores mostraram que a distância de 50,0 mm entre eles é a mais adequada, considerando as taxas de crescimento específicas e produtividades de biomassa por metro quadrado de coletor solar. Nessas condições, obteve-se maior eficiência no aproveitamento da energia fotônica em comparação ao teste com tubo único. Portanto, o uso desses novos PBRs para o crescimento de microalgas pode representar uma redução geral do custo do processo, além de levar a uma maior produção de células e, consequentemente, maximizar a remoção de nutrientes em efluentes industriais.

Palavras-chave: Biorremediação. Fotobiorreator. Microalgas. Produção de biomassa. Remoção de nutrientes. Tratamento de águas residuárias.

RESUMO EXPANDIDO

Introdução

As microalgas são micro-organismos fotossintéticos, que têm sido amplamente estudadas nos últimos anos, tendo em vista o seu elevado potencial econômico. Uma das principais aplicações da biomassa de microalgas é a extração de lipídios para a produção de biocombustíveis. No entanto, outros compostos de interesse comercial também podem ser extraídos, tais como: carboidratos, vitaminas, polissacarídeos, proteínas, ácidos nucleicos, carotenoides e polímeros. As microalgas também podem ser empregadas em processos de biorremediação (captura de CO_2 e remoção de contaminantes de águas residuárias). No entanto, apesar de ser uma matéria-prima flexível e com alta produtividade, alguns desafios precisam ainda ser superados, para uma produção em larga escala, sustentável e competitiva. É necessário desenvolver sistemas de cultivo mais eficientes, reduzir os custos de processo e a pegada ambiental. Neste caso, o crescimento de microalgas em águas residuárias, promovendo concomitantemente sua biorremediação, é uma importante estratégia a ser explorada, que permite reduzir a demanda por água doce e os custos com fertilizantes (fonte externa de nutrientes). Nas indústrias de papel e celulose, assim como em outros segmentos industriais, grandes quantidades de água são empregadas durante os estágios de fabricação e, consequentemente, grandes volumes de efluentes são gerados. Nos aterros sanitários, em decorrência das chuvas que percolam os aterros e da decomposição dos próprios resíduos, estes também representam uma importante fonte de efluentes líquidos (lixiviados). Entre os contaminantes presentes nos efluentes da indústria de papel e celulose, o fósforo é de particular preocupação, pois subsiste nos efluentes mesmo após a etapa de tratamento secundário e é um dos principais contribuintes para o fenômeno de eutrofização. Além disso, os métodos físico-químicos, normalmente, empregados para reduzir a concentração de fósforo, tendem a ser caros e a produzir grandes quantidades de lodo contaminado. Já em relação aos lixiviados de aterro, considerando as tecnologias atualmente disponíveis, é necessário o emprego de um conjunto de processos físicos, químicos e biológicos para o seu tratamento. No entanto, o efluente resultante ainda apresenta altas concentrações de nitrogênio, que precisam ser reduzidas antes de ser descartado nos corpos d'água receptores. Portanto, a busca por soluções ecologicamente corretas para o tratamento destes efluentes é de extrema importância. Neste contexto, como o fósforo e o nitrogênio são nutrientes essenciais para o crescimento de microalgas, o uso destas como tratamento terciário sustentável tem-se mostrado uma alternativa viável aos métodos convencionais. Deste modo, considerando a matriz complexa dos efluentes, a sua viabilidade de aplicação deve ser estudada quanto ao efeito dos parâmetros bióticos e abióticos (i) no metabolismo celular; (ii) nas cinéticas de crescimento e remoção de nutrientes; (iii) na composição da biomassa produzida. Alguns compostos, mesmo sendo nutrientes essenciais (amônia e metais, por exemplo) para o crescimento de microalgas, quando presentes em altas concentrações, podem ser tóxicos para as mesmas. Além disso, a cor e turbidez dos efluentes também podem limitar o crescimento destes micro-organismos, pois dificultam a penetração da luz no meio de cultura. Neste caso, como solução, a maioria dos estudos sugere o uso de efluentes altamente diluídos, o que pode inviabilizar a aplicação em larga escala. Por outro lado, nitrogênio e fósforo também podem estar presentes em baixas concentrações ou em uma proporção desequilibrada. Neste caso, o meio deve ser suplementado com uma fonte externa destes nutrientes, de modo a atingir uma razão molar (N:P) ótima. Além de todas as questões relacionadas à composição dos efluentes, os processos também demandam (i) uma seleção adequada das espécies; (ii) a otimização dos parâmetros operacionais; (iii) e o desenvolvimento de fotobiorreatores (PBRs). Sendo a luz

um parâmetro essencial para o crescimento foto-autotrófico das microalgas, um adequado projeto de PBR deve melhorar a eficiência de utilização da luz. A transparência dos materiais empregados e a ampliação da área iluminada do reator são fatores importantes a serem considerados, visando uma maior absorção e uma boa distribuição da luz na cultura. Além disso, os PBRs, normalmente, se baseiam na redução do caminho da luz até as células e, por isso, representam uma alternativa mais adequada para superar as restrições de disponibilidade de luz em comparação aos sistemas abertos. Por outro lado, a exposição a altas irradiâncias pode acabar inibindo o crescimento das microalgas (foto-inibição). Este problema, no entanto, pode ser minimizado pela mistura de células, movendo-as entre as zonas claras e escuras do PBR, o que resulta em uma distribuição da luz mais homogênea. O uso de uma fonte de luz artificial também permite um melhor controle da intensidade da radiação luminosa sobre o sistema. Já em relação à área iluminada, esta pode ser maximizada otimizando o arranjo da fonte de luz ou acoplando refletores ópticos ao PBR. Deste modo, o projeto de PBRs compostos por uma superfície refletora acoplada a um tubo cilíndrico de vidro de borossilicato parece ser uma alternativa promissora, e ainda pouco explorada, para ampliar a área de iluminação do reator e reduzir as perdas de energia. Dependendo da geometria e do material do refletor, quase toda a luz que chega até à sua superfície pode ser coletada e disponibilizada para o cultivo das microalgas.

Objetivos

Este trabalho teve como objetivo principal desenvolver um sistema inovador de culturas de microalgas, visando à produção de biomassa e à remoção de nutrientes (tratamento terciário). Para alcançar tal objetivo, os seguintes objetivos específicos foram traçados: (i) avaliar a capacidade de absorção de nitrogênio e fósforo e os possíveis efeitos inibitórios dos efluentes empregados como meio de cultura sobre o crescimento de microalgas; (ii) testar um PBR tubular inovador acoplado a um refletor óptico como uma plataforma de cultivo em lixiviado do aterro; e (iii) comparar a eficiência de diferentes configurações deste mesmo PBR, quanto à geometria dos refletores (plano (F), parábola dupla simples (SP) e parábola dupla tradicional (DP)) e materiais de superfície refletora (alumínio anodizado com (MS) e sem (R85) revestimento protetor e aço inoxidável (SS)), visando maiores rendimentos de biomassa e pegadas ambientais significativamente menores.

Metodologia

Dois efluentes (efluente da indústria de papel (PIE) e lixiviado de aterro (LL)), previamente caracterizados, foram utilizados como meio de cultura alternativo para duas espécies de microalgas (*C. vulgaris* e *T. obliquus*). O efluente da indústria do papel foi coletado após a fase de tratamento secundário numa empresa papeleira portuguesa. Devido à baixa concentração de nitrogênio no efluente, quando comparado às necessidades nutricionais típicas de microalgas, este foi suplementado com $NaNO_3$. Já o lixiviado foi obtido a partir de um aterro de resíduos urbanos localizado no norte de Portugal. O efluente foi coletado após a etapa de tratamento biológico. Nesse caso, o mesmo foi suplementado com uma fonte externa de fósforo (KH_2PO_4), pois sua caracterização indicava uma limitação desse nutriente. Os experimentos em batelada, com ambos os efluentes, foram realizados durante 11 dias, utilizando diferentes diluições dos mesmos para avaliar o seu efeito inibitório sobre as microalgas. Parâmetros operacionais, como pH e temperatura, foram monitorados diariamente. O crescimento de microalgas e a concentração de nutrientes (PO_4-P, NO_3-N e NO_2-N) também foram avaliados. Ainda nos ensaios com lixiviado de aterro, após definir as condições que permitiram as maiores produtividades de biomassa e eficiências de remoção de nutrientes, estas foram reproduzidas em um inovador PBR tubular. Este PBR é caracterizado

por uma superfície refletora de alumínio anodizado (R85) em parábola dupla (coletor parabólico composto (CPC)) em torno de um tubo cilíndrico de vidro de borossilicato. Por fim, para comparar a eficiência de diferentes configurações deste mesmo PBR, foram realizados cultivos com duração de sete dias, em batelada, usando um volume de trabalho de ~520 mL. Refletores com diferentes materiais (alumínio anodizado Mirosun com capa protetora - MS, alumínio anodizado Reflective 85 sem camada protetora - R85, e aço inoxidável polido - SS) e geometrias (plano - F, parábola dupla simples - SP, e parábola dupla truncada tradicional - DP) foram testados. Para o refletor plano com o melhor material, também foram realizados testes com dois tubos absorvedores em distâncias diferentes (12,5 mm, 25,0 mm, 50,0 mm e 75,0 mm) entre eles. O desempenho de cada superfície refletora foi, inicialmente, comparado por meio da potência radiante incidente (RP_i, J s^{-1}) na superfície tubular do PBR, potência radiante (RP, J s^{-1}) que atinge a solução actinométrica e razão de concentração óptica (CR_O). Além disso, para o cálculo dos parâmetros de crescimento, a concentração celular das microalgas foi monitorada diariamente por densidade óptica a 680 nm (DO_{680}), usando um espectrofotômetro UV-6300 PC (VWR, Estados Unidos). A relação entre os valores de DO_{680} e a concentração de massa seca de biomassa (X, mg_{dw} L^{-1}) foi obtida por regressão linear, de acordo com a lei de Lambert-Beer. Da mesma forma, para o cálculo dos parâmetros de remoção de nutrientes, as concentrações de nitrogênio (N) e fósforo (P) foram avaliadas nos meios de cultura com efluentes. Todas as culturas foram continuamente expostas a (i) radiação fotossinteticamente ativa entre 30-69 μmol m^{-2} s^{-1}, usando um painel de LED branco de 34 W; e (ii) ar atmosférico filtrado com membranas de nylon de 0,45 μm (Specanalitica, Portugal), injetado a ~90 L h^{-1}, usando bombas de ar Trixie AP 180 (Trixie, Tarp, Alemanha).

Resultados e discussão

Os resultados obtidos mostraram que, nas condições estudadas, tanto *C. vulgaris* quanto *T. obliquus* conseguiram crescer nos meios de cultura alternativos com diferentes diluições de efluentes. No entanto, os efluentes não diluídos inibiram o crescimento das microalgas, indicando que o processo de biorremediação é possível após uma diluição adequada do efluente. A diluição do efluente minimiza a toxicidade do mesmo e reduz a cor/turbidez que dificultam a passagem da luz através da cultura. Assim, em relação aos cultivos em efluente da indústria de papel, os maiores valores de taxas específicas de crescimento (0,16±0,02 d^{-1}) e de produtividades máximas de biomassa (30±6 mg_{dw} L^{-1} d^{-1}) foram obtidos nos ensaios com maior diluição. Nestes ensaios, no entanto, foi observado um comportamento diferente em relação aos demais testes, pois as microalgas cresceram na forma de flocos. Este fato pode representar uma real vantagem econômica para a etapa de recuperação da biomassa (processo de colheita), embora, por outro lado, resulte em produtividades mais baixas. Nos ensaios em lixiviado de aterro com *C. vulgaris*, os valores também aumentaram conforme a concentração do efluente diminuiu, atingindo assim os seguintes resultados para taxas específicas de crescimento e produtividades máximas de biomassa: 0,15±0,03 d^{-1} e 93±39 mg_{dw} L^{-1} d^{-1}, respectivamente. Já para *T. obliquus*, a concentração de lixiviado não apresentou um efeito significativo ($p>0,05$) nas taxas específicas de crescimento (os valores variaram entre 0,129±0,002 d^{-1} e 0,146±0,03 d^{-1}). Porém, em termos de produtividades máximas de biomassa, os resultados alcançados foram estatisticamente diferentes entre si ($p<0,05$) e o maior valor (86±17 mg_{dw} L^{-1} d^{-1}) foi obtido para uma concentração de efluente intermediária (15% (v/v)). As espécies também indicaram ter capacidade de promover a remoção de nitrogênio e fósforo. Nos estudos com efluente da indústria de papel, resultados promissores foram alcançados, principalmente, nos experimentos conduzidos com o efluente mais diluído. De modo geral, observou-se um aumento nas eficiências de remoção conforme a concentração

de efluente diminuiu, com valores variando de 24±10% a 80±4% para nitrogênio e de 13,0±0,9% a 54±1% para fósforo. Nos ensaios com lixiviado de aterro, as eficiências máximas de remoção de nitrogênio variaram de 7±2% a 65±1% para *C. vulgaris*, e de 3,0±0,3% a 56±1% para *T. obliquus*, enquanto que as eficiências de remoção de fósforo obtidas em culturas de *C. vulgaris* e *T. obliquus* variaram de 12±1% a 31±2% e de 10,7±0,6% a 29,9±0,7%, respectivamente. Ainda em relação ao lixiviado de aterro, após a seleção de uma concentração adequada de efluente nos testes realizados em frascos de 1 L, também foram realizados os testes em um fotobiorreator tubular (PBR) inovador. Este PBR é conhecido por sua capacidade de melhorar a absorção e distribuição da luz dentro do meio reacional, um parâmetro crucial para os processos fotossintéticos. Uma melhoria significativa nas taxas específicas de crescimento de *C. vulgaris* (de 0,15±0,04 d^{-1} a 0,61±0,04 d^{-1}) e nas eficiências de remoção de nitrogênio (um ganho de aproximadamente 26% e 33% para *C. vulgaris* e *T. obliquus*, respectivamente) foi alcançada neste teste preliminar, confirmando que o desenvolvimento de novos PBRs também é uma estratégia importante a ser explorada. Com base nisso, estudos para avaliar a eficiência do PBR tubular sob diferentes configurações também foram realizados. Em função do tempo, resultados superiores foram alcançados em sistemas com refletores parabólicos (SP e DP) feitos de materiais com maior refletância especular (R85 e MS), o que foi concordante com uma maior potência radiante incidente (RP_i). Assim, a taxa específica de crescimento máxima (μ, d^{-1}) foi de 0,230±0,005 d^{-1}, com RP_i de 0,167±0,005 J s^{-1}, em ensaio com refletor R85-DP. Em termos de energia, houve uma pequena queda nas taxas específicas de crescimento, com o aumento do fluxo de fótons, o que pode estar associado à baixa transmissibilidade das suspensões de microalgas, principalmente quando se atinge maior densidade celular. Testes utilizando dois tubos absorvedores (com espaçamento entre eles de 12,5, 25,0, 50,0 e 75,0 mm) e refletor R85-F também foram realizados. Os resultados mostraram que a distância de 50,0 mm levou ao melhor compromisso entre as taxas de crescimento específicas e produtividades de biomassa por metro quadrado de coletor solar. Nessas condições, obteve-se maior eficiência no aproveitamento da energia fotônica em comparação ao teste com tubo único. Enquanto no ensaio com um tubo absorvedor e refletor R85-F, a taxa específica de crescimento foi de apenas 0,177±0,006 d^{-1}, com dois tubos, este valor passou para 0,202±0,003 d^{-1}.

Conclusões

Este estudo mostrou a viabilidade de utilização de dois efluentes (efluente da indústria de papel e lixiviado de aterro) como meio de cultura alternativo para *C. vulgaris* e *T. obliquus*, em paralelo à remediação dos mesmos. Ambas as espécies de microalgas foram capazes de crescer nas diferentes diluições de efluentes estudadas e promover a remoção de nitrogênio e fósforo. No entanto, de modo geral, resultados superiores foram obtidos à medida que reduzimos a concentrações de efluentes no meio. Assim, podemos concluir que os efluentes estudados têm um efeito tóxico sobre as microalgas cultivadas, o que se deve tanto a sua composição quanto a redução da eficiência fotossintética devido a coloração e/ou turbidez dos mesmos. Deste modo, o aumento significativo na produção de biomassa e na remoção de nutrientes para *C. vulgaris*, cultivada em PBR tubular, em comparação aos estudos em frascos de 1 L, pode estar associada a uma melhor eficiência de utilização da luz incidida (distribuição e captação) neste sistema. Os resultados positivos obtidos com o uso deste PBR tubular abrem novas perspectivas no uso desses reatores para o crescimento de microalgas em diferentes águas residuárias, especialmente em efluentes altamente coloridos/turvos. Os ensaios com as diferentes configurações estudadas deste PBR tubular também confirmaram um desempenho superior em comparação às culturas cultivadas no PBR sem refletor. Isso indica que o aumento da área iluminada do tubo absorvedor e da captação da luz incidida foi

benéfico ao processo em termos de produção de células, devido à maior disponibilidade de luz. Os resultados obtidos também deixaram claro que as mudanças na geometria e nos materiais das superfícies refletoras têm um impacto direto no uso da energia luminosa. Enquanto os refletores com geometrias parabólicas (SP e DP) permitem ampliar a área iluminada do tubo absorvedor, os materiais com maior refletância especular (R85 e MS) resultam em um maior fluxo de fótons atingindo o meio de cultura. Assim, nestes casos, tem-se uma maior energia acumulada no sistema, o que beneficiou a eficiência fotossintética do processo, resultando em uma maior produção celular. Também é possível afirmar que não ocorreu inibição do crescimento celular devido à luz incidente excessiva (fotoinibição). Tal comportamento, provavelmente, se deve ao uso de um painel de LED de baixa intensidade luminosa como fonte de luz. Por outro lado, apesar do efeito benéfico observado no crescimento celular com o aumento da energia acumulada, os resultados baseados na energia mostram uma perda de eficiência neste aspecto. Já nos testes com dois tubos absorvedores pode-se concluir que a distância de 50,0 mm entre eles leva a uma maior eficiência no aproveitamento da energia fotônica e do espaço ocupado pelo sistema. Assim, nestas condições, temos uma produtividade máxima por área superior aos testes com um tubo absorvedor. Em suma, conclui-se que o uso de refletores afeta positivamente o desempenho de crescimento das culturas de *C. vulgaris* no interior do tubo absorvedor. Além disso, os coletores solares planos mostraram-se uma alternativa promissora, em termos de área ocupada e custos associados, quando se considera uma expansão em escala.

Palavras-chave: Produção de biomassa. Microalgas. Tratamento de águas residuárias. Biorremediação. Remoção de nutrientes. Fotobiorreator.

ABSTRACT

Microalgae are seen as a flexible and very promising raw material and, therefore, have been widely studied in recent years in different applications: CO_2 capture, biomass/biofuel production and nutrients uptake from wastewater. However, it is necessary to make the process more efficient and cheaper. In this context, microalgal growth in wastewater, concomitantly promoting its bioremediation, has been proposed as a viable alternative to reduce the costs and environmental footprint. Thus, in this study, a landfill leachate and paper industry effluent, concentrated and in dilutions different, were evaluated as culture medium for *Chlorella vulgaris* and *Tetradesmus obliquus*. As phosphorus and nitrogen are essential nutrients for microalgae, their concentration was monitored throughout the cultivations in terms of phosphate, nitrate and nitrite. Under the conditions studied, both strains were able to grow on these media and promote the removal of these nutrients. The maximum removal efficiencies obtained were 83±1% and 56±1% for nitrogen and phosphorus, respectively. However, it was observed that undiluted effluents inhibited the growth of the species. Thus, it can be concluded that the bioremediation process is possible after an adequate effluent dilution. The effluent dilution minimizes the toxicity and reduces color/turbidity. In *T. obliquus* cultivation in the paper industry effluent, a different behavior was observed in relation to the other tests, as the microalgae grew in the form of flakes. This fact can represent a real economic advantage for the biomass recovery stage (harvest process). Although, on the other hand, it results in lower productivities. Concerning landfill leachate, after selecting an adequate effluent concentration in the tests carried out in 1 L flasks, tests on an innovative tubular photobioreactor (PBR) were also carried out. The configuration employed was composed by a borosilicate glass absorber tube and a reflecting surface with a double parabola geometry (DP) made of anodized aluminum material (R85). This PBR is known for its ability to improve the absorption and distribution of light inside the reaction medium, a crucial parameter for photosynthetic processes. Significant improvement in specific growth rates of *C. vulgaris* (from 0.15±0.04 d^{-1} to 0.61±0.04 d^{-1}) and nitrogen removal efficiencies (a gain of approximately 26% and 33% for *C. vulgaris* and *T. obliquus*, respectively) were achieved in this preliminary test, confirming that the development of new PBRs is also an important strategy to be explored. Based on this, studies were carried out with different configurations of this PBR, where the following aspects were evaluated: (i) reflective surface geometries (flat (F), simple double parabola (SP) and traditional double parabola (DP)) and

(ii) the materials used in their manufacture (anodized aluminum with protective coating MIRO-SUN® (MS), anodized aluminum without protective coating (R85) and stainless steel (SS)). To determine the impact of these parameters on the use of light energy, actinometric tests were carried out, and the growth of *C. vulgaris* was determined as the function of the cultivation time and the energy accumulated in the system. As a function of time, superior results were achieved with systems with parabolic reflectors (SP and DP) made of materials with higher specular reflectance (R85 and MS), which is in agreement with a higher incident radiant power (RP_i) obtained under these conditions, according to the actinometric tests. Thus, the maximum specific growth rate (μ, d^{-1}) obtained was 0.230±0.005 d^{-1} in PBR R85-DP assay, with RP_i of 0.167±0.005 J s^{-1}. There was a small drop in specific growth rates on the energy basis as the photon flux increased, which may be associated with the low transmissibility of microalgal suspensions, especially when a higher cell density is reached. The tests using two absorber tubes showed that the distance of 50.0 mm between them is the most adequate, considering the specific growth rates and biomass productivity per square meter of solar collector. Under these conditions, higher efficiency on the photonic energy usage was attained comparing to the test with a single tube. Therefore, the use of these novel PBRs towards microalgal growth may represent an overall process cost reduction, in addition to leading to greater cell production and, consequently, maximizing nutrient recovery in industrial effluents.

Keywords: Biomass production. Bioremediation. Microalgae. Nutrients removal. Photobioreactor. Wastewater treatment.

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1 INTRODUCTION

Microalgae are seen as a flexible raw material and with high economic potential. Compounds of commercial interest with added-value can be extracted from microalgal biomass, such as carbohydrates proteins, lipids, nucleic acids, carotenoids and polymers (SPOLAORE *et al*., 2006). These photosynthetic organisms produce biomass and oxygen using light (solar or artificial) as an energy source, carbon dioxide and/or organic carbon, among other micro and macro nutrients, such as nitrogen and phosphorus (BARBOSA *et al*., 2003; UGGETTI *et al*., 2014; ZHANG *et al*., 2014; SUGANYA *et al*., 2016). Thus, these microorganisms have been widely studied in recent years in a vast variety of applications: (i) CO_2 capture (WANG *et al*., 2008; KUMAR *et al*., 2010; RAZZAK *et al*., 2017); (ii) biomass/biofuel production (ODJADJARE *et al*., 2017; RIZWAN *et al*., 2018); and (iii) nutrients uptake from wastewater (RAWAT *et al*., 2011; ALCÁNTARA *et al*., 2020; SHAHID *et al*., 2020). One of the main applications of microalgal biomass is the extraction of lipids for the production of biofuels (third-generation). When compared to other terrestrial cultures, commonly used for the production of biofuels (first and second-generation), microalgae stand out for their high productivity, adaptability and for dispensing with the use of agricultural land for their production (CHISTI, 2007; BRENNAN and OWENDE, 2010; SUGANYA *et al*., 2016).

On the other hand, microalgal cultivation requires a substantial amount of water to keep them in suspension for their survival and cell proliferation (SALAMA *et al*., 2017). Thus, the use of wastewater for microalgal cultivation is an important way to reduce the demand for freshwater and fertilizers (such as a source of nutrients) (BARROS *et al*., 2015). The nutrients present in the effluents can be assimilated by microalgal cells through enzymatic degradation and bioaccumulation (RATH, 2011; BILAL *et al*., 2018; SHAHID *et al*., 2020). From an environmental point of view, the removal of anthropogenic nutrients from wastewaters, before their discharge, is essential to avoid the contamination of aquatic ecosystems, eutrophication and to protect potable water reserves (CONLEY *et al*., 2009; CHANG *et al*., 2019).

In consequence, several studies have evaluated the growth of microalgae in domestic (ZHANG *et al*., 2014; GAO *et al*., 2016; DO *et al*., 2020; MA *et al*., 2020; MOONDRA *et al*., 2020) and industrial wastewaters from different sectors (USHA *et al*., 2016; BHATTACHARYA *et al*., 2017; LIN *et al*., 2017; RAJWAR *et al*., 2017;

WU *et al*., 2017; MAZHAR *et al*., 2019; OYEBAMIJI *et al*., 2019; WU *et al*., 2020). In almost all studies, microalgae were able to efficiently remove the monitored nutrients, and simultaneously achieve high values for biomass and lipid productivity. The extensive increase in the generation of residual flows, as well as the complex and variable composition of these effluents (KJELDSEN *et al*., 2002; POKHREL and VIRARAGHAVAN, 2004; PASKULIAKOVA *et al*., 2016; USHA *et al*., 2016; FAZAL *et al*., 2018), demands for the development of ecologically correct treatment solutions. Thus, the combination of microalgal biomass production processes with wastewater treatment is an interesting strategy to increase the competitiveness of microalgae biofuels against fossil fuels, while promoting environmental sustainability (EISBERG, 2006; GOMEZ *et al*., 2008; CHANG *et al*., 2019).

However, for the process to be economically sustainable and reach an industrial scale, it is necessary to make it more efficient and cheaper. For this, using wastewater as a culture medium, the following approaches must be adopted and studied (BRASIL *et al*., 2017; MORENO-GARCIA *et al*., 2017; FAZAL *et al*., 2018): (i) optimize cultivation parameters; (ii) evaluate the effect of biotic and abiotic parameters on cell production and nutrients removal kinetics, as well as on the composition of the biomass produced; (iii) develop ways to reduce the impact of wastewater toxicity on microalgal cells; (iv) promote an adequate selection of cultivated strains; (v) evaluate the cultivation systems on a larger scale and; (vii) develop new photobioreactors (PBR) for microalgal cultivation, taking into account some critical points, such as the light distribution.

Based on what was exposed, this study sought to evaluate different species of microalgae, grown in the paper industry and landfill leachate effluents, regarding their productivity and bioremediation capacity (nitrogen and phosphorus removal). The paper industry effluent was obtained from a secondary-treated effluent of a Portuguese paper company, and the pre-treated leachate was collected in a landfill located in northern Portugal. The studies with the Portuguese effluents were carried out during my research stay (PhD Sandwich, PDSE / CAPES) at the Faculty of Engineering of the University of Porto (FEUP), Portugal. Cultivations were carried out, mostly, in flasks with different concentrations of effluents, to evaluate their toxicity and the ideal initial nutritional conditions. Subsequently, considering that effluent color and turbidity can also limit microalgal growth, due to the reduction on the light penetration in the culture medium (CHEUNG *et al*., 1993), a tubular PBR was used in the cultivation of *Tetradesmus*

obliquus and *Chlorella vulgaris* in the landfill leachate. This PBR consists of a reflective surface placed below a borosilicate glass absorber tube. The application of this new technology allows a more homogeneous light distribution around the entire perimeter of the absorber tube (GOMES *et al*., 2018), thus promoting better light absorption by microalgal cells. In this way, it was also evaluated the efficiency of this PBR with different reflectors, geometries and materials, targeting the evaluation of the influence of these aspects on the use of light energy and their effects on biomass yields.

1.1 OBJECTIVES

In this section, the general objective and the specific objectives of this doctoral thesis are described.

1.1.1 General Objective

To develop an innovative system of microalgal cultures with reduce the costs and environmental footprint, for biomass production and effluents' treatment.

1.1.2 Specific Objectives

To achieve the proposed general objective, the following specific objectives were defined:

i. evaluate the nitrogen and phosphorus absorption capacity and the possible inhibitory effects of the effluents used as a culture medium on the microalgae growth;
ii. testing the PBR tubular, characterized by a reflective surface of anodized aluminum (R85) in double-parabola geometry (DP) around a cylindrical borosilicate glass tube, as a culture platform in landfill leachate;
iii. compare the efficiency of different configurations of this same PBR, regarding the geometry and materials of the reflective surfaces, aiming at higher biomass yields and environmental footprints.

THESIS OUTLINE

This thesis is structured in seven chapters, including this introductory one, Chapter 1, which provides the scope and objectives of this work. Chapter 2 brings a brief literature review about microalgae and their applications, mainly concerning the treatment of effluents.

The experimental methodology and materials employed are presented in Chapter 3, including a description of the experimental procedures, experimental units and analytical methods used.

The subsequent three chapters (Chapters 4 to 6) present the experimental results obtained during my PhD Sandwich at FEUP (PDSE/CAPES). In Chapter 4, the growth of *C. vulgaris* in a paper industry effluent was evaluated, coupling biomass production with nutrients removal. In Chapter 5, the potential of two microalgal species (*C. vulgaris* and *T. obliquus*) for nutrients removal from landfill leachate was assessed. In Chapter 6, the efficiency of an innovative tubular PBR was studied. Different reflector geometries and materials were evaluated, targeting high biomass yields, better use of luminous energy provided by LEDs, and significantly smaller footprints.

Finally, Chapter 7 summarizes the final remarks, where the most relevant results and conclusions are reported. Some suggestions for future work are also presented.

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2 LITERATURE REVIEW

This chapter contextualizes relevant aspects to the work through a bibliographic survey. Thus, the following review discusses the application of microalgae as a promising technology for the treatment of effluents and the production of biofuels. Initially, it seeks to understand the characteristics of these organisms and the differences between species. Then, it deals with the environmental problem and the economic aspects pertinent to their large-scale application. Also noteworthy, the influence of cultivation parameters on the growth of microalgae and the removal of contaminants is discussed. Finally, it addresses the differences between the commonly used culture systems.

2.1 MICROALGAE

Microalgae are photosynthetic unicellular organisms, which can be divided into eukaryotic and prokaryotic (cyanobacterial) (MORENO-GARCIA *et al*., 2017; SHAHID *et al*., 2020). These microorganisms can also be grouped into Phyla, the main ones being: green algae (Chlorophyta), golden-brown algae (Heterokontophyta), red algae (Rhodophyta), dinoflagellates (Dinophyta), in addition to blue-green algae or cyanobacteria (Cyanophyta) (GRAHAM *et al*., 2016). Despite the wide variety of species already discovered (20-800 thousand), there are about 40-50 thousand species that have not yet been studied (SUGANYA *et al*., 2016). Microalgae biomass is mainly composed of proteins (8-71%), carbohydrates (9-64%), lipids (2-75%), and nucleic acids (up to 5%), being these percentages variable between species and strongly dependent on culture conditions (AFZAL *et al*., 2017; LI *et al*., 2019).

Microalgae are found in different aquatic systems, such as freshwater and marine environments and in residual water, due to their structure and simple nutritional requirements (CHISTI, 2007; SCOTT *et al*., 2010; MORENO-GARCIA *et al*., 2017). Their size can vary according to the species, from a few micrometers to a few hundred micrometers. Unlike plants, algae have no roots, stems or leaves. (SUGANYA *et al*., 2016). Because of their metabolism and based on their energy and carbon source requirements, they can be further divided into photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic (BHARATHIRAJA *et al*., 2015; SAMBUSITI *et al*., 2015; SUGANYA *et al*., 2016).

Through the photosynthetic process, microalgae produce biomass and oxygen, making use of sunlight (energy source), carbon dioxide (carbon source), among other nutrients such as nitrogen and phosphorus (BARBOSA *et al*., 2003; UGGETTI *et al*., 2014; ZHANG *et al*., 2014; SUGANYA *et al*., 2016). Through this process, microalgae produce about half of the atmospheric oxygen (SUGANYA *et al*., 2016). Also, these microorganisms are one of the most important sources of biomass and have undeniable economic and environmental potential (SURESH KUMAR *et al*., 2015; SUGANYA *et al*., 2016). They can be used as bioindicators (O'NEILL *et al*., 2019) and in bioremediation processes for carbon sequestration and wastewater treatment (SHAHID *et al*., 2020). Microalgae can also be employed in various industries as an alternative to traditional feedstocks, including the production of biofuels, cosmetics, biofertilizers, medicines and food supplements (SURESH KUMAR *et al*., 2015; SUGANYA *et al*., 2016; AFZAL *et al*., 2017).

2.2 ENVIRONMENTAL APPLICATIONS

The urbanization and industrialization process led to an increase in emissions of polluting gases into the atmosphere, causing severe climate change. With this, the United Nations Conference on climate change, held in Paris, 2015, outlined a series of goals for 2030 and 2050, seeking to curb emissions and their negative impacts on the climate. Governments agreed that stronger and more ambitious action was urgently needed. In this scenario, the decarbonization of the energy sector is essential to achieve these goals (HÜBLER and LÖSCHEL, 2013; FRAGKOS *et al*., 2017). The energy transition leads to the need to develop new technologies and solutions where the use of renewable energy sources and other alternative fuels, replacing fossil fuels, play an important role. However, although there are several alternative sources of clean energy, such as solar, geothermal, wind and biomass energy, the economic aspects and their applicability are still a challenge (MABEE *et al*., 2005; SUGANYA *et al*., 2016). In this context, biofuels, produced by biological or biochemical processes, have gained prominence as a substitute energy source for fossil fuels (SCOTT *et al*., 2010; YUSUF *et al*., 2011; SUGANYA *et al*., 2016). Their production has been growing in the last decades and the tendency is for this process to continue. Currently, biofuels already represent about 77.4% of the total renewable energy produced in the world (DORADO

et al., 2006; HAAS *et al*., 2006; CARLOS and BA KHANG, 2008; BERNAL *et al*., 2012; SUGANYA *et al*., 2016).

These fuels can be classified as the first, second or third generation, according to the raw material used in their production. First-generation biofuels, already produced on a commercial scale, use food crops as raw material, a fact that can affect biodiversity and impact land use in detriment of food production, leading to an increase in food prices (GOH and LEE, 2010; NAIK *et al*., 2010; SUGANYA *et al*., 2016). On the other hand, second-generation biofuels are obtained from vegetable biomass, that is, non-food lignocellulosic materials (SUGANYA *et al*., 2016). Despite the ability of biofuels from plant biomass to compete economically with fossil fuels, the use of agricultural by-products can supply only part of the growing demand (EISBERG, 2006; GOMEZ *et al*., 2008). On the other hand, third-generation biofuels, produced from algae or cyanobacteria, are considered a renewable, economical method and environmentally sustainable. This technology has enormous potential to meet global demand, replacing fossil fuels and at the same time avoiding the use of food cultures for this purpose (MIAO and WU, 2006; CHISTI, 2007; JOHN *et al*., 2011; SUGANYA *et al*., 2016). Among the advantages of microalgae over traditional cultures, one can mention superior growth speed, adaptability to different growth media, higher photosynthetic efficiency (about 40-50%), higher harvesting index, highest rate of carbon fixation, simpler nutritional requirements and higher lipid content (40-80% dry weight) (CHEN *et al*., 2015; SURESH KUMAR *et al*., 2015; SUGANYA *et al*., 2016; KHAN *et al*., 2017; NG *et al*., 2017; KHAN *et al*., 2018). As an example, microalgae can produce up to 300 times more lipids than traditional cultures (CHISTI, 2007; RITTMANN, 2008). Besides, biofuels produced from microalgae biomass are highly biodegradable and do not contain sulfur or other toxic compounds (SCHENK *et al*., 2008).

Microalgae can also contribute to mitigating CO_2 emissions since about half of the dry weight of microalgal biomass is carbon. Some species are tolerant to high amounts of CO_2. Thus, through the bio-fixation process (where approximately 1.83 kg of CO_2 are consumed per 1 kg of microalgal biomass produced), microalgae can also promote the treatment of flue gases (CHISTI, 2007; MORENO-GARCIA *et al*., 2017; NG *et al*., 2017). From microalgal biomass, it is also possible to extract proteins, carbohydrates, and other compounds, used to produce several other co-products (CHRISTENSON and SIMS, 2011; YU *et al*., 2017). Carbohydrates are used to produce bioethanol and methane, while residual biomass can be used to generate heat

and electricity, as feed for livestock or as an organic fertilizer (WANG *et al*., 2008; MATA *et al*., 2010; SUGANYA *et al*., 2016). Thus, the simultaneous production of biofuels and fine chemicals (fatty acids and pigments, for example) in a microalgal biorefinery can considerably improve the process economy (BHARATHIRAJA *et al*., 2015; YU *et al*., 2017; AZIZ *et al*., 2019).

However, the large-scale production of microalgal biofuels is still economically unviable. At present, algal biofuels cannot compete with the prices of fossil fuels (DASAN *et al*., 2019). Thus, several researchers have been developed to optimize the process conditions to reduce operating costs and improve biomass production, directing the balance towards the production of compounds of interest (NG *et al*., 2017). An appropriate selection of the cultivated strain can also be determinant to produce cost-competitive algal products (BRASIL *et al*., 2017; MORENO-GARCIA *et al*., 2017). Another important strategy is the use of wastewater as an alternative growth medium (low cost), aiming to supply the water demand and reduce the costs with nutrients supply, and concomitantly, the bioremediation of these effluents (MCGINN *et al*., 2011; KOMOLAFE *et al*., 2014; MORALES-AMARAL *et al*., 2015; SUGANYA *et al*., 2016; ROOSTAEI and ZHANG, 2017; YU *et al*., 2017; YADAV *et al*., 2019).

2.2.1 Effluents Treatment

Worldwide, about 3881 billion m³ of freshwater was consumed in 2017 (WORLD BANK, 2017). Most water is employed in human activities (in the industry, agricultural and domestic use), which generate exorbitant volumes of wastewater (DINIZ *et al*., 2017). According to QADIR *et al*. (2020), about 380 billion m³ of wastewater is generated annually worldwide. The study further estimates that global production will increase 24% by 2030 and 51% by 2050 over the current level.

Micropollutants, nutrients, heavy metals and organic pollutants represent the main contaminants that can be found in wastewater (SALAMA *et al*., 2017). These contaminants can cause water eutrophication and the destruction of ecosystems, besides render them unfit for human consumption (GODFRAY *et al*., 2010; MORÉE *et al*., 2013). Usually, due to the inherent limitations of each method, it is necessary to apply a set of treatment steps to achieve an adequate removal of the contaminants (SHAHID *et al*., 2020). Conventional wastewater treatment methods are subdivided into chemical, physical and biological processes. The selection of these methods depends on the

required treatment complexity and the purpose that will be given to the treated effluent (DVOŘÁK *et al*., 2014). The methods may present certain deficiencies, such as demand for large land areas, high-energy requirements, extensive maintenance and operational costs, instability in the treatment, carbon emission, excess sludge discharge and recyclable resource wasting (QU and FAN, 2010; JIN *et al*., 2014; UDAIYAPPAN *et al*., 2017; LI *et al*., 2019). As a result, high concentrations of several kinds of ionic compounds continue to be released into the environment, such as phosphorus, nitrogen and metal ions (ZENG *et al*., 2015).

That way, to meet increasingly stringent discharge standards and protect the environment, it is required to develop more sustainable treatment technologies and promote wastewater reuse. In this context, microalgae-based bioremediation (phycoremediation) represents a safe and efficient treatment alternative to achieve a high-quality effluent that can be reused or discharged into aquatic compartments. The application of phycoremediation has several positive points, including vast microalgae strain availability, efficient nutrient consumption, resource recovery and the possibility of acting in parallel with the production of microalgal biomass (LAM *et al*., 2012; YADAV *et al*., 2019; SHAHID *et al*., 2020). With this, several studies have evaluated the ability of the microalgae strains to promote: CO_2 fixation (DING *et al*., 2020; SONG *et al*., 2020), nitrogen and phosphorus uptake (GAO *et al*., 2016), toxic metals (MATAMOROS *et al*., 2015; KHAN *et al*., 2017) and organic pollutants (BHATTACHARYA *et al*., 2017) removal from wastewater (MATA *et al*., 2010). Biorefinery approaches have also been proposed, integrating the cultivation of microalgae with the already existing wastewater treatment infrastructure (LAM *et al*., 2012; GILL *et al*., 2013; XIE *et al*., 2019; YADAV *et al*., 2019). The cultivation of microalgae requires a substantial amount of water both to keep the cells in suspension and to their survival and proliferation (MURPHY and ALLEN, 2011). Thus, the use of wastewater as a culture medium is an important way to minimize freshwater consumption and nutrients requirements in microalgae cultivation (BARROS *et al*., 2015).

Microalgae remediation can replace conventional treatment methods or be integrated with them for the final polishing of the effluent. In conventional wastewater treatment plants, the first treatment step generally consists of a primary settler to promote the removal of sedimentable solids from the raw wastewater (GULDHE *et al*., 2017). The effluent from this phase contains a high load of organic carbon that

microalgae can use in a mixotrophic or photoheterotrophic metabolic route (ZHOU *et al*., 2011). However, the primary effluent also contains a high concentration of microorganisms, which compete with microalgae for nutrients and organic carbon. On a large scale, promoting the removal of pathogens at this stage of treatment may be impractical (ABDEL-RAOUF *et al*., 2012). Thus, most investigations have focused on the use of secondary and tertiary effluents, generated after biological treatment and after disinfection, respectively. The secondary effluent contains a reduced concentration of toxic substances and organic carbon (OLGUÍN, 2012; GULDHE *et al*., 2017). However, to increase the productivity of microalgal biomass, it may be necessary to supplement it due to low nutrient concentrations. On the other hand, nitrogen depletion condition e.g., also can provide a greater lipid accumulation in microalgae (CHINNASAMY *et al*., 2010).

In the last decades, several studies have been carried out to evaluate the ability of microalgae in the treatment of domestic effluents, industrial wastewater from different sources and agro-industrial wastewaters (Chart 2.1). In almost all studies, microalgae were able to efficiently remove the monitored nutrients, with promising values of biomass and lipid productivity still being achieved. The scale expansion and application of microalgae wastewater treatment technology is also associated with the need to improve culture systems so that they operate at high flow rates, considering the exorbitant volume of effluents generated daily. In this context, new reactors have been developed, aiming at better process control, cell productivity and efficiency in the uptake of contaminants.

Chart 2.1 – Studies realized to evaluate the ability of microalgae species in the treatment of wastewater from different sources.

Wastewater	**Sector**	**References**
Domestic	-	RAWAT *et al*. (2011); MOSTAFA *et al*. (2012); ZHANG *et al*. (2014); GAO *et al*. (2016); CALICIOGLU and DEMIRER (2019); DO *et al*. (2020); MA *et al*. (2020); MOONDRA *et al*. (2020).
Industrial	Brewery	MATA *et al*. (2012); MATA *et al*. (2014); FERREIRA *et al*. (2017); (SONG *et al*., 2020).
	Textile	BHATTACHARYA *et al*. (2017); WU *et al*. (2017); OYEBAMIJI *et al*. (2019); WU *et al*. (2020).
	Pharmaceutical	XIE *et al*. (2019).
	Slaughterhouse	HERNÁNDEZ *et al*. (2016); AKIZUKI *et al*. (2019); AZIZ *et al*. (2019); AZAM *et al*. (2020)
	Palm oil factory	KAMYAB *et al*. (2015); HARIZ and TAKRIFF (2017); CHEAH *et al*. (2018b; 2018a); SASONGKO *et al*. (2018); CHIA *et al*. (2020); DING *et al*. (2020); EMPARAN *et al*. (2020)
	Paper and oil mill	TARLAN *et al*. (2002); GENTILI (2014); KOUHIA *et al*. (2015); POLISHCHUK *et al*. (2015); USHA *et al*. (2016); TAO *et al*. (2017); PORTO *et al*. (2020).
Agro-industrial	-	CALIXTO *et al*. (2016); KOUTRA *et al*. (2018); MARKOU *et al*. (2018); GUPTA *et al*. (2019); ALCÁNTARA *et al*. (2020); DE MEDEIROS *et al*. (2020); KOUTRA *et al*. (2021).

Author (2021).

2.3 CULTURE SYTEMS

Microalgal culturing can be performed in closed (photobioreactors) or open systems. However, most industrial cultivation systems are promoted in open ponds, since the construction and operation of these systems are less expensive, in addition to having greater production capacity compared to other systems at an equivalent cost (SINGH and SHARMA, 2012; WANG *et al*., 2012) On the other hand, large-scale production typically results in low cells density, which increases the costs of the biomass harvesting process. Thus, among the system's disadvantages, it is possible to mention the difficulty in distributing nutrients and light, which can be overcome with the addition of mixing equipment. Another disadvantage is related to difficulties with species control and contamination risks. In this case, species that grow in highly selective environments are more suitable (SUH and LEE, 2003).

Open systems can be categorized into natural waters (lakes, lagoons, ponds) and artificial ponds (SINGH and SHARMA, 2012). Usually called raceway ponds, these ponds are shallow to ensure that there is no limitation due to lack of light. Paddlewheels provide the flow of cultures around a racetrack and keep them in suspension (SINGH and SHARMA, 2012). To overcome the limitations of open systems, the tanks can be closed with a transparent or translucent barrier, which transforms them into a greenhouse. Closed tanks allow for better temperature control, minimize the risk of contamination from monocultures and increase the availability of CO_2, thus increasing the growth rates of microalgae (SINGH and SHARMA, 2012).

Photobioreactors (PBRs) consist of an enclosed to the environment and illuminated culture vessel design. Thus, there is no gas exchange directly with the environment and the risk of contamination is lower. On the other hand, the associated costs are higher (SINGH and SHARMA, 2012). Despite this, several other advantages of these systems can be highlighted, among them (SINGH and SHARMA, 2012; WANG *et al*., 2012): (i) control of culture conditions and growth parameters; (ii) less risk of contamination; (iii) prevent water evaporation and CO_2 losses; (iv) higher photosynthetic efficiency; (v) higher biomass productivity and cell concentrations; (vi) enable the production of biopharmaceuticals; and (vii) promote higher contaminants removal from wastewater. A large variety of different PBRs has been developed in the last decades for microalgal cultivation. The PBRs can be classified according to their geometry in a vertical tubular photobioreactor (e.g., bubble column, airlift), flat panel, horizontal tubular, helical type, stirred tank and hybrid PBR (SINGH and SHARMA, 2012; WANG *et al*., 2012).

According to TSOGLIN *et al*. (1996); WANG *et al*. (2012), an efficient PBR project must have the following characteristics: (i) maximum use of light energy; (ii) adequate operational control; (iii) high transfer rate of mass and CO_2 (without the damage microalgal cells or suppress their growth); (iv) permit the cultivation of different microalgal species; (v) reduce or prevent the fouling of the reactor; (vi) work under conditions of intense foaming; and (vii) present low cost and energy consumption. In this context, new technologies have been developed to improve these key parameters. For example, about the capture and distribution of light, there are spectral shifting, internal illumination (XUE *et al*., 2013) and coupling of optical reflectors (PORTO *et al*., 2020), whereas, about mass transfer, new membrane PBRs have been developed (GAO *et al*., 2016; LUO *et al*., 2017; CHANG *et al*., 2019); and to

minimize costs, cheaper materials have been employed in the construction of the reactors (e.g., plastic bag PBRs) (WANG *et al*., 2012). For example, GAO *et al*. (2016) used a membrane photobioreactor (MPBR) to promote the treatment of domestic secondary wastewater in a continuous flow operation mode with *Chlorella vulgaris*. The membrane module of the reactor prevented the washing of microalgal cells, enabling the reactor to operate at a high supply rate. Thus, biomass production was highly efficient as well as the removal of nutrients and metal ions. The maximum growth biomass was 1.64 times higher than the conventional photobioreactor (CSTR).

2.4 FACTORS AFFECTING NUTRIENTS RECOVERY, GROWTH AND MICROALGAL BIOMASS COMPOSITION

Microalgal growth and nutrients removal efficiency can be significantly affected by several factors, both biotic and abiotic. Biotic factors include the presence of pathogens (bacteria, fungi and viruses) and other competing microalgae. Abiotic factors include light, temperature, pH, salinity, nutritional profile, presence of toxic compounds and dissolved oxygen concentration (GONÇALVES *et al*., 2017). The manipulation of these factors affects biomass productivity and plays a fundamental role in redirecting cell metabolism for the product of interest (SHAHID *et al*., 2020). Likewise, operational conditions (e.g., hydraulic residence time (HRT), harvesting rates, mixing, CO_2 availability, shear rates and light) also influence microalgal growth (KUMAR *et al*., 2010; MASOJÍDEK *et al*., 2013; BARSANTI and GUALTIERI, 2014; YEN *et al*., 2019).

2.4.1 Light Effect

The light supply is a fundamental factor in the photoautotrophic microalgae growth since microalgal use it as an energy source. Several studies have already shown that the efficiency of nutrient removal, growth rate and productivity of microalgal biomass as well as the synthesis of co-products (accumulation of lipids, fatty acid profile and pigment production) are affected by lighting (LI *et al*., 2012; LEE *et al*., 2015; GONÇALVES *et al*., 2019; HWANG and MAIER, 2019). Three parameters related to light have a strong impact on the photosynthetic efficiency of cultures: quantity and quality of light (intensity and wavelength) and light-dark cycle

(photoperiod) (KIM *et al*., 2014; LEE *et al*., 2015; SINGH and SINGH, 2015; SUTHERLAND *et al*., 2015; MORENO-GARCIA *et al*., 2017).

When the irradiance of light is less than the saturation point of the cells, the photosynthetic activity is proportional to the same. However, if this saturation point is exceeded, the photosynthetic process and, consequently, the growth of microalgae may be inhibited (MORENO-GARCIA *et al*., 2017). The ideal amount of light can differ between species, as well as it can be affected by cultivation conditions and the system configuration itself (XUE *et al*., 2013; PEGALLAPATI *et al*., 2014; SINGH and SINGH, 2015). AKIZUKI *et al*. (2019) achieved an ammonia removal of 80.4% under half-day illuminated conditions at 140 μmol photons m^{-2} s^{-1}. On the other hand, the authors observed that prolonged light exposure and/or high light intensity (at least 1573 μmol photons m^{-2} s^{-1}) with excess free ammonia (ranging from 3.3 to 59.4 mg L^{-1}) can lead to severe inhibition of nitrification by the microalgal-bacterial consortium. In this case, it is necessary to develop light mitigation strategies, such as using granular sludge.

2.4.2 Temperature Effect

The temperature strongly affects the growth of microalgae, the cellular chemical composition, the absorption of nutrients and CO_2. Up to a certain limit, higher temperatures can improve metabolic activity, while lower temperatures inhibit microalgal growth (SINGH and SINGH, 2015). On the other hand, under extreme temperature conditions, the metabolic activity of microalgae can even be interrupted. The optimum temperature varies between the different microalgal species, but many species have an optimum temperature between 28 and 35 °C (PARK *et al*., 2011). However, according to the region where they are found, some microalgae are adapted to lower temperatures (<10 °C), others grow at moderate temperatures (10 - 20 °C), while others still grow at temperatures above 30 °C (MICHEL *et al*., 1989; TEOH *et al*., 2004; GONÇALVES *et al*., 2017).

2.4.3 pH Effect

The pH affects the physiology of microalgae, influences the enzyme activity, increases triglyceride accumulation, and is responsible for the availability and solubility of nutrients, contaminants, and CO_2 (JUNEJA *et al*., 2013; YING *et al*., 2014; SHAHID

et al., 2020). Besides, pH is a fundamental parameter in controlling invading organisms (predators or competitors) (SHAHID *et al*., 2020). However, it should be noted that CO_2 supplementation decreases the pH of the medium, due to the chemical balance between CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}. Thus, the control of this parameter is crucial to avoid the loss of culture by extreme pH values (KUMAR *et al*., 2010; HU, 2013; YEN *et al*., 2014; GONÇALVES *et al*., 2017).

Generally, the ideal pH for most species is between 7 and 9. However, tolerance varies between species, mainly due to the natural environmental conditions of the habitats in which they are found. Some species are alkaliphilic, for example, such as *Arthrospira platensis* (pH> 9), while others are acidophilic, such as *Chlorococcum littorale* (pH 5-6) (BELKIN and BOUSSIBA, 1991; KUMAR *et al*., 2010; HU, 2013; YEN *et al*., 2014; GONÇALVES *et al*., 2017).

2.4.4 Salinity Effect

Changes in the salinity of the medium can cause osmotic stress, ionic stress and/or changes in permeability in a membrane system (GLASS, 1983; GONÇALVES *et al*., 2017). In open systems, both rainfall and natural evaporation can cause changes in salinity. However, this parameter can be controlled by adding freshwater or salts in the medium to reach an adequate level for microalgae (MATA *et al*., 2010; GONÇALVES *et al*., 2017).

2.4.5 CO_2 Effect

Microalgae use the available CO_2 in the atmosphere for their growth. However, the extra supply of CO_2 contributes to increasing the lipid content in the biomass. Several studies have shown that this is a crucial factor to maximize lipid productivity (NAKANISHI *et al*., 2014; WANG *et al*., 2014; MORENO-GARCIA *et al*., 2017). From an environmental point of view, this process is also interesting, since CO_2 can be captured from flue gas emissions, thus contributing to the mitigation of greenhouse gas emissions into the atmosphere (MORENO-GARCIA *et al*., 2017).

2.4.6 Mixing Effect

Among the operational conditions, the mixture of the medium is a parameter that deserves to be highlighted. Adequate homogenization of the medium prevents the formation of stagnant zones, sedimentation and thermal stratification of the cells, in addition to improving gas transfers, the distribution of light and nutrients (KUMAR *et al*., 2010; BARSANTI and GUALTIERI, 2014; GONÇALVES *et al*., 2017; MORENO-GARCIA *et al*., 2017). The mixture of the medium causes the cells to alternate between light and dark areas, preventing the photo-inhibition of some cells, and photo-limitation of others (MORENO-GARCIA *et al*., 2017).

2.4.7 Nutrients Concentration Effect

The concentration of certain nutrients can affect the growth rate of microalgae and, consequently, biomass productivity. For example, autotrophic species require inorganic carbon to perform photosynthetic reactions, while nitrogen and phosphorus are essential for the synthesis of nucleic acids and proteins (GONÇALVES *et al*., 2017). In addition to these, some vitamins and micronutrients, especially metals (Mg, Ca, Mn, Zn, Cu and Mo), are also required and benefit cell growth (BECKER, 1994; KUMAR *et al*., 2010; GONÇALVES *et al*., 2017). On the other hand, the biotic stress of certain nutrients, mainly nitrogen and phosphorus, can increase the biomass productivity and induce the metabolic routes of microalgae towards the products of interest, such as proteins, lipids and pigments (CHEN *et al*., 2017; SHAHID *et al*., 2020). This form of stress can be caused using wastewater, which may have a low concentration or an unbalanced nutrient ratio (SHAHID *et al*., 2020).

As previously mentioned, nitrogen is an essential nutrient for the growth of microalgae. About 1-20% of microalgae dry cell matter is composed of nitrogen, which acts in the synthesis of nucleic acid, protein, energy-carrying molecules and enzymes (JUNEJA *et al*., 2013; SATPATI and PAL, 2018). KAMYAB *et al*. (2015) promoted *Chlamydomonas incerta* cultivation in palm oil mill effluent (POME) with different dilutions factors. After 28 days, COD removal was about 67.35% in 250 mg L^{-1} of POME concentration. The optimum lipid content and productivity were achieved at the carbon-total nitrogen 100:7 (C:TN) ratio. Nitrogen demonstrated a crucial role in lipid accumulation in microalgal cells. Supplementation of extra sources of nitrogen and

carbon (glucose, urea and glycerol) also had a positive effect on biomass (1.68 g L^{-1}) and lipid (15.07%) productions in cultures of *Chlorella sorokiniana CY-1* in POME (CHEAH *et al*., 2018b). On the other hand, N-stress has been considered an important strategy to trigger the accumulation of lipid, triglyceride and carotenogenesis (JUNEJA *et al*., 2013; MINHAS *et al*., 2016; SHI *et al*., 2017). After reaching a high cell concentration, the depletion of the supply of nitrogen, for example, causes all the carbon present in the medium to be converted into lipids at the expense of protein production (MORENO-GARCIA *et al*., 2017).

Phosphorus is another important macronutrient for microalgae, although it makes up only <1% of its total dry mass (MINHAS *et al*., 2016). This nutrient works in the synthesis of protein and transcription, and the carbon cycle (MÜHLROTH *et al*., 2017). In addition, it is part of RNA, DNA backbone, ATP, phospholipids, phosphoproteins, polyphosphates and NADPH (JUNEJA *et al*., 2013). CHU *et al*. (2014) observed an increase in lipid productivity with the addition of phosphorus above standard conditions. PASKULIAKOVA *et al*. (2016) also achieved higher growth rates with the addition of phosphorus. However, the combined stress of nitrogen and phosphorus also allows achieving higher biomass and lipid productivities (CHEN *et al*., 2017). WU *et al*. (2017) observed an increase in the accumulation of lipid in *Chlorella sp.* G23 when grown in raw textile wastewater (without dilution). According to the results obtained, adding extra phosphate and nitrogen sources could also enhance the pollutant removal efficiency and fatty acid methyl ester (FAME) production. That way, maximum FAMEs accumulation (20.0±4.0%) in *Chlorella sp.* G23 biomass was achieved when cultivated at pH 9–11 with the addition of K_2HPO_4 (4 mg L^{-1}) and urea (1 g L^{-1}) to the raw wastewater.

Microalgae consume sulfur, mainly in the form of sulfate (SHAHID *et al*., 2020). This macronutrient is present in high concentrations in wastewater from the paper and cellulose, pharmaceutical, mining and food processing industries (LV *et al*., 2017). Several cellular processes are affected by the presence of sulfur, such as assimilation, secondary metabolic pathways, responses to oxidative stress, flavonoids and nitrogen metabolism (SHAHID *et al*., 2020). For microalgae, the assimilation of sulfur can inhibit the process of photosynthesis, while the lack of it induces the expression of proteins associated with stress (GIORDANO and RAVEN, 2014; SHAHID *et al*., 2020). Moreover, sulfur deprivation leads to the accumulation of starch efficiency (VITOVA *et al*., 2015). This is because while sulfur hunger inhibits energy

consumption, growth and cell division, it also alters the metabolic pathways for starch accumulation (ANTAL *et al*., 2014). However, the impact of the presence of sulfate in the culture medium may not be the same for different strains of microalgae. Thus, a more detailed assessment is needed, both in relation to sulfur stress alone and in combination with other nutrients (SHAHID *et al*., 2020).

Regarding metals, several microalgae species have already been shown to remove heavy metals from wastewater by biosorption and bioaccumulation, in an environmentally friendly and efficient process, compared to conventional treatment techniques (SHAHID *et al*., 2020). Some metals can be beneficial for the growth of microalgae (such as Cu, Zn, Ni, Mn and Co), while others can positively affect the nutritional quality and other important functions of the living system (such as Mn, Cu, I, Zn, Fe, Pb) (KUMAR, 2015; WELLS *et al*., 2017). On the other hand, certain heavy metals (including Pb, Cd, Cr and Hg) can affect the metabolic processes, the physical structure of algae and can cause toxicity, mutagenesis and allergenicity (MIKULEWICZ *et al*., 2017); in addition to stimulating the formation of reactive oxygen species (ROS), causing oxidative damage to cells and, consequently, inhibiting the growth and production of pigments (SHAHID *et al*., 2020).

2.5 CURRENT CHALLENGES OF MICROALGAL CONSORTIA AND RESEARCH NEEDS

The economic viability of microalgal cultures, that is, the costs of the associated processes, is the major challenge for the technology to be applied at full scale. For this, three research paths can be followed: (i) use wastewater as a culture medium, reducing footprints in fresh water, and use of fertilizers as nutrients source; (ii) apply low-cost harvesting processes; and (iii) intensify the concentration and extraction of products of commercial interest from the microalgal biomass.

Although microalgae-based effluent remediation has many benefits (in the provision of low-cost growth media, wastewater cleaning, reducing the environmental impact and the water footprint), as well as the production of biofuels from microalgal biomass, compared to the first- and second-generation biofuels, several aspects need to be better explored so that their full potential is reached (ZHOU, HU, *et al*., 2012; YADAV *et al*., 2019). One of the main challenges of cultivation in wastewater is the limitation of certain nutrients in the water from secondary and tertiary treatment units,

while, on the other hand, raw water can contain an excessive load of contaminants, in addition to having high color and turbidity, thus inhibiting the photosynthetic process of microalgae. A strategy to circumvent this problem consists of diluting them with wastewater from other sources, balancing the nutritional composition and reducing the toxic load of the effluent, without increasing the costs with chemical supplementation of nutrients and neither the demand for freshwater (ZHOU, MIN, *et al*., 2012; GULDHE *et al*., 2017). Besides, manipulating biotic and abiotic factors and stress conditions can improve biomass productivity and redirect cell metabolism towards the product of interest (CHEN *et al*., 2017; GONÇALVES *et al*., 2017; SHAHID *et al*., 2020). However, the mechanisms of nutrient assimilation and the correlation of process parameters with microalgae growth and with bioremediation of effluents are not yet fully understood (NAWAZ *et al*., 2020). A broader understanding of these processes is a critical point to enable the implementation of microalgae biorefineries in large-scale units (CHEN *et al*., 2017; GONÇALVES *et al*., 2017; SHAHID *et al*., 2020).

An adequate selection of microorganisms can also contribute to the effective treatment of specific contaminants, without reducing the photosynthetic activity of microalgal cells. For this, it is necessary to explore the integration of consortia and the genetic manipulation of strains, aiming to improve the robustness and long-term effectiveness of cultures. The positive symbiosis between different species of algae can be used to minimize the toxicity of the medium and increase the growth of some strains in the consortium. This is due to the exchange of metabolites and molecular signals between species and the division of functions (SUBASHCHANDRABOSE *et al*., 2011; JAGMANN and PHILIPP, 2014; GONÇALVES *et al*., 2017; NAWAZ *et al*., 2020). For example, in the case of a bacterial-algal consortium, bacteria can decompose several nitrogen compounds in forms that microalgae can directly assimilate. Besides, through the cellular respiration process, microalgae provide bacteria with O_2 and receive CO_2 from them (ZHAO *et al*., 2014; NAWAZ *et al*., 2020). MOONDRA *et al*. (2020) evaluated the optimum concentration of a microalgal-bacterial consortium for raw domestic wastewater treatment. Three concentrations (20, 30, and 40%) were tested in cultivations with 8 h and 16 h hydraulic retention time (HRT). The maximum removal efficiencies of phosphate, ammonia, biological oxygen demand (BOD) and chemical oxygen demand (COD) were obtained for 30% consortia (99.79%, 94.85%, 89.02% and 88.96%, respectively) at HRT of 8 h. This study indicated that there is a positive symbiotic relationship between algae and bacteria, enhancing the cost-effective

efficiency by lowering the HRT. Likewise, HARIZ *et al*. (2019) demonstrated the capability of the two indigenous strains (*Scenedesmus sp.* and *Chlorella sp.*) in promoting the treatment of POME and capturing CO_2 from the flue gas an integrated system. The maximum CO_2 fixation rate and contaminant removal (total nitrogen (TN), reactive phosphate (PO_4^{3-}), total organic carbon (TOC) and chemical oxygen demand (COD)) were the following: 0.829 g of CO_2 per liter per day, 86%, 85%, 77% and 48%, respectively. According to the authors, the integrated system and the use of two microalgal species with different metabolic traits contributed to more CO_2 fixed and nutrients removal compared to the individual treatment. On the other hand, some aspects still need to be improved, such as light penetration through POME and microalgae acclimatization to shorten the lag growth phase. Also, the presence of bacteria limits lipid productivity, reducing the quality of the biofuels that will be produced; for this reason, new studies must be carried out to determine adequate speciation of the consortia (ZHAO *et al*., 2014; NAWAZ *et al*., 2020).

Another approach is to develop mathematical models that correctly represent the behavior of cultures, which will allow to reliably predict the ideal operating conditions to obtain more efficient results, both in the mitigation of pollutants and in the production of microalgal biomass (BORDEL *et al*., 2009; GONÇALVES *et al*., 2017). However, more studies must be carried out to develop assay protocols, thus minimizing the inconsistent results. This will allow explaining and predicting mathematically the performance of cultivation systems. These protocols must yet present a good correlation between the small-scale systems with the corresponding medium and large-scale systems (NAWAZ *et al*., 2020).

The harvest stage is another bottleneck for the scale-up of the process and represents one of the main costs. MATA *et al*. (2014) evaluated the potential of microalga grown in brewery wastewater to produce biomass and biodiesel. The results of this study showed that the recovery of the biomass produced is one of the main obstacles and that the associated costs are still unfeasible. For this reason, improved productivity and increased lipid content are essential factors in making microalgal biofuels competitive. Likewise, harvesting techniques also need to be improved to effectively promote the removal of dead biomass over cultures, separating it from growing biomass (BARROS *et al*., 2015; ZHAO *et al*., 2015; FAYAD *et al*., 2017). Dead biomass occupies space inside the reactors, blocking the passage of light and can

also promote the growth of wild microorganisms, negatively impacting the system's performance (NAWAZ *et al*., 2020).

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3 MATERIALS AND METHODS

This third chapter describes the analytical methodologies and experimental procedures adopted in this thesis. In this way, the materials, equipment and experimental units employed are described in this section. This chapter aims to provide relevant information to understand the experiments performed, both using effluents as a culture medium and evaluating the different configurations tested for tubular photobioreactors (PBRs). The experimental work was developed in the Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), at the Department of Chemical Engineering (DEQ), Faculty of Engineering of the University of Porto (FEUP).

3.1 MATERIALS

3.1.1 Wastewater

Two effluents were evaluated as an alternative culture medium for microalgal biomass production. Their results are divided into 2 chapters: Chapter 4 for paper industry effluent (PIE) and Chapter 5 for landfill leachate (LL). According to the methodologies described in Section 3.4 of this chapter, the referred effluents were first characterized for their physicochemical properties.

The paper industry effluent (PIE) was collected after the secondary treatment step from a Portuguese paper company. Due to the low nitrogen concentration in the effluent, when compared with the typical nutritional requirements of microalgae, it was supplemented with $NaNO_3$ to achieve N:P molar ratios ranging between 6:1 and 9:1. Ratios between 5:1 and 30:1 have been considered adequate for several microalgal species (LARSDOTTER, 2006; SILVA *et al*., 2015).

The leachate (LL) sample was obtained from an urban waste landfill located in northern Portugal. As the proposed microalgal treatment aimed to promote nutrients removal as part of the tertiary treatment, the effluent was collected after the biological treatment step. The dilution solutions were supplemented to achieve an adequate N:P molar ratio (12:1). In this case, an external source of phosphorus (KH_2PO_4) was used, since its characterization indicated a limitation of this nutrient.

3.1.2 Microalgae and growth medium

Chlorella vulgaris (CCAP 211/11B) was used in the following cultivation experiments: (i) in novel tubular PBRs using modified culture medium as described by SANTOS *et al*. (2019); and (ii) in batch systems with paper industry effluent (PIE) and landfill leachate (LL). In the case of tests with LL, the microalgae *Tetradesmus obliquus* (CCAP 276/3A) was also employed. Both species were obtained from the Culture Collection of Algae and Protozoa (CCAP, UK) and maintained in the modified Organization for Economic Co-operation and Development (OECD, 2011) culture medium described by SANTOS *et al*. (2019).

The modified version of the OECD Test medium, used as a growth and maintenance medium for microalgal cultivation in this study, presented the following final composition (values in mg L^{-1} - nutrient): 119 - KNO_3; 12 - $MgCl_2 \cdot 2H_2O$; 18 - $CaCl_2 \cdot 2H_2O$; 15 - $MgSO_4 \cdot 7H_2O$; 20 - KH_2PO_4; 0.08 - $FeCl_3 \cdot 6H_2O$; 0.1 - $Na_2EDTA \cdot 2H_2O$; 0.185 - H_3BO_3; 0.415 - $MnCl_2 \cdot 4H_2O$; 0.003 - $ZnCl_2$; 0.0015 - $CoCl_2 \cdot 6H_2O$; 0.00001 - $CuCl_2 \cdot 2H_2O$; 0.007 - $Na_2MoO_4 \cdot 2H_2O$; and 100 - Na_2CO_3.

3.2 EXPERIMENTAL UNITS

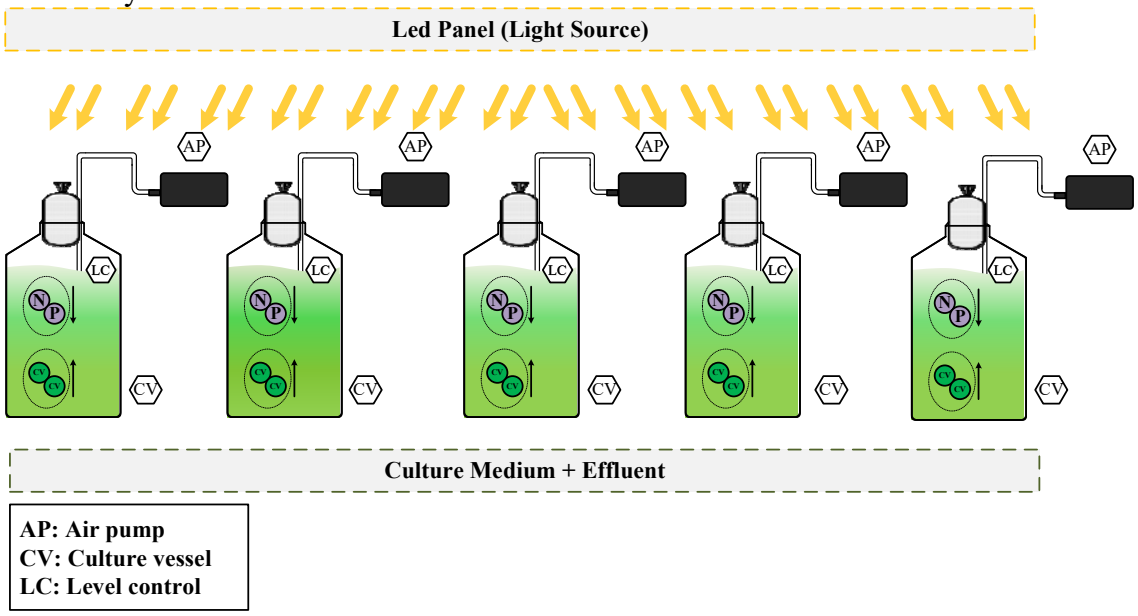
In this section, the various sets of experiments carried out for the preparation of this thesis will be described, subdivided into tests with PIE (Section 3.2.1), with LL (Section 3.2.2) and with tubular PBRs with different configurations (Section 3.2.3).

3.2.1 Experimental setup for paper industry effluent

Batch experiments were performed in 1 L borosilicate glass flasks (VWR, Portugal) with a working volume of 950 mL for 11 days. The effluent (assay 1) and four different dilutions with freshwater (assays 2-5) were used as the culture medium for microalgal growth, corresponding to concentrations of 100%, 80%, 60%, 40% and 20% (v/v). In these conditions, the nitrogen concentrations ranging between 12.7 mg_N L^{-1} and 34.2 mg_N L^{-1} and phosphorus concentrations ranging between 4.01 mg_P L^{-1} and 12.3 mg_P L^{-1}. The medium was inoculated with 250 mL of *C. vulgaris* inoculum to obtain an initial biomass concentration of ~ 68 mg_{dw} L^{-1}. The cultures were continuously exposed to (i) photosynthetically active radiation between 30-40 μmol m^{-2} s^{-1}, using a 34 W

white LED panel (120 cm long and 30 cm wide); and (ii) atmospheric air filtered with 0.22 µm cellulose acetate membranes (Specanalitica, Portugal), injected at ~90 L h^{-1}, using Trixie AP 180 air pumps (Trixie, Tarp, Germany). The experimental setup is shown in Figure 3.1.

Figure 3.1 – Schematic representation of the experimental setup used for assay with paper industry effluent concentration and landfill leachate in different concentrations.



Author (2021).

The tested conditions were performed in duplicate. Operational parameters, such as pH and temperature, were daily monitored using a SympHony SB90M5 pH-meter (VWR, Portugal). Microalgal growth and nutrients (PO_4-P, NO_3-N and NO_2-N) concentration were also evaluated, according to the methods described in Sections 3.3.2 and 3.3.3, respectively.

3.2.2 Experimental setup for landfill leachate

To offset the possible toxic effects of the landfill leachate, as well as a light limitation due to the high turbidity of the effluent, five dilution factors were evaluated in this study (4×, 5×, 7×, 10× and 20×), corresponding to leachate concentrations of 25%, 20%, 15%, 10% and 5% (v/v). These dilutions resulted in turbidity values ranging between 14.5 and 72.5 NTU, and initial nitrogen concentration values ranging between 197 mg_N L^{-1} to 41.7 mg_N L^{-1}. Dilution factors were selected, taking into account the

study proposed by CHOI *et al*. (2018). In this study, the authors showed that the growth of *C. vulgaris* on a dairy effluent presenting turbidity of 190 NTU was strongly inhibited, but dilution factors of 5× and 10× (corresponding to turbidities of 38 NTU and 19 NTU, respectively) significantly enhanced *C. vulgaris* specific growth rates. To avoid growth inhibition due to the low phosphorus concentration present in the leachate, the diluted effluent solutions were supplemented with KH_2PO_4 to achieve a N:P molar ratio of approximately 12:1.

The diluted leachate samples (with phosphorus supplementation) were transferred to 1 L borosilicate glass flasks (VWR, Portugal) and inoculated with both algal suspensions to achieve initial biomass concentrations (in terms of dry weight, *dw*) of ~77 mg_{dw} L^{-1} and ~104 mg_{dw} L^{-1}, for *C. vulgaris* and *T. obliquus*, respectively. The cultures were allowed growing in batch mode for 11 days, under the following conditions: (i) continuous light supply (photosynthetically active radiation, PAR, of about 30-40 μmol m^{-2} s^{-1}), provided by a 34 W white LED panel (120 cm long and 30 cm wide); and (ii) continuous injection of atmospheric air (~90 L h^{-1}), filtered with 0.22 μm cellulose acetate membranes (Specanalitica, Portugal), using Trixie AP 180 air pumps (Trixie, Tarp, Germany). The schematic representation of the experimental setup is similar to that employed for the PIE (Figure 3.1 from Section 3.3.1).

Two independent experiments were performed for all tested conditions. During the experiments, microalgal growth and nutrients (PO_4-P, NO_3-N and NO_2-N) concentration in the culture media were periodically monitored, according to the methods described in Sections 3.3.2 and 3.3.3, respectively. The temperature and pH of the culture media were also monitored (daily) using a SympHony SB90M5 pH-meter (VWR, Portugal).

3.2.2.1 Experiments in the tubular PBR

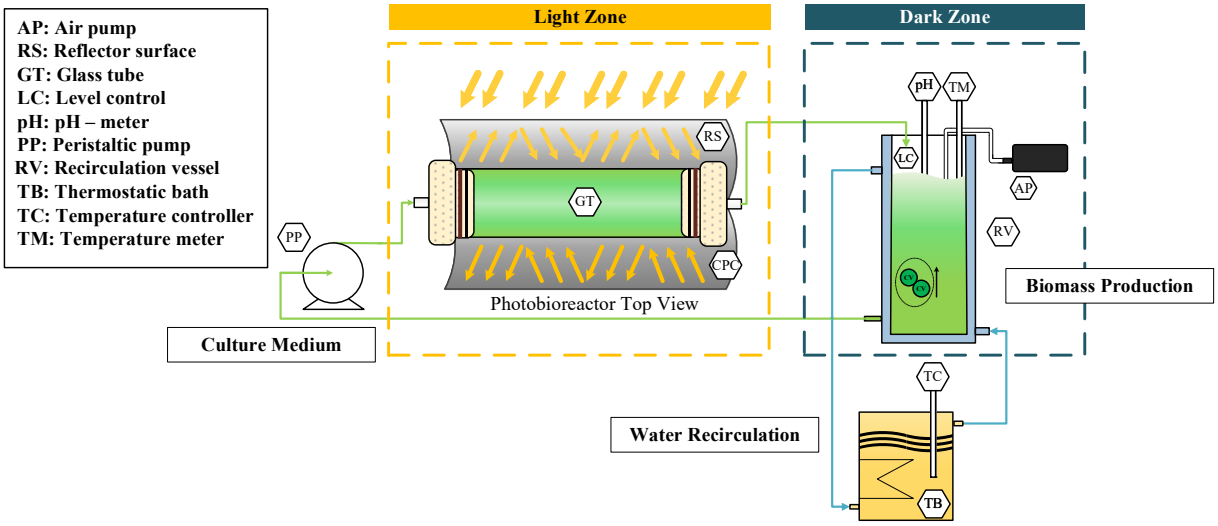
After defining the leachate concentration that allowed the highest biomass productivities and nutrients removal efficiencies for both studied microalgae, the selected conditions for each species were reproduced in a tubular PBR, characterized by a reflective surface of anodized aluminum (R85) in double-parabola geometry (compound parabolic collector) around a cylindrical borosilicate glass tube (GOMES *et al*., 2018). The system is described in Section 3.3.3, and the schematic representation of this experimental setup is presented in Figure 3.2. During the experiments, the pH and

temperature were monitored once a day and biomass and nutrients concentrations present in the culture medium were quantified according to the methodologies described in Sections 3.3.2 e 3.3.3 respectively.

3.2.3 Photobioreactor and cultivation conditions

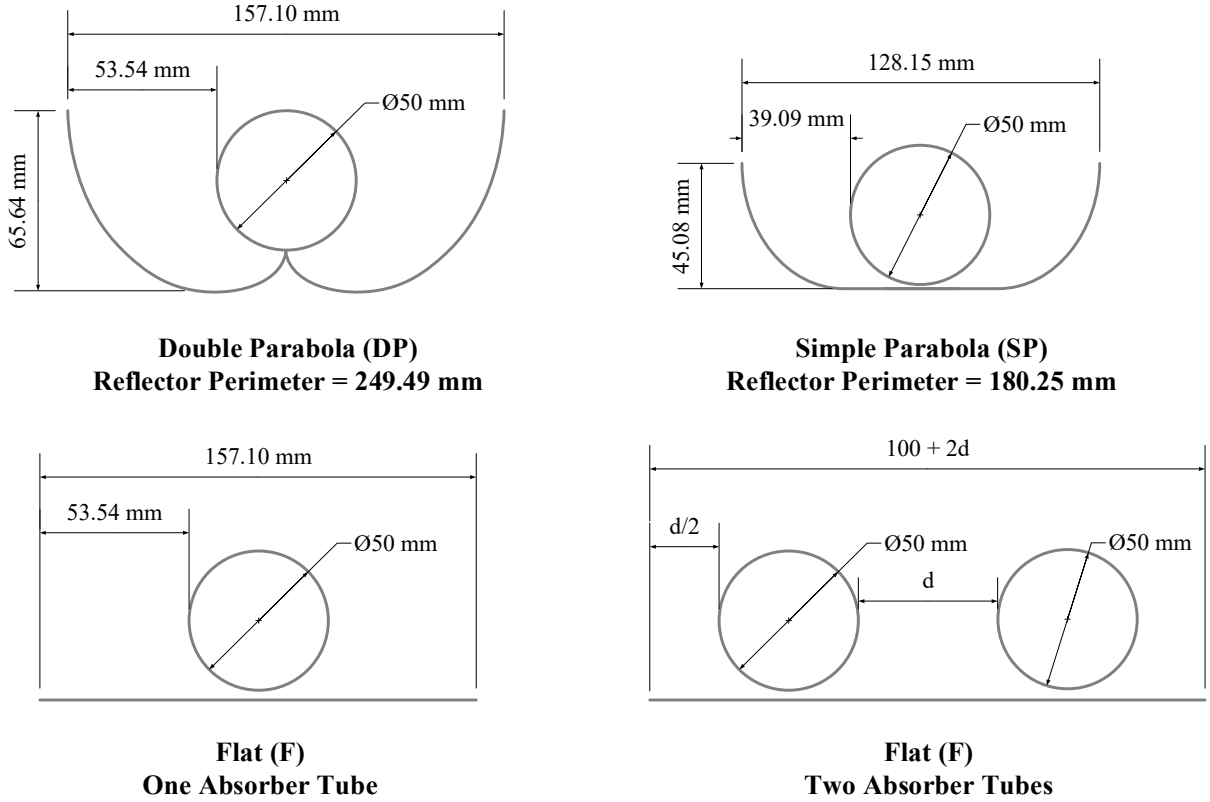
The cultivation assays were performed in a PBR (Figure 3.2), consisting of a reflective surface placed below a borosilicate glass absorber tube (Schott-Duran type 3.3, Germany, cut-off at 280 nm, total length of 200 mm, useful length of 160 mm, internal diameter of 46.4 mm and thickness of 1.8 mm) with 270 mL of illuminated volume. Different reflector's materials (anodized aluminum Mirosun with a protective coating – MS, anodized aluminum Reflective 85 without protective coating – R85, and polished stainless steel – SS) and geometries (flat – F, simple double parabola – SP, and traditional truncated double parabola – DP) were tested. Figure 3.3 shows the cross-section of each studied geometry together with the respective dimensions. Further specifications of the reflective surfaces can be found in GOMES *et al*. (2018).

Figure 3.2 – Schematic representation of the experimental setup used for *C. vulgaris* cultivation in tubular PBRs.



Adapted from GOMES *et al*. (2018).

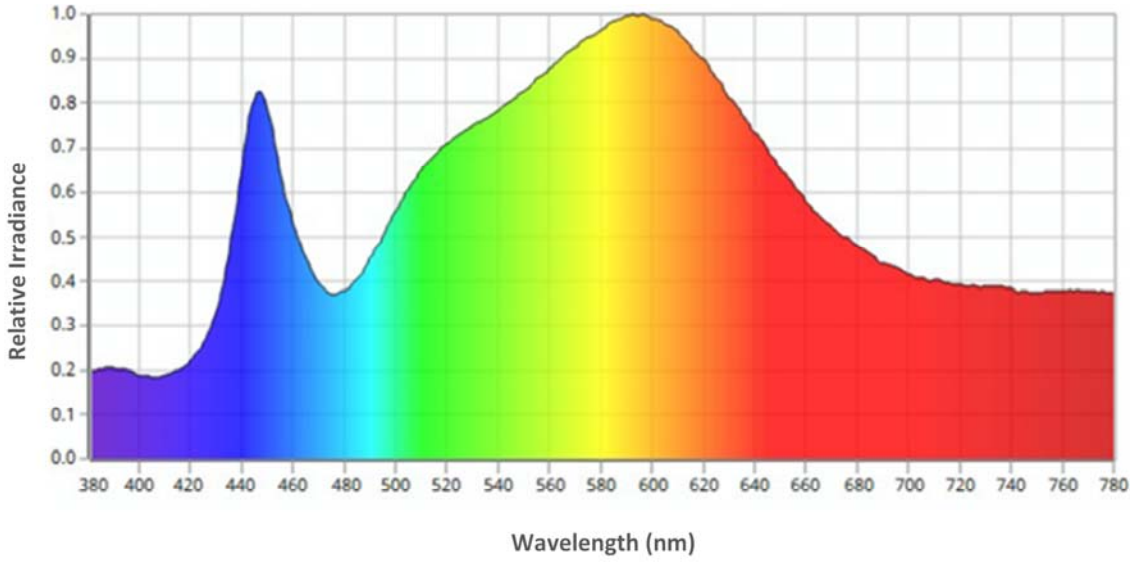
Figure 3.3 – Sketch of the geometries adopted for the reflective surfaces' optics.



Adapted from (GOMES *et al*. (2018)).

Microalgal cultivation experiments were conducted in batch mode for seven days, using a working volume of ~520 mL and an initial biomass concentration of ~200 mg_{dw} L^{-1}. The absorber tube was continuously exposed to a PAR of ~69 μmol m^{-2} s^{-1} (the equivalent to 13.4 W m^{-2} in the range of 400-700 nm), supplied by a 34 W LED panel (EGLO Connect RGB/Tunable Whites, Austria) with 120 cm long and 30 cm wide, located 25 cm above the reactor top surface. Figure 3.4 displays the LED spectrum for the wavelength range from 380 to 780 nm. PAR was measured by a radiometer HD 2102.2 (Delta OHM, Italy).

Figure 3.4 – **S**pectrum of the 34 W LED panel (EGLO Connect RGB/Tunable Whites, Austria) for the wavelength range from 380 to 780 nm.



EGLO Lights (2019).

Microalgal suspensions were recirculated (at a flow rate of 50 L h^{-1}) between the absorber tube (light zone) and a glass vessel (dark zone), using a peristaltic pump (Ismatec VC-381, Germany). Atmospheric air provided by a Trixie AP 180 air pump (Trixie, Tarp, Germany), filtered through 0.22 μm cellulose acetate membranes (Specanalitica, Portugal), was injected (~ 90 L h^{-1}) into the glass vessel to prevent cells' sedimentation and to supply CO_2 to the cultures. Suspensions' pH (between 8-9) and temperature (from 25 ºC to 30 ºC) were maintained constant by means of pH buffers and a thermostatic bath (CW-05G, Lab Companion, Korea), respectively. Therefore, their influence on microalgal growth could be ignored. Biomass concentration in the culture medium was quantified throughout the assays according to the methodology described in Section 3.3.2.

3.2.3.1 Experimental procedure for cultivation assays

The experimental work consisted of thirteen batch-mode assays, using different reflective surfaces, and a control one, without a reflector. In the first ten cultivation assays, the effect of the reflective surfaces' material and geometry was evaluated employing one absorber tube. Finally, for the flat reflector featuring the best material, tests were also carried out with two absorber tubes at different distances: $d/4$, $d/2$, d, and $3d/2$, where d is the external diameter (50 mm) of the borosilicate tube.

3.2.3.2 Actinometric tests

The performance of each reflective surface was, initially, compared through the radiant power incident (RP_i, J s^{-1}) on the tubular PBR surface, the radiant power (RP, J s^{-1}) reaching the actinometric solution, and the optical concentration ratio (CR_O). These parameters were estimated through ferrioxalate actinometry ([Fe^{3+}] = 0.006 M, with a [Fe^{3+}]:[oxalic acid] molar ratio of 1:5 to avoid iron precipitation during the trials), as reported in GOMES *et al*. (2018). This method is based on the photochemical reduction of ferric ions into ferrous ions, and it is suitable for measurements in the UV-Vis region up to 580 nm (the limit for which ferrioxalate complex can absorb radiation) (GOMES *et al*., 2018). It is worth mentioning that the photon flux emitted by the LEDs panel was determined considering the wavelength range between 380 – 580 nm (lower limit of LEDs – upper limit of ferrioxalate absorption), which is lower than that of the PAR, i.e., 400–700 nm. Therefore, it should be bearing in mind that microalgal cells could absorb more radiation than what was quantified, since LEDs used in this study were emitting radiation beyond 580 nm.

Actinometric tests started by adding 1 L of the actinometric solution to the recirculation vessel and switching on the magnetic stirring. During the first 10 min, the solution was recirculated between the PBR and the glass vessel, by means of a peristaltic pump, under dark conditions for complete homogenization. Afterwards, a control sample was taken, and the LEDs panel was turned on, thus beginning the photoreduction of iron (III) into iron (II). Samples were collected at predefined times during the period corresponding to 25% of the actinometer's conversion, which differed according to the reflector's efficiency. Ferrous ions concentration, generated during the irradiation period, was measured by conventional conversion to the colored tris-phenanthroline complex (ε = 11100 L mol^{-1} cm^{-1} at μ_{max} = 510 nm) in line with ISO 6332:1988. The total volume of the samples collected within a single experimental run did not change by more than 5% of the initial liquid volume.

RP and RP_i were determined based on the models proposed by BOSSMANN *et al*. (1998) and RIOS-ENRIQUEZ *et al*. (2004). CR_O, or the ratio between the irradiance incident on the absorber tube (which is given by dividing RP_i by the absorber tube side area, i.e., 0.025 m^2) and the irradiance incident on the collector aperture (which is given by converting the PAR measured within the range of 400-700 nm into the LEDs radiation intensity within the range of 380-580 nm, i.e., 8.5 W m^{-2}) was estimated using

the methodology described by GOMES *et al*. (2018). Furthermore, *RP* (J s^{-1}) parameter was used to calculate the accumulated energy ($Q_{380\text{-}580nm,n}$, kJ L^{-1}) per unit of water volume during the microalgal cultivation trials, as stated in Equation 3.1:

$$Q_{380-580nm,n} = Q_{380-580nm,n-1} + \frac{RP \times (t_n - t_{n-1})}{1000 \times V_s} \quad (3.1)$$

where t_n (s) and $t_{n\text{-}1}$ (s) are the times corresponding to the n and n-1 water samples, respectively, and V_s (L) is equivalent to the microalgal suspension volume.

3.3 ANALYTICAL METHODS

In this section, there are described the analytical methodologies employed for effluents characterization (item 3.3.1), microalgal growth monitoring (item 3.3.2) and anions concentration determination (item 3.3.3). Anions were also determined in the previous characterization of the effluents. During cultivation assays, the pH and temperature were daily monitored and biomass and nutrients concentrations present in the culture medium were quantified. In addition, the respective calculation realized for determining the parameters of microalgal growth, nutrients removal and effluents characterization are described.

3.3.1 Effluents characterization

3.3.1.1 Soluble chemical oxygen demand (COD_S)

The chemical oxygen demand (COD) expresses the amount of oxygen that reacts with oxidizable substances, present in 1 L of water, and under specific operating conditions. For the PIE and LL effluents, the COD_S was determined using the 5220-D method, according to the Standard Methods for the Examination of Water and Wastewater (RICE *et al*., 2012), using the photometer Spectroquant NOVA 60A, Merck Millipore®,

3.3.1.2 Turbidity determination

The turbidity, which is related to the transparency of fluid, was determined according to the Standard Methods for the Examination of Water and Wastewater, through the 2130-B test. In this case, a turbidimeter, model HI88703, HANNA Instruments®, was used, and the results are expressed in nephelometric turbidity unit (NTU).

3.3.1.3 Dissolved organic carbon (DOC) determination

Dissolved organic carbon (DOC) was determined by NDIR spectrometry in a TC-TOC-TN analyzer equipped with ASI-V autosampler (Shimadzu, model TOCVCSN). DOC was given by the difference between TDC (Total Dissolved Carbon) and DIC (Dissolved Inorganic Carbon).

3.3.1.4 Total suspended solids (TSS) determination

Total suspended solids (TSS) were determined by the gravimetric method (2540-D test), as described in the Standard Methods for Examination of Water & Wastewater (RICE *et al*., 2012).

3.3.2 Microalgal growth monitoring and growth parameters

C. vulgaris and *T. obliquus* growth was daily monitored by optical density at 680 nm (OD_{680}), using a UV-6300 PC spectrophotometer (VWR, United States). The relationship between OD_{680} and biomass dry weight concentration (X, $mg_{dw}\ L^{-1}$) was determined by using previously established correlation curves. The correlation curves obtained for both microalgae are shown in Figure A1 from Appendix A. To obtain these curves, the OD680 and cell dry weight of 20-mL microalgal suspensions, with different biomass concentrations, were determined, and the relationship between both variables was obtained by linear regression, according to the Lambert-Beer law. The linear regression expressions obtained for *C. vulgaris* and *T. obliquus* calibration curves are expressed by Equations 3.2 and 3.3, respectively. The estimated linear regression parameters (and respective errors) and quality of the model fit to the experimental data,

as well as an analysis of variance (ANOVA) to the results obtained can be found in Appendix A (Tables A1 and A2, respectively).

$$X = 0.0024 \times OD_{680} + 0.0030 \qquad R^2 = 0.9999 \qquad (3.2)$$

$$X = 0.0033 \times OD_{680} + 0.0105 \qquad R^2 = 0.9995 \qquad (3.3)$$

For cultures in LL and PIE, before taking the OD680 measurements, to eliminate the interference of effluent color and turbidity, the collected samples were centrifuged (at 4000 rpm, for 10 min), the supernatant discarded, and the cells washed and resuspended in the same volume of distilled water, as described by HODAIFA *et al*. (2008).

3.3.2.1 Growth parameters calculated for assays using tubular PBRs

Biomass concentration values over time, obtained for cultures in the tubular PBRs, were used to calculate the following growth parameters: (i) specific growth rates as a function of time (μ, d^{-1}) and accumulated energy (μ', L kJ^{-1}); and (ii) areal biomass productivities (P_A, mg_{dw} m^{-2} d^{-1}). The average specific growth rates were determined by applying a pseudo-first-order kinetic model to biomass concentration values over the cultivation time, as shown in Equations 3.4 and 3.5:

$$\frac{dX}{dt} = \mu X \leftrightarrow X_t = X_0 \exp^{\mu \times t} \qquad (3.4)$$

$$\frac{dX}{dQ} = \mu X \leftrightarrow X_Q = X_0 \exp^{\mu' \times Q_{380-580\,nm}} \qquad (3.5)$$

where X_0 and X_t *or* X_Q are initial biomass concentration (mg_{dw} L^{-1}) and biomass concentration obtained within the time t (d) or accumulated energy $Q_{380\text{-}580nm}$ (kJ L^{-1}), respectively, corresponding to the exponential phase of microalgal growth curves.

Areal biomass productivities (P_A, mg_{dw} m^{-2} d^{-1}) were determined through Equation 3.6:

$$P_A = \frac{(X_{z+1} - X_z) \times V_S}{(t_{z+1} - t_z) \times A_I} \quad (3.6)$$

where V_s is the solution volume (L), A_i is the PBR aperture area (m²), X_z represents the biomass concentration (mg_{dw} L^{-1}) at time t_z (d), and X_{z+1} corresponds to the biomass concentration (mg_{dw} L^{-1}) at time t_{z+1} (d). The maximum value of areal biomass productivity ($P_{A,max}$ in mg_{dw} m^{-2} d^{-1}) for each experiment was computed for comparison purposes.

3.3.2.2 Growth parameters calculated in the assays with paper industry effluent and landfill leachate

Growth kinetics of both species, employed in the assays with LL and PIE, was analyzed in terms of specific growth rate (μ, d^{-1}), as described in Section 3.5.1.

The biomass concentration values were also used to determine maximum and average biomass productivities (P_{max} and P_{aver}, mg_{dw} L^{-1} d^{-1}). Maximum biomass productivity for each tested condition corresponds to the maximum value of biomass productivity (P, mg_{dw} L^{-1} d^{-1}) calculated for each pair of consecutive points, as represented in Equation 3.7:

$$P = \frac{X_{z+1} - X_z}{t_{z+1} - t_z}, \quad (3.7)$$

where X_z represents the biomass concentration (mg_{dw} L^{-1}) at time t_z (d) and X_{z+1} corresponds to the biomass concentration (mg_{dw} L^{-1}) at time t_{z+1} (d). On the other hand, average biomass productivities were determined according to Equation 3.8:

$$P_{aver} = \frac{X_f - X_I}{t_f - t_I}, \quad (3.8)$$

where X_f and X_i correspond to the biomass concentrations (mg_{dw} L^{-1}) in the final (t_f, d) and initial (t_i, d) times of the cultivation period, respectively.

3.3.3 Anions concentration determination

The concentration of the anions (chlorides, nitrates, nitrites and phosphates) was determined in the raw effluents (LL and PIE) for their characterization by ion chromatography. In the same way, the nutrients were evaluated in the culture media (LL and PIE samples at different concentrations) throughout the cultivation experiments in terms of nitrogen (N), in the forms of NO_3-N and NO_2-N, and phosphorus (P), in the form of PO_4-P. From each experiment, 5-mL samples were periodically collected (on days 0, 1, 2, 4, 7, 9 and 11, for the effluent concentration experiments, and on days 0, 1, 2, 3, 4 and 7, for the tubular PBR experiments). These samples were centrifuged (at 4000 rpm, for 10 min), and the supernatants were filtered through 0.22 μm nylon membranes (Specanalitica, Portugal). NO_3^-, NO_2^- and PO_4^{3-} concentrations, as well as other anions, were determined by ion chromatography (ICS-2100, Dionex) equipped with an anion analytical column (4× 250 mm, AS11-HC) and a self-regeneration suppressor (4 mm, AERS 500).

3.3.3.1 Nutrients removal

With N and P concentration values determined at different time points, the following nitrogen and phosphorus removal parameters were calculated: (i) removal efficiencies (*%R*, %), represented by Equation 3.9; (ii) average removal rates (*RR*, mg L^{-1} d^{-1}), according to Equation 3.10; and (iii) mass removal (*R*, mg L^{-1}), as indicated in Equation 3.11.

$$\%R = \frac{S_i - S_f}{S_i} \times 100, \tag{3.9}$$

$$RR = \frac{S_i - S_f}{t_f - t_i}, \tag{3.10}$$

$$R = S_i - S_f, \tag{3.11}$$

In these expressions, S_f and S_i represent the N or P concentrations (mg L^{-1}) in the final (t_f, d) and initial (t_i, d) times of the cultivation period, respectively.

3.4 STATISTICAL ANALYSIS

The parameters presented in this topic were expressed as average and standard deviation. Tukey HSD (honestly significant difference) statistical test was used to investigate whether differences between the obtained results could be considered significant, according to the significance level (p) of 0.05. The software Statistica 8.0 (StatSoft Inc., USA) was used to generate the statistical analyses.

To establish the adjustment models to the experimental data and extract the adjusted parameters, as well as estimated values for kinetic constants, the Fig.P (Biosoft®, UK) software was used. The fit quality of the models was evaluated through the ANOVA test.

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4 MICROALGAL GROWTH IN PAPER INDUSTRY EFFLUENT: COUPLING BIOMASS PRODUCTION WITH NUTRIENTS REMOVAL

This Chapter is based on the research article: “PORTO, B. *et al*. Microalgal Growth in Paper Industry Effluent: Coupling Biomass Production with Nutrients Removal. Applied Sciences, Vol. 10, n. 9, p. 3009, 2020." The research was developed at the Associate Laboratory LSRE-LCM, Faculty of Engineering, University of Porto (FEUP), as part of my Sandwich PhD (PDSE/CAPES).

ABSTRACT

Paper and pulp industries generate effluents with a high phosphorus concentration, requiring adequate treatment before their discharge into water compartments. The use of microalgae for this purpose has attracted the attention of the scientific community due to two main points: (i) microalgae can assimilate phosphorus, which is one of the main nutrients for their growth; and (ii) growing on effluents can significantly reduce the costs and environmental impact of microalgal biomass production. This study evaluated the growth and ability of *Chlorella vulgaris* to remove phosphorus from a secondary-treated effluent of a Portuguese paper company. Batch experiments were performed for 11 days using different effluent dilutions to evaluate its inhibitory effect on microalgae. Results showed that the non-diluted effluent inhibited microalgal growth, indicating that this bioremediation process is possible after a proper dilution of the effluent. Promising results were achieved regarding phosphorus removal, especially in the experiments conducted with the most diluted effluent. Another interesting finding of this study was the growth of microalgae in flakes’ form. This was mainly due to the effluent compounds and the pH values achieved, which can be an important economic advantage for biomass recovery after the remediation step.

Keywords: Biomass production. *Chlorella vulgaris*. Microalgae. Nutrients removal. Paper industry effluent. Effluent treatment.

4.1 INTRODUCTION

Paper and pulp industries require large amounts of water during their manufacturing stages. For example, 1 kg of paper production requires 10 to 50 L of water (USHA *et al*., 2016). At the same time, large amounts of effluents (about 2000 m^3 d^{-1}) are generated, presenting as main features (BUZZINI and PIRES, 2007; REID *et al*., 2008; SINGH *et al*., 2008; SOLOMAN *et al*., 2009; KAMALI *et al*., 2016; USHA *et al*., 2016; RAJWAR *et al*., 2017; PING *et al*., 2019): (i) high chemical oxygen demand (COD, 1000-13000 mg O_2 L^{-1}); (ii) high contents of total suspended solids (TSS, 0-7150 mg L^{-1}); (iii) non-biodegradable organics; (iv) color (from 1660.0 to 7317.2 platinum-cobalt units); (v) phenolic compounds (from 0.535 to 1440.010 mg L^{-1}).; (vi) high contents of total phosphorus (TP, 1.31-5920.40 mg_P L^{-1}); and (vii) low nitrogen concentration (TN, 0.01-137.00 mg_N L^{-1}). Due to the large volumes involved and respective compositions, the discharge of these effluents without proper treatment can have a negative impact on the aquatic ecosystems (POKHREL and VIRARAGHAVAN, 2004; USHA *et al*., 2016): (i) colored effluents can affect aesthetics, water transparency and gas solubility in water bodies; (ii) the presence of toxic compounds can affect aquatic flora and fauna; and (iii) nutrient rich effluents can result in the eutrophication with consequent decrease of dissolved oxygen concentration and pH oscillations. Therefore, the proper treatment of these effluents is required before their discharge into the aquatic compartments.

Among the contaminants present in these effluents, phosphorus is of particular concern, as it subsists in the effluents after the secondary treatment step and is one of the main contributors to the eutrophication phenomenon (PIAO *et al*., 2016). Currently applied methods to reduce phosphorus concentration in these effluents include physicochemical methods, such as precipitation using aluminum and iron salts. However, these techniques tend to be costly and produce large amounts of sludge contaminated with the referred chemical compounds, requiring further treatment (SINGH and THOMAS, 2012; ZANG *et al*., 2015). Therefore, microalgal cultures have appeared as a feasible alternative to conventional physicochemical methods. These microorganisms have shown their ability to remove color effectively, nutrients, such as nitrogen and phosphorus, trace metals and other compounds from the culture medium (TARLAN *et al*., 2002; LI *et al*., 2019).

Microalgae are fast-growing photosynthetic microorganisms that have gained much attention in the last decades, due to their high potential in a wide variety of applications. During photosynthesis, microalgae uptake CO_2 from the atmosphere or flue gas emissions, contributing to the reduction of the atmospheric concentration of this greenhouse gas (PIRES *et al*., 2012). These microorganisms also require inorganic sources of nitrogen and phosphorus as macronutrients, enabling the use of microalgal cultures as a tertiary treatment stage (when significant concentrations of these nutrients persist after previous treatment processes) (RAWAT *et al*., 2011). Finally, microalgal biomass presents a very rich composition in polysaccharides, lipids, proteins, vitamins and other valuable compounds, which make microalgae a valuable resource for several applications (ODJADJARE *et al*., 2017; RIZWAN *et al*., 2018), such as the production of natural colorants or dyes, bioenergy and biofertilizers. Also, the use of microalgae for the treatment of wastewaters has several advantages (RAWAT *et al*., 2011): (i) reduction of nitrogen and phosphorus concentrations to levels below the discharge limits (EU Directives 1991/271/EEC and 1998/15/EC); (ii) recovery/recycle of these nutrients, which production presents negative environmental impacts; (iii) increase of the oxygen concentration in the treated effluent; (iv) production of biomass that can be integrated into the value chain of the company; and (v) reduction of net carbon dioxide emissions.

Despite the need to search for eco-friendly and cost-effective remediation strategies, only a few studies have reported the treatment of pulp and paper industry effluents using microalgae (USHA *et al*., 2016). TARLAN *et al*. (2002) evaluated the removal of color, absorbable organic xenobiotic (YU *et al*.) and COD from an effluent resulting from a wood-based pulp and paper Turkish company using a mixed microalgal culture (composed by *Chlorella* and diatoms). The initial composition of this effluent in terms of color, AOX and COD was: 4018 Pt-Co, 46.3 mg L^{-1} and 1248 mg L^{-1}, respectively. Operating in batch mode and using three different dilutions of this effluent, resulting from the process of pulp production using red pine, the authors reported removal efficiencies of 84%, 80% and 58% for color, AOX and COD, respectively. GENTILI (2014) reported the growth of microalgae on a wastewater resulting from the mixture of municipal, dairy and pulp and paper effluents to achieve the dual goal of nutrients removal and lipids production. The use of mixtures of pulp and paper industry effluents with municipal and dairy ones was evaluated to (i) promote microalgal growth without previous dilution with fresh water; and (ii) provide the

required nutrients for biomass production without the need for nutrients supplementation. These mixtures presented an ammonium-nitrogen (NH_4-N) concentration ranging from 14.75 mg_N L^{-1} to 22.35 mg_N L^{-1}, a nitrate-nitrogen (NO_3-N) concentration between 1.6 mg_N L^{-1} and 10.1 mg_N L^{-1} and a phosphate-phosphorus (PO_4-P) concentration ranging between 1.06 mg_P L^{-1} and 1.25 mg_P L^{-1}. Trials were performed in batch mode using a tubular reactor. The authors demonstrated that the microalgae *Scenedesmus* sp., *Scenedesmus dimorphus* and *Selenastrum minutum* were able to achieve nitrogen and phosphorus removal efficiencies of 96-99% and 91-99%, respectively. Finally, in the study performed by USHA *et al*. (2016), a mixed microalgal culture (composed by two *Scenedesmus* species) was grown in different dilutions (0-95%) of a pulp and paper mill effluent, resulting from an Indian company, with the following composition: (i) ~10 mg_N L^{-1} of NO_3-N; (ii) ~ 30 mg_P L^{-1} of PO_4-P; (iii) ~ 3000 mg L^{-1} of COD; and (iv) ~ 2944 mg L^{-1} of biochemical oxygen demand (BOD). The experiments, aimed at evaluating both biomass production and nutrients removal efficiencies, were performed in batch mode for 28 days, using open ponds as cultivation system (outdoor conditions). Regarding nutrients uptake, the most promising results were obtained with a dilution factor of 40%: (i) 65% for NO_3-N; (ii) 81% for PO_4-P; and (iii) 75% for COD; and (iv) 82% for BOD.

The main goal of the present study was to evaluate biomass production and phosphorus removal from a secondary-treated effluent of a Portuguese paper company using the microalga *Chlorella vulgaris*. Different dilutions were performed to evaluate possible inhibitory effects of the effluent on microalgal growth and phosphorus uptake ability.

4.2 MATERIALS AND METHODS

The materials, analytical methods and experimental unit used in the development of the current study can be assessed in Chapter 3. Section 3.2.1 presents in detail the experimental setup and operational conditions employed.

A paper industrial effluent was used as the culture medium for *C. vulgaris*, acting as a nutrients source. The analytical methods used for the wastewater characterization (Section 3.3.1), monitoring of microalgae growth and nutrients removal (Sections 3.3.2 and 3.3.3), as well as the kinetic growth parameters (Sections 3.3.2.2) and nutrient removal parameters (Section 3.3.3) were also described in Chapter 3.

4.3 RESULTS AND DISCUSSION

4.3.1 Paper industry effluent

The paper industry effluent characterization results are presented in Table 4.1. It is possible to observe that the effluent has low turbidity, thus allowing light to be transmitted through the medium, which is vital for the photosynthetic process of the microalgae. In addition, the natural pH is close to neutral (7.02), making it suitable for the culture of *C. vulgaris*. And in nutritional terms, there was the presence of essential elements, but it was necessary to supplement nitrogen in the medium to achieve an adequate N:P ratio, since its concentration was low compared to phosphorus. As previously mentioned, an N:P ratio between 5:1 and 30:1 has been considered adequate for several microalgal species (LARSDOTTER, 2006; SILVA *et al*., 2015). Thus, the medium was supplemented with NaNO3 reaching N:P molar ratios ranging between 6:1 and 9:1.

Table 4.1 – Physicochemical characterization of the paper industry effluent used in this study.

Parameters	**Values**	**Unit**
Turbidity	1.55	NTU[1]
pH	7.02	-
Dissolved organic carbon (DOC)	296	mg L^{-1}
Total dissolved carbon (TDC)	369	mg L^{-1}
Dissolved inorganic carbon (DIC)	72.5	mg L^{-1}
Soluble chemical oxygen demand (COD_S)	323	mg L^{-1}
Chlorides (Cl^-)	671	mg L^{-1}
Sulphates (SO_4^{2-})	808	mg L^{-1}
Phosphate-phosphorus (PO_4-P)	12.3	mg L^{-1}
Nitrate-nitrogen (NO_3-N)	8.73	mg L^{-1}
Nitrite-nitrogen (NO_2-N)	3.42	mg L^{-1}

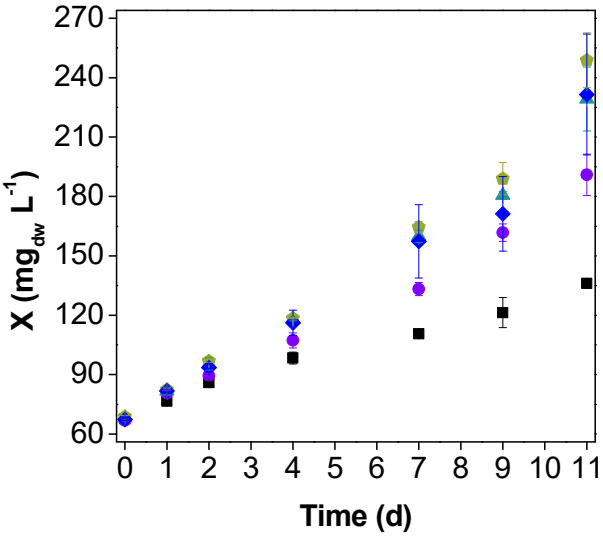
[1] Nephelometric turbidity unit.

Author (2021).

4.3.2 Microalgal growth

The *C. vulgaris* growth curves in raw and diluted paper industry effluent are shown in Figure 4.1. These results evidence the inexistence of an adaptation phase for all assays and an exponential growth phase that lasted approximately four days. Besides, no cell decay was observed during the 11-day cultivation period, indicating that the cultures could be extended for a longer period. The increase of biomass concentration during the cultivation period, as well as the lack of an adaptation phase, shows that *C. vulgaris* was able to grow in this effluent. However, biomass concentrations achieved in non-diluted effluent (assay 1) were statistically lower (p<0.05) than those achieved in more diluted effluents from assays 3-5.

Figure 4.1 – *C. vulgaris* cultures growth curves in raw and diluted secondary-treated paper industry effluent: 100% (v/v) – ■, 80% (v/v) – ●, 60% (v/v) – ▲, 40% (v/v) – ⬟ and 20% (v/v) – ◆ assays. Error bars correspond to the standard deviation of the mean obtained from two independent experiments.



Author (2021).

To complement the analysis from growth curves, microalgal growth parameters, such as specific growth rate, maximum biomass concentration and maximum and average biomass productivities, were determined and presented in Table 4.2. From these data, it is possible to see a general increase in growth parameters from assay 1 to assay 5, from the non-diluted effluent to the more diluted one. Regarding specific growth rates, values ranged from 0.093±0.007 d^{-1} to 0.16±0.02 d^{-1} in assays 1 and 5, respectively. The highest values of maximum biomass concentrations were also obtained in more diluted effluents from assays 4 and 5: 249±14 mg_{dw} L^{-1} and 231±31

mg_{dw} L^{-1}, respectively. Similar behavior was observed for both maximum and average biomass productivities. Maximum biomass productivities/average biomass productivities obtained in assays 4 and 5 were 30±3/16±1 mg_{dw} L^{-1} d^{-1} and 30±6/15±3 mg_{dw} L^{-1} d^{-1}, respectively. In opposition, maximum and average biomass productivities obtained in assay 1 were 9.8±0.2 mg_{dw} L^{-1} d^{-1} and 6.22±0.09 mg_{dw} L^{-1} d^{-1}, respectively.

Table 4.2 – Specific growth rates (μ, in d^{-1}), maximum biomass concentrations (X_{max}, in mg_{dw} L^{-1}) and biomass productivities (P_{max} and P_{aver}, in mg_{dw} L^{-1} d^{-1}) determined for *C. vulgaris* grown in non-diluted and diluted secondary-treated paper industry effluent

Assay	**(NO_3+NO_2)-N (mg_N L^{-1})**	**PO_4-P (mg_P L^{-1})**	**μ (d^{-1})**	**X_{max} (mg_{dw} L^{-1})**	**P_{max} (m_{dw} L^{-1} d^{-1})**	**P_{aver} (m_{dw} L^{-1} d^{-1})**
1	34.2	12.7	0.093±0.007 [a]	136±1 [a]	9.8±0.2 [a]	6.22±0.09 [a]
2	28.3	8.55	0.11±0.01 [ab]	191±10 [ab]	15±2 [ab]	11±1 [ab]
3	21.5	6.04	0.136±0.004 [bc]	229±16 [b]	24±7 [ab]	15±1 [b]
4	16.7	4.22	0.134±0.002 [bc]	249±14 [b]	30±3 [b]	16±1 [b]
5	12.7	4.01	0.16±0.02 [c]	231±31 [b]	30±6 [b]	15±3 [b]

Author (2021).

In contrast to what was observed by GENTILI (2014), the increment in nitrogen and phosphorus concentration did not contribute to an increase in kinetic growth parameters. Accordingly, these results may indicate inhibitory effects of the effluent on microalgae, which can influence microalgal cultures in different ways (POLISHCHUK *et al*., 2015; TAO *et al*., 2017; MOLINUEVO-SALCES *et al*., 2019): (i) the effluent color may act as a barrier to light penetration, thus limiting microalgal access to light and photosynthetic activity; and (ii) paper industry effluents are characterized by the presence of lignin, humic acids, furans and dioxins and by high levels of aluminum and manganese, which exhibit toxic effects on microalgae.

Most studies regarding the bioremediation of paper industry effluents with microalgae focus on the removal of contaminants, and only a few report biomass production yields. POLISHCHUK *et al*. (2015) reported that the maximum specific growth rate obtained for *Nannochloropsis oculata* grown in effluents resulting from the pulp and paper industry was 0.405 d^{-1}. TAO *et al*. (2017) revealed that maximum biomass concentrations achieved by *Scenedesmus acuminatus* and *C. vulgaris* grown in paper industry effluents were 291 mg L^{-1} and 822 mg L^{-1}, respectively. Considering the values referred in the literature, microalgal growth parameters obtained in this study were significantly lower, which can be attributed to the inhibitory effects promoted by the effluent used (in assays 1-3) and to the low concentration of some essential nutrients

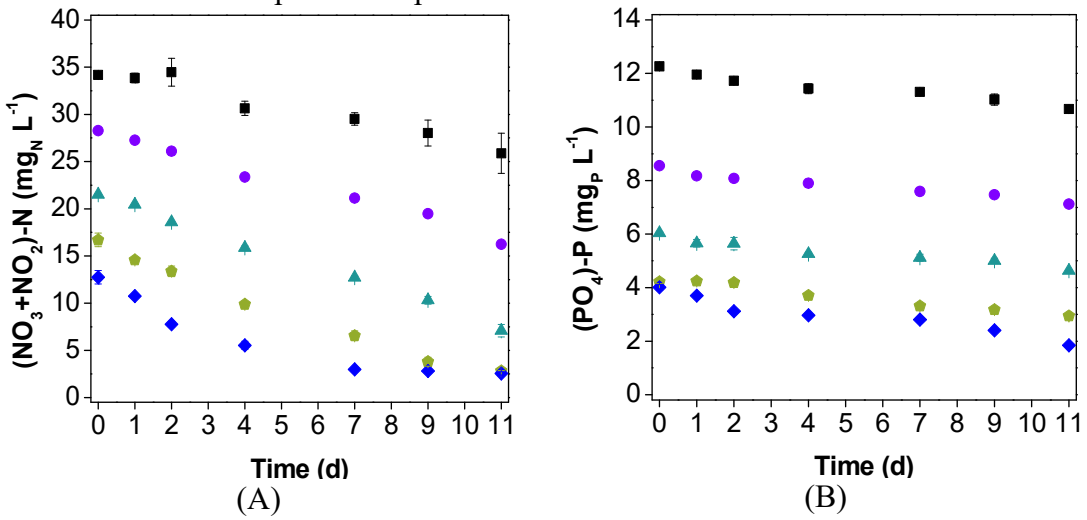
(in more diluted effluents of assays 4 and 5). Another explanation for the low biomass concentrations and productivities achieved may be related to the phenomenon of flakes formation observed within the cultivation period (autoflocculation). Cells' agglomeration can affect the accurate measurement of OD_{680} and, on the other hand, it can reduce light absorption efficiency by cells incorporated within flakes, thus resulting in lower photosynthetic activity. In this study, this phenomenon occurred due to the increase of culture pH (from 7.8 to 8.6) or due to the presence of certain compounds in the effluent, which can induce a change in the surface charge of the cells and affect suspensions' stability (BARROS *et al*., 2015). Despite the low microalgal growth rates, the flakes formation enables a cost-effective biomass removal after effluent remediation. The density similar to water and small size of microalgal cells difficult the harvesting process and make this step one of the most expensive within microalgal biomass production processes (GERDE *et al*., 2014; BARROS *et al*., 2015). However, when cells agglomerate, their density and size increase is observed, contributing to higher settling rates and allowing biomass recovery using the least expensive harvesting method: sedimentation.

4.3.3 Nutrients removal

In this study, nitrogen (in the forms of NO_3-N and NO_2-N) and phosphorus (in the form of PO_4-P) concentrations were monitored within the cultivation time to evaluate the potential of *C. vulgaris* to uptake these nutrients from a paper industry effluent with different concentrations of both nitrogen and phosphorus. Figure 4.2 shows the variation of nitrogen and phosphorus concentration in each assay. Regarding nitrogen removal (Figure 4.2A), this element was readily assimilated by *C. vulgaris* in the diluted effluents (assays 2-5). In the raw effluent (corresponding to assay 1), a two-day delay was observed in nitrogen assimilation, which may be related to the adaptation of the microalga to these conditions. Regarding the assimilation patterns observed in assays 2-5, these were approximately linear for assays 2-4, with nitrogen concentration decreasing gradually during the cultivation time. On the other hand, in assay 5, corresponding to the more diluted effluent experiments, nitrogen concentration decreased until the seventh day of culturing and then it was maintained approximately constant. This behavior may be attributed to a decrease in photosynthetic activity, as nitrogen concentration decreased, and explains the lower biomass concentrations

achieved in assay 5 when compared to the one obtained in assay 4 (according to Table 4.2, 231±31 mg_{dw} L^{-1} and 249±14 mg_{dw} L^{-1}, respectively). Also, at the end of the cultivation time, nitrogen concentration remaining in cultures corresponding to assays 4 and 5 was approximately the same (2.81±0.05 mg_N L^{-1} and 2.6±0.2 mg_N L^{-1}, respectively), indicating a limitation of this nutrient in the last days of assay 5. As for nitrogen concentration, phosphorus concentration also decreased within the cultivation time (Figure 4.2B), but to a lesser extent, which is related to microalgal nutritional requirements, as given by its typical elemental biochemical composition: $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (CHISTI, 2007). The reduction observed in nitrogen and phosphorus concentration in the studied effluent (diluted or not) shows that *C. vulgaris* can promote an efficient uptake of both nutrients. However, except for nitrogen concentration in assay 5, total depletion of these nutrients did not occur after the 11 days of culturing, reiterating what was stated about cell growth, that the cultures could be extended for an increased period to further improve nutrients removal efficiencies. Another similarity with the microalgal growth parameters already described is the higher variations in nitrogen and phosphorus concentrations observed in the experiments where the effluent was previously diluted (assays 2-5), which indicates that these conditions were more favorable for *C. vulgaris* photosynthetic activity.

Figure 4.2 – Time variation of nitrogen (A) and phosphorus (B) concentration determined in *C. vulgaris* cultures grown in raw and diluted secondary-treated paper industry effluent (Assays: 100% (v/v) – ■, 80% (v/v) – ●, 60% (v/v) – ▲, 40% (v/v) – ⬟ and 20% (v/v) – ◆). Error bars correspond to the standard deviation of the mean obtained from two independent experiments.



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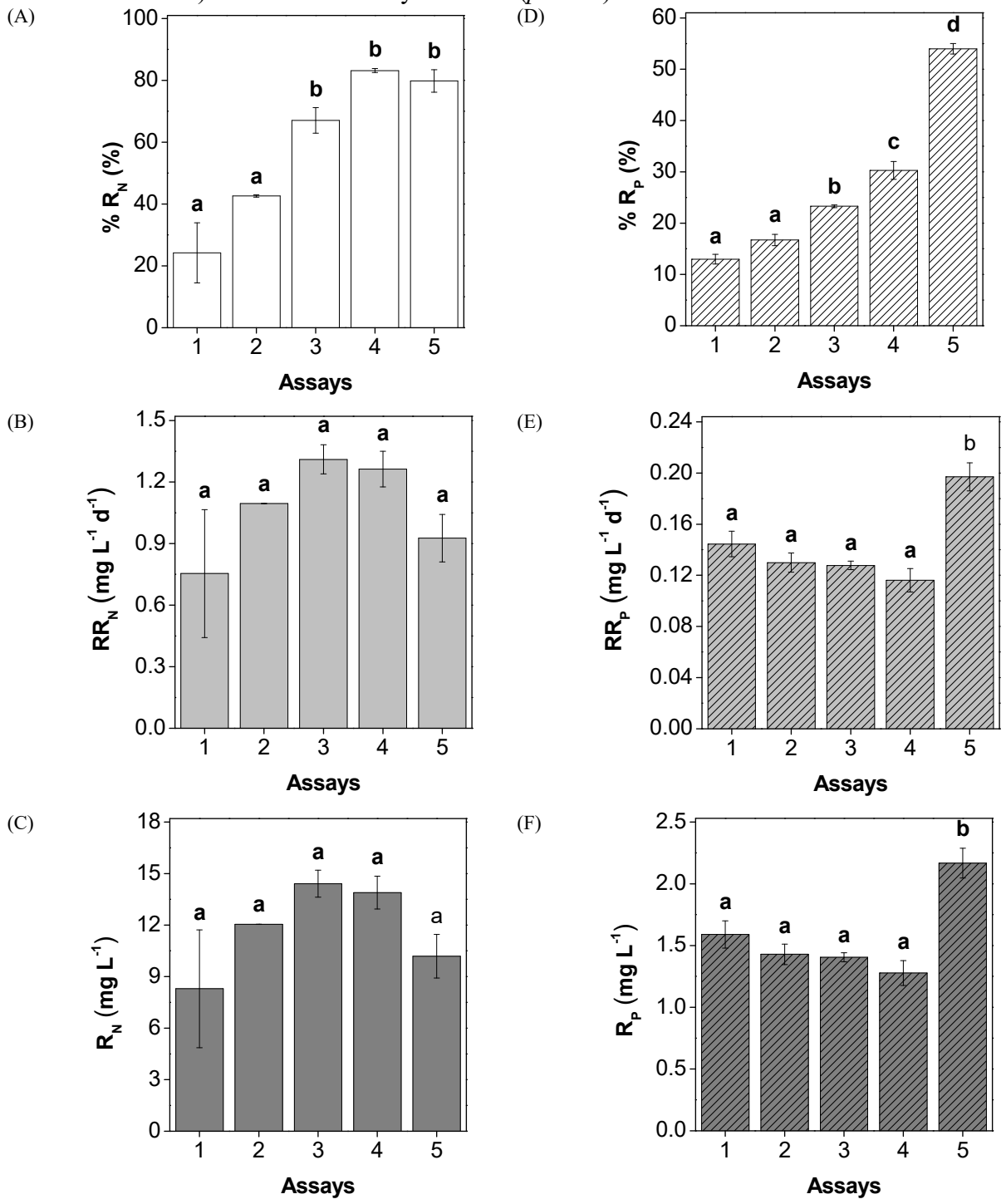
Similarly to microalgal growth parameters, a general increase in nutrients removal efficiencies was observed from assay 1 to 5, with values ranging from 24±10% to 80±4% for nitrogen (Figure 4.3A) and from 13.0±0.9% to 54±1% for phosphorus (Figure 4.3D). However, Figure 4.3A shows that there was no statistical difference (p>0.05) in nitrogen removal efficiency between assays 4 and 5, which can be explained by the low concentration achieved in assay 5 (the one corresponding to the most diluted effluent) that might have been limiting for microalgal growth. In fact, according to Table 4.2, the maximum biomass concentration achieved in assay 4 was higher than that in assay 5, indicating that the highest dilution applied in this study may have contributed to nitrogen limitation to *C. vulgaris*, with effects on their growth and nutrients removal parameters. Regarding nitrogen removal rates (Figure 4.3B) and mass removal (Figure 4.3C), the highest values were determined in assays 3 and 4 and no statistical differences were observed (p>0.05): (i) average removal rates were 1.31±0.07 $mg_N\ L^{-1}\ d^{-1}$ and 1.26±0.08 $mg_N\ L^{-1}\ d^{-1}$, respectively; and (ii) mass removal values were 14.4±0.8 $mg_N\ L^{-1}$ and 13.9±0.9 $mg_N\ L^{-1}$, respectively. These results are following the maximum biomass concentration achieved and indicate higher photosynthetic activity of *C. vulgaris* in these intermediate conditions. A different behavior was observed for phosphorus. In this case, average removal rates and mass removal values determined for assays 1 to 4 were not statistically different (p>0.05), but values determined for assay 5 were statistically higher (p<0.05), reaching an average removal rate of 0.20±0.01 $mg_P\ L^{-1}\ d^{-1}$ and mass removal of 2.2±0.1 $mg_P\ L^{-1}$.

Figure 4.3 – Nitrogen (nitrate + nitrite) and Phosphorus (phosphate) removal obtained by *C. vulgaris* cultures grown in raw and diluted secondary-treated paper industry effluent (assays 1-5): (A and D) removal efficiency (%R); (B and E) average removal rate (RR); and (C and F) mass removal (R). Error bars correspond to the standard deviation of the mean obtained from two independent experiments. The letters a, b, c and d (shown above the bars) represent the statistical significance of the results, as given by the Turkey HSD test: mean values sharing at least one common letter (shown above the bars) are not statistically different (p>0.05).



Author (2021).

Nutrients removal from paper industry effluents has already been reported in the literature. Table 4.3 highlights nitrogen and phosphorus removal efficiencies and removal rates obtained in these studies. According to these data, removal efficiencies reported by TAO *et al*. (2017) and GENTILI (2014) are significantly higher than those obtained in this study, whereas values reported by USHA *et al*. (2016) were closer to those obtained in the present study, especially in assays 3-5. The lower removal efficiencies obtained in this study when compared with those reported by TAO *et al*. (2017), may be associated with the higher N:P molar ratio used in the reference study, which was ~ 66:1. On the other hand, the higher removal efficiencies reported by GENTILI (2014) may be associated with the use of other effluents to achieve the dual role of providing the required nutrients for microalgal growth while contributing to a reduction in the toxicity of the paper industry effluent. Another explanation for the increased efficiencies obtained in these studies is the nitrogen source used. As in the present study, USHA *et al*. (2016) cultivated microalgae in an effluent with nitrate-nitrogen as the main nitrogen source. On the other hand, TAO *et al*. (2017) tested an effluent with ammonium as the main nitrogen source (digestate obtained from the treatment of a pulp and paper industry effluent), and GENTILI (2014) evaluated this treatment with both nitrogen forms present. According to several studies, although nitrate-nitrogen is the most thermodynamically stable form (and the most commonly found in aquatic environments), ammonia is directly assimilated and converted into proteins by microalgae, while nitrate must be reduced to nitrite and then to ammonia before being assimilated by microalgal cells (GONÇALVES *et al*., 2017). However, for an adequate comparison of nutrients removal performance, it is important to determine the average removal rate, as this parameter takes into account initial nutrients concentrations and cultivation/treatment time. Comparing average removal rates obtained in the present study and in the reference studies, values in the same order of magnitude were obtained, except in what concerns ammonium-nitrogen removal in the studies performed by TAO *et al*. (2017) and GENTILI (2014). In these cases, the higher removal rates obtained may be associated with the higher ability of microalgae to assimilate ammonium-nitrogen than nitrate-nitrogen. Considering the values of average removal rates, it is possible to conclude that promising results were obtained in this study. Moreover, differences found in experimental conditions used in this study and the studies reported in the literature, show that these results can be significantly enhanced. Besides increasing N:P molar ratio and providing an ammonium-nitrogen

source, the increase of light supply should also be considered, as values reported in the literature correspond to cultures grown under light intensities of 130-800 μmol m^{-2} s^{-1}, whereas results reported in the present study were obtained with light intensities of 30-40 μmol m^{-2} s^{-1}.

In summary, the results obtained in this study, for both nitrogen and phosphorus removal, evidence that the remediation of paper industry effluents using microalgae is possible when a proper dilution is performed to avoid inhibitory effects related to the presence of strong color or high concentrations of toxic compounds, typically associated with effluents resulting from this industrial sector (POLISHCHUK *et al*., 2015; TAO *et al*., 2017). Considering the results obtained for nitrogen removal, the dilution factors employed in assays 3 and 4 are the most adequate. In these conditions, nitrogen concentrations were significantly reduced, reaching values of 7.1±0.7 mg_N L^{-1} and 2.81±0.05 mg_N L^{-1}, respectively, corresponding to the highest average removal rates: 1.31±0.07 mg_N L^{-1} d^{-1} and 1.26±0.09 mg_N L^{-1} d^{-1}, respectively. Regarding phosphorus removal, the highest removal rate was obtained for the conditions tested in assay 5: 0.20±0.01 mg_P L^{-1} d^{-1}.

Despite the promising nitrogen and phosphorus removal rates, the results obtained in this study demonstrated that the cultures were limited by nitrogen, as nitrogen and phosphorus were assimilated by *C. vulgaris* at a N:P molar ratio ranging from 10:1 to 24:1. Considering these results and the N:P molar ratios used in this study (between 6:1 and 9:1), nutrients uptake could be enhanced by increasing nitrogen supply. Another alternative to achieve an adequate N:P molar ratio and reduce the toxicity of this effluent would be to dilute it with other effluents, as proposed in other studies (GENTILI, 2014). Finally, the remediation process could be further improved by modulating microalgal cultivation conditions. According to GONÇALVES *et al*. (2017), light conditions, temperature, and pH are also important parameters that can influence microalgal growth and, hence, the efficiency of the bioremediation process.

Table 4.3 – Comparison between nutrients removal efficiencies (%R, in %) and average removal rates (RR, in mg L^{-1} d^{-1}) obtained in this study and other studies reporting microalgal growth in effluents resulting from pulp and paper industries.

(to be continued)

Operational Conditions	Effluent	Microalgae	Culture time (d)	Element / Form	Initial concentration (mg L^{-1})	%R (%)	RR (mg L^{-1} d^{-1})	Ref.
CS: 1 L borosilicate glass flasks; OM: batch; WV: 0.95 L; PAR: 30-40 µmol m^{-2} s^{-1}; L/D: 24/0; T: 23-28 °C; pH: 7.4-8.7.	Paper	*C. vulgaris*	11	(NO_3+NO_2)-N	34.2	24	0.75	This study
				PO_4-P	12.3	13	0.14	
				(NO_3+NO_2)-N	28.2	43	1.1	
				PO_4-P	8.55	17	0.13	
				(NO_3+NO_2)-N	21.5	67	1.3	
				PO_4-P	6.04	23	0.13	
				(NO_3+NO_2)-N	16.7	83	1.3	
				PO_4-P	4.22	30	0.12	
				(NO_3+NO_2)-N	12.7	80	0.93	
				PO_4-P	4.01	54	0.20	
CS: 1 L glass bottle; OM: batch; WV: 0.7 L; PAR: 150 µmol m^{-2} s^{-1}; L/D: 24/0; T: 22±2 °C; pH: 7.5-8.0.	Pulp and paper mill	*C. vulgaris*	14	NH_4-N	240	100	17	(TAO *et al.*, 2017)
				PO_4-P	8.00	97	0.55	
CS: 0.05L plastic tube; OM: batch; WV: 0.04 L; PAR: 130 µmol m^{-2} s^{-1}; L/D: 24/0; T: 21.7-32.2 °C; pH: 7.4-7.7.	Pulp and paper with dairy sludge and municipal	*Scenedesmus* sp. *Scenedesmus dimorphus* *Selenastrum minutum*	6	NH_4-N	22.4	99	3.7	(GENTILI, 2014)
				NO_3-N	1.06	27-53	0.048-0.094	
				PO_4-P	10.1	96-98	1.6-1.7	
				NH_4-N	14.8	96-98	2.3-2.4	
				NO_3-N	1.08	41-46	0.074-0.083	
				PO_4-P	1.60	96-97	0.25-0.26	

(conclusion)

CS: 30 L outdoor open circular ponds; OM: batch; WV: 30 L; PAR: 250-800 µmol m^{-2} s^{-1}; T: 23±3 °C; pH: 8.0-10.	Paper mill	*Scenedesmus* sp.	28	NH_4-N	21.0	99	3.5	(USHA *et al*., 2016)
				NO_3-N	1.25	27-43	0.056-0.090	
				PO_4-P	2.99	90-94	0.45-0.47	
				NO_3-N	2.24	65	0.052	
				PO_4-P	9.86	71	0.25	

CS: cultivation system; OM: operation mode; WV: working volume; PAR: photosynthetically active radiation; L/D: light/dark period; T: temperature.
Author (2021).

4.4 CONCLUSIONS

This study showed the feasibility of using *C. vulgaris* for the bioremediation of a paper industry effluent fortified with a nitrogen source, targeting phosphorus removal. *C. vulgaris* was able to grow in all studied effluent conditions (in non-diluted and diluted ones). However, it was possible to conclude that growing on non-diluted effluent resulted in lower biomass productivities, which was also reflected in nitrogen and phosphorus removal efficiencies. From microalgal growth and nitrogen removal points of view, the effluent dilutions used in assays 3 and 4 (intermediate dilutions) seem to be the most adequate, as microalgal growth was not inhibited in these conditions and nitrogen mass removal was quite satisfactory, achieving final concentrations of 7.1±0.7 $mg_N\ L^{-1}$ and 2.81±0.05 $mg_N\ L^{-1}$, respectively. Regarding phosphorus removal, concentrations achieved in the last day of culturing in assays 3 and 4 were higher (4.63±0.04 $mg_P\ L^{-1}$ and 2.940±0.005 $mg_P\ L^{-1}$, respectively) than the one obtained in assay 5 (1.85±0.02 $mg_P\ L^{-1}$). However, the results obtained in assay 5 suggest a growth limitation, mainly related to nitrogen concentration. Accordingly, the obtained results indicate that these values can be further improved by studying different N:P molar ratios, different microalgal cultivation conditions, dilution with other effluents, among others. Improving the remediation performances can significantly contribute to the development of an effective microalgae-based remediation process of pulp and paper industry effluents.

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5 ASSESSING THE POTENTIAL OF MICROALGAE FOR NUTRIENTS REMOVAL FROM A LANDFILL LEACHATE USING AN INNOVATIVE TUBULAR PHOTOBIOREACTOR

This Chapter is based on the research article: “PORTO, B. *et al*. Assessing the potential of microalgae for nutrients removal from landfill leachate using an innovative tubular photobioreactor. Chemical Engineering Journal, p. 127546, 2020. ISSN 1385-8947." The research was developed at the Associate Laboratory LSRE-LCM, Faculty of Engineering, University of Porto (FEUP), as part of my Sandwich PhD (PDSE/CAPES).

ABSTRACT

Landfill leachate treatment relies on the adoption of a multi-barrier strategy, involving primary, secondary, and tertiary processes. Due to their ability to grow in a wide variety of environmental conditions, and their nutritional requirements, especially in terms of nitrogen and phosphorus, microalgae appear as a promising alternative for the tertiary treatment of landfill leachate. Despite these advantages, only a few studies have promoted landfill leachate treatment using microalgae, most of them emphasizing the need for effluent dilution to minimize the toxicity and high color/turbidity of these effluents. An option to avoid the use of freshwater for effluent dilution would be the development of novel photobioreactors (PBRs), able to improve light absorption and, hence, light utilization efficiency by microalgae. This study evaluated the potential of *Chlorella vulgaris* and *Tetradesmus obliquus* on nitrogen (in the forms of nitrate and nitrite) removal from pre-treated landfill leachate. Preliminary experiments using different concentrations of the pre-treated leachate were carried out in 1 L flasks. These results have shown that microalgal growth and nitrogen removal were higher in more diluted conditions. After the selection of the adequate effluent concentration, a tubular PBR coupled to an optical reflector was used for biomass production. This innovative PBR is known to improve light distribution along the tube perimeter, enhancing microalgal photosynthetic activity. In fact, a significant improvement in *C. vulgaris* biomass productivities and nitrogen species removal efficiencies was observed in this PBR, confirming its potential as an effective platform for microalgal biomass production and tertiary treatment of landfill leachate.

Keywords: Biomass production. Bioremediation. Landfill leachate. Microalgae. Nitrogen species removal. Photobioreactor.

5.1 INTRODUCTION

Landfills represent the main method used for the final disposal of solid waste, especially due to their economic advantages. However, the generation of complex liquids from landfilling, as so-called “leachates”, remains inevitable, mainly due to rainwater percolation through the landfill and waste decomposition (BILGILI *et al*., 2007; CASTRILLÓN *et al*., 2010; EL-GOHARY and KAMEL, 2016; CHANG *et al*., 2018; TAGLIAFERRO *et al*., 2019). Leachates may be characterized as a strongly-odored dark water-based solution. Its composition can widely vary regarding the landfill age, climatic conditions and stored waste type (KJELDSEN *et al*., 2002; PASKULIAKOVA *et al*., 2018a), being mainly composed of: dissolved organic matter (including recalcitrant and toxic compounds), inorganic macro components (ammonia, sulfate, sulfide/sulfite, etc.) and heavy metals (Pb, Ni, etc.) (KJELDSEN *et al*., 2002). The increase of anthropogenic activities has contributed to an increase in the generation of solid waste and, consequently, in leachate production. For this reason, the search for environmentally-friendly solutions for landfill leachate treatment are of utmost importance. Currently available technologies for leachate treatment rely on the adoption of a treatment train, integrating a set of physical, chemical and biological processes (ZAINOL *et al*., 2012; EL-GOHARY and KAMEL, 2016; TAGLIAFERRO *et al*., 2019). Among these processes, the following stand out (GAO *et al*., 2015; NAIR *et al*., 2019): (i) physical and chemical methods (e.g., coagulation/flocculation, advanced oxidation processes, activated carbon adsorption, membrane filtration, ion exchange and electrochemical advanced oxidation processes), responsible for the removal of recalcitrant organic and inorganic substances, and reduction of color and particulates; and (ii) biological processes (e.g., aerated lagoons, activated sludge reactors) that can be highly effective in nitrogen and biodegradable organics removal from the leachate. Although commonly applied biological processes can be quite effective in the removal of biodegradable organic matter and nitrogen species through nitrification and denitrification reaction mechanisms, high amounts of an external carbon source (e.g., methanol) are requested for the denitrification step (i.e., the conversion of nitrates and nitrites species into nitrogen gas). Thus, the effluent resulting from this step still

presents high nitrogen concentrations (mainly in oxidized forms), which need to be reduced before being disposed into the receiving water bodies. The increase of nutrients concentration in aquatic environments may lead to the complete degradation of these ecosystems due to the eutrophication phenomenon, which is responsible for oxygen depletion, pH shifts, and cyanotoxins production (CHRISTENSON and SIMS, 2011; KHANZADA, 2020). As nitrogen is one of the essential nutrients for microalgal growth, the use of microalgae as a sustainable tertiary treatment of landfill leachates has been gaining prominence in the last decades (GAO *et al*., 2015; NAIR *et al*., 2019). Besides nitrogen (in the forms of ammonium, NH_4-N, nitrate, NO_3-N, or nitrite, NO_2-N), other contaminants present in the leachates (e.g., metals and phosphate-phosphorus, PO_4-P) can be used by microalgae as a source of nutrients. In this way, microalgae can be grown in landfill leachate as a culture medium, promoting its remediation and working as a nitrogen carrier for agriculture use as fertilizer, contributing to the development of a sustainable and profitable agricultural production in the long term. Microalgae can also be used to mitigate CO_2 emissions, as these microorganisms can uptake this gas from the atmosphere or biogas produced in landfills and from the emissions resulting from biogas burning in thermoelectric power plants, for example (AWFA *et al*., 2018; LIAO *et al*., 2018; TAGLIAFERRO *et al*., 2019). Moreover, microalgal biomass is a promising raw material for the production of biofuels (e.g., bioethanol and biodiesel) (ŠOŠTARIČ *et al*., 2012). Therefore, the bioremediation of landfill leachates using microalgae may be an interesting alternative to protect water resources, while minimizing the environmental impact of burning fossil fuels, and meeting the growing energy demand (CHANG *et al*., 2019).

Considering the multiple applications described for microalgae, several researchers have focused their studies on the use of these microorganisms for contaminants removal from raw and biologically-treated landfill leachates coupled to biomass and lipids production. Aiming at maximizing nutrients uptake and biomass and lipids productivities, different photobioreactors (PBRs) have been described. KHANZADA (2020) evaluated the bioremediation of raw landfill leachate (containing an initial NH_4-N concentration of 1100 mg L^{-1}) by the microalgae *Chlorella vulgaris* and *Chlamydomonas reinhardtii* using a membrane PBR. In this study, the authors assessed the effect of PO_4-P supplementation (from 15 mg L^{-1} to 100 mg L^{-1}) on nitrogen removal efficiencies, concluding that the highest NH_4-N removal efficiency, 69.03%, was observed in the landfill leachate supplemented with 100 mg L^{-1} of PO_4-P

(which corresponds to a N:P molar ratio 11:1). These results demonstrate a positive effect of phosphorus supplementation on NH_4-N removal. However, the supplied phosphorus was completely consumed in these experiments, suggesting that an increase in phosphorus concentration (i.e., lower N:P molar ratios) could further improve nitrogen uptake by the studied microalgae. Similar conclusions on phosphorus supplementation had already been highlighted by PASKULIAKOVA *et al*. (2016). In experiments carried out with 10% (v/v) of raw leachate (with ~100 mg L^{-1} of NH_4-N), phosphorus supplementation corresponding to a N:P molar ratio of 16:1 resulted in an increase in NH_4-N removal efficiencies, from 51.7% to 90.7%. Although phosphorus concentration was not monitored throughout the experiments, the low final phosphorus concentrations indicated that phosphorus is a limiting factor in the bioremediation of landfill leachates using microalgae. Several authors have also observed that highly concentrated landfill leachates were not beneficial for microalgal growth (LIN *et al*., 2007; ZHAO *et al*., 2015; TAGLIAFERRO *et al*., 2019), which can be related to the presence of nutrients at high (and inhibitory) concentrations, mainly in the form of NH_4-N (1000–5000 mg L^{-1}) and other toxic substances (e.g., phenols, sulfates and trace metals) (CHEUNG *et al*., 1993; PEREIRA *et al*., 2016; KHANZADA and ÖVEZ, 2017; PASKULIAKOVA *et al*., 2018b; HERNÁNDEZ-GARCÍA *et al*., 2019). Although some of these compounds are essential nutrients for microalgal growth (e.g., NH_4-N and certain trace metals), when present at high concentrations, they can have severe toxic effects, interfering with cells' metabolism (ARUNAKUMARA and ZHANG, 2008; SURESH KUMAR *et al*., 2015). The effluent color and turbidity can also limit microalgal growth, as they difficult light penetration into the culture medium, reducing the amount of light available for microalgal photosynthesis (CHEUNG *et al*., 1993). To reduce the toxic effect of landfill leachates, some authors have suggested effluent dilution in freshwater or wastewater (CHEUNG *et al*., 1993; HERNÁNDEZ-GARCÍA *et al*., 2019). For instance, TAGLIAFERRO *et al*. (2019) examined the effect of effluent concentration, raw landfill leachate at 5-10% (v/v), on the cultivation of *Chlorella minutissima* in a continuous concentric tube airlift PBR, concluding that the maximum biomass productivity was obtained for a landfill leachate concentration of 7.5% (v/v). The increase in effluent concentration had a negative effect on cell productivity, as well as nitrogen removal. The highest NO_3-N removal efficiency (100%) was achieved with only 5% (v/v) of leachate (initial NO_3-N concentration in these conditions was 20.1±0.6 mg L^{-1}). As an alternative to effluent dilution, CHANG *et*

al. (2019) studied *C. vulgaris* growth, lipid productivity, and nutrients removal from biologically-treated landfill leachate in two closed PBRs (a membrane and a traditional one). The membrane PBR aimed at reducing the toxic effect of the effluent on microalgal cells, by avoiding the contact between the cells and the landfill leachate (in this system only inorganic ions of interest were continuously transported across the membrane, while other suspended solids could hardly pass through). The effluent presented the following composition in terms of nitrogen and phosphorus: 105±3 mg L^{-1} of NH_4-N, 143±2 mg L^{-1} of NO_3-N, and 5.0±0.3 mg L^{-1} of PO_4-P. Regarding the performance of the studied cultivation systems, biomass concentration increased from 0.66 g L^{-1} to 0.95 g L^{-1} in cultures carried out in the membrane PBR. The membrane PBR also promoted higher nitrogen and phosphorus removal efficiencies from the landfill leachate (48.1% and 96.2%, respectively), as well as an improvement in the quality of the produced lipids.

The results achieved so far have demonstrated the potential of microalgae grown on landfill leachates for both biomass production and contaminants removal. However, the majority of these research studies refer to effluent toxicity and intense color/turbidity as the major limitations to microalgal growth in these effluents, suggesting the use of highly diluted effluents, which may be unfeasible for large-scale applications. The need for an external phosphorus source to provide adequate N:P molar ratios for microalgal growth was also evaluated in these studies, but its usage can cause an external source of contamination, besides the overall increase in process costs. Considering these limitations, the success of nutrients removal from landfill leachates using microalgae relies on the following steps: (i) the identification of microalgal species that can thrive in leachates without a significant pre-treatment; (ii) the understanding of the impact of different process variables (e.g., effluent concentration, pH and N:P ratio); and (iii) the development of novel PBR designs to improve light absorption within the culture and offset the negative impact of effluent color/turbidity. Accordingly, the present study evaluated the growth and nutrients removal performance (tertiary treatment) of two microalgal species commonly used in wastewater treatment processes (C. vulgaris and Tetradesmus obliquus) on different dilutions pre-treated leachate collected in a landfill located in northern Portugal. To further improve nutrients removal efficiencies, an innovative tubular PBR coupled with an optical reflector, known for its ability to enhance light distribution throughout the cultivation system and avoid the light limitation due to effluent color/turbidity, was used as a cultivation

platform for microalgal growth in the landfill leachate.

5.2 MATERIALS AND METHODS

The materials, analytical methods and experimental units employed in the current study are described in Chapter 3. Sections 3.2.2 and 3.2.2.1 present in detail the experimental setup (for the flask and tubular PBR assays) and operational conditions employed.

A landfill leachate was used as culture medium for *C. vulgaris*, acting as a nutrients source. The analytical methods used for the wastewater characterization (Section 3.3.1), monitoring of microalgae growth and nutrients removal (Sections 3.3.2 and 3.3.3), as well as the kinetic growth parameters (Sections 3.3.2.2) and nutrient removal parameters (Section 3.3.3) were also described in Chapter 3.

5.3 RESULTS AND DISCUSSION

5.3.1 Landfill leachate characterization

Leachate obtained from an urban waste landfill was collected after the biological treatment step and used as a culture medium for microalgal growth. Table 5.1 presents the main effluent characteristics.

The leachate sample presented high turbidity (Table 5.1), which may limit the photoautotrophic growth of microalgae due to difficulties in light penetration. Moreover, nitrogen concentration was also excessively high, when compared to commonly used microalgal growth media, such as BG11, Bold's Basal Medium (BBM), Detmer's and Modified Detmer's Medium (DM), Modified Bristol's Medium (MBM) and OECD test medium (WATANABE, 1960; BISCHOFF and BOLD, 1963; STANIER *et al*., 1971; ANDERSEN, 2005; OECD, 2011). Accordingly, the landfill leachate was not directly used for microalgal cultivation experiments, being previously diluted with distilled water. In addition, data from Table 5.1 show that the N:P molar ratio of the collected effluent was ~177:1, which indicates that phosphorus concentration in the effluent may be limiting for microalgal growth. According to several authors, N:P molar ratios above 30:1 are associated with microalgal growth inhibition due to phosphorus limitation (WATANABE, 1960; LARSDOTTER, 2006).

Considering these assumptions, the diluted effluent was also supplemented with an external source of phosphorus.

Table 5.1 – Physicochemical characterization of the landfill leachate used in this study

Parameter	Values	Unit
Turbidity	290	NTU[1]
pH	8.40	-
Total suspended solids (TSS)	735	mg L^{-1}
Total dissolved carbon (TDC)	411	mg L^{-1}
Dissolved organic carbon (DOC)	328	mg L^{-1}
Dissolved inorganic carbon (DIC)	83	mg L^{-1}
Chemical oxygen demand (COD)	663	mg L^{-1}
Chlorides (Cl^-)	2630	mg L^{-1}
Phosphate-phosphorus (PO_4-P)	7.95	mg L^{-1}
Nitrate-nitrogen (NO_3-N)	624	mg L^{-1}
Nitrite-nitrogen (NO_2-N)	12.6	mg L^{-1}

[1] Nephelometric turbidity unit

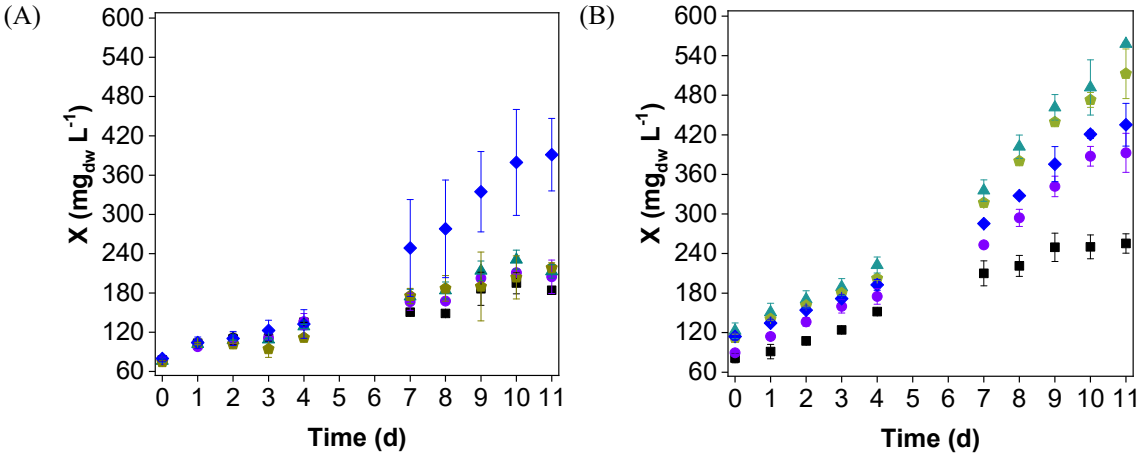
Author (2021).

5.3.2 Effect of effluent concentration on microalgal growth

Considering the increase observed in biomass concentration within the cultivation period for all studied conditions (Figure 5.1), it is possible to conclude that both microalgae (*C. vulgaris* and *T. obliquus*) were able to grow in the landfill leachate under different effluent concentrations (ranging from 5% (v/v) to 25% (v/v)). However, these experiments revealed an improved growth performance for *T. obliquus*, as biomass concentrations achieved by this microalga were higher than those achieved by *C. vulgaris*. Regarding the effect of effluent concentration on *C. vulgaris growth*, similar slow growth rates were obtained for effluent concentrations ranging from 10% (v/v) to 25% (v/v). Additionally, the growth curves demonstrate also lower growth ability in the experiments performed in the highest effluent concentrations, especially for the 25% (v/v) concentration. A small cell decay was observed on the last day of cultivation for the assays with leachate concentrations of 15-25% (v/v). On the other hand, the faster growth rate was obtained with the lowest effluent concentration, 5% (v/v). After an initial adaptation phase of approximately 3 days, the growth rate increased significantly until the tenth day of the experiments and then a slight decrease was observed on the last day of culture. The analysis of the growth curves obtained for 5% (v/v) and 25% (v/v) of landfill leachate evidences a decrease in the exponential growth phase and an increase in the adaptation phase. These results may be associated

with some sort of growth limitation, either by the excessive color or turbidity of the effluent, which hinders light penetration into the culture and leads to light limitation, or by the presence of high concentrations of potentially toxic compounds (LEE and SHEN, 2004).

Figure 5.1 – *C. vulgaris* (A) and *T. obliquus* (B) growth curves obtained under different effluent concentrations of the landfill leachate: 5% (v/v) – ◆, 10% (v/v) – ⬟, 15% (v/v) – ▲, 20% (v/v) – ●, and 25% (v/v) – ■. Error bars correspond to the standard deviation of the mean obtained from two independent experiments.

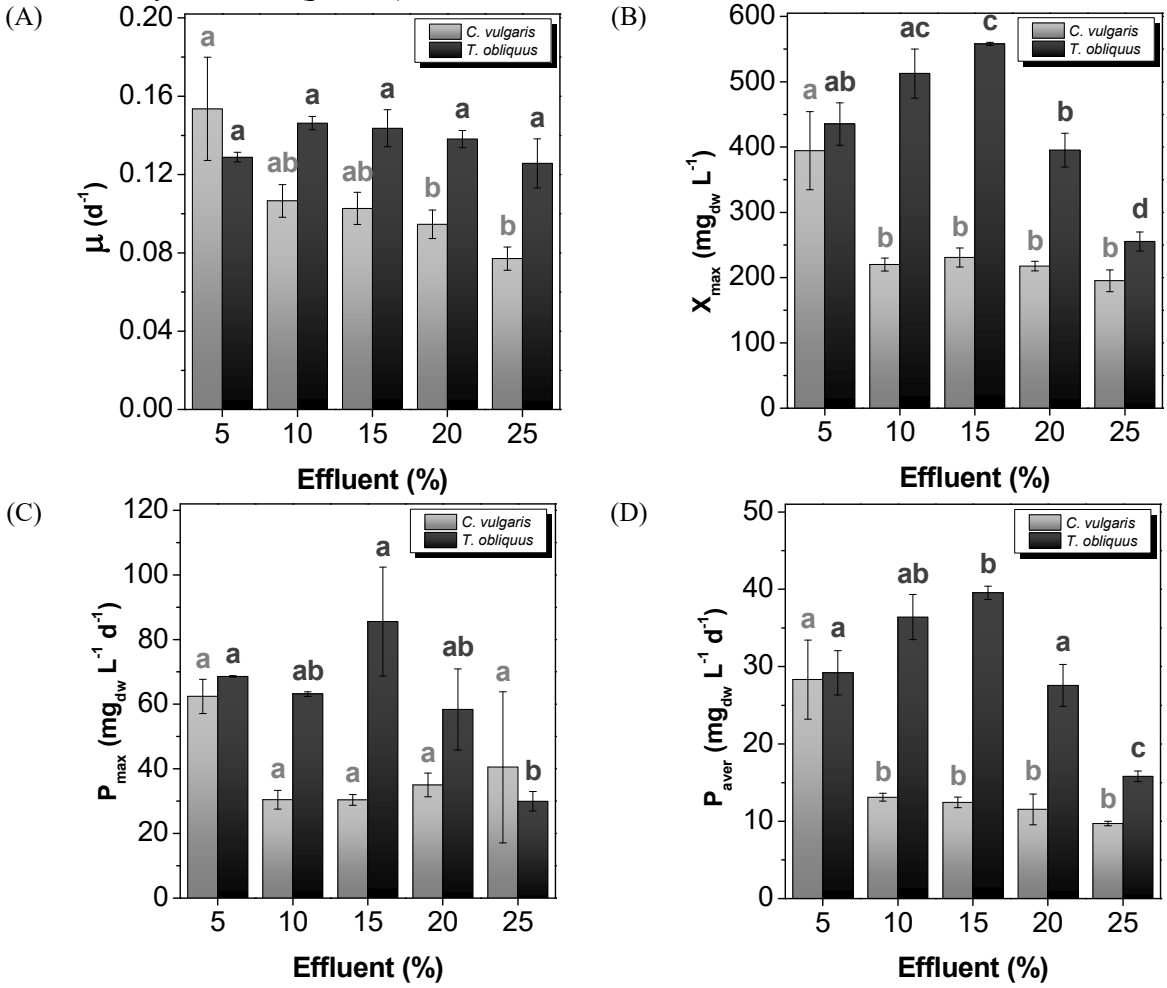


Author (2021).

In *T. obliquus* cultures (Figure 5.1B), a smaller lag phase and a more pronounced growth were observed for all tested conditions. Moreover, no cell decay was observed in these cultures. Similar to what was found for *C. vulgaris* growth curves, high effluent concentrations, in this case between 20% (v/v) and 25% (v/v), resulted in lower biomass concentrations and growth rates for the microalga *T. obliquus*. As already explained previously, these results may be due to the high color and/or turbidity of the landfill leachate in these concentrations, which hinders light penetration and, consequently, decreases the photosynthetic rate and biomass productivities (LEE and SHEN, 2004; CHANG *et al.*, 2018). Another reason for this effect may be associated with the presence of certain compounds that, in high concentrations, can be toxic to microalgae (LEE and SHEN, 2004). On the other hand, an improvement in *T. obliquus* growth was observed when effluent concentration increased from 5% (v/v) to 15% (v/v). The highest growth performance achieved for the intermediary effluent concentration (15% (v/v)) can be linked to the higher ability of this microalga to withstand higher levels of the studied landfill leachate. These results were also confirmed by the higher biomass productivities achieved by *T. obliquus* in comparison

with those obtained for *C. vulgaris*, regardless of the effluent concentration. Some studies have already reported the growth of *T. obliquus* in different effluents, emphasizing its robustness and the ability for nutrients and metals removal (RUGNINI *et al*., 2019; MA *et al*., 2020). In a comparative study with *C. vulgaris*, TEJIDO-NUÑEZ *et al*. (2019) also pointed out that *T. obliquus* grown in non-sterilized aquaculture effluent had a better performance, thus being more suitable for real effluent applications. Regarding microalgal growth parameters, Figure 5.2 presents the results from specific growth rates, maximum biomass concentrations and biomass productivities determinations, emphasizing the statistical significance of the obtained results through the letters *a*, *b* and *c*. As indicated in Section 3.7, the comparison between the different studied conditions was performed through the Tukey HSD test. According to Figure 5.2, a general increase in *C. vulgaris* growth parameters was observed as the effluent concentration decreased. Deviations from this trend were observed for: (i) maximum biomass concentrations (Figure 5.2B) obtained in the assays with 15% (v/v) and 10% (v/v) of landfill leachate, where obtained values were not statistically different ($p>0.05$); and (ii) maximum biomass productivities (Figure 5.2C), where similar values ($p>0.05$) were determined for all effluent concentrations. Except for maximum biomass productivities (Figure 5.2C), the values achieved in the experiments using 5% (v/v) of landfill leachate were statistically higher ($p<0.05$) than those achieved in the experiments performed with the highest effluent concentration evaluated in this study. Regarding specific growth rates (Figure 5.2A), the obtained values ranged from 0.077±0.006 d^{-1} to 0.15±0.03 d^{-1}, corresponding to effluent concentrations of 25% (v/v) and 5% (v/v), respectively. Similarly, maximum biomass concentration values (Figure 5.2B) increased as the effluent concentration decreased, ranging from 195±17 mg_{dw} L^{-1} to 394±60 mg_{dw} L^{-1}. The highest values of maximum and average biomass productivities (Figure 5.2C and Figure 5.2D, respectively) were also obtained for the lowest effluent concentration: 93±39 mg_{dw} L^{-1} d^{-1} and 28±5 mg_{dw} L^{-1} d^{-1}, respectively.

Figure 5.2 – Growth parameters obtained by *C. vulgaris* and *T. obliquus* cultures in different concentrations of the landfill leachate. Error bars correspond to the standard deviation of the mean obtained from two independent experiments. The letters a, b, c and d (shown above the bars) represent the statistical significance of the results, as given by the Turkey HSD test: mean values sharing at least one common letter are not statistically different (p>0.05).



Author (2021).

In this way, in general, growth parameters determined from the evolution of biomass concentration within the cultivation period (Figure 5.2) also evidence that microalgal biomass production was favored by a decrease in effluent concentration and, hence, in nitrogen and phosphorus concentration in the culture medium. Similar to what was observed by LIN *et al*. (2007); ZHAO *et al*. (2014); PEREIRA *et al*. (2016), the increase of nitrogen concentration in the culture medium above certain levels did not contribute to an increase in kinetic growth parameters. Also, microalgal growth parameters determined under the highest studied effluent concentration, 25% (v/v), confirm that high concentrations of landfill leachate have an inhibitory effect on microalgal growth, which was also noted by ZHAO *et al*. (2014). Besides the excessive

nitrogen load, other characteristics of the landfill leachate that may have a toxic effect on microalgal growth include (ZHAO *et al*., 2014): (i) the presence of various types of organic compounds; (ii) salinity; and (iii) reduction in light penetration due to the effluent color and/or turbidity. Usually, by increasing the dilution factor of the effluent in water, these effects can be minimized, resulting in a better adaptation of microalgal species to the culture medium. Despite the differences observed in specific growth rates, maximum biomass concentrations and average biomass productivities in response to different effluent concentrations, maximum biomass productivities determined in the different conditions were not statistically different (p>0.05), as this parameter corresponds to the maximum value of the individual daily productivities. Therefore, it is possible to obtain similar daily productivities in different periods of the cultivation time, even for different culturing conditions. However, if one looks at the average biomass productivities, which represent an overall value from the entire cultivation period, the differences between the different effluent concentrations are clear.

Regarding *T. obliquus* cultures, improved growth performance was observed in all tested conditions. Regarding the effect of effluent concentration on specific growth rates of this microalga, Figure 5.2A shows that different effluent loads have not significantly influenced (p>0.05) this kinetic growth parameter, with values ranging between 0.129±0.002 d^{-1} and 0.146±0.03 d^{-1}. In terms of maximum biomass concentrations (Figure 5.2B), the obtained values ranged from 255±15 mg_{dw} L^{-1} to 558±3 mg_{dw} L^{-1}, being these values (minimum and maximum) statistically different (p<0.05). However, for this microalga, the highest value of maximum biomass concentration was obtained for an effluent concentration of 15% (v/v). Similarly, the highest values achieved for maximum (Figure 5.2C) and average (Figure 5.2D) biomass productivities were obtained in the experiments carried out with 15% (v/v) of landfill leachate: 86±17 mg_{dw} L^{-1} d^{-1} and 39.5±0.8 mg_{dw} L^{-1} d^{-1}, respectively. Contrary to what was observed in *C. vulgaris* cultures, *T. obliquus* growth parameters increased with the rise of effluent concentration (for the concentration range between 5% (v/v) and 15% (v/v)). However, for effluent concentrations above 15% (v/v), *T. obliquus* growth was also negatively affected. Deviations from this behavior were observed for: (i) maximum biomass productivities (Figure 5.2C) determined for experiments carried out with 5% (v/v) and 10% (v/v) of landfill leachate, where the determined values were not statistically different (p>0.05); and (ii) specific growth rates (Figure 5.2A) determined for 10% (v/v) and 15% (v/v), which were not statistically different (p>0.05), as well.

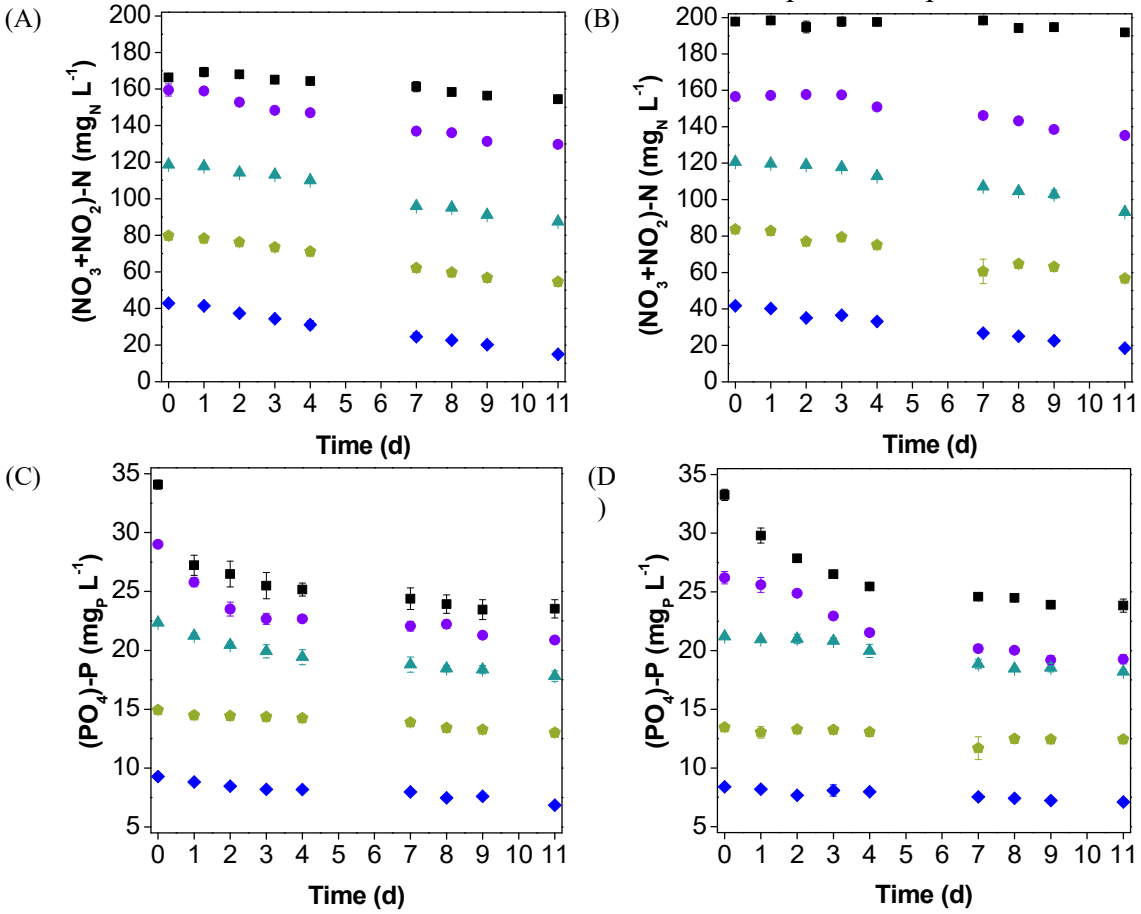
Although both microalgae were able to grow in the landfill leachate, the results obtained in this study for microalgal growth were inferior to the majority of values reported in the literature, which can be attributed to the toxicity of the studied effluent and the conditions employed in reference studies (e.g., light availability, the concentration of landfill leachate, the use of microalgal consortia, and the use of optimized PBRs for microalgal growth). For example, when growing the microalgae *Chlorella* cf. *ellipsoidea* and *Scenedesmus* cf. *rubescens* in 125 mL Erlenmeyer flasks exposed to a continuous PAR of 125 μmol m^{-2} s^{-1} using 10% (v/v) of landfill leachate, EDMUNDSON and WILKIE (2013) reported specific growth rates of 0.67 d^{-1} and 0.83 d^{-1}, respectively. When growing *Chlorella pyrenoidosa* in the same concentration of landfill leachate, in 500 mL flasks exposed to a PAR of ~108 μmol m^{-2} s^{-1} and a light:dark ratio of 20:4, ZHAO *et al*. (2014) reported a specific growth rate of 0.28 d^{-1}. More recently, TIGHIRI and ERKURT (2019) achieved a specific growth rate of 0.5 d^{-1} for a microalgal-bacterial consortium (containing the cyanobacteria *Microcystis* sp. and *Oscillatoria* sp., and the microalgae *Chlorella* sp., *Scenesdesmus* sp. and *Stigeoclonium* sp.) grown in 10% (v/v) of landfill leachate, using a bubble column PBR operating in sequencing batch mode and exposed to a PAR of 76 μmol m^{-2} s^{-1}. In another study, TAGLIAFERRO *et al*. (2019) obtained a specific growth rate of 0.30 d^{-1} for *C. minutissima* cultured in a concentric-tube airlift PBR supplied with a PAR of 80-90 μmol m^{-2} s^{-1} and using 5% (v/v) landfill leachate as culture medium. On the other hand, the specific growth rates obtained in this study were close to those reported by PASKULIAKOVA *et al*. (2016). When growing *Chlamydomonas* sp. in 250-mL Erlenmeyer flasks exposed to a PAR of 22 μmol m^{-2} s^{-1} and a light:dark ratio of 14:10, and fed with 10% (v/v) of landfill leachate, the specific growth rate reported by the authors was 0.19 d^{-1}.

5.3.3 Effect of effluent concentration on nutrients removal

Figure 5.3 shows the variation of nitrogen (in the forms of NO_3-N and NO_2-N) and phosphorus (in the form of PO_4-P) concentrations within the cultivation time for both microalgae in all studied conditions. Comparing the curves obtained for both microalgae, the results evidence a similar behavior between them. Additionally, nitrogen concentrations decreased gradually, without stabilizing at the end of the cultivation period in all studied conditions, indicating that this nutrient was not supplied

in limiting concentrations. This behavior was already expected due to the N:P molar ratios employed in this study (~12:1), which are considered adequate for microalgal growth (not compromising nitrogen and phosphorus availability). In fact, according to LARSDOTTER (2006), nitrogen limitation to microalgal growth may occur for N:P molar ratios below 5:1, whereas phosphorus limitation can occur for N:P molar ratios above 30:1.

Figure 5.3 – Temporal variation of nitrogen (A and B) and phosphorus (C and D) concentrations determined in *C. vulgaris* (A and C) and *T. obliquus* (B and D) cultures grown in different concentrations of the landfill leachate: 5% (v/v) – ◆, 10% (v/v) – ⬟, 15% (v/v) – ▲, 20% (v/v) – ●, and 25% (v/v) – ■. Error bars correspond to the standard deviation of the mean obtained from two independent experiments.



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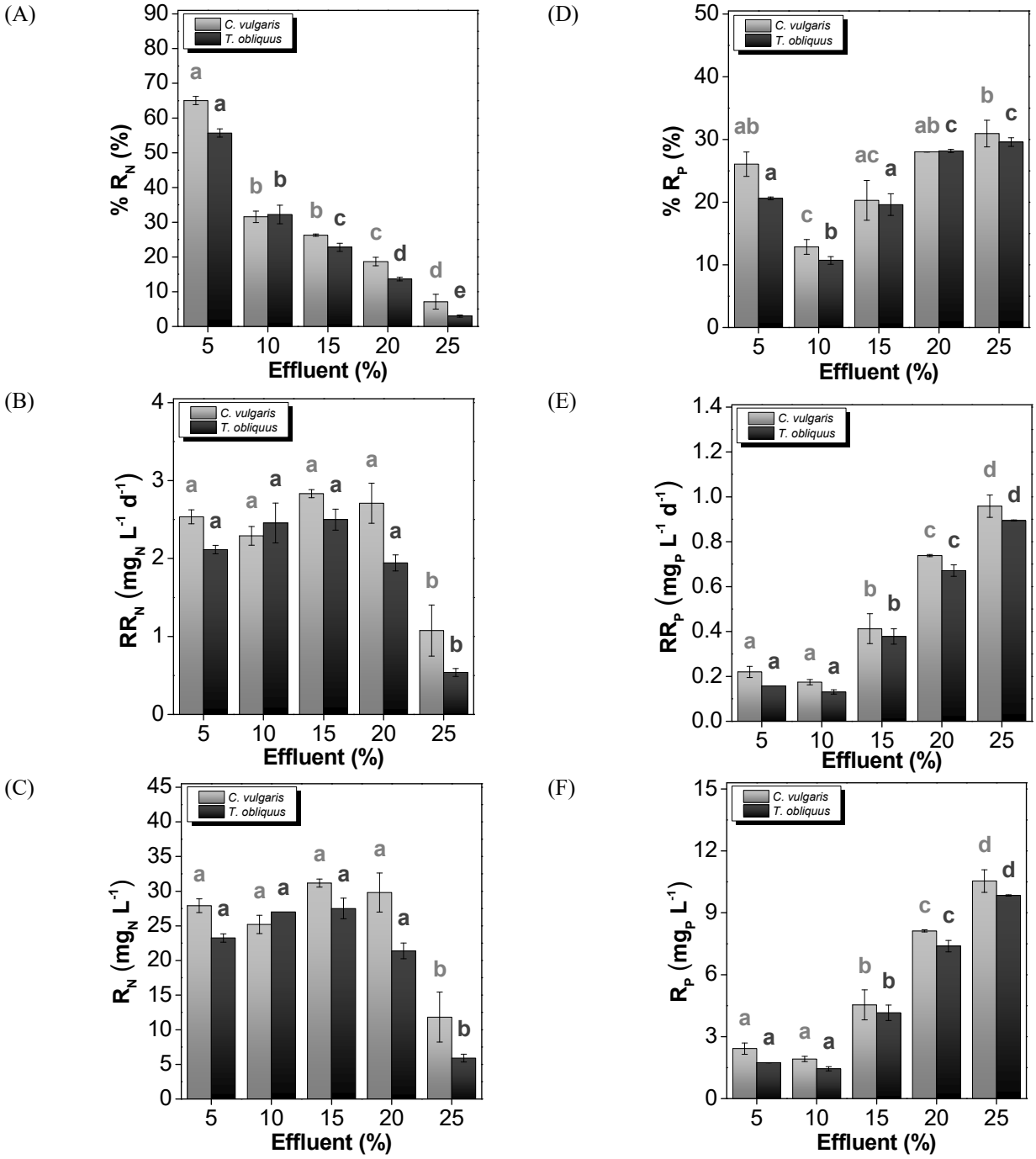
On the other hand, the rate of nutrients assimilation differed depending on the element and on the effluent concentration. Regarding nitrogen uptake by *C. vulgaris* (Figure 5.3A), an almost linear decrease in nitrogen concentration was observed in all studied conditions. However, a two-day delay was observed in the experiments performed under the highest effluent concentrations (20% (v/v) and 25% (v/v) of

landfill leachate). Similar behavior was observed in nitrogen assimilation by *T. obliquus* (Figure 5.3B). In the experiments carried out under the lowest effluent concentrations, 5-15% (v/v), a linear decrease in nitrogen concentration was obtained, whereas for 25% (v/v) of landfill leachate, nitrogen concentration remained approximately constant, and for 20% (v/v), a 3-day delay was observed in the assimilation of this element. Moreover, Figs. 5A and 5B show that the decrease in nitrogen concentration was more prominent as the effluent concentration decreased (i.e., from the 25% (v/v) experiments to the 5% (v/v) ones). Under the lowest effluent concentration evaluated in this study, the final nitrogen concentrations achieved in *C. vulgaris* and *T. obliquus* cultures were 15.0±0.2 mg_N L^{-1} and 18.5±0.3 mg_N L^{-1}, respectively. Regarding phosphorus concentration, a decrease within the cultivation time was also observed for *C. vulgaris* and *T. obliquus* cultures (Figs. 5C and 5D, respectively). However, the assimilation pattern observed for different effluent concentrations was different from the one reported for nitrogen consumption: phosphorus uptake occurred in a greater extent for the experiments performed with the highest effluent concentrations (i.e., in the experiments carried out with 20% (v/v) and 25% (v/v) of landfill leachate). Therefore, only a slight decrease in phosphorus concentration was observed in 5% (v/v) landfill leachate experiments: (i) from 9.3±0.2 mg_P L^{-1} to 6.85±0.06 mg_P L^{-1} in *C. vulgaris* cultures; and (ii) from 8.41±0.06 mg_P L^{-1} to 7.11±0.03 mg_P L^{-1} in *T. obliquus* cultures. Also, in the experiments carried out with higher effluent dilutions, phosphorus concentration decreased until the $6^{th}/7^{th}$ day of culture, and then it was maintained approximately constant.

As for microalgal growth parameters, nitrogen and phosphorus removal parameters are presented in Figure 5.4, respectively, being the statistical differences determined through the Tukey HSD test denoted by the letters *a*, *b*, *c*, *d* and *e*. Regarding nitrogen removal efficiencies (Figure 5.4A), an increase was observed as the effluent concentration decreased, ranging from 7±2% to 65±1% for *C. vulgaris*, and from 3.0±0.3% to 56±1% for *T. obliquus*. Figure 5.4A shows that there was a statistical difference ($p<0.05$) in nitrogen removal efficiencies obtained for different effluent concentrations, except between the experiments performed with 15% (v/v) and 10% (v/v) of landfill leachate. On the other hand, the maximum phosphorus removal efficiencies were achieved by both microalgae in the highest effluent concentration (25% (v/v)), whereas the minimum values were obtained for an effluent concentration of 10% (v/v). Minimum and maximum phosphorus removal efficiencies obtained in *C.*

vulgaris and *T. obliquus* cultures (Figure 5.4D) were statistically different (p<0.05), ranging from 12±1% to 31±2%, and from 10.7±0.6% to 29.9±0.7%, respectively. Regarding nitrogen removal rates (Figure 5.4B) and nitrogen mass removal (Figure 5.4C), the lowest values were obtained in the highest effluent concentration, being statistically lower (p<0.05) than those obtained in the other studied conditions. However, no statistical difference (p>0.05) was observed in the experiments performed with effluent concentrations ranging between 5% (v/v) and 20% (v/v). The highest nitrogen removal rates were obtained in 15% (v/v) of landfill leachate for both *C. vulgaris* and *T. obliquus*: 2.83±0.05 mg_N L^{-1} d^{-1} and 2.5±0.1 mg_N L^{-1} d^{-1}, respectively. The highest nitrogen mass removal values obtained for both microalgae, 31.2±0.6 mg_N L^{-1} and 27±1 mg_N L^{-1}, were also obtained in these conditions. A different behavior was observed for phosphorus. In this case, average removal rates (Figure 5.4E) and mass removal values (Figure 5.4F) declined as the effluent concentration decreased, being statistically different (p<0.05) in all studied conditions, except between the 5% (v/v) and 10% (v/v) concentrations. Accordingly, the highest values were obtained for the highest effluent concentration evaluated in this study (25% (v/v)): (i) maximum phosphorus removal rates were 0.96±0.05 mg_P L^{-1} d^{-1} and 0.895±0.003 mg_P L^{-1} d^{-1} (for *C. vulgaris* and *T. obliquus*, respectively); and (ii) maximum mass removal values were 10.5±0.5 mg_P L^{-1} and 9.84±0.03 mg_P L^{-1} (for *C. vulgaris* and *T. obliquus*, respectively).

Figure 5.4 – Nitrogen (NO_3-N and NO_2-N) and Phosphorus (PO_4-P) removal parameters (A, B, C and D, E, F, respectively) obtained by *C. vulgaris* and *T. obliquus* cultures grown in different concentrations of the landfill leachate. Error bars correspond to the standard deviation of the mean obtained from two independent experiments. The letters *a*, *b*, *c* and *d* (shown above the bars) represent the statistical significance of the results, as given by the Turkey HSD test: mean values sharing at least one common letter are not statistically different (p>0.05).



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Still, regarding nitrogen uptake (Figure 5.4), the lower effectiveness observed for the highest effluent concentrations (20% (v/v) and 25% (v/v)) is in line with the lower biomass productivities observed in these conditions and can be related to reduced

photosynthetic activity in highly concentrated landfill leachates. On the other hand, phosphorus concentration decreased just until the $7^{th}/8^{th}$ day of culturing and its uptake occurred to a greater extent in the highest effluent concentration evaluated in this study. According to KHANZADA (2020), the removal of one nutrient is associated with the availability of the other. For instance, some studies have shown that nitrogen removal is increased by supplementing the cultures with an additional source of phosphorus (PASKULIAKOVA *et al*., 2016; PEREIRA *et al*., 2016; CHANG *et al*., 2019). Likewise, sufficiently high nitrogen concentrations guarantee effective removal of phosphorus from wastewater (BEUCKELS *et al*., 2015; KHANZADA, 2020), which justifies a more significant drop in phosphorus concentration for the experiments carried out with higher effluent concentrations and, consequently, with higher nitrogen levels. According to the results present in Figs. 5.4, which refer to the nitrogen and phosphorus removal parameters, it is possible to conclude that a total depletion of these nutrients did not occur. Moreover, these results confirm that the cultures were not limited by nitrogen, as the removal efficiencies increased with a decrease in the effluent concentration. The higher photosynthetic efficiencies obtained for the lower effluent concentrations may have contributed to the higher nitrogen removal efficiencies obtained in these conditions (5% (v/v) and 10% (v/v)). On the other hand, the phosphorus removal parameters confirm that phosphorus uptake is closely linked to nitrogen availability: the use of higher effluent loads, with higher nitrogen concentrations, favored phosphorous removal.

Nutrients remediation by microalgae from landfill leachates has already been reported in the literature. Table 5.2 highlights nitrogen and phosphorus removal efficiencies and removal rates obtained in different research studies. The data presented in Table 5.2 indicate higher removal efficiencies in the studies reported in the literature when compared to the data obtained in this study. These results can be attributed to differences in the experimental conditions (e.g., cultivation system, working volume, light supply, cultivation period and nutrients supply) employed in the different studies. First, the results reported in the literature were achieved for significantly higher cultivation periods than those used in this study. As nitrogen and phosphorus have not been completely depleted and the growth curves have not reached the stationary growth phase, it is believed that the obtained efficiencies could be further improved by increasing the cultivation period. Another issue affecting nutrients recovery efficiencies and biomass productivity is the N:P molar ratio (KHANZADA, 2020). In this study, the

selected N:P molar ratio was ~12:1, while PASKULIAKOVA *et al*. (2016) and PEREIRA *et al*. (2016), who obtained superior results in terms of nitrogen removal, employed N:P molar ratios of 16:1 and 35:1, respectively. These results indicate that a N:P molar ratio higher than the one adopted in this study could further improve nitrogen uptake from the landfill leachate. Another important factor that should be considered when evaluating nitrogen removal efficiencies is the supplied nitrogen source, as microalgae assimilate different nitrogen forms at different rates. According to microalgal assimilation mechanisms, higher nitrogen removal efficiencies are commonly observed in cultures supplied with NH_4-N because it is directly assimilated and converted into proteins by microalgae, while other forms (e.g., NO_3-N and NO_2-N) must be reduced before being assimilated (LIN *et al*., 2007; GONÇALVES *et al*., 2017). Except for CHANG *et al*. (2019) and TAGLIAFERRO *et al*. (2019), all other authors evaluated NH_4-N removal, as it is the main nitrogen source present in raw and primary-treated landfill leachates. In this way, higher nitrogen removal efficiencies were already expected in these studies. However, when comparing the average removal rates, which consider the initial concentrations of nutrients and the cultivation/treatment time in their calculation, it is observed that the values obtained in the reference studies are in the same order of magnitude as those obtained in this study, except in what concerns the studies carried out in PBRs and in the study performed by KHANZADA (2020), who evaluated the removal of NH_4-N exclusively. The higher average removal rates obtained in the study carried out by KHANZADA (2020) may be associated with the higher ability of microalgae to assimilate NH_4-N rather than NO_3-N. Moreover, this study promoted effluent treatment using a microalgal consortium composed of the microalgal species *C. vulgaris* and *C. reinhardtii*, which may have contributed to an improved nutrients uptake performance. According to GONÇALVES *et al*. (2017), in microalgal consortia, cooperative interactions between species can occur, improving biomass production and nutrients uptake, as also evidenced in the studies performed by KOREIVIENĖ *et al*. (2014) and CHINNASAMY *et al*. (2010). Moreover, the use of microalgal consortia tends to make wastewater treatment systems more resistant to environmental oscillations (GONÇALVES *et al*., 2017): the growth of one species of the consortium can compensate for a possible loss of the population of other strain due to environmental and nutritional stress conditions (BHATNAGAR *et al*., 2011). Considering the values reported in the literature, the results for nutrients recovery obtained in this study can be considered promising, as some improvements may be

achieved by increasing the N:P molar ratio and the cultivation period. Moreover, optimized PBRs for microalgal growth may improve photosynthetic efficiency and, hence, nutrient uptake rates. Therefore, preliminary experiments in an innovative tubular PBR coupled to an anodized aluminum reflective surface (with a double-parabola geometry) were carried out using the lowest effluent concentration evaluated in this study: 5% (v/v).

Table 5.2 – Comparison between nutrients removal efficiencies (*%R*, in %), average removal rates (*RR*, in mg L^{-1} d^{-1}) and average biomass productivities (P_{aver}, mg_{dw} L^{-1} d^{-1}) obtained in this study and other studies reporting microalgal growth in landfill leachates (LL).

(to be continued)

Operational Conditions	LL (v/v)	Microalgae	Culture Time (d)	Element / Form	Initial Concentration (mg L^{-1})	%R (%)	RR (mg L^{-1} d^{-1})	P_{aver} (mg_{dw} L^{-1} d^{-1})	Ref.
CS: Borosilicate glass flasks; OM: batch; WV: 1 L; PAR: 30-40 µmol m^{-2} s^{-1}; L/D: 24/0; T: 24±3 °C	5%	*Chlorella vulgaris*	11	(NO_3+NO_2)–N	42.9	65±1	2.54±0.09	28±5	This study
	25%			PO_4–P	34.1	31±2	0.96±0.05	9.7±0.3	
	5%	*Tetradesmus obliquus*	11	(NO_3+NO_2)–N	41.7	56±1	2.11±0.05	29±3	
	25%			PO_4–P	33.3	29.6±0.7	0.895±0.003	15.8±0.7	
CS: Borosilicate glass flasks; OM: batch; WV: 1 L; PAR: 32–42 µmol m^{-2} s^{-1}; L/D: 24/0; T: 16±2 °C	-	*Chlorella vulgaris*	11	(NH_4+NO_3)–N	159	34	5.1	95±6	(PEREIRA *et al*., 2016)
				PO_4–P	~10	100	0.87	101±7	
CS: Erlenmeyer flasks; OM: batch; WV: 0.15 L; PAR: 23 µmol m^{-2} s^{-1}; L/D: 14/10; T: 15 °C	10%	*Chlamydomonas* sp.	30	NH_4–N	~100	90.7	~3.8	-	(PASKULIAKOVA *et al*., 2016)
				PO_4–P	~6.5	~1	0.2	-	

(conclusion)

CS: Erlenmeyer flasks; OM: batch; WV: 0.15 L; PAR: 22 µmol m^{-2} s^{-1}; L/D: 14/10; T: 15 °C	30%	*Chlamydomonas* sp.	40	NH_4–N	130.3±4.2	99.8	3.25	-	(PASKULIAKOVA *et al*., 2018a)
				PO_4–P	17.6±0.6	97.7	0.43	-	
CS: Glass bottles; OM: batch; WV: 0.5 L; PAR: 55 µmol m^{-2} s^{-1}; L/D: 24/0; T: 24-25 °C	-	*Chlorella vulgaris* and *Chlamydomonas reinhardtii*	30	NH_4–N	1100	69	25.3	-	(KHANZADA, 2020)
CS: PBR coupled to an optical reflector; OM: batch; WV: ~0.52 L; PAR: 55-65 µmol m^{-2} s^{-1}; L/D: 13/11; T: 24±3 °C	5%	*Chlorella vulgaris*	8	(NO_3+NO_2)–N	35.4	91.1	4.61	110±1	This study
				PO_4–P	7.42	70.1	0.74		
		Tetradesmus obliquus		(NO_3+NO_2)–N	37.8	88.7	4.80	18.4±0.1	
				PO_4–P	9.75	56.0	0.78		

CS: cultivation system; OM: operation mode; WV: working volume; PAR: photosynthetically active radiation; L/D: light/dark period; T: temperature.
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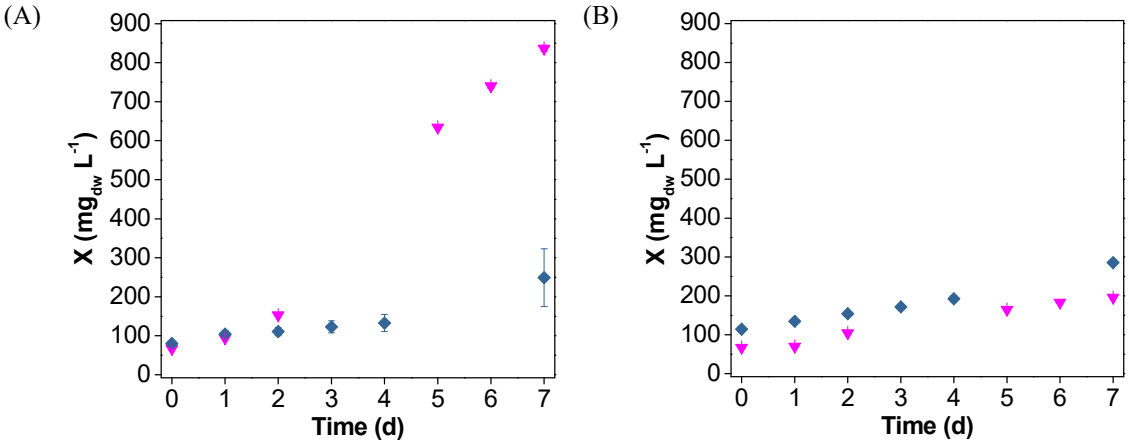
5.3.4 Microalgal growth performance in the tubular PBR

The growth curves obtained in cultures grown in 1 L flasks and the tubular PBR are presented in Figure 5.5. The daily monitoring of biomass concentration in the tubular PBR experiments allowed the comparison of microalgal growth behavior in both cultivation systems (Figure 5.5).

Figure 5.5 – Growth curves obtained for *C. vulgaris* (A) and *T. obliquus* (B) cultures grown in 1-L flasks (◆) and in the tubular PBR (▼), using 5% (v/v) of landfill leachate. Error bars correspond to the standard deviation of the mean obtained from two independent experiments.



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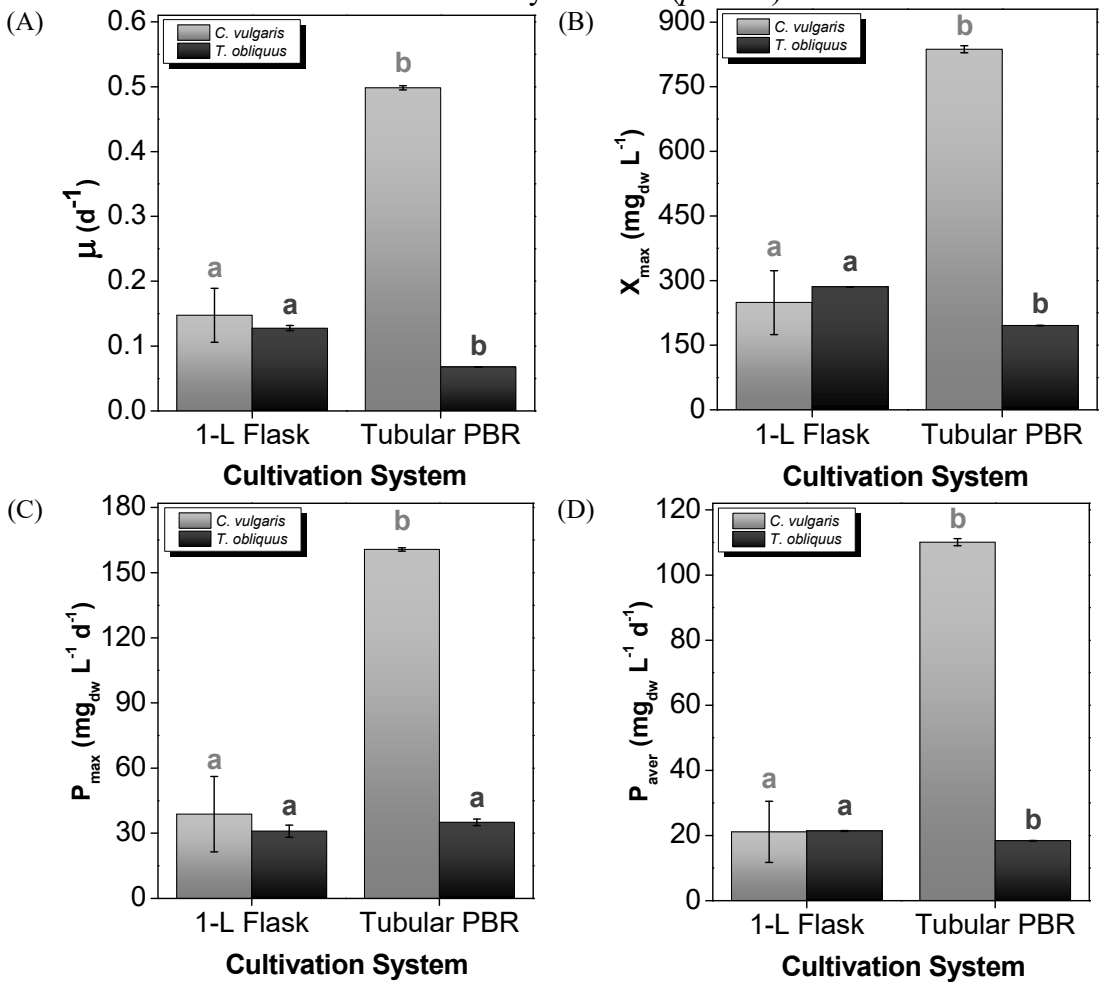
According to these data, the cultivation of *C. vulgaris* in the tubular PBR (Figure 5.5A) improved significantly microalgal growth. On the 7th day of culture, biomass concentration in the tubular PBR, 837±8 mg_{dw} L^{-1}, was significantly higher (p<0.05) than biomass concentration determined in 1 L flask cultures, 249±74 mg_{dw} L^{-1}, which represents an increase of ~236%. Moreover, cultivation in the tubular PBR significantly reduced the adaptation phase of *C. vulgaris* to just one day, and the exponential growth phase extended until the last day of the experiments. Short adaptation phases were already reported in the literature for *C. vulgaris* cultures grown in different effluents (PEREIRA *et al*., 2016; PORTO *et al*., 2020). The maintenance of exponential growth until the last day of culturing suggests that nutrients were not limiting after the 7 days of culturing and that the experiments could be extended for a longer period.

Figure 5.6 presents microalgal growth parameters determined for *C. vulgaris and T. obliquus* grown in both cultivation systems. Comparison of the results obtained

in both cultivation systems was performed according to the Tukey HSD statistical test. Based on these data, it is possible to state that *C. vulgaris* growth in the tubular PBR was significantly higher ($p<0.05$) than the one observed in 1 L flasks. For example, specific growth rates (Figure 5.6A) determined for *C. vulgaris* increased from 0.15±0.04 d^{-1} in 1 L flasks to 0.498±0.003 d^{-1} in the tubular PBR. Additionally, the specific growth rate achieved by *C. vulgaris* in the tubular PBR, 0.61±0.04 d^{-1}, was higher than the values reported in the literature (PASKULIAKOVA *et al*., 2016; PEREIRA *et al*., 2016; PASKULIAKOVA *et al*., 2018a; NAIR *et al*., 2019; TAGLIAFERRO *et al*., 2019; TIGHIRI and ERKURT, 2019; KHANZADA, 2020). For example, the maximum specific growth rate reported by PASKULIAKOVA *et al*. (2018a) PASKULIAKOVA *et al*. (2018a) for *Chlamydomonas* sp. cultures grown in different concentrations of landfill leachate (in 250-mL Erlenmeyer flasks) was 0.19±0.01 d^{-1}. Lower specific growth rates were also reported for the microalgal-bacterial consortium studied by TIGHIRI and ERKURT (2019): 0.5 d^{-1}. These results suggest that the studied tubular PBR had a positive effect on *C. vulgaris* growth. One possible reason for this benefic effect may be associated with a higher absorption capacity and more homogeneous light distribution around the absorber tube perimeter, promoted by the reflective surface coupled to the tubular PBR. Considering the radiant power (*RP*) that reaches the solution (determined by ferrioxalate actinometry ($[Fe^{3+}]$ = 6.0 mM; $[C_2O_4^{2-}]$ = 30 mM), as previously reported by GOMES *et al*. (2018)), the working volume and cultivation time of both reaction systems, the accumulated energy determined in the tubular PBR 191.0 kJ L^{-1} was at least 4 times greater than the energy accumulated in 1 L flasks 42.9 kJ L^{-1}. Thus, the increase in light energy captured by the tubular PBR may have contributed positively to the increase in biomass productivity.

Figure 5.6 – Growth parameters obtained by *C. vulgaris* and *T. obliquus* cultures grown in 1 L flasks and in the tubular PBR, using 5% (v/v) of landfill leachate. Error bars correspond to the standard deviation of the mean obtained from two independent experiments. The letters *a*, *b* and *c* (shown above the bars) represent the statistical significance of the results, as given by the Turkey HSD test: mean values sharing at least one common letter are not statistically different (p>0.05).



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Moreover, the values of specific growth rates and average biomass productivities as a function of the accumulated energy determined for each cultivation system, did not present a significant difference (p>0.05): (i) specific growth rates (in terms of accumulated energy) determined for *C. vulgaris* in 1 L flasks and the tubular PBR were 0.024±0.007 L kJ^{-1} and 0.017±0.001 L kJ^{-1}, respectively; and (ii) *C. vulgaris* average biomass productivities achieved per accumulated energy were 4±2 mg kJ^{-1} and 4.031±0.005 mg kJ^{-1}, for experiments carried out in 1 L flasks and in the tubular PBR, respectively. These similar results suggest that no photo-saturation occurred in the tubular PBR, being all energy used by microalgae for biomass production (i.e., the

conversion of light energy into chemical energy in the form of biomass). Therefore, it is possible to conclude that within a certain range of light irradiance values, the tubular PBR couple with a double-parabola optical reflector promotes a better light utilization efficiency by microalgae.

As it is well known, light distribution, intensity and quality are factors that strongly influence the photosynthetic activity and, hence, microalgal biomass production (MORENO-GARCIA *et al*., 2017). Although PBRs are commonly designed taking into account the reduction of the light path and the improvement of light distribution, when cells are located in a dark zone (e.g., in a non-illuminated area), the photosynthetic activity may be reduced due to light limitation (BRINDLEY *et al*., 2011). In this study, the existence of dark zones was offset by coupling the tubular PBR with a double-parabola optical reflector made of anodized aluminum. According to GOMES *et al*. (2018), if light reaches a double-parabola optical reflector at an angle of 90º, the estimated illuminated area for the absorber tube is 100%. GOMES *et al*. (2018) further point out that the selected reflective surface material (anodized aluminum) has a high specular reflectance in the electromagnetic spectrum, thus increasing the availability of light in the system.

Contrary to what was observed for *C. vulgaris* cultures, cultivation in the tubular PBR did not favor *T. obliquus* growth, as biomass concentrations and microalgal growth parameters obtained in this cultivation system were significantly lower ($p<0.05$) than those obtained in 1 L flasks' experiments. The concentrations achieved in the 7th day of culturing in 1 L flasks and the tubular PBR were 285.32±0.07 mg_{dw} L^{-1} and 195.6±0.5 mg_{dw} L^{-1}, respectively. Considering the improvement in light penetration promoted by this PBR, an increase in biomass production of *T. obliquus* was also expected. The following hypotheses can explain these unexpected results: (i) cell photoinhibition, due to the increase in the amount of light reaching the tubular PBR; (ii) achievement of low biomass concentrations, as a result of the low initial biomass concentrations used in the PBR experiments, when compared to those used in 1 L flasks; and (iii) low biomass concentrations in the suspended form due to cells' attachment in the PBR walls. Considering the three hypotheses, culture observations and the obtained results, it is more likely that the low biomass concentrations achieved by *T. obliquus* in the tubular PBR may be a result of biofilm formation in the PBR walls. *T. obliquus* is known for its self-flocculating properties, which facilitate microalgal settling and adhesion and, hence, its biofilm formation ability (GUO *et al*.,

2013). For this reason, biomass concentration measurements based on OD_{680} may be underestimated. Samples for biomass concentration measurements were collected from the glass vessel, where biomass recirculation occurred, and cells attached to the reactor walls did not detach from this surface. Accordingly, results from OD_{680} measurements in these conditions have not included biomass concentration of the attached cells.

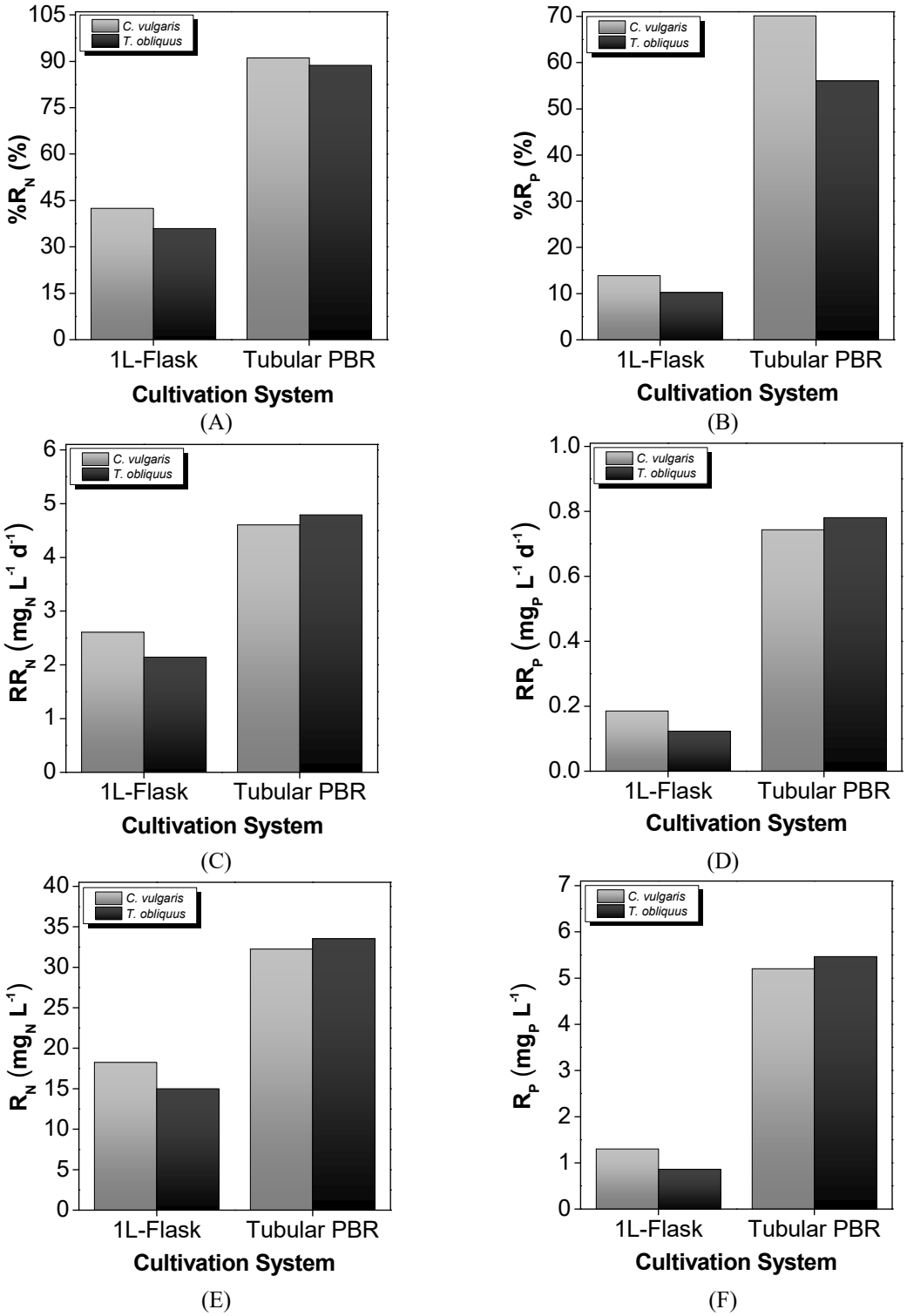
Comparing the growth performance of the selected species in the tubular PBR, the growth parameters obtained for *C. vulgaris* were significantly higher ($p<0.05$) than those obtained for *T. obliquus*: (i) specific growth rates (Figure 5.6A) obtained for *C. vulgaris* and *T. obliquus* were 0.498±0.003 d^{-1} and 0.0679±0.0005 d^{-1}, respectively; (ii) maximum biomass concentrations (Figure 5.6B) were 837±8 mg_{dw} L^{-1} and 195.6±0.5 mg_{dw} L^{-1}; (iii) maximum biomass productivities (Figure 5.6C) were 160.7±0.8 mg_{dw} L^{-1} d^{-1} and 35±1 mg_{dw} L^{-1} d^{-1}; and (iv) average biomass productivities (Figure 5.6D) were 110±1 mg_{dw} L^{-1} d^{-1} and 18.4±0.1 mg_{dw} L^{-1} d^{-1}.

5.3.5 Nutrients removal performance in the tubular PBR

Nitrogen (in the forms of NO_3-N and NO_2-N) and phosphorus (in the form of PO_4-P) concentrations within the cultivation period were also monitored in the tubular PBR experiments, allowing the determination of nutrients removal parameters and further comparison with the values obtained in 1 L flasks. The results obtained in both cultivation systems for *C. vulgaris* and *T. obliquus* are presented in Figure 5.7. Although *C. vulgaris* and *T. obliquus* growth in the tubular PBR has shown a different behavior, nitrogen (NO_3-N and NO_2-N) and phosphorus (PO_4-P) removal parameters obtained for both species were higher in the experiments carried out in the tubular PBR (Figure 5.7). These contradictory results support the hypothesis of underestimation of biomass concentration results for *T. obliquus*, since a high nutrients uptake should have been promoted by higher biomass concentrations than those measured from microalgal suspensions. Regarding nitrogen, removal efficiencies achieved for *C. vulgaris* and *T. obliquus* (Figure 5.7A) in the tubular PBR were 91.1% and 88.7%, respectively, whereas values obtained in 1 L flasks did not exceed 43% and 36%, respectively. Moreover, it is noteworthy that average removal rates (Figure 5.7C) obtained in the PBR were almost twice as large (4.6 mg_N L^{-1} d^{-1} and 4.8 mg_N L^{-1} d^{-1} for *C. vulgaris* and *T. obliquus*, respectively) as those achieved in 1 L flasks (2.6 mg_N L^{-1} d^{-1} and 2.1 mg_N L^{-1} d^{-1} for *C. vulgaris* and *T. obliquus*, respectively). In the same way, the mass of

nitrogen removed in the tubular PBR experiments was also higher: 32.2 mg_N L^{-1} by *C. vulgaris* and 33.5 mg_N L^{-1} by *T. obliquus* (Figure 5.7E). Contrary to what was observed in terms of biomass production, nitrogen uptake by *T. obliquus* grown in the tubular PBR was superior to the one achieved by *C. vulgaris.* As for nitrogen removal, higher phosphorus removal efficiencies were observed in microalgal cultures grown in the tubular PBR: according to Figure 5.7B, phosphorus removal efficiencies obtained for *C. vulgaris* and *T. obliquus* in the tubular PBR were 70.1% and 56.0%, respectively, whereas the values obtained in 1 L flasks were 13.9% and 10.2%, respectively. Similarly, higher phosphorus removal rates (Figure 5.7D) and mass removal values (Figure 5.7F) were obtained in cultures grown in the PBR. Regarding average removal rates, the values obtained for *C. vulgaris* and *T. obliquus* were, respectively, 0.74 mg_P L^{-1} d^{-1} and 0.78 mg_P L^{-1} d^{-1} (in the tubular PBR), and 0.18 mg_P L^{-1} d^{-1} and 0.12 mg_P L^{-1} d^{-1} (in 1 L flasks). On the other hand, mass removal values determined for *C. vulgaris* and *T. obliquus* in the tubular PBR were 5.20 mg_P L^{-1} and 5.46 mg_P L^{-1}, respectively, whereas the same values determined in 1 L flasks were 1.30 mg_P L^{-1} and 0.86 mg_P L^{-1}, respectively.

Figure 5.7 – Nitrogen (NO_3-N and NO_2-N) and phosphorus (PO_4-P) removal parameters (A, B, C and D, E, F, respectively) obtained by *C. vulgaris* and *T. obliquus* cultures grown in 1 L flasks and the tubular PBR, using 5% (v/v) of landfill leachate



Author (2021).

Nutrients removal from landfill leachates using microalgae grown in different PBRs has already been reported in the literature. Table 5.4 shows nitrogen and

phosphorus removal efficiencies and removal rates obtained in these studies. In general, values achieved in the tubular PBR were in the same order of magnitude as those obtained in the reference studies. However, nitrogen removal rates obtained by CHANG *et al*. (2018); CHANG *et al*. (2019); TIGHIRI and ERKURT (2019) and TIGHIRI and ERKURT (2019) were much higher, which seems to be associated with the nitrogen source supplied (NH_4-N or NH_4-N + NO_3-N). Microalgae can directly assimilate NH_4-N, while NO_3-N must be primarily reduced to NO_2-N and NH_4-N before being assimilated by microalgal cells (GONÇALVES *et al*., 2017). Accordingly, microalgae preferably uptake NH_4-N. Nitrogen and phosphorus removal efficiencies reported in the literature were also higher than those obtained in this study. However, these results may be related to the higher cultivation periods promoted in the reference studies. Consequently, it is possible to conclude that the remediation potential of the proposed system could be further enhanced. First, the cultivation period could have been extended to increase the removal efficiencies. The use of microalgal-bacterial consortia could also be a promising approach. As demonstrated by TIGHIRI and ERKURT (2019), the presence of bacteria favored microalgal growth and contaminant removal. The same beneficial behavior has also been highlighted for different effluent types (SU *et al*., 2011; GONÇALVES *et al*., 2016; 2017). Besides, further studies on a larger scale are needed. The use of flat reflectors instead of double-parabola ones should also be evaluated, as these devices allow a reduction in manufacturing costs and land area requirements, as noted by GOMES *et al*. (2018).

Regarding the feasibility and sustainability of diluting the effluent to avoid its toxicity, the dilution of landfill leachates (up to 10–20 times) to reduce their inhibitory effects on microalgae is a widely adopted strategy (ZHAO *et al*., 2014; PASKULIAKOVA *et al*., 2016). For example, TAGLIAFERRO *et al*. (2019) observed a reduction in nitrate removal efficiency, from 100% to 87%, with the increase in the landfill leachate concentration from 5% (v/v) to 10% (v/v). The same behavior occurred for biomass productivity, where the best result, 232±8 mg L^{-1} d^{-1}, was obtained for an effluent concentration of just 7.5% (v/v). These results demonstrate that the remediation of landfill leachates using microalgae strongly depends on effluent dilution. However, full-scale landfill leachates remediation demands a lot of water, which could restrict the use of microalgal cultures for this application (PASKULIAKOVA *et al*., 2016). One possible alternative to the use of freshwater may be the use of other effluent types to promote landfill leachates dilution. According to GENTILI (2014), this strategy enables

the achievement of an adequate N:P molar ratio for microalgal growth and minimizes effluent toxicity, while reducing the demand for freshwater. Another alternative to avoid effluent toxicity and promote landfill leachates remediation without previous dilution would be the use of membrane PBRs similar to those reported by CHANG *et al*. (2019). In this study, the authors proposed a scalable tubular PBR embedded with membrane modules (SM-PBR) for the remediation of untreated landfill leachate. The difference between this PBR and a traditional PBR (T-PBR) is in the form of contact between the effluent and the cells: in T-PBRs the cells contact directly with the effluent to be treated, whereas, in the SM-PBR, the fixed membranes separate microalgal cells from the landfill leachate stream. Using both configurations, nutrients removal efficiencies and rates reported with the SM-PBR were higher when compared to the results obtained with a T-PBR. While in the SM-PBR, a mass removal of nitrogen and phosphorus equivalent to 645.4 mg L^{-1} and 43.7 mg L^{-1}, respectively, was achieved, in the T-PBR, these values were only 181.1 mg L^{-1} and 0.83 mg L^{-1}. Despite the improvement in nutrients removal, the high cost associated with a membrane system and the complex operation could make the remediation process unfeasible. Therefore, the effectiveness of the system proposed in this study for the treatment of landfill leachates is highlighted, as it promoted nitrogen and phosphorus removal employing a simpler reactor and at a relatively lower cost when compared to a membrane system similar to the one reported by CHANG *et al*. (2019).

Thus, further studies in the proposed tubular PBR should be carried out to further improve the treatment capacity of landfill leachates and also microalgal biomass productivities. The proposed approaches (e.g., increase of cultivation period, the study of flat optical reflectors and leachate dilution using other effluents) must be considered to ensure the sustainability of process scale-up.

5.4 CONCLUSIONS

In this study, both microalgal strains (*C. vulgaris* e *T. obliquus*) were able to grow in the studied effluent compositions (from 5% (v/v) to 25% (v/v)). However, the landfill leachate exhibited an inhibitory effect on the studied microalgae: the highest biomass productivities were obtained in the experiments carried out with lower effluent concentrations. Similar behavior was observed for nitrogen uptake, as the highest removal efficiencies (65±1% and 56±1% for *C. vulgaris* and *T. obliquus*, respectively)

were obtained for the lowest effluent concentration studied. Taking into account biomass production and nitrogen uptake results, the experiments with 5% (v/v) of landfill leachate were reproduced in a tubular PBR equipped with a double-parabola optical reflector made of anodized aluminum. According to these results, a significant increase was observed in both biomass production and nutrients removal for *C. vulgaris*. Specific growth rates increased from 0.15±0.04 d^{-1}, in 1 L flasks experiments, to 0.61±0.04 d^{-1}, in the tubular PBR experiments. Regarding nitrogen and phosphorus removal efficiencies, there was an increment (49% and 57%, respectively) in the experiments performed in the tubular PBR. The improved performance observed in the PBR experiments may be associated with a better light distribution in this system, which was achieved by a reduction in the optical path of the light to the cells and by the use of an optical reflector. The results obtained in this study demonstrate the potential of the selected microalgae in nutrients removal from landfill leachates. Moreover, the positive results obtained from the use of this tubular PBR constitutes an important advance to improve light penetration within microalgal cultures, opening new perspectives on the use of these types of PBRs for microalgal growth in different wastewaters, especially in highly colored/turbid effluents.

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6 NOVEL TUBULAR PHOTOBIOREACTOR DESIGNS ILLUMINATED BY LEDS TO BOOST MICROALGAL BIOMASS PRODUCTION

This Chapter is based on the research article: “PORTO, B. *et al*. Novel tubular photobioreactor designs illuminated by LEDs to boost microalgal biomass production. Chemical Engineering Journal, under revision." The research was developed at the Associate Laboratory LSRE-LCM, Faculty of Engineering, University of Porto (FEUP), as part of my Sandwich PhD (PDSE/CAPES).

ABSTRACT

This work proposes novel configurations for tubular photobioreactors (PBRs) illuminated with specific and adequate light wavelength provided by LEDs. These configurations enable a uniform (spatial and temporal) light distribution inside the culture vessel and a low heat generation, aiming for a higher light utilization efficiency and photosynthetic activity. The proposed PBRs are characterized by an involute/flat reflective surface around/below a cylindrical borosilicate glass tube that is illuminated by a LED panel located above the collector. Depending on the reflector design, almost all the light arriving at the collector aperture can be collected and available for microalgal cultivation. *C. vulgaris* growth was evaluated as a function of the reflective surface geometry (flat (F), simple double parabola (SP) and traditional double parabola (DP)) and material (anodized aluminum with (MS) and without (R85) protective coating and stainless steel (SS)). *C. vulgaris* growth as a function of time was found to be in good agreement with the actinometric results, where the parabolic reflectors (SP and DP) made of higher specular reflectance materials (R85 and MS) were the most efficient systems. Conversely, energy-based specific growth rates slightly increased as the photon flux decreased, signaling an energetic efficiency loss due to the low transmissibility of microalgal suspensions. Additional tests using two absorber tubes (spaced between 12.5 and 75.0 mm) over the R85-F reflector were also carried out, showing that the distance of 50.0 mm LED to the best compromise between the specific growth rates and biomass productivities per square meter of solar collector. Under these conditions, higher efficiency on the photonic energy usage was attained comparing to the test with a single tube.

Keywords: Biomass production; Microalgae; Illumination efficiency; Tubular photobioreactor; Reflector optics.

6.1 INTRODUCTION

Microalgae are photosynthetic microorganisms found in different aquatic environments due to their simple structure (unicellular or simple multicellular) and basic requirements (light, inorganic carbon, macronutrients, such as N and P, and micronutrients like Fe, Cu, Co, among others) (CHISTI, 2007; MATA *et al*., 2010; MORENO-GARCIA *et al*., 2017; YU *et al*., 2017; AWFA *et al*.). The presence of proteins, carbohydrates, lipids, carotenoids, and polymers in microalgal biomass, as well as their high growth rates, make these organisms an interesting renewable raw material for several industrial sectors (SPOLAORE *et al*., 2006; MATA *et al*., 2010; SINGH and SINGH, 2015; MORENO-GARCIA *et al*., 2017; ELRAYIES, 2018). Thus, microalgae have been widely studied in recent years for different applications (ODJADJARE *et al*., 2017; UDAIYAPPAN *et al*., 2017; RIZWAN *et al*., 2018), including: (i) CO_2 capture; (ii) nutrients uptake from wastewater; and (iii) extraction of interest compounds, which can be converted into biofuels and several bio-products (e.g., cosmetics, nutraceuticals, pharmaceuticals, fertilizers, human food and animal feed). However, the large-scale production of microalgae is still facing several economic constraints (MORENO-GARCIA *et al*., 2017). To reduce process costs and energetic requirements while maximizing biomass production, several studies have been dedicated to developing more efficient cultivation systems (BHARATHIRAJA *et al*., 2015).

Light supply is essential for the photoautotrophic growth of microalgae because it provides the necessary energy to convert the inorganic carbon (usually in CO_2 form) into organic carbon (GONÇALVES *et al*., 2017). In this context, light-related aspects must be taken into account, such as spatial distribution, intensity, light/dark cycle, and light spectral quality (MORENO-GARCIA *et al*., 2017). Although artificial light is one of the main process costs, the use of sunlight can be quite ineffective, as only 50% of sunlight is composed of photosynthetically active radiation (PAR) (WANG *et al*., 2012). In addition, its use is limited to regions where sunlight supply is constant and intense enough to support microalgae growth. On the other hand, in regions with high radiation intensity, photoinhibition can occur when reaching the

maximum sunlight level and consequently, to a drop in the biomass growth rate (CHISTI, 2007). Variation in light intensity and photoperiod cycle also impact the composition of produced biomass (total lipid, pigments, fatty acid profile, cell size, and co-products synthesis), making it difficult to maintain the properties of the final product (MORENO-GARCIA *et al*., 2017). Therefore, light-emitting diodes (LEDs) have often been used in closed photobioreactor (PBR) systems. With narrow light emission spectra, these low energy consumption devices allow an adequate light supply for microalgal cultures and improved control over cell metabolism (HWANG and MAIER, 2019). Therefore, several studies have been focused on developing novel closed PBR designs that allow higher light absorption and transportation efficiencies, a critical parameter in microalgal photosynthesis (BOROWITZKA, 1999; RINKEVICH, 1999; WANG *et al*., 2012; MORENO-GARCIA *et al*., 2017; RAZZAK *et al*., 2017).

Usually, closed PBR designs are based on reducing the light path to cells. Therefore, compared to open systems, closed PBRs represent a more suitable alternative to overcome light availability restrictions. Conversely, the exposition to high irradiances must be avoided. It can cause luminous stress, a decrease in the photosynthetic efficiency (photoinhibition), and, consequently, in microalgal growth (GONÇALVES *et al*., 2017; MORENO-GARCIA *et al*., 2017). Notwithstanding, this issue can be minimized by cells' mixture, moving them cyclically between the light and dark zones of the PBR. Near the surface, it occurs a direct exposure to light (light zone), while in the central part of the PBR, the photosynthetic process is limited by the lack of light (dark zone) (WANG *et al*., 2012; MORENO-GARCIA *et al*., 2017). Therefore, promoting the culture mixing, the time spent between these zones is reduced (light/dark cycle), and light is distributed in a more homogeneous way, which is beneficial for cell growth and light utilization efficiency (BARBOSA *et al*., 2003; WANG *et al*., 2012). Other important factors to consider in closed PBRs' design, aiming at a good light distribution, include the materials' transparency and the illuminated area. Materials such as glass, plexiglass, polyvinylchloride (PVC), acrylic-PVC, and polyethylene have been widely used in PBRs construction, due to their transparency and mechanical properties (WANG *et al*., 2012). On the other hand, the illuminated area can be maximized by optimizing the arrangement of the light source or coupling optical reflectors to the PBR. According to WANG *et al*. (2012), flat-panel and tubular PBRs are the most promising designs for the industrial production of microalgal biomass, primarily due to the wide surface to volume ratio. Another alternative entails the use of internally illuminated

PBRs. SUN, HUANG, *et al*. (2016) obtained a 23.4% increase in biomass production by incorporating transparent rectangular chambers in a flat-plate PBR, providing a secondary light source in light-deficient regions. Another study proposed to dope the planar waveguide with light scattering nanoparticles (SUN, LIAO, *et al*., 2016). As a result, there was an increase in the illuminated volume fractions (21-41%) and an increase of 220% in microalgal biomass production. The same reactor was still optimized by LIAO *et al*. (2017) regarding the distance between waveguides and light intensity. However, these systems are complex and present a significant footprint (WANG *et al*., 2016; LIAO *et al*., 2017). In this context, the design of new PBRs composed of a reflective surface coupled to a cylindrical borosilicate glass tube seems to be a promising alternative, and yet little explored, to expand the lighting area of the reactor and reduce energy losses. Compound parabolic collectors (CPCs) have been used to promote solar-driven advanced oxidation processes, targeting the capture of the UV-visible fraction of the solar spectrum to treat water and air streams (MONTEIRO *et al*., 2015; GOMES *et al*., 2018). Despite the need to improve photosynthetic efficiency in cultivation systems for sustainable production of microalgal biomass, only PORTO *et al*. (2020) and LOPES *et al*. (2020) employed CPCs for this purpose. In the first work (LOPES *et al*., 2020), the use of CPCs to capture solar light showed some limitations in microalgal cultivation due to high UV light intensity exposure (photoinhibition) and temperature. Therefore, the application of CPCs may be beneficial for microalgal biomass production in countries with higher latitude (with lower solar irradiance levels) or to induce environmental stress (high irradiance) on microalgae, aiming to modify their biochemical composition for the accumulation of metabolites with high commercial value. In the second work (PORTO *et al*., 2020), CPCs coupled with LEDs showed positive results for microalgal growth and nutrients uptake from highly colored wastewater (secondary-treated leachate). These conditions allowed a homogenous light distribution in the entire tube perimeter, reducing the light optical path and enhancing microalgal photosynthetic activity. According to GOMES *et al*. (2018), the performance of optical reflectors depends on the material used in their construction, due to variations in specular reflectance. Moreover, the geometry of these surfaces directly interferes with the light distribution and illuminated area of the absorber tube.

Therefore, this work aims to compare the efficiency of PBRs, illuminated with specific and adequate light wavelength provided by LEDs, composed by different reflectors geometries (flat (F), single double parabola (SP), and traditional double

parabola (DP)) and reflective surface materials (anodized aluminum with (MS) and without (R85) protective coating and stainless steel (SS)), targeting higher biomass yields and significantly smaller footprints. The efficiency of the different PBR designs will be compared through the determination of the: (i) optical concentration ratio (CR_O) and radiant power incident (RP_i) on the absorber tube, using ferrioxalate actinometry; and (ii) biomass (*Chlorella vulgaris*) yields.

6.2 MATERIALS AND METHODS

The materials, analytical methods and experimental units employed in the current study are described in Chapter 3. Section 3.2.3 presents in detail the experimental setup and operational conditions employed.

A modified version of the OECD Test medium, described by SANTOS *et al*. (2019), was used as a growth medium for *C. vulgaris* CCAP 211/11B cultivation. The methodologies employed in the actinometric tests (Section 3.2.3.2) and for the microalgae growth monitoring (Section 3.3.2), as well as the growth parameters in function of time and accumulated energy (Section 3.3.2.1), also can be assessed in Chapter 3.

6.3 RESULTS AND DISCUSSION

6.3.1 Actinometric measurements

6.3.1.1 One absorber tube

Actinometric measurements for the different reflectors' materials and geometries were performed under the same experimental conditions, using a LED panel (34 W of rated power and 8.5 W m^{-2} in the range 380-580 nm reaching the collector aperture) as a light source. The obtained results are presented in Table 6.1.

Table 6.1 – Illumination efficiency parameters (*RP*, RP_i, and CR_0) obtained from ferrioxalate actinometric tests, total accumulated energy ($Q_{380-580nm}$) and growth kinetics parameters (μ and μ') along with fitting intervals of time (Δt) and accumulated energy ($\Delta Q_{380-580nm}$) obtained for *C. vulgaris* cultures in different tubular PBR configurations.

Absorber tube		**Reflective surface**		**RP**[c] **(J s^{-1})**	**RP$_i$**[d] **(J s^{-1})**	**CR$_0$**[e]	**Q$_{380-580nm}$**[f] **(kJ L^{-1})**	**Growth over time**		**Growth over energy**	
Number	**d**[a] **(mm)**	**Type**	**Aa**[b] **(m²)**					**Δt (d)**	**μ (d^{-1})**	**ΔQ$_{380-580nm}$ (kJ L^{-1})**	**μ' (L kJ^{-1})**
1	n.a.	R85-DP	0.025	0.152±0.004	0.167±0.005	0.79±0.02	191	0-5	0.230±0.005	0-131	0.0088±0.0002
	n.a.	MS-DP	0.025	0.151±0.005	0.166±0.006	0.78±0.03	190	0-5	0.215±0.008	0-130	0.0083±0.0003
	n.a.	SS-DP	0.025	0.106±0.004	0.116±0.004	0.55±0.02	133	0-5	0.182±0.009	0-91.4	0.0100±0.0005
	n.a.	R85-SP	0.020	0.128±0.003	0.141±0.004	0.67±0.02	161	0-5	0.205±0.009	0-110	0.0093±0.0005
	n.a.	MS-SP	0.020	0.127±0.006	0.140±0.006	0.66±0.03	160	0-5	0.203±0.003	0-109	0.0093±0.0002
	n.a.	SS-SP	0.020	0.104±0.002	0.114±0.002	0.54±0.01	131	0-5	0.18±0.02	0-90.0	0.0102±0.0007
	n.a.	R85-F	0.016	0.106±0.005	0.117±0.006	0.55±0.03	133	0-5	0.177±0.006	0-91.4	0.0097±0.0004
	n.a.	MS-F	0.016	0.107±0.004	0.118±0.004	0.56±0.02	134	0-5	0.171±0.007	0-92.3	0.0093±0.0004
	n.a.	SS-F	0.016	0.100±0.004	0.110±0.005	0.52±0.02	126	0-5	0.170±0.005	0-86.2	0.0099±0.0003
	n.a.	No-RS	-	0.082±0.001	0.090±0.001	0.425±0.006	103	0-5	0.156±0.004	0-70.7	0.0111±0.0003
2	12.5	R85-F	0.020	0.192±0.006	0.211±0.007	0.50±0.03	241	0-5	0.150±0.002	0-81.2	0.00925±0.0009
	25.0	R85-F	0.024	0.192±0.004	0.211±0.005	0.50±0.02	241	0-5	0.152±0.003	0-81.2	0.0094±0.0002
	50.0	R85-F	0.032	0.212±0.005	0.233±0.005	0.55±0.03	267	0-5	0.202±0.003	0-89.6	0.0113±0.0002
	75.0	R85-F	0.040	0.215±0.004	0.237±0.005	0.56±0.02	270	0-5	0.186±0.004	0-90.9	0.0103±0.0002

[a] Distance between the absorber tubes;
[b] Reflector aperture area;
[c] Radiant power reaching the reactional medium;
[d] Radiant power incident on the absorber tube;
[e] Optical concentration ratio;
[f] Accumulated energy in the range of 380-580 nm during the entire cultivation period (seven days).

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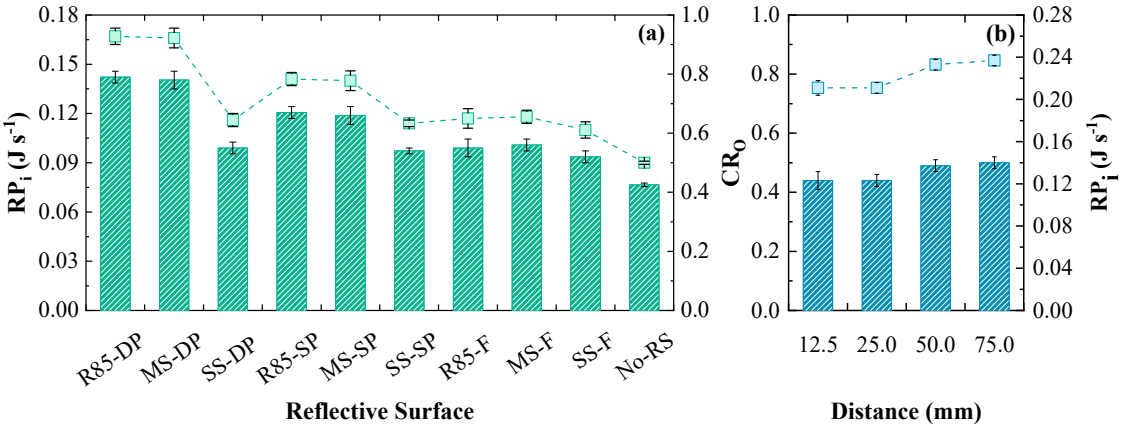
As expected, coupling a reflective surface with the borosilicate tube boosted the incident radiant power (RP_i) and, consequently, the optical concentration ratio (CR_O) of the PBR, as shown in Figure 6.1a. Therefore, the lowest RP_i value (0.090±0.001 J s^{-1}) was obtained for the PBR without reflector (No-RS). On the other hand, the PBR with the R85-DP surface presented the highest RP_i value (0.167±0.005 J s^{-1}), followed by the MS-DP (0.166±0.006 J s^{-1}), R85-SP (0.141±0.004 J s^{-1}) and MS-SP (0.140±0.006 J s^{-1}). Between the studied parabolic reflectors, the SS-SP presented the lowest RP_i, only 0.114±0.002 J s^{-1}, which represents a 1.4 fold decrease when compared to R85-DP. Still, the value achieved with SS-SP was 1.3 times higher than the one with No-RS. Considering the flat geometry reflectors, the RP_i values were very similar for the three materials, with slightly higher values (about 6-7%) for R85 and MS when compared to SS. These results indicate that the reflectors' material has a more prominent role in the parabolic reflectors.

Figure 6.1 – Radiant power incident (RP_i, points) and optical concentration ratio (CR_O, bars) for the different tubular PBR configurations: (a) one absorber tube over different reflective surfaces; (b) two absorber tubes over R85-F reflector at different distances. Error bars correspond to the standard error obtained from the linear model fitting used to estimate RP_i and CR_O.



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The similarity between the RP_i values for R85 and MS materials is associated with the average specular reflectance of these materials in the visible light wavelengths (emission range of the LED panel) that is very close: according to GOMES *et al.* (2018), 72.9% and 73.4%, respectively. On the other hand, the average specular reflectance for SS in the visible region is only 46.8%, leading to a substantial decrease in RP_i values (ca. 30%) within the parabolic reflectors.

Regarding the geometries, considering the same reflective material, the RP_i values are closely linked to the illuminated area of the absorber tube. The larger the illuminated area, the higher the RP_i value, and, therefore, the parabolic surfaces stand out over the F geometry, as can be observed in Figure 6.1a.

Bearing in mind that microalgae use light as an energy source, it is plausible to state that the analysis of the irradiance incident on the absorber tube can be a good predictive tool of the culture performance in the tubular PBR with different configurations. As expected, for the same irradiation time, the increment of accumulated energy accompanies the increase in radiant power for the different tested configurations, ranging from 126 to 191 kJ L^{-1} (Table 6.1). In this way, it is possible to assume that the increase in accumulated energy will benefit the photosynthetic efficiency of the process, and, with that, a higher cell production will be obtained.

The performance of the different reflective surfaces can also be assessed through the CR_O, which corresponds to the amount of irradiance emitted by the light source that reaches the absorber tube of the PBR. The efficiency of light reflection by the tested reflective surfaces increases in the following sequence (Figure 6.1a and Table C1 from Appendix C): SS-F<SS-SP<MS-F≈R85-F≈SS-DP<MS-SP<R85-SP<MS-DP<R85-DP. Therefore, the maximum use of the light energy emitted by the LEDs lamps was achieved for the PBR with the R85-DP and MS-DP reflectors, corresponding to about 79-78% of the irradiance emitted by the LED panel. In contrast, the PBR without any reflective surface (No-RS) features the lowest efficiency. In this configuration, only 42% of the irradiance reaches the external walls of the absorber tube, which makes sense since only its superior circumference perimeter is effectively illuminated.

6.3.1.2 Two absorber tubes using a flat reflector

Actinometric tests were also performed using two absorber tubes over the R85-F reflector, with different distances between them (Figure 6.1b and Table B from Annex B). The minimal distance of 12.5 mm was adopted to be easier the replacement of tubes and to prevent the accumulation of dirt, which would reduce the light reflection efficiency and, consequently, the amount of light reaching the absorber tube. The higher distance was selected based on the results reported by GOMES *et al*. (2018), where illumination efficiency began to decay for distances above 50 mm.

The RP_i value remained very similar as the distance between the tubes increased from 12.5 to 25.0 mm (Figure 6.1b), corresponding to an increment of the reflective area from 0.020 to 0.024 m^2. When the tubes are near each other, a shadow effect can exist between them, in addition to a reduced reflector area for light reflection. Thus, as the distance between the tubes increases, this effect is expected to decrease until it becomes null. In this way, for distances >50.0 mm, the shadow effect is minimized, resulting in a higher RP_i value. However, GOMES *et al*. (2018) observed a decrease in the RP_i value for the distances of 75.0 mm and 100 mm (aperture areas of 0.040 and 0.048 m^2). This was attributed to the fixed position of the lamp inside a Suntest® chamber, where the experiments were carried out. According to TAYLOR (2000), light irradiance decreases inversely with the distance and with the cosine law related to the incident angle between the light source and the receiving surface (reflective surface). Such behavior was not detected in the present work since a LED panel (1200 × 300 mm) was used above the PBR, allowing a uniform light distribution over the entire collectors' surface area.

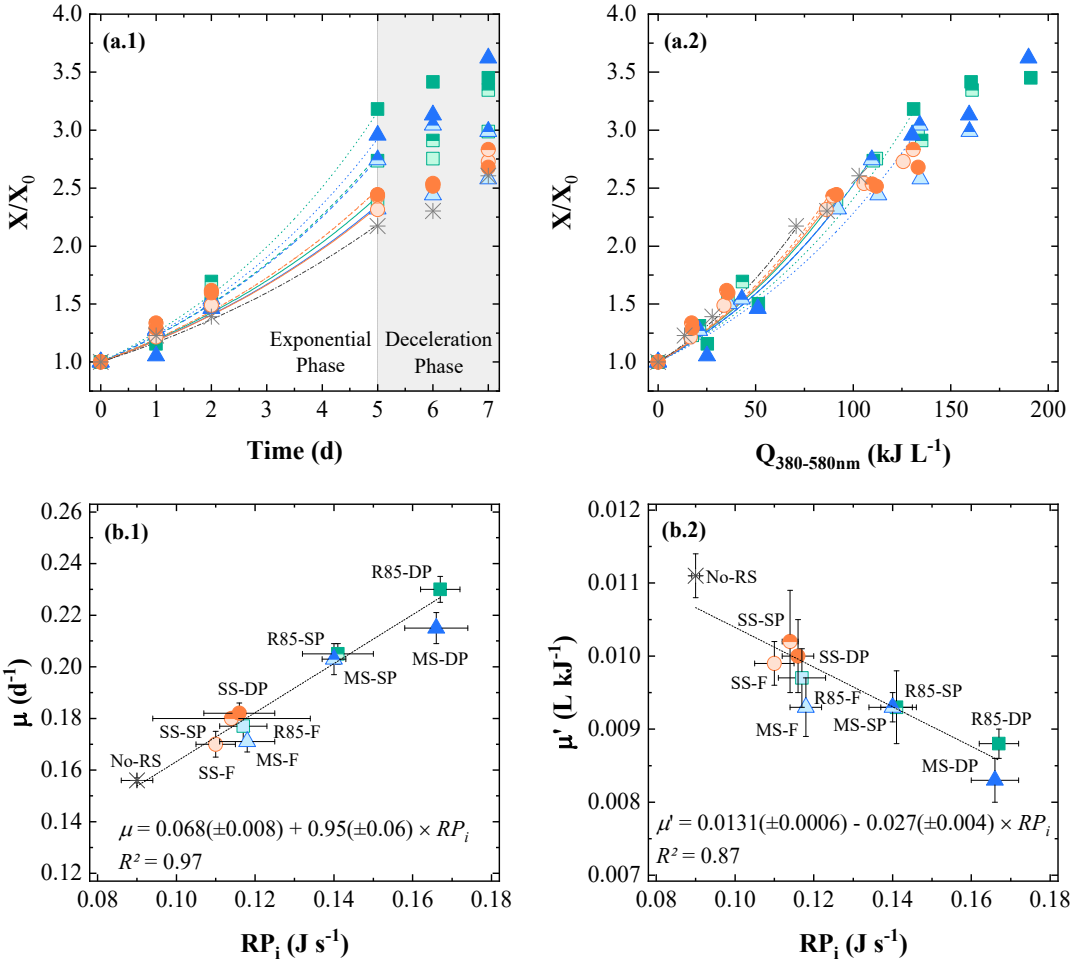
As expected, the RP_i value for a distance of 50 or 75 mm is twice as superior to the one with the same reflector (R85-F) using only one tube (Figure 6.1a), since the illuminated volume doubled when using two absorber tubes.

6.3.2 Microalgal growth

6.3.2.1 Reflective surface effect

Microalgal growth curves in the different PBRs, with and without reflectors, are shown in Figure 6.2 as a function of time (Figure 6.2a.1) and accumulated energy (Figure 6.2a.2). The results evidence a short adaptation phase (one day) followed by an exponential growth phase that lasted approximately until the fifth day for all assays. From this day forward, the beginning of the deceleration phase was observed. Figure 6.2a.1 shows a more prominent growth in cultures grown using the parabolic surfaces, mainly after the second day. On the other hand, the growth curves based on the accumulated energy are nearly superimposed (Figure 6.2a.2), with slightly lower performances for the parabolic reflectors. These results indicate that almost all the absorbed energy was used for biomass production.

Figure 6.2 – Representation of the (a) normalized biomass dry weight concentration (points) along with the respective *C. vulgaris* growth fitting curves (lines) as a function of time (.1) and accumulated energy (.2), and (b) specific growth rates based on time (.1) and accumulated energy (.2) as a function of RP_i, for the assays performed with tubular PBRs composed by one absorber tube over different reflectors: R85-DP (■), MS-DP (▲), SS-DP (●), R85-SP (▬), MS-SP (△), SS-SP (◓), R85-F (□), MS-F (△), SS-F (○), and No-RS (✳). Error bars correspond to the standard error obtained from the linear and non-linear model fitting used to estimate the RP_i and specific growth rates, respectively.



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Such behavior can also be verified through the specific growth rates obtained for *C. vulgaris* cultures, as shown in Table 6.1. The estimated linear regression parameters (and respective errors) and model quality fit the experimental data. ANOVA analysis of the obtained specific growth rates can also be found in Appendix B (Tables B1 and B2).

Considering all PBR configurations, the specific growth rates as a function of time (μ, d^{-1}) present a good linear correlation with RP_i ($R^2 = 0.97$) (Figure 6.2b.1). The highest μ values were obtained for the parabolic geometries (DP > SP) made of aluminum-based materials (R85 > MS): 0.230±0.005 d^{-1} (R85-DP) > 0.215±0.008 d^{-1}

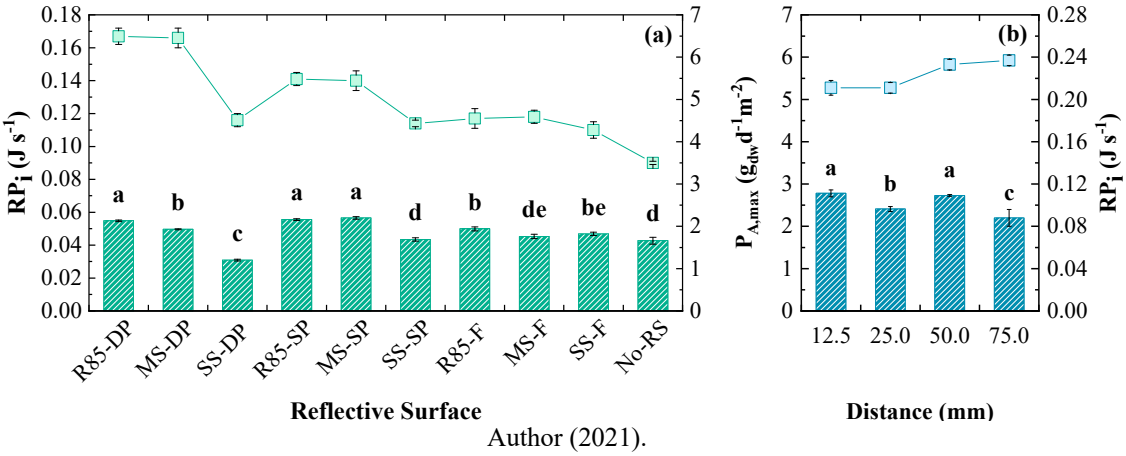
(MS-DP) > 0.205±0.009 d^{-1} (R85-SP) > 0.203±0.003 d^{-1} (MS-SP). It is also possible to affirm that the cultures grown in PBRs composed of aluminum flat reflectors (F) has a 20-23% decrease in the time-based specific growth rate (μ, d^{-1}) when compared with the traditional parabolic collectors (DP). This is mainly related to the gain in the illuminated area of the absorber tube when located in the focus of a double parabola. Regarding the materials used, a higher/similar performance was already expected for reflectors made of R85 and MS, in contrast with the SS reflectors. This is due to the remarkable/similar reflectance properties in the visible spectrum emission range of the aluminum materials in comparison with SS, which features lower specular reflectance. Furthermore, as the complexity of the geometry decreases from DP to F, the effect of the material's reflectance becomes smaller. This can be seen by the increasing proximity of the specific growth rates, which indicates that the photonic flux entering the absorber tube over flat plates is mainly provided by direct radiation. Therefore, within the studied conditions, it can be concluded that the increase in the illuminated area and the use of materials with a higher reflectance had a beneficial effect *C. vulgaris* growth over time, as disclosed in Table 6.1 and Figures 6.2a.1 and 6.2b.1.

On the other hand, Figure 6.2b.2 shows that the specific growth rates based on the accumulated energy (μ', L kJ^{-1}) present a slightly downward trend as the RP_i increases (R^2 = 0.87), with F geometries being 9-11% more efficient than DP ones, among the aluminum-made reflectors. This small decrease in the μ' values pointed out a slightly energetic efficiency loss as the photon flux reaching the absorber tube increased, which is in compliance with the low transmissibility of microalgal suspensions. Notwithstanding, it is also possible to assume that, under the employed conditions, there was no inhibition of microalgal growth due to excess of light (photo-inhibition). According to DEGEN *et al*. (2001), the light saturation point for *C. vulgaris* is about 250 μmol m^{-2} s^{-1}, well above (about 3.6-fold) the value employed in this work.

Similar behavior to the time-based specific growth rates was also observed for the maximum biomass productivities per square meter of solar collector (Figure 6.3a). It is possible to verify that the drop in the maximum areal productivity is also associated with the reduction in the RP_i values, within the same geometry and, as such, the same reflector aperture area. Furthermore, it is noticed that the values of maximum areal productivities are very close to each other (with no significant difference – $p>0.05$) among the tests with lower RP_i, namely: (i) F geometry; (ii) SS material, excepting the reflector with DP geometry; and (iii) without reflective surface (No-RS). For the flat

geometry, the reflector material practically did not affect biomass production because almost all light that reaches the absorber tube is given by direct exposure and not by reflection. Thus, it is possible to verify that there is no significant difference ($p>0.05$) in the maximum areal productivity between the tests performed with the R85-F and SS-F reflectors, as well as between the tests performed with the MS-F and SS-F reflectors. The MS-F test also showed no significant difference ($p>0.05$) in relation to other tests featuring low RP_i values: SS-SP and No-RS. On the other hand, there was a slight general gain in the maximum areal productivity for the SP and DP surfaces (which provide the absorber tube with the highest illuminated areas), with R85 and MS materials (with the highest reflectivity), reaching an average value of 2.1 g m^{-2} d^{-1}, which is only 14% higher than the average value obtained for the flat reflectors. However, according to the technical-economic study presented by GOMES *et al.* (2018), the construction of 100 m^2 of solar collectors constituted by DP reflectors can be around 30-31% more expensive than 100 m^2 of a flat-based solar plant, making the application of the last configuration more appealing for microalgal production.

Figure 6.3 – Maximum areal biomass productivities ($P_{A,max}$, bars) and radiant power incident (RP_i, points) for the different tubular PBR configurations: (a) one absorber tube over different reflective surfaces; (b) two absorber tubes over R85-F reflector at different distances. Error bars correspond to the standard deviation of the mean obtained from triplicate samples. The letters *a*, *b*, *c*, *d* and *e* (shown above the bars) represent the statistical significance of the results, according to the Turkey HSD test: mean values sharing at least one common letter are not statistically different ($p>0.05$).



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6.3.2.2 Effect of the distance between two absorber tubes using a flat reflector

C. vulgaris production was also evaluated in the tubular PBR with two absorber tubes using a flat reflector, under the same experimental conditions as the tests

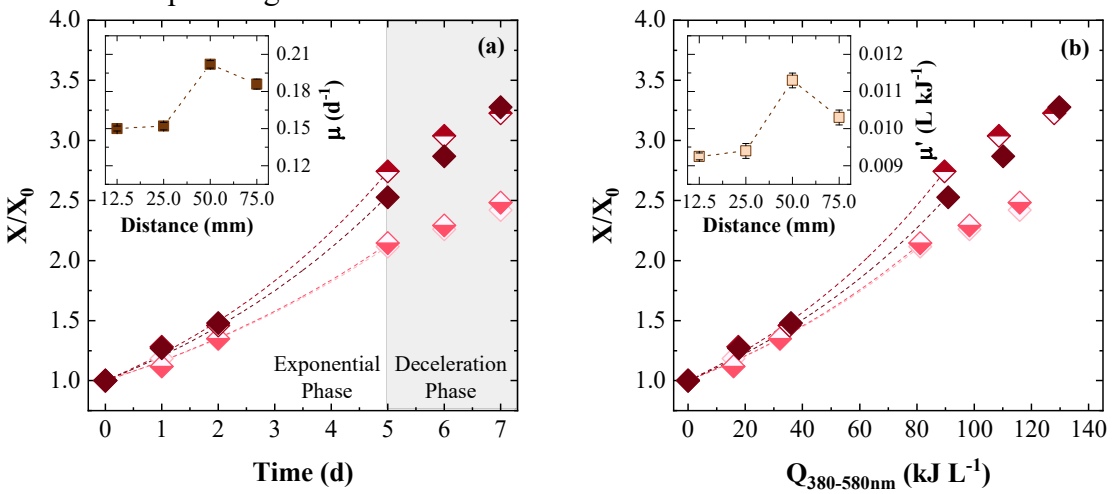
carried out with one absorber tube. On real-scale applications, the large land areas requirements for microalgal cultures are one of the main limiting factors (VASUMATHI *et al*., 2012; UDAIYAPPAN *et al*., 2017; ELRAYIES, 2018; GOMES *et al*., 2018). Therefore, the use of flat reflectors with the absorber tubes in parallel can be a good option to reduce land area requirements to implement such cultivation systems. Besides, flat reflectors require less construction and maintenance complexity, which represents a cost reduction in the cultivation units.

The reaction volume was doubled for these experiments, and two peristaltic pumps (at a flow rate of 50 L h-1) were used, thus maintaining the ratio between light and dark zones. The reflector R85-F was selected for these tests due to the superior results achieved in terms of illumination efficiency, biomass areal productivity, and specific growth rate compared to the other two materials studied.

Microalgal growth curves as a function of time and accumulated energy in the assays with two absorber tubes are shown in Figures 6.4a and 6.4b, respectively. As a function of time, until the second day of cultivation, microalgal growth curves overlap. From this day forward, superior performance was observed in the experiments where the tubes were separated by the distances of 50.0 and 75.0 mm. These observations are in agreement with the actinometric results (Table 6.1). For distances below 50.0 mm, a shorter exponential phase and a more pronounced deceleration process are spotted, which is related to the lower amount of accumulated energy (Figure 6.4b).

When microalgal growth curves are compared as a function of the accumulated energy, the overlapping of growth curves is even more evident. Moreover, it is also possible to verify a more prominent biomass production when using tube distances of 50.0 and 75.0 mm. The same kind of conclusions can be withdrawn from the values of specific growth rates (Table 6.1 and Figure 6.4: inset) as a function of the time (μ, d^{-1}) and accumulated energy (μ', L kJ^{-1}). The estimated linear regression parameters (and respective errors) and quality of the model fit the experimental data and ANOVA analysis of the obtained specific growth rates, also can be found in Appendix B (Tables B3 and B4).

Figure 6.4 – Representation of the (a) normalized biomass dry weight concentration (points) along with the respective *C. vulgaris* growth fitting curves (lines) as a function of time (a) and accumulated energy (b), as well as the specific growth rates based on time (a: inset) and accumulated energy (b: inset) for assays performed with tubular PBRs composed of two absorber tubes over R85-F reflector separated by different distances: 12.5 mm (◇), 25.0 mm (◆), 50.0 mm (◆), and 75.0 mm (◆). Error bars correspond to the standard error obtained from the non-linear model fitting used to estimate the specific growth rates.



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The radiant power increases as the absorber tubes are further apart due to the higher reflective surface area and the lower shadow effects. For distances between tubes higher than 50.0 mm, the increment on the reflective surface area does not substantially affect the radiant power (Table 6.1) because part of the reflected light is not oriented to the absorber tubes. Hence, considering the biomass production data, the distance between tubes of 50.0 mm is more favorable without considering the economic aspects involved.

When comparing the time-based specific growth rate (μ, d^{-1}) (shown in Table B1), there was a significant difference ($p<0.05$) between using one absorber tube or two 50.0 mm apart, considering the same reflector (R85-F) and the same illuminated volume to total volume ratio. The values achieved for the respective assays were 0.177±0.006 d^{-1} and 0.202±0.003 d^{-1}. Slightly higher values for the two tubes can also be observed through microalgal growth curves (Figure 6.2a.1 and 6.4a), indicating a higher efficiency in using the photonic energy reaching the PBR. This affirmation is supported by the energy-based specific growth rate improvement: from 0.0097±0.0004 to 0.0113±0.0002 L kJ^{-1}.

In terms of areal productivity (Figure 6.3b), maximum values of 2.78±0.08 and 2.73±0.02 g_{dw} d^{-1} m^{-2} were obtained for distances between tubes of 12.5 and 50.0 mm,

respectively. Although the test with 50.0 mm spacing showed a higher specific growth rate (Figure 6.4a), the productivity per occupied area was not significantly different ($p>0.05$) than the one obtained in the test with 12.5 mm of distance because its reflective surface area is lower. These results were also higher than the values obtained in the tests with one absorber tube, which ranged from 1.20±0.02 to 2.20±0.03 g_{dw} d^{-1} m^{-2}. Thus, it is possible to affirm that using F reflectors with spacing between 12.5 mm and 50.0 mm is more advantageous for microalgal production than the other studied conditions. Moreover, these reflectors can also represent an advantage concerning the costs associated with the manufacture of the reflective surfaces, deployment area, and maintenance of the culture system.

6.4 CONCLUSIONS

In this study, *C. vulgaris* was able to grow in all studied tubular PBR configurations, with no inhibition in cell growth due to excessive incident light (photoinhibition). Nonetheless, superior performances were attained for microalgal cultures growing in the cylindrical borosilicate glass tube assembled with a reflective surface (in opposition to cultures grown in the PBR devoid of a reflector). This indicates that the increase in the absorber tube illuminated area was beneficial to the process in terms of cell production, due to higher light availability. Comparing the different geometries tested (F, SP, and DP), *C. vulgaris* specific growth rates as a function of time were higher when parabolic surfaces were used, which is explained by a better distribution of light across the cultures. Regarding the reflectors' materials (SS, MS and R85), it was found that those featuring the highest average specular reflectance (R85 and MS) LED to the best results in terms of microalgal biomass production over time. However, this effect is most prominent when the DP geometry was employed. When F reflectors are used, the material's reflective properties ends up not affecting the results in such an expressive way due to the lower reflection efficiency. Accordingly, through the actinometric tests, it was observed that as the tube illuminated area was expanded and materials with greater reflectance were used, the greater the radiant power incident on the absorber tube, and the greater the photosynthetic efficiency as a function of time. Thus, within the limits of the current study, it was demonstrated that the increase in the lighting efficiency parameters culminated in higher growth rates. In contrast, energy-based specific growth rates slightly increased as the photon flux provided by the

different reflective surfaces decreased, pointing out to an energetic efficiency loss due to the low transmissibility of microalgal suspensions.

Further tests using two absorber tubes disposed in parallel over the R85-F reflective surface showed that the distance of 50.0 mm LED to the best compromise between specific growth rates and maximum biomass productivity per square meter of the reflector. Under these conditions, higher efficiency on the photonic energy usage and greater maximum areal productivity were attained compared to the tests with one absorber tube, even when the most efficient R85-DP reflector was used around it. Taking into account that the area occupied by a certain system can be a limiting factor when a full-scale facility is envisioned, the use of flat reflectors as an alternative to the traditional R85-DP collectors can be a more interesting approach. Besides, due to the constructive complexity of the parabolic reflectors, higher construction costs are expected for these reflectors.

In short, it is concluded that the use of reflectors positively affects the growth performance of *C. vulgaris* cultures inside the absorber tube due to the improvement of light distribution across it. Also, flat-based solar collectors showed to be a promising alternative, in terms of occupied area and associated costs, when considering a scale expansion.

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7 FINAL REMARKS

7.1 CONCLUSIONS

The present thesis was essentially divided into two major stages, namely: (i) assessment of the application of two effluents (paper industry effluent and landfill leachate) at different dilutions as culture medium for microalgal cultures (*C. vulgaris* and *T. obliquus*); and (ii) assessment of different configurations of an innovative tubular PBR, regarding its reflective surface geometry and material.

Based on the data presented, it is possible to conclude that under the conditions studied, both microalgae were able to grow in the media containing the diluted effluents. Thus, effluents from the paper industry and landfill leachate can represent a viable source of low-cost nutrients, in addition, to reduce the demand for fresh water in the process. Furthermore, the microalgae were able to reduce phosphorus and nitrogen concentrations, which is crucial for coupling microalgal biomass production with effluent remediation. However, in general, it was observed that the increase in their concentration in the medium had an inhibitory effect on the growth of microalgae.

To overcome this gap, this thesis also investigated the use of an innovative PBR. In the cultivation with the landfill leachate using the tubular PBR, a significant improvement in *C. vulgaris* biomass productivities and nitrogen removal efficiencies were observed. This enhancement is associated with the known ability of this reactor to improve light distribution within the culture medium.

In the modified OECD medium, where different configurations of the tubular PBR were evaluated, the results obtained make clear that changes in geometry and reflective surfaces materials directly impact the use of light energy. The reflectors with parabolic geometries made of materials with higher specular reflectance (R85 and MS) allowed a higher illuminated area of the absorber tube and photon flux to reach the culture medium. In this way, considering that light is the main source of energy for microalgal growth, it is assumed that the increase in accumulated energy would benefit the photosynthetic efficiency of the process and higher cell production. In fact, the highest specific growth rate of *C. vulgaris* as a time function was in good agreement with the illumination efficiency parameters obtained in the actinometric tests. However, it should be emphasized that if the light irradiance is greater than the saturation point of the cells, the photosynthetic process and, consequently, the growth of microalgae may

be inhibited. This photoinhibition was not observed in this study, probably due to the use of a low luminous intensity LED panel as a light source.

On the other hand, despite the beneficial effect observed on cell growth with an increase in accumulated energy, the energy-based results show a loss of efficiency in this regard. This means that not all the energy reaching the absorber tube is used by cells for their growth. In this context, the renewal of part of the microalgal suspension can be an interesting alternative, since the low transmissibility of microalgal suspensions hinders the passage of light through the cultures when reaching a high density.

The tubular PBR with flat-based solar collectors represents a promising alternative for the implementation of the system on a larger scale. Often, the costs and complexity of installation, maintenance and operation, as well as the demand for large land areas to be implemented, can end up making the project unfeasible. However, it is possible to obtain an optimized relationship between production and area occupied by the system in this case. Systems with R85-F reflectors, despite resulting in lower specific growth rates compared to the traditional R85-DP collectors, show a better productivity per unit of the occupied area. This is due to the fact that, for the same occupied area, it is possible to associate more absorber tubes when using flat reflectors. Besides, due to the constructive complexity of the parabolic reflectors, higher construction and maintenance costs are expected for these reflectors.

7.2 SUGGESTIONS FOR FUTURE WORKS

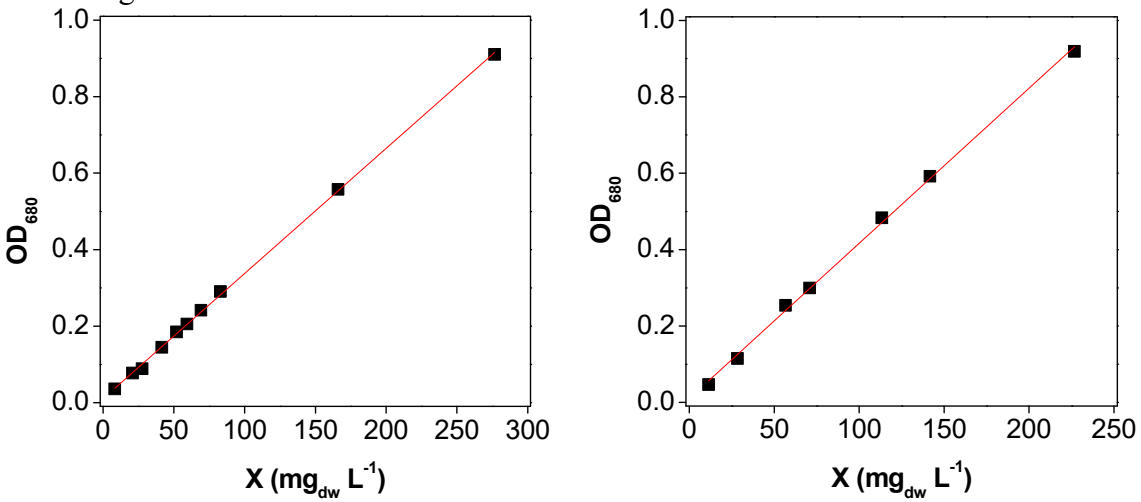
Several challenges must be overcome for a sustainable application of the proposed technology. In this context, further investigations must be carried out:

- Identification of compounds present in the effluents that may have a toxic effect on the microalgal cells and how this influences biomass production and composition.
- Promote studies in stress conditions (nutritional and luminous) in order to direct microalgal metabolism to produce high added-value products.
- Carry out flocculation tests, mainly in view of the self-flocculating properties of some species, such as *T. obliquus*. As observed, the formation of flakes can have a negative effect on the production of biomass. However, this phenomenon can facilitate biomass recovery (harvest), thus minimizing its costs.

- Evaluate the chemical composition of the microalgae biomass, which will allow inferring its commercial value. For application on an industrial scale, it is essential to carry out an economic balance, which will determine the configuration of the cultivation system itself.
- Develop mathematical models that correctly represent the behavior of cultures, allowing the transposition of bench tests to a larger scale.

APPENDIX A – *C. vulgaris* (a) and *T. obliquus* (b) calibration curves: linear regression parameters, model fit, variance analysis and Turkey test

Figure A1 – *C. vulgaris* (A) and *T. obliquus* (B) calibration curves relating biomass concentration with OD_{680} measurements. The straight lines (—) represent the linear regression lines obtained for OD_{680} as a function of biomass concentration.



Author (2021).

Table A1 – Linear regression parameters and quality of the model fit (given by the adjusted R-Square) estimated for *C. vulgaris* and *T. obliquus* calibration curves relating OD_{680} measurements with biomass concentration

Microalgae	Linear regression coefficients				Adj. R-Square
	Intercept (b)		Slope (a)		
	Value	Standard Error	Value	Standard Error	
C. vulgaris	0.010	0.008	0.00406	0.00007	0.998
T. obliquus	0.010	0.003	0.003	0.00003	0.999

Author (2021).

Table A2 – Analysis of variance (ANOVA) results obtained for *C. vulgaris* and *T. obliquus* calibration curves relating OD_{680} measurements with biomass concentration

Microalgae	Source	DF[1]	Sum of Squares	Mean Square	F Value	Prob>F
C. vulgaris	Model	1	0.550	0.550	3752	2.19E-08
	Error	5	0.0007	0.0001		
	Total	6	0.551			
T. obliquus	Model	1	0.646	0.646	15979	1.72E-14
	Error	8	0.0003	0.00004		
	Total	9	0.646			

[1] Degrees of freedom.

Author (2021).

APPENDIX B – Kinetics parameters (μ and μ') obtained from the pseudo-first-order kinetic model adjusted to the experimental data during the exponential *C. vulgaris* growth phase, in the tests carried out with tubular PBRs. Analysis of variance (ANOVA) results and quality of the model fit.

Table B1 – Kinetics parameters (μ and μ') and quality of the model fit ((i) standard error (S); (ii) residual variance (S^2_R); and (iii) coefficient of determination (R^2)) obtained from the pseudo-first-order kinetic model adjusted to the experimental data during the exponential *C. vulgaris* growth phase, in the tests carried out with tubular PBRs composed of one absorber tube over different reflector surfaces.

Reflective Surface	Kinetics parameters							
	Growth over time				Growth over energy			
	μ (d^{-1})	S_μ	S^2_R ($m^2\ L^{-2}$)	R^2	μ' ($L\ kJ^{-1}$)	$S_{\mu'}$	S^2_R ($m^2\ L^{-2}$)	R^2
R85-DP	0.230	0.005	0.017	0.994	0.0088	0.0002	0.015	0.996
MS-DP	0.215	0.008	0.041	0.983	0.0083	0.0003	0.051	0.986
SS-DP	0.182	0.009	0.035	0.970	0.0100	0.0005	0.013	0.966
R85-SP	0.205	0.009	0.045	0.974	0.0093	0.0005	0.023	0.970
MS-SP	0.203	0.003	0.004	0.998	0.0093	0.0002	0.006	0.996
SS-SP	0.18	0.02	0.053	0.953	0.0102	0.0007	0.036	0.947
R85-F	0.177	0.006	0.012	0.989	0.0097	0.0004	0.011	0.987
MS-F	0.171	0.007	0.019	0.980	0.0093	0.0004	0.059	0.977
SS-F	0.170	0.005	0.008	0.991	0.0099	0.0003	0.039	0.989
No-RS	0.156	0.004	0.004	0.994	0.0111	0.0003	0.007	0.991

Author (2021).

Table B2 – Analysis of variance (ANOVA) results, obtained for the regression models established to describe the exponential growth phase of *C. vulgaris*, in the tests carried out with tubular PBRs composed of one absorber tube over different reflector surfaces.

(to be continued)

Reflective Surface	ANOVA									
	Source	DF[1]	Growth over time				Growth over energy			
			Sum of Squares	Mean Square	F Value	Prob>F	Sum of Squares	Mean Square	F Value	Prob>F
R85-DP	Regression	1	14.71	14.70	2521	1	14.71	14.71	3329	1
	Residual	3	0.017	0.006			0.013	0.004		
	Total	4	14.72				14.72			
MS-DP	Regression	1	12.94	12.94	935.7	1	12.94	12.94	1078	1
	Residual	3	0.041	0.014			0.036	0.012		
	Total	4	12.98				12.98			
SS-DP	Regression	1	11.15	11.15	966.4	1	11.15	11.15	846.4	1
	Residual	3	0.035	0.012			0.040	0.013		
	Total	4	11.15				11.19			
R85-SP	Regression	1	13.02	13.02	876.9	1	13.02	13.02	767.1	1
	Residual	3	0.045	0.015			0.051	0.017		
	Total	4	13.07				13.07			
MS-SP	Regression	1	12.52	12.52	8745	1	12.52	12.52	5833	1
	Residual	3	0.004	0.001			0.006	0.002		
	Total	4	12.53				12.53			
SS-SP	Regression	1	11.26	11.26	637.3	1	11.25	11.25	571.4	1
	Residual	3	0.053	0.018			0.059	0.020		
	Total	4	11.31				11.31			
R85-F	Regression	1	10.58	10.58	2653	1	10.58	10.58	2163	1
	Residual	3	0.012	0.004			0.015	0.005		
	Total	4	10.60				10.60			

(conclusion)

MS-F	Regression	1	10.26	10.26	1607	1	10.26	10.26	1364	1
	Residual	3	0.019	0.006			0.023	0.008		
	Total	4	10.28				10.28			
SS-F	Regression	1	10.04	10.04	3528	1	10.04	10.04	2809	1
	Residual	3	0.009	0.003			0.011	0.004		
	Total	4	10.05				10.05			
No-RS	Regression	1	9.162	9.162	6318	1	9.005	9.005	3941	1
	Residual	3	0.004	0.001			0.007	0.002		
	Total	4	9.167				9.012			

[1] Degrees of freedom

Author (2021).

Table B3 – Kinetics parameters (μ and μ') and quality of the model fit ((i) standard error (S); (ii) residual variance (S^2_R); and (iii) coefficient of determination (R^2)) obtained from the pseudo-first-order kinetic model adjusted to the experimental data during the exponential *C. vulgaris* growth phase, in the tests carried out with tubular PBRs composed of two absorber tubes over the R85-F reflector at different distances.

Distance (mm)	**Kinetics parameters**							
	Growth over time				**Growth over energy**			
	μ (d^{-1})	**S_μ**	**S^2_R (m^2 L^{-2})**	**R^2**	**μ' (L kJ^{-1})**	**$S_{\mu'}$**	**S^2_R (m^2 L^{-2})**	**R^2**
12.5	0.150	0.002	23.4	0.999	0.0092	0.0009	29.8	0.999
25.0	0.152	0.003	99.1	0.997	0.0094	0.0002	84.0	0.998
50.0	0.202	0.003	195	0.997	0.0113	0.0002	202	0.997
75.0	0.186	0.004	233	0.996	0.0103	0.0002	273	0.996

Author (2021).

Table B4 – Analysis of variance (ANOVA) results, obtained for the regression models established to describe the exponential growth phase of *C. vulgaris*, in the tests carried out with tubular PBRs composed of two absorber tubes over the R85-F reflector at different distances.

Distance (mm)	ANOVA									
	Source	DF[1]	Growth over time				Growth over energy			
			Sum of Squares	Mean Square	F Value	Prob>F	Sum of Squares	Mean Square	F Value	Prob>F
12.5	Regression	1	398.8	398.8	51.06	1	398.8	398.8	40.10	1
	Residual	3	23.40	7.810			29.80	9.940		
	Total	4	398.8				398.8			
25	Regression	1	403.3	403.3	12.20	1	403.3	403.3	14.41	1
	Residual	3	99.10	33.00			84.00	28.00		
	Total	4	403.3				403.4			
50	Regression	1	500.5	500.5	7.706	1	500.7	500.5	7.428	1
	Residual	3	195.0	65.00			202.0	67.40		
	Total	4	500.5				500.7			
75	Regression	1	505.6	505.6	6.519	1	505.5	505.5	5.559	1
	Residual	3	233.0	77.50			273.0	90.90		
	Total	4	505.8				505.8			

[1] Degrees of freedom
Author (2021).