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UTILIZAÇÃO DE MICROALGAS NA REMOÇÃO DE NUTRIENTES DE UMA LAGOA EUTROFIZADA DA CIDADE DE MANAUS-AM

RAIZE CASTRO MENDES

MANAUS-AM

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Orientador: Dr. Edinaldo Nelson dos Santos Silva

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DEDICATÓRIA

Dedico esta tese:

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Ponha numa cuia açu
ou numa cuia mirim
burnida de cumatê:
camarões secos, com casca,
folhas de jambu cozido
e goma de tapioca.
Sirva fervendo, pelando,
o caldo de tucupi,
depois tempere a seu gosto:
um pouco de sal, pimenta
malagueta ou murupi.
Quem beber mais de 3 cuias
bebe fogo de velório.
Se você gostar me espere
na esquina do purgatório.
(Receita de Tacacá, de Luiz Bacellar 1928-2012)

RESUMO

O descarte dos efluentes domésticos sem um tratamento adequado podem elevar os níveis de nitrogênio e fósforo nos corpos hídricos naturais. Nós testamos se as microalgas *Scenedesmus acuminatus*, *Chlorella vulgaris* e *Planktothrix isothrix* reduzem de forma desigual as concentrações de nutrientes dissolvidos em diferentes diluições de água eutrofizada com efluente doméstico. Utilizamos água eutrofizada de uma lagoa urbana e a submetemos em três condições: água não diluída, diluída em 50% e diluída em 90%. Adicionamos a biomassa das microalgas a estas diferentes condições. Os organismos de *C. vulgaris* e *S. acuminatus* atingiram o pico de crescimento no 7º e 9º dia, respectivamente. Os organismos de *C. vulgaris* tiveram maior produção de biomassa e maior taxa específica de crescimento em PW0 (10,4 ± 9,4 mg L^{-1} e 0,96 d^{-1}) e em PW50, (10 ± 8,5 mg L^{-1} e 0,85 d^{-1}). O crescimento de *P. isothrix* foi baixo comparado ao das microalgas verdes. As três espécies foram igualmente eficientes na remoção de amônia em PW0. A eficiência de remoção de nitrato foi mais alta com *C. vulgaris* em PW0, e mais alta com *C. vulgaris* e *P. isothrix* em PW50 e PW90. A eficiência de remoção de ortofosfato foi mais alta com *S. acuminatus* e *C. vulgaris* em PW0, igualmente eficiente para as três espécies em PW50, e mais alta com *C. vulgaris* e *P. isothrix* em PW90. Concluímos que a água eutrofizada por efluente doméstico sem diluição é a melhor opção como meio alternativo para o cultivo de microalgas verdes planctônicas. As três espécies de microalgas testadas são eficientes na remoção da amônia. *Scenedesmus acuminatus* não foi ideal para a remoção de nitrato. *Planktothrix isothrix* foi eficiente na remoção de nutrientes quando a água residual doméstica é diluída. *Chlorella vulgaris* foi eficiente na remoção de nutrientes de águas residuais domésticas, estando diluída ou não.

ABSTRACT

The disposal of domestic effluents without an adequate treatment may increase nitrogen and phosphorus levels in natural water bodies. We tested whether the microalgae *Scenedesmus acuminatus*, *Chlorella vulgaris* and *Planktothrix isothrix* unevenly reduced dissolved nutrient concentrations in different dilutions of eutrophicated water with domestic effluent. We used eutrophicated water from an urban and subjected it to three conditions: undiluted water, diluted by 50%, and diluted by 90%. We added microalgae biomass to these different conditions. *C. vulgaris* and *S. acuminatus* reached their peak growth on the 7th and 9th day, respectively. *C. vulgaris* had higher biomass production and higher specific growth rate at PW0 (10.4 ± 9.4 mg L^{-1} and 0.96 d^{-1}) and at PW50 (10 ± 8.5 mg L^{-1} and 0.85 d^{-1}). The growth of *P. isothrix* was low compared to that of green microalgae. The three species were equally efficient in removing ammonia in PW0. Nitrate removal rate was highest for *Chlorella vulgaris* in PW0, and higher for *C. vulgaris* and *P. isothrix* in PW50 and PW90. Orthophosphate removal efficiency was higher for *S. acuminatus* and *C. vulgaris* in PW0, equally efficient for the three species in PW50, and higher for *C. vulgaris* and *P. isothrix* in PW90. We conclude that water eutrophicated by undiluted domestic effluent is the best option as an alternative medium for growing planktonic green microalgae. We concluded that the three species of microalgae tested are efficient in removing ammonia. *Scenedesmus acuminatus* was not an ideal species for nitrate removal. *Planktothrix isothrix* was efficient in removing nutrients when domestic wastewater is diluted. *Chlorella vulgaris* was efficient in removing nutrients from domestic wastewater whether diluted or not.

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INTRODUÇÃO GERAL

O saneamento básico é o controle de todos os fatores do ambiente que tem o potencial de afetar adversamente o bem-estar físico, mental e social da população. O saneamento caracteriza o conjunto de ações sócioeconômicas que tem por objetivo alcançar salubridade ambiental (OMS, 2019). Um dos desafios primordiais enfrentados pelos países em desenvolvimento é assegurar o acesso ao saneamento apropriado.

Na cúpula das Nações Unidas sobre o Desenvolvimento Sustentável em 2015, em uma reunião que envolveu a participação de várias nações, foi estabelecida agenda mundial composta por 17 Objetivos de Desenvolvimento Sustentável (ODS) e 169 metas a serem alcançadas até o ano de 2030 (UN 2015; Anício *et al*., 2022).

Um dos objetivos específicos dos ODS, que é o sexto, concentra-se em assegurar a água potável e saneamento seguro para todos e tem como metas: Até 2030 melhorar a qualidade da água, reduzindo a poluição, eliminando despejo inadequado, minimizando a liberação de substâncias prejudiciais, diminuindo pela metade as águas residuais não tratadas e aumentando significativamente a reciclagem e reutilização segura em todo o mundo (Meta 6.3). Até 2030, fortalecer a cooperação internacional e o apoio à capacitação em água e saneamento para países em desenvolvimento, abrangendo áreas como coleta de água, dessalinização, uso eficiente de recursos hídricos, tratamentos de efluentes, reciclagem e tecnologias de reutilização (Meta 6.A) (Agenda 2030).

Porém o cenário que vemos atualmente (2023), não é um dos melhores para o cumprimento dessas metas. De acordo com Organização Mundial da Sáude (2023), as seguintes informações são considerados fatos: a) Mais de 1,7 milhões de pessoas ainda não têm acesso a serviços de saneamento básico, como banheiros, privadas ou latrinas; b) Dentre esses, 494 milhões ainda defecam ao ar livre, por exemplo nas sarjetas das ruas, atrás de arbustos ou em corpos d'água desprotegidos e c) Em 2020, que foi marcado pela pandemia de Covid-19, aproximadamente 45% das águas residuais domésticas geradas em todo o mundo foram descartadas sem passar por tratamento seguro. Além disso, a falta de saneamento seguro expõe a população a infecções e doenças incluindo diarreia, doenças tropicais e doenças transmitidas por vetores (OMS, 2020).

Considerando a América do Sul, especificamente no Brasil, em 1970 havia um programa de apoio ao saneamento básico chamado de Plano Nacional de Saneamento Básico (PLANASA), com o objetivo de atender 80% da população urbana com serviço

de água e 50% com serviço de esgoto até 1980. Um dos motivos pelo qual esse programa não teve mais progresso foi a maior ênfase ao atendimento nos sistemas de abastecimento de água e, a não contribuição para diminuir o déficit de coleta e tratamento de esgoto (Turolla, 2002). A partir da década de 80 após a extinção da PLANASA, outros programas de saneamento básico surgiram como PRONURB, PRO-SANEAMENTO, PASS, PROSEGE, FUNASA-SB, PMSSI, PMSSII, PNCDA, FCP/SAN, PROPAR E PROSAB (Turolla 2002; Leoneti *et al*., 2011).

Apesar do surgimento destes programas de saneamento básico, segundo Leoneti *et al*. (2011) o Brasil estava marcado por uma grande desigualdade e escassez em relação à coleta e ao tratamento de esgoto. Este panorama não teve mudanças pois, 12 anos depois segundo o levantamento realizado por Kligerman *et al*. (2023) o Brasil ainda é marcado pela falta de acesso aos serviços de saneamento, que inclui tanto a coleta quanto o tratamento de águas residuais. A situação piora para a região Norte do país onde apenas 13,1% da população tem coleta de esgoto (Kligerman *et al*., 2023). Segundo um levantamento realizado por Vasconcelos e Albuquerque (2019) considerando a coleta e o tratamento de esgoto no ano de 2015 no SNIS e do Instituto Trata Brasil, a região Norte do Brasil tem a pior taxa de tratamento de esgoto do país (16,42%).

Sabe-se que a região norte do Brasil, é composta por uma diversidade de corpos d'água como o rio Solimões, rio Negro, rio Amazonas e igarapés que tem a nascente dentro da cidade e as atravessam. A cidade de Manaus, capital do Amazonas, é composta por vários igarapés os principais são, o igarapé do Educandos e o de São Raimundo, tendo como limites o rio Negro a sudoeste e o igarapé do Tarumã-Açú a oeste (Santos-Silva e Silva, 1993). Na bacia do Educandos, estão situados o igarapé do Quarenta e seus afluentes. A bacia do São Raimundo é formada pelo igarapé do Mindu, nascendo na Zona Norte (Reserva Duque), atravessando a Zona Leste, desaguando no igarapé do São Jorge, além do igarapé dos Franceses, Bindá e Franco, onde se encontram algumas áreas críticas de alagamento da cidade (Fonseca, 2008).

A bacia do Tarumã é formada pelos igarapés do Gigante, Tabatinga e o próprio Tarumã, considerado ainda uma bacia "conservada", sem grandes indícios de poluição. Já a Bacia do Puraquequara, que é composta do rio Puraquequara e seus afluentes, também tem grande parte de sua extensão localizada na área de expansão urbana da cidade (Fonseca, 2008).

Grobe (2014) fez uma revisão sobre a ocupação da cidade de Manaus e afirma que

o abastecimento de água e os sistemas de esgoto, prioridades colocadas nos discursos dos construtores e administradores da cidade e reivindicadas pela população, tiveram um longo e complicado processo. Pois, o sistema de abastecimento teve início no ano de 1881 com a exploração do igarapé da Cachoeira Grande, em 1889. Em 1898, a empresa de capital estrangeiro *Manáos Raliway Company*, firmou contrato com o governo para executar o bombeamento de água dos Mananciais da Cachoeira Grande para o Mocó e da Castelhana, utilizando energia elétrica da usina que atende o serviço de viação, bombas turbinas e casas pertencentes ao Estado, localizadas na casa de Máquinas na Cachoeira Grande. Em 1900, no governo de José Cardoso Ramalho Junior, as propostas de lançamento de dejetos da cidade determinam o rio Negro como receptor dos produtos de esgoto. Em 1906 o projeto baseado no *Separate System* é escolhido por ser o mais econômico. Em 1907, a Companhia *Manáos Improvements*, assume o abastecimento de água e a implantação dos serviços de esgotos. É perceptível que a atenção é dada sempre ao abastecimento de água e não ao tratamento de esgoto, além disso, essas medidas adotadas relacionadas ao abastecimento de água acabavam não atendendo a população residente nos bairros mais afastados.

O crescimento populacional em Manaus sem organização e sem planejamento proporcionou a instalação de casas nas margens de igarapés da cidade. Atualmente (2023), a maior parte dos igarapés que atravessam a cidade encontram-se poluídos. Quando a água baixa (seca) é perceptível à demanda de lixo sólido (latas, garrafas plásticas, garrafas de vidro, pneus, geladeiras, fogão etc.) que ficam nos igarapés, e quando a água sobe (enchente) o mesmo lixo fica debaixo d'água, tornando – se um ciclo. A prefeitura de Manaus tem contribuído apenas na retirada do lixo "bruto" através de arrastos de redes, para amenizar a situação.

Porém, sabe-se que além do lixo, há várias fontes de efluentes (efluentes industriais, efluentes agropecuários e efluentes domésticos) que são despejados nos corpos d'água. Esses efluentes podem conter alto teor de nutrientes, principalmente nitrogênio e fósforo que são carreados pelas chuvas dos locais circundantes e, despejados de forma indevida nos corpos d'água.

Alguns estudos já foram realizados para identificação da concentração de fósforo nos igarapés de Manaus como o estudo de Silva (2008) que identificou os principais tipos de ortofosfato presentes nos igarapés do Educandos, São Raimundo e Tarumã-Açú, onde as concentrações de ortofosfato totais e dissolvidos tiveram como principal característica,

uma alta variabilidade com valores mínimos e máximos de 39,6 a 71,6 mg/L-1 e, 38,5 a 63,3 mg/L-1 respectivamente.

Oliveira e Valle (2009) estudando a lagoa do Japiim, uma lagoa da cidade de Manaus, constataram as seguintes concentrações de nutrientes: fosfato (5,7±0,2 mg/L), nitrato (62,4±0,4 mg/L) e amônia (36,3±0,2 mg/L). Pinheiro (2010) com objetivo de investigar os diferentes tipos de ortofosfatos presentes nos igarapés do Saõ Raimundo, Educandos, Tarumã, Puraquequara, lago do Aleixo e no rio Negro, identificou variabilidade nas concentrações de ortofosfatos. Essa variabilidade se destacou particularmente entre os períodos de seca e cheia, com valores de 0,004 a 39,1 mg L^{-1} e 0,03 a 4,73 mg L^{-1} respectivamente.

No estudo realizado por Correa e Cunha (2011), foram registrados os seguintes valores de nutrientes nos igarapés do Mindu e do Quarenta. No igarapé do Mindu, foram registrados níveis de 805 mg/L de fósforo total e 12,3 mg/L de nitrogênio total. Por outro lado, no igarapé do Quarenta, as concentrações foram mais elevadas, com 2280 mg/L de fósforo total e 23,35 mg/L de nitrogênio total. Com base nesses valores, ambos os igarapés foram classificados como ambientes eutrofizados. Já no igarapé Mestre-Chico (Tarumã) o fósforo total variou de 0,201 a 3,7 mg/L, a amônia de 0,01 a 22,03 mg/L e o nitrato de 0,1 a 6,81 mg/L (Normando, 2014).

Nota-se que alguns igarapés já foram classificados como ambientes eutrofizados. De acordo com Tundisi e Matsumura-Tundisi (2008) o aumento excessivo de nutrientes no ambiente aquático traz sérias consequências como: Anoxia, que causa mortalidade em massa de peixes e invertebrados e libera um gás odorífero, muitas vezes tóxico (H2S e CH4); *Blooms* de algas e crescimento descontrolado de plantas aquáticas, especialmente macrófitas; Produção de toxinas por algumas espécies tóxicas de algas; Níveis elevados de matéria orgânica, que, se tratados com cloro, podem produzir carcinógenos; Declínio acentuado da biodiversidade e número de espécies vegetais e animais; Alterações na composição das espécies de peixes, com diminuição do seu valor comercial; Diminuição da concentração de oxigênio dissolvido e redução de estoque de peixes causada pelo esgotamento de oxigênio dissolvido.

Com base nesse contexto, é essencial adotar medidas para controlar e eliminar os nutrientes, promovendo assim, a restauração dos ecossistemas aquáticos. Isso inclui a necessidade do tratamento de esgoto antes dos efluentes serem descartados nos ambientes aquáticos. Geralmente os tratamentos de esgoto ocorrem em níveis, onde a etapa inicial

busca apenas a remoção de sólidos grosseiros. Após essa remoção inicial, há o tratamento primário, destinado a retirar sólidos sedimentáveis e matéria orgânica, tratamento secundário, voltado para a remoção de nutrientes (e.g. nitrogênio e fósforo) e o tratamento terciário, cujo objetivo é a remoção de outros poluentes ou a retirada dos nutrientes que não foram removidos eficazmente no tratamento secundário (Osvald *et al*., 1988a; Cunha *et al*., 2018).

Muitas vezes a segunda etapa dos tratamentos de esgoto são negligenciadas onde os efluentes são descartados nos ambientes aquáticos com altos níveis de nitrogênio e fósforo, isso porque os custos associados às etapas subsequentes dos tratamentos são extremamente elevados. Umas das alternativas para mitigar este gargalo é a utilização de alguns organismos como por exemplo as microalgas (Lourenço, 2006). De fato, o cultivo de microalgas em efluentes tem se destacado pela elevada eficácia na remoção dos nutrientes, dessa forma é vantajoso a utilização destes organismos para este fim (Lam *et al*., 2018). É vantajoso porque as microalgas assimilam naturalmente nitrogênio e fosforo da água e utilizam para o seu crescimento. Além disso, após a remoção dos nutrientes a sua biomassa pode ser utilizada para produção de biofertilizantes e produção de biodiesel (Priyadarshani e Rath, 2012; Lam *et al*., 2018).

Geralmente, as microalgas utilizadas para remoção de nutrientes são provenientes do próprio meio natural (e.g. lago ou lagoa) ou, podem ser provenientes de cultivos para serem aplicadas como removedoras de nutrientes. Esses sistemas de cultivo podem ser em laboratório (e.g. pequena escala), em bateladas ou estanques, cultivos semicontínuos, cultivos contínuos e cultivos massivos (Lourenço, 2006).

Os sistemas de cultivo em massa podem ser instalados em locais abertos (e.g. cultivos em tanques e cultivos em cascatas) e em locais fechados (e.g. fotobiorreatores). A vantagem dos sistemas de cultivo fechado é o controle dos fatores ambientais havendo possibilidade de ajustes (Lourenço, 2006). Porém, os custos de infraestrutura tornam-se dispendiosos quando comparados aos sistemas abertos (Demirbas e Demirbas, 2011).

Também existem os chamados HRAPs (*High rate algal ponds*), desenvolvidos em 1950, são sistemas em lagoas para o tratamento de águas residuais e recuperação de recursos, onde a remoção de nutrientes é feita principalmente através do crescimento e assimilação de nutrientes por algas (Craggs *et al*., 2012).

Craggs *et al*. (2012) desenvolveram quatro sistemas de HRAPs para o tratamento de águas residuais na Nova Zelândia onde, deixou as espécies de algas se desenvolverem naturalmente e, verificou que a composição de espécies de algas foi similar nos quatro sistemas de tratamento, onde a remoção de ortofosfato foi de 14 – 24% e de amônia 5,6 – 67,4%.

Já Bai *et al*. (2012) cultivou algas em estrume de porco para gerar um método econômico de produção de algas a ser operado em pequenas fazendas de animais na Hungria. Testou quatro espécies de algas (*Chlorella vulgaris*, *Scenedesmus quadricauda*, *Scenedesmus dimorphus* e *Arthrospira platensis*) em diferentes diluições e, concluiu que *Chlorella vulgaris* é a espécie mais adequada para remoção de nutrientes e que o "estrume líquido filtrado" é o recomendado para produção de algas.

De acordo com Lourenço (2006), algumas espécies de algas são utilizadas em tanques de tratamento de esgoto dentre elas: *Oscillatoria*, *Scenedesmus, Chlorella*, *Cymbella*, *Euglena*, *Ankistrodesmus, Anabaena* e *Micractinium*. A espécie *Chlorella vularis* pertence ao grupo das Chlorophyta (Bicudo e Bicudo, 2006; Lourenço, 2006) e é utilizada para produção de biodiesel, alimentação humana, alimentação para os organismos zooplanctônicos, para remoção de nutrientes de ambientes eutrofizados (Liu e Chen, 2016). Tem a capacidade de depositar dióxido de carbono de forma eficiente e remover nutrientes como nitrogênio e fósforo, tornando-se uma excelente espécie para biomedicamento de gases de efeito estufa e biorremediação de águas residuais. Além disso, tem alto teor de óleo, culturas de massa ao ar livre e são "fáceis" de manter (Liu e Chen, 2016). Segundo Safi *et al*. (2014) os organismos de *Chlorella vulgaris* removem 28 – 96 % de fósforo do ambiente. Segundo Wang *et al*. (2010) os organismos de *Chlorella* sp. assimilam 90,6 % de fósforo de águas residuais, tornando-se eficiente na remoção desse nutriente.

Outra espécie de alga utilizada na remoção de nutrientes é *Scenedesmus* sp., também pertencente ao grupo das Chlorophyta (Bicudo e Bicudo, 2006; Lourenço, 2006). Os organismos desta espécie são eficientes para alimentação dos organismos zooplanctônicos (Hardy e Castro, 2000; Sipaúba-Tavares e Rocha, 2003), produção de biodiesel e na remoção de nutrientes (Wong *et al*., 2015).

Wong *et al*. (2015) testaram a eficiência de *Scenedesmus quadricauda* na remoção de nutrientes e na produção de lipídios em águas residuais em diferentes diluições e, verificou que essa espécie remove mais de 90% de ortofosfato e 80% de fosfato nas

diluições de 25% e 50%, concluindo que *Scenedesmus quadricauda* é viável para o tratamento de águas residuais e produção de lipídios.

Mara (2003) afirma que quando a sustentabilidade é considerada em relação ao tratamento de águas residuais domésticas nos países em desenvolvimento, as seguintes questões são relevantes: baixo custo, tanto em termos de capital quanto de operação e manutenção, simplicidade de operação e manutenção, baixo uso de energia, preferencialmente zero, essencial para baixos custos operacionais e uso baixo de produtos químicos, especialmente cloro ou outros desinfetantes prejudiciais para o meio ambiente.

Portanto, levando em consideração a problemática relacionada ao saneamento básico e tratamento de esgoto, principalmente na região amazônica que continua sendo negligenciada junto a sua população, a necessidade de promover a sustentabilidade tornou-se urgente. Dessa forma, utilizamos o efluente doméstico de uma lagoa eutrofizada para o crescimento da biomassa de *Scenedesmus acuminatus*, *Chlorella vulgaris* e *Planktothrix isothrix*, bem como testamos se estas três espécies reduzem de forma desigual os nutrientes (nitrato, amônia e ortofosfato). Com a perspectiva de, no futuro integrar as microalgas à segunda fase dos procedimentos de tratamentos de esgoto das águas amazônicas.

OBJETIVOS

Geral:

Testar se a biomassa de *S. acuminatus*, *C. vulgaris* e *P. isothrix* reduz de forma desigual a concentração de nutrientes de águas eutrofizadas

Específicos:

- Caracterizar a biomassa de *S. acuminatus*, *C. vulgaris* e *P. isothrix*
- Caracterizar as quantidades de nitrato, amônia e ortofosfato nas diluições de água eutrofizada
- Caracterizar a eficiência de remoção de *S. acuminatus*, *C. vulgaris* e *P. isothrix*
- Testar se a biomassa de *S. acuminatus*, *C. vulgaris* e *P. isothrix* reduzem de forma desigual a quantidade de nitrato, amônia e ortofosfato de águas eutrofizadas

MATODOLOGIA GERAL

Para responder aos objetivos específicos montamos um delineamento amostral e estes estão apresentados nos capítulos ao longo da tese. Dessa forma aqui apresentamos de maneira geral, a metodologia relacionada a área de estudo, ao cultivo das microalgas antes de serem colocadas nos experimentos e outros procedimentos gerais.

Área de estudo

A lagoa do Japiim (figura 1a) fica localizada no Bairro Japiim e está incluída no parque da Lagoa do Japiim, inaugurado no dia 27 de dezembro de 2008, zona Sul da cidade de Manaus. Foi construída pelo barramento de um pequeno igarapé próximo a sua nascente e, faz limites com os bairros do Coroado, Petrópolis, Raiz e Distrito Industrial. Antes de se tornar um parque, a lagoa foi utilizada para criação de peixes em uma área particular e depois ficou abandonada (Ribeiro, 2011).

A lagoa do Japiim possui um "ladrão", à montante, que leva a água dessa lagoa a um igarapé que nasce na área do campus da Universidade Federal do Amazonas (UFAM) e é afluente do igarapé do Quarenta, este por sua vez deságua no igarapé do Educandos. A lagoa do Japiim tem aproximadamente 155 m de comprimento e 45 m de largura e sua maior profundidade é de aproximadamente 4,8 m (Castro-Mendes, 2013). Atualmente, a lagoa do Japiim é um ambiente eutrofizado recebendo tanto a água proveniente das chuvas quanto do esgoto doméstico *in natura*, proveniente dos moradores dos bairros mais próximos (figura 1b) e os organismos de *Planktothrix isothrix* (cianobacteria) são predominantes formando o *Bloom* (figura 1c).

Figura 1: Lagoa do Japiim, localizada no bairro Japiim, Manaus Am. a) Parque Lagoa do Japiim. Foto: Bruno Barreto (2019); b) Casas localizadas ao lado do Parque Lagoa do Japiim. Foto: Pinheiro (2019); c) "Bloom" da cianobactéria *Planktothrix isothrix* na Lagoa do Japiim. Foto: Santos-Silva (2019).

Cultivo das microalgas em laboratório

Nós isolamos (do meio natural) e cultivamos os organismos de *Chlorella vulgaris* e *Scenedesmus acuminatus* em laboratório. Estas espécies de microalgas foram cultivadas no fertilizante comercial NPK na proporção (20:05:20) ureia, superfosfato triplo e cloreto de potássio, respectivamente. Estes organismos foram mantidos em volumes de 1 L (figura 2) sob as seguintes condições: aeração constante, temperatura a 28ºC, intensidade luminosa 1761 lux e fotoperíodo 12 h claro e 12 h escuro. Estas microalgas foram mantidas nestas condições até o começo dos experimentos. Por estarem em abundância e serem predominantes formando uma camada de biomassa, os organismos de *Planktothrix*

isothrix foram coletados diretamente da própria água da lagoa para serem incluídos nos experimentos.

Figura 2: Cultivo das microalgas no laboratório em volume de 1L no meio de cultivo comercial NPK. Foto: Arquivo pessoal.

Filtragem da água da lagoa

Coletamos 60 L de água da lagoa introduzindo verticalmente um tubo de PVC de 1,5 m de comprimento e 5 cm de diâmetro, com uma válvula de retenção da água acoplada em sua extremidade. Este volume de água foi levado para o laboratório para ser filtrado e removido toda a matéria orgânica em suspensão. Para isto, um filtro foi construído utilizando uma garrafa PET (tereftalato de polietileno) com o volume de 20 L, que teve seu fundo removido e foi utilizada de forma invertida. No interior da garrafa foram colocadas várias camadas de material filtrante (figura 3a, 3b e 3c). Após a filtragem, foi adicionado 0.5 ml de hipoclorito de sódio por litro de água filtrada. Após este procedimento a água foi armazenada e mantida no escuro por 24 horas.

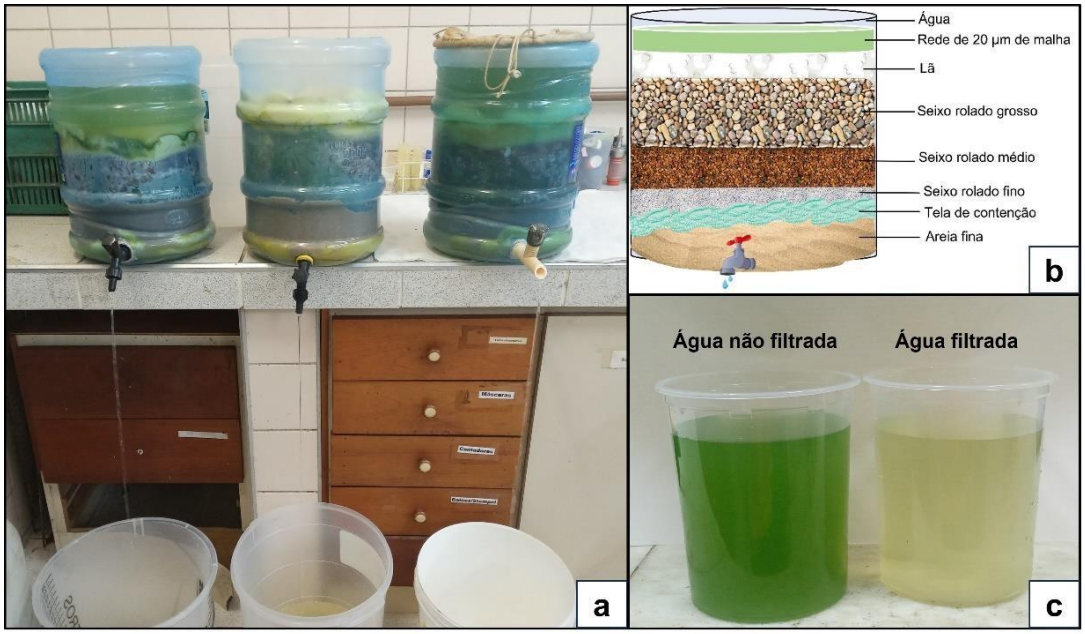

Figura 3: Representação dos filtros caseiros. a) Filtros construídos para a filtragem da água da lagoa. Foto: Arquivo pessoal; b) Representação esquemática dos compartimentos do filtro. Fonte: Arquivo pessoal (2023); c) Comparação da água da lagoa não filtrada e após a filtração. Foto: Arquivo pessoal.

Biovolume

Os organismos de *C. vulgaris*, *S. acuminatus* e *P. isothrix* tem tamanhos diferentes por isso, nós escolhemos o biovolume como estimativa de biomassa. Nós separamos 15 organismos para a medida do biovolume inicial do inóculo dos organismos de cada espécie. As células destes organismos foram medidas em um microscópio óptico com ocular micrometrada. Para o cálculo do biovolume de cada organismo utilizamos o software "*BioCalc*" e, calculamos a média (Santos-Silva *et al*., 2019). Em uma câmera de Sedgewick Rafter fizemos a contagem do número de células. O biovolume dos organismos de cada espécie foi calculado multiplicando a média do volume celular pelo número de organismos em 100 ml. O biovolume inicial do inóculo dos organismos de cada espécie foi 0.741mg L^{-1}.

Condições da água da lagoa utilizadas nos experimentos

Após a filtragem (pré-tratamento) a água da lagoa foi utilizada nos experimentos em 100%, 50% e 10%. A água da lagoa sem diluição (100%) foi composta apenas com a água da lagoa sem adição de água destilada. A diluição 50%, foi composta por 50% de

água da lagoa mais 50% de água destilada. A segunda diluição foi composta por 10% de água da lagoa e 90% de água destilada.

Análise dos nutrientes

Para análises dos nutrientes, foram filtrados 300 ml em filtros de fibra de vidro com uma bomba à vácuo com 0,17kW de potência. Para medir as concentrações de nitrato (NO_3^-) e ortofosfato (PO_4^3) foi utilizado o método descrito por Golterman *et al.* (1978) e para amônia (NH_4^+) utilizamos análise por injeção em fluxo (FIA) (Ruzicka e Hansen, 1975; Stewart, 1976). Para ambos os métodos foram utilizadas curvas de calibração com padrões específicos e utilizamos técnica de espectrofotometria para a leitura.

Após as diluições, determinação das concentrações de nutrientes iniciais e adições dos inóculos nos recipientes nos experimentos (figura 4a e 4b), estes foram levados para sala de cultivo sob condições controladas de fotoperíodo (12 horas claro e 12 horas escuro) e temperatura (28 ºC). Todos os recipientes foram mantidos com aeração constante, isso foi feito para obtermos o CO_2 e para evitar que as células das microalgas sedimentassem no fundo do recipiente. O pH variou de 7.6 a 7.8. A cada 24 horas retiramos um recipiente de cada tratamento, do qual retiramos uma alíquota de 5 mL para a determinação do biovolume (Santos-Silva *et al.*, 2019), que foi utilizado como estimativa de biomassa dos organismos.

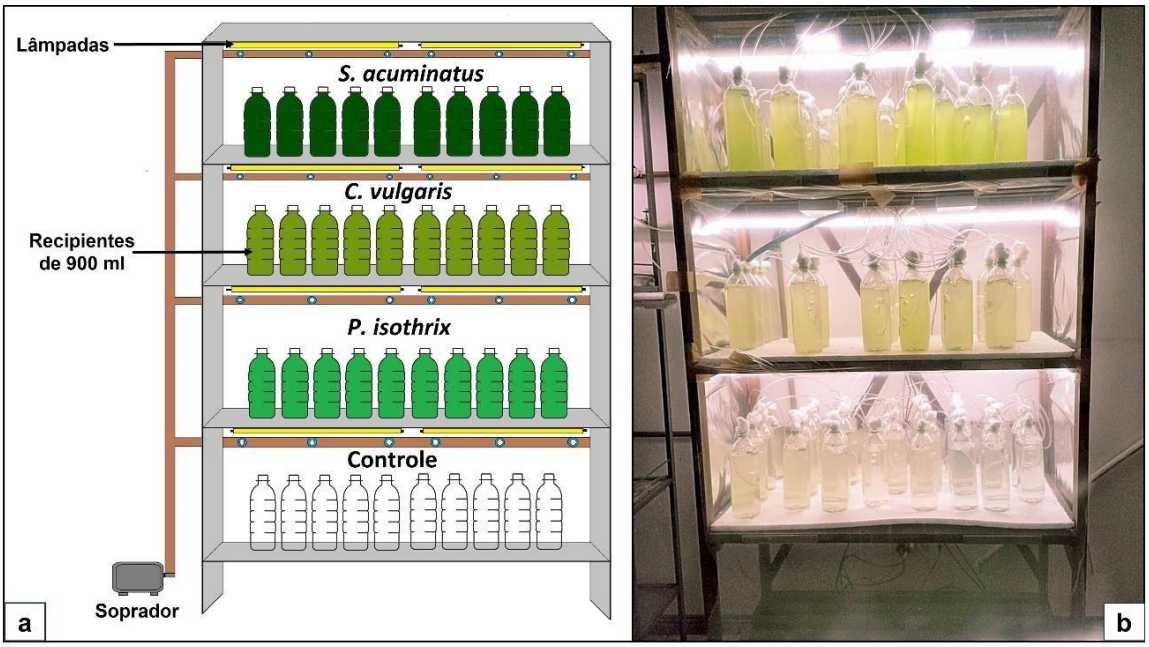

Figura 4: Experimento sob condições laboratoriais. a) Representação esquemática do experimento em laboratório. Fonte: Arquivo pessoal e b) Imagem real do experimento em laboratório. Foto: Arquivo pessoal.

ORGANIZAÇÃO DA TESE

Esta tese está dividida em dois capítulos que se encontram em formato de artigo científico, ambos formatados conforme as regras das revistas nas quais foram submetidos, Hoehnea e Acta Amazônica. O capítulo I, trata-se de um estudo de caracterização da produção da biomassa de *S. acuminatus*, *C. vulgaris* e *P. isothrix* cultivadas em efluente doméstico da Lagoa do Japiim. O capítulo II, trata-se de um estudo sobre o efeito de dois tratamentos (espécie e condições da água da lagoa) sob a concentração dos nutrientes.

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CAPÍTULO I

CASTRO-MENDES, R. C.; NASCIMENTO, R. G.; SANTOS-SILVA, E. N. 2023. To dilute or not to dilute? That is the question: Use of domestic effluent as an alternative culture medium for microalgae. Hoehnea.

To dilute or not to dilute? That is the question: Use of domestic effluent as an alternative culture medium for microalgae

Raize Castro MENDES*, Renan Gomes do NASCIMENTO, Edinaldo Nelson dos SANTOS-SILVA

Instituto Nacional de Pesquisas da Amazônia – INPA, Laboratório de Plâncton, Av. Bem-Te-Vi, 8-406 – Petrópolis, Manaus, AM, Brazil

*Corresponding author: raize.mendes@gmail.com; https://orcid.org/0000-0003-1215-7702

ABSTRACT – (To dilute or not to dilute? That is the question: Use of domestic effluent as an alternative culture medium for microalgae). In this study, we used domestic effluent as an alternative culture medium for freshwater microalgae. The green microalgae *Scenedesmus acuminatus* and *Chlorella vulgaris* and the cyanobacteria *Planktothrix isothrix* were cultivated in three concentrations (PW0, PW50 and PW90) of domestic effluent from an urban pond. *Chlorella vulgaris* and *S. acuminatus* reached their peak growth on the 7th and 9th day, respectively. *Chlorella vulgaris* had higher biomass production and higher specific growth rate at PW0 (10.4 ± 9.4 mg L^{-1} and 0.96 d^{-1}) and at PW50 (10 ± 8.5 mg L^{-1} and 0.85 d^{-1}). *Scenedesmus acuminatus* had higher biomass production and higher specific growth rate at PW0 (8.15 ± 5.8 mg L^{-1} and 0.25 d^{-1}) and at PW50 (8.72 ± 7.0 mg L^{-1} and 0.28 d^{-1}). The growth of *P. isothrix* was low compared to that of green microalgae. We conclude that water eutrophicated by undiluted domestic effluent is the best option as an alternative medium for growing planktonic green microalgae. The organisms of *C. vulgaris* are the best option for biomass usage.

Keywords: chlorophyte cultivation, cyanobacterial cultivation, microalgae production, specific growth rate

RESUMO – (Utilização de efluente doméstico como meio de cultivo alternativo para microalgas). Neste estudo nós adotamos a abordagem da utilização de efluente doméstico como meio de cultivo alternativo para microalgas de água doce. As microalgas verdes *Scenedesmus acuminatus* e *Chlorella vulgaris* e a cianobactéria *Planktothrix isothrix* foram cultivadas em três concentrações (PW0, PW50 e PW90) de efluente doméstico de uma lagoa urbana. Os organismos de *C. vulgaris* e *S. acuminatus* atingiram o pico de crescimento no 7º e 9º dia, respectivamente. Os organismos de *C. vulgaris* tiveram maior produção de biomassa e maior taxa específica de crescimento em PW0 (10.4 ± 9.4 mg L^{-1} e 0.96 d^{-1}) e em PW50, (10 ± 8.5 mg L^{-1} e 0.85 d^{-1}). Os organismos de *S. acuminatus* tiveram maior produção de biomassa e maior taxa específica de crescimento em PW0 (8.15± 5.8 mg L^{-1} e 0.25 d^{-1}) e em PW50 (8.72± 7.0 mg L^{-1} e 0.28 d^{-1}). O crescimento de *P. isothrix* foi baixo comparado ao das microalgas verdes. Concluímos que a água eutrofizada por efluente doméstico sem diluição é a melhor opção como meio alternativo para o cultivo de microalgas verdes planctônicas. Os organismos de *C. vulgaris* são a melhor opção para utilização de sua biomassa.

Palavras-chave: cultivo de clorofíceas, cultivo de cianobactéria, produção de microalgas, taxa específica de crescimento

Introduction

Microalgae are autotrophic, photosynthetic organisms found abundantly in aquatic environments. Microalgae naturally need sunlight, CO_2 and nutrients, mainly nitrogen and phosphorus, for growth (Andersen 2005). These organisms assimilate nutrients (e.g., nitrogen and phosphorus) through protein complexes in the plasmatic membrane (Flores & Herrero 2005, Glibert *et al*. 2015, Lin *et al*. 2016, Takabayashi *et al*. 2005). Nitrogen is used to structure proteins, nucleic acids and photosynthetic

pigments. Phosphorus is used to transfer energy and compose structural molecules (Lourenço 2006).

Microalgae have been widely cultivated because they grow easily under natural conditions of light, temperature, and nutrients. Their biomass has been tested in different applications, such as production of biodiesel, biofertilizers for agriculture, animal feed, human food, pharmaceuticals, cosmetics, and natural dyes (Priyadarshani & Rath 2012, Dos Santos *et al*. 2022).

Some cultivation methods to obtain microalgae biomass include open systems (e.g., open ponds) and closed systems (e.g., photobioreactors) (Alabi *et al*. 2009, Cruz *et al*. 2013). The types of cultures that can be a) autotrophic, in which cells receive light energy and assimilate CO_2, fixing carbon in the form of glyceraldehyde-3-phosphate, which in turn enters the glycolytic pathway through the Calvin cycle, b) heterotrophs, in which growth occurs in the absence of light using organic substrates as a carbon source for biosynthesis and energy, and c) mixotrophic, in which microalgae simultaneously have organic compounds, light and CO_2 as a source of carbon and energy (Zhang *et al*. 2012). The methods are chosen depending on the application of the biomass (Cruz *et al*. 2013). However, there is a great challenge regarding the culture media used. Commercial culture media are those that meet the needs of microalgae growth, such as Blue Green (BG-11), Tris Acetate Phosphate (TAP), Bold's Basal Medium (BBM), Chu-10, and D-Medium (Pandey *et al*. 2023). The nutrient composition of commercial growth media varies between brands and each species of microalgae responds differently to these media, mainly with regard to production, composition and nutritional quality of biomass (Pandey *et al*. 2023).

Commercial culture media are appropriate and meet the demands for the isolation of some species, maintenance of strains, and small-scale cultivation for experimental

purposes (Sipaúba-Tavares & Rocha 2003). When large-scale production is required, larger amounts of nutrients are needed, increasing the cost of microalgae production (Lourenço 2006), and water consumption is also highly relevant, especially if the water is not reused (Acién *et al*. 2017). To minimize these problems, some alternatives have been evaluated, such as the use of effluents (swine, pharmaceutical, fish farming, industrial, domestic) as a culture medium for the growth of organisms.

The use of effluents as a culture medium are evaluated for the growth of *Chlorella vulgaris* and *Scenedesmus* sp. using swine effluents (Bai *et al*. 2012, Abou-Shanab *et al*. 2013, Wang *et al*. 2015, Zhao *et al*. 2022), pharmaceutical effluents (Nayak & Ghosh 2019), fish farm effluents (Trivedi *et al*. 2019), industrial effluents (Mercado *et al*. 2020, Rodrigues-Souza *et al*. 2021, Wu *et al*. 2020) and domestic effluents (Yin-Hu *et al*. 2013, Trevisam *et al*. 2014, Zhang *et al*. 2014, Fernández-Linares *et al*. 2017, Jebali *et al*. 2018, Pandey *et al*. 2019, Thangam *et al*. 2021, Wang *et al*. 2022).

However, the optimal microalgae growth performance depends on the type of effluent, if is pre-treatment, if there is dilution and filtration, and also the concentration of nutrients present in the effluent. For example, Zhang *et al*. (2014) observed that the secondary domestic effluent (effluent that went through a pre-treatment or primary treatment) replaces the commercial culture medium (BG11), saving inputs (nutrients N and P) and reusing water. Bai *et al*. (2012) observed the growth of the microalgae *Chlorella vulgaris*, *Scenedesmus quadricauda*, *Scenedesmus dimorphus* and *Arthrospira platensis* in filtered swine effluent at 1%, 5%, 10% and 25% dilutions. The best dry yields (g m^3) are obtained at 10% dilution. However, Jebali *et al*. (2018) cultivated *Scenedesmus* sp. at different nutrient concentrations in sewage treatment plants and observed a greater biomass and tolerance (g m^2) to high nutrient conditions (60%).

Thus, considering that the growth response and biomass production may vary according to the effluent and nutrient concentration, we characterized the growth of the biomass of two green microalgae (*Scenedesmus acuminatus* and *Chlorella vulgaris*) and of a cyanobacterium (*Planktothrix isothrix*) using different nutrient concentrations of eutrophicated water by domestic effluent, in order to determine the biomass production of these three organisms under these conditions.

Material and methods

To characterize the biomass production of *Scenedesmus acuminatus* (Lagerheim) Chodat, 1902 (Chlororophyta, Scenedesmaceae), *Chlorella vulgaris* Beijerinck, 1890 (Chlororophyta, Chlorellaceae) and *Planktothrix isothrix* Anagnostidis & Komárek, 1988 (Cyanobacteria, Oscillatoriales) grown at different concentrations of nutrients in water eutrophicated by domestic effluent, was used water from a pond located in the city of Manaus, Brazil (Lagoa do Japiim). This pond receives domestic effluents from adjacent houses and there is a predominance of the cyanobacteria *P. isothrix*. The strains of green microalgae (chlorophytes), *S. acuminatus* and *C. vulgaris*, used in this experiment came from the Plankton laboratory of the Instituto Nacional de Pesquisas da Amazônia – INPA. The organisms of *P. isothrix* came from the pond itself, from which we used the water.

We collected 60 L of water from the pond by vertically introducing a PVC pipe 1.5 m long and 5 cm in diameter, with a water retention valve attached to its end. This volume of water was taken to the laboratory to be filtered, and all suspended organic matter was removed. For this, a filter was built using a PET bottle (polyethylene terephthalate) with a volume of 20 L, which had its bottom removed and was used upside down. Several layers of filtering material were placed inside the bottle (figure 1). After filtering, 0.5 ml of sodium hypochlorite was added per liter of filtered water. After this procedure, the water was stored and kept in the dark for 24 hours.

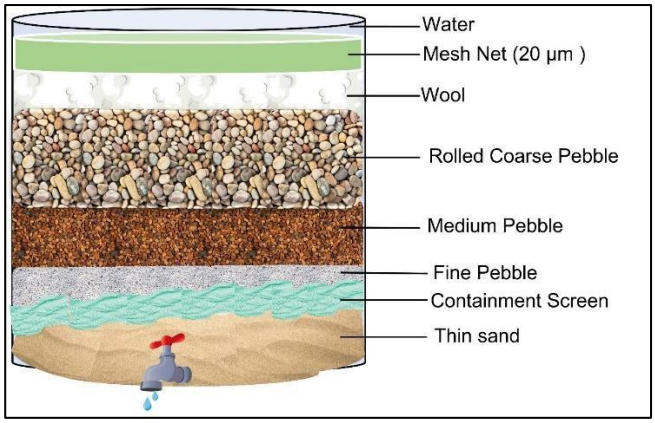

Figure 1: Representation of the filter built from a PET bottle. Upper layer consisting of a 20 µm mesh mesh. The second layer consists of wool-based fabric. The third layer of coarse rolled pebble (38 to 19 mm), medium pebble (12,7 a 6,35 mm) and fine pebble (6,35 a 3,36 mm). The fourth layer consists of a plastic screen containing the pebbles (0.27 mm) and, finally, a layer of fine sand.

Chlorella vulgaris, *S. acuminatus* and *P. isothrix* organisms have different sizes, therefore we chose biovolume as the biomass estimate. Were separated 15 organisms to measure the initial inoculum biovolume of the organisms of each species. The cells of these organisms were measured in an optical microscope with a micro-metered eyepiece. To calculate the biovolume of each organism, we used the software "*BioCalc*" and calculated the average (Santos-Silva *et al*. 2019). In a Sedgewick Rafter camera, we counted the number of cells. The biovolume of organisms of each species was calculated by multiplying the mean cell volume by the number of organisms in 100 mL. The initial inoculum biovolume of the organisms of each species was 0.741 mg L^{-1}.

The experiment was carried out in a culture room at the Plankton laboratory of the Instituto Nacional de Pesquisas da Amazônia - INPA. For this, three treatments consisted of three different concentrations of nutrients in pond water (show Table 1).

We considered the conditions of the pond water as the treatment (three levels) and used distilled water for the dilutions. The pond water was subjected to the following conditions: i) treatment PW0, undiluted pond water, ii) treatment PW50, pond water diluted to 50% with distilled water, and iii) treatment PW90, pond water diluted to 90% with distilled water. Ten containers with a volume of 900 ml were used for each treatment. There were ten repetitions for each treatment (n = 90). Table 2 shows the nutrients concentrations in each treatment.

After performing the dilutions to determine nutrient concentrations and addition of inoculums in the recipients, these were taken to the culture room under controlled conditions of photoperiod (12 hours of light and 12 hours of dark) and temperature (28 ºC). All containers were kept with constant aeration. This was done to obtain CO_2 and to prevent microalgae cells from sedimenting at the bottom of the container. The pH ranged from 7.6 to 7.8. Every 24 hours was removed aliquot from each treatment to determine the biovolume (Santos-Silva *et al*. 2019), which was in turn used as an estimate of the organisms' biomass.

Table 1: Initial volumes of filtered pond water and initial biovolume of microalgae added in each treatment. Where, PW0 = undiluted pond water; PW50 = 50% diluted pond water and, PW90 = 90% diluted pond water.

Treatments	***S. acuminatus***	***C. vulgaris***	***P. isothrix***
PW0	Filtered pond water (873 mL) + *S. acuminatus* concentrate (27 mL).	Filtered pond water (800 mL) + *C. vulgaris* concentrate (100 mL).	Filtered pond water (821 mL) + *P. isothrix* concentrate (79 mL)

PW50	Filtered pond water (436.5 mL) + distilled water (436.5 mL) + *S. acuminatus* concentrate (27 mL).	Filtered pond water (400 mL) + distilled water (400 mL) + *C. vulgaris* concentrate (100 mL).	Filtered pond water (410.5 mL) + distilled water (410.5 mL) + *P. isothrix* concentrate (79 mL)
PW90	Filtered pond water (87.3 mL) + distilled water (785.7 mL) + *S. acuminatus* concentrate (27 mL).	Filtered pond water (80 mL) + distilled water (720 mL) + *C. vulgaris* concentrate (100 mL).	Filtered pond water (82.1 mL) + distilled water (738.9 mL) + *P. isothrix* concentrate (79 mL)

Table 2: Nutrient amounts of filtered pond water in each treatment. Where, PW0 = undiluted pond water; PW50 = 50% diluted pond water and, PW90 = 90% diluted pond water.

Nutrients	**PW0 (mg L)**	**PW50 (mg L)**	**PW90 (mg L)**
NH_4^+ - N	2.94	1.32	0.24
NO_3^- - N	0.11	0.06	0.01
PO_4^{3-} - P	0.48	0.22	0.03

The characterization of biomass production of each species in each treatment was determined regarding specific growth rate, biomass growth, and total biomass. To characterize the growth phases, were plotted three graphs containing the microalgae biomass growth curves considering all phases (e.g., lag, exponential, stationary, and senescence) in the three treatments over time. To determine the total biomass, was organized a table with the means of total biomass of all species in each treatment. Were specific growth rate (equation 1) was determined using the set of points relative to the exponential growth phase of the microalgae:

$$\mu = \frac{In\,(X_i\,/X_0)}{t_i - t_0} \quad (1)$$

where X_i and X_0 are biomass concentration (mg L^{-1}) at times t_i and t_0, the end and beginning of the exponential growth, respectively.

Results

Table 3 shows the results of specific growth rates of each species in each treatment. The specific growth rates of *C. vulgaris* were higher in PW0 and PW50 (0.96, 0.85 d^{-1}), respectively. The two species of chlorophytes showed a lower specific growth rate in the PW90 treatment. *P. isothrix* did not show phases of biomass growth curve defined in the PW50 and PW90 treatments. Therefore, we did not consider the results of specific growth rates for this species in these treatments.

Figure 2a shows the biomass growth curve of *S. acuminatus*, *C. vulgaris* and *P. isothrix* in the PW0. In this treatment, we observed a biomass growth of *S. acuminatus* and *C. vulgaris* during the experiment. However, the organisms of *P. isothrix* showed less growth in relation to the two species of chlorophytes. As for the biomass growth of *C. vulgaris*, the lag phase occurred between the 1st and 4th day, followed by an exponential phase between the 4th and 7th day, reaching the highest growth peak on the 7th day. As for the biomass growth of *S. acuminatus*, the lag phase occurred between the 1st and the 3rd day, followed by an exponential phase between the 3rd and the 9th day, reaching the highest growth peak on the 9th day. Despite the less growth of *P. isothrix*, in this treatment we still observed a lag phase between the 1st and 3nd day, followed by an exponential phase between the 3rd and 5th day. The highest biomass production was by *C. vulgaris*, with a mean of 10.4 ± 9.4 mg L^{-1} (Table 3).

Figure 2b shows the biomass growth curve of *S. acuminatus*, *C. vulgaris* and *P. isothrix* from the PW50. In this treatment, we observed a biomass growth of *S.*

acuminatus and *C. vulgaris* during the experiment. However, the organisms of *P. isothrix* showed less growth in relation to the two species of chlorophytes. As for the biomass growth of *C. vulgaris*, the lag phase occurred between the 1st and 4th day, followed by an exponential phase between the 4th and 7th day, reaching the highest growth peak on the 7th day, followed by a phase of cell death. As for the biomass growth of *S. acuminatus*, the lag phase occurred between the 1st and the 3rd day, followed by an exponential phase between the 3rd and 9th day, reaching the highest growth peak on the 9th day, followed by a cell death. The highest biomass production was by *C. vulgaris*, with a mean of 10.0 ± 8.5 mg L^{-1} (Table 3).

Figure 2c shows the biomass growth curve of *S. acuminatus*, *C. vulgaris* and *P. isothrix* from the PW90. The organisms of the three species showed a low growth in relation to PW0 and PW50. As for the biomass growth of *C. vulgaris*, the lag phase occurred between the 1st and 4th day, followed by an exponential phase between the 4th and 8th day, reaching the highest growth peak on the 8th day, followed by a cell death. As for the biomass growth of *S. acuminatus*, the lag phase occurred only on the 1st day, followed by an exponential phase between the 2nd and 5th day, in which they reached a stationary phase between the 5th and 9th day. In this treatment, the production of biomass of *C. vulgaris* and *S. acuminatus* were similar, means of 4.1 ± 3.1 mg L^{-1} and 4.2 ± 1.6 mg L^{-1} (Table 3).

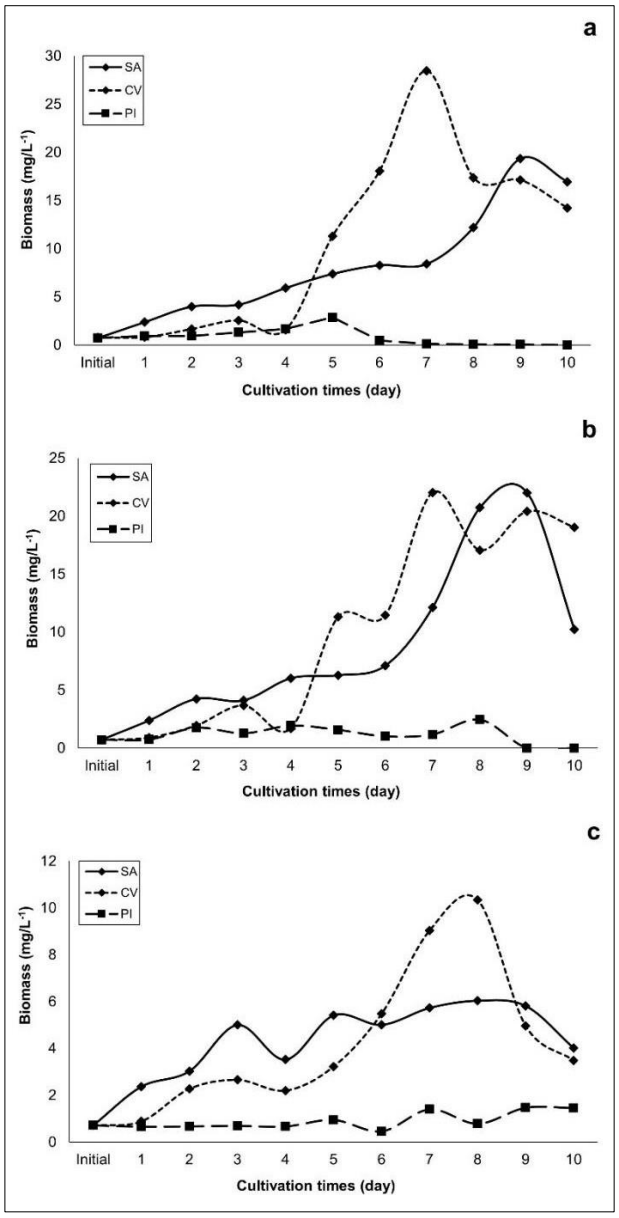

Figure 2: Microalgae biomass growth curve in domestic effluent. a) Microalgae biomass growth curve in the PW0 treatment (undiluted pond water); b) Microalgae biomass growth curve in the PW50 treatment (50% diluted pond water); c) Microalgae biomass growth curve in the PW90 treatment (90% diluted pond water.). *SA = *Scenedesmus acuminatus*, CV = *Chlorella vulgaris*, PI = *Planktothrix isothrix*.

Table 3: The reported values correspond to the mean biomass production (mg L^{-1}) ± standard deviation (SD) and specific growth rate (μ day^{-1}) of microalgae in the treatments. Where, PW0 = undiluted pond water; PW50 = 50% diluted pond water and, PW90 = 90% diluted pond water.

Treatments	Microalgae	Biomass (mg L^{-1})	μ (dia^{-1})
PW0	*S. acuminatus*	8.15 ± 5.88	0.25
	C. vulgaris	10.4 ± 9.46	0.96
	P. isothrix	0.82 ± 0.87	0.39
PW50	*S. acuminatus*	8.72 ± 7.05	0.28
	C. vulgaris	10.0 ± 8.55	0.85
	P. isothrix	1.15 ± 0.76	-
PW90	*S. acuminatus*	4.24 ± 1.69	0.22
	C. vulgaris	4.11 ± 3.13	0.38
	P. isothrix	0.90 ± 0.36	-

Discussion

Chlorella vulgaris specific growth rates were higher compared to those of *S. acuminatus* in the PW0 and PW50. This means that *C. vulgaris* achieved faster growth than *S. acuminatus*. We compared the results of specific growth rates of *C. vulgaris* and *S. acuminatus* with the results from the literature (Table 4). Our results for the specific growth rates of the two species of chlorophytes corroborate those of the literature, mainly for domestic effluents.

In our results, the chlorophytes *S. acuminatus* and *C. vulgaris* showed biomass growth curves with phases (lag/adaptation, growth/exponential, and senescence/death) and follow the standard growth curve observed for microalgae (Sipaúba-Tavares & Rocha 2003, Lourenço 2006). The time of growth phases vary according to species, volume, type of cultivation, and culture medium. For example, Ansari *et al*. (2019)

cultivated *Scenedesmus obliquus* in a municipal effluent for 16 days and observed the lag phase on the 1st day, followed by an exponential phase until the 13th day, with the beginning of the stationary phase from the 14th day onwards. They did not follow the senescence phase. On the other hand, Jasni *et al*. (2023) cultivated *Scenedesmus* sp. in palm oil effluent (POME) for 30 days and observed the lag phase until the 20th day, followed by an exponential phase between the 21st and 25th day, followed by a stationary phase. The authors did not follow the senescence phase. Júnior *et al*. (2013) cultivated *C. vulgaris* in different dilutions of swine effluent (10%, 1%, 0.5%, and 0.3%) for 60 days and observed that there was no specific development of all phases beyond the exponential phase. On the other hand, AlMomani & Örmeci (2016) cultivated *C. vulgaris* in primary, secondary and tertiary effluent from a wastewater treatment plant for 40 days and observed a lag, an exponential and a stationary phase. The period of the lag phases in the primary (6 days), secondary (15 days) and tertiary (19 days) effluents were different. According to AlMomani & Örmeci (2016), the organisms of *C. vulgaris* need an adaptation period to wastewater from sewage treatments.

Table 4: Specific growth rate of *C. vulgaris* and *S. acuminatus* in different types of effluent compared to the literature.

Microalgae	**Medium**	**μ (day^{-1})**	**References**
C. vulgaris	Domestic wastewater	0.92	He *et al*. (2013)
C. vulgaris	Domestic wastewater	1.37	Ji *et al*. (2013)
C. vulgaris	Municipal wastewater	0.20 – 0.37	Li *et al*. (2013)
C. vulgaris	Vinasse wastewater	0.76	Marques *et al*. (2013)
C. vulgaris	Vinasse wastewater	0.53 – 0.52	Candido *et al*. (2015)
S. obliquus	Domestic wastewater	1.14	Ji *et al*. (2013)
Scenedesmus sp.	Synthetic urban wastewater	0.58	Wang *et al*. (2016)
S. obliquus	Domestic wastewater	0.42 ± 0.02	Ansari *et al*. (2019)

Scenedesmus sp.	Wet market wastewater	0.19	Apandi *et al*. (2021)
Scenedesmus sp.	Domestic wastewater	0.25	Jasni *et al*. (2023)

The monitoring of growth phases is always necessary since the subculture of microalgae in a culture system must always be carried out at the exponential phase, as the cells tend to present a better physiological state, which contributes to a faster adaptation to the desired experimental conditions (Lourenço 2006). Thus, our results indicate that in the PW0 and PW50, the subculture of *S. acuminatus* and *C. vulgaris* organisms could occur between the 3th and 9th day and between the 4th and 7th day, respectively. This makes it clear that depending on the type of application carried out using the biomass of these microalgae species, *C. vulgaris* grows faster than *S. acuminatus*.

Despite the successful growth of *P. isothrix* in the pond water, under these controlled experiment conditions its growth was low. There is little evidence regarding the cultivation of organisms of *P. isothrix* mainly using eutrophic water as a culture domestic effluent, such as the study by Silva-Benavides and Torzillo (2012), where the organisms of this species had an optimal growth when cultivated together with *C. vulgaris*. In commercial culture media such as WC (Guillard & Lorenzen, 1972) and BG-11 (Rippka, 1979), rich in micronutrients and vitamins, and under controlled conditions *P. isothrix* has a high growth rate and an optimal biomass yield (Sassi *et al.*, 2019).

The domestic effluent used at different concentrations of nutrients was not sufficient for the growth of *P. isothrix*. The fact that *P. isothrix* organisms showed less growth may be due to other factors (association with other organisms such as bacteria, fungi and other species of algae) that were not measured in this study since pH, temperature, amount of nutrients, and photoperiod are in accordance with the ideal

conditions for the general growth of cyanobacteria as mentioned in the literature (Talbot *et al*. 1991, Markou & Georgakakis 2011).This study is one of the pioneers in the use of *P. isothrix* at different concentrations of nutrients from a domestic effluent in a controlled way and, with the results obtained, questions arise that deserve to be explored: What is the determining factor for the success of the growth of *P. isothrix* in addition to those used in this study? Is there a relevant association between *P. isothrix* and other organisms that affect its growth since there is evidence about growth in consortium with cyanobacteria and other organisms? Does the growth optimization of *P. isothrix* depend on the concomitant growth of other microalgae species?

The biomass production of *S. acuminatus* and *C. vulgaris* were similar in all three treatments. However, *C. vulgaris* had the highest biomass production in the PW0 and PW50. Table 5 compares the biomass production of *S. acuminatus* to the biomass production of *Scenedesmus* sp. cultivated in various types of effluents. We observed that the biomass production of *S. acuminatus* found in this study is similar as the biomass production of *Scenedesmus* sp. grown in domestic effluent. This confirms the potential use of this effluent as an alternative culture medium for growth and biomass yield for this species.

We observed that *S. acuminatus* organisms showed a good growth performance and biomass production in PW0 and PW50. As the biomass production was similar in these treatments, our results indicate that it is not necessary to dilute the effluent for the growth of this species. Therefore, our result corroborates those of Wang *et al*. (2016), who tested the cultivation of *Scenedesmus* sp. at different levels of dilution of urban effluent (100%, 75%, 50%, 25% and BG11) and observed greater biomass production at 100% (4.12×10^6 cells/mL) and a higher biomass than those of the commercial medium BG11. Ambiental *et al*. (2017) tested a synthetic domestic effluent at different

concentrations of total nitrogen and total phosphorus and observed that of the 25 species of microalgae tested, only *Scenedesmus* sp. grew efficiently at the highest concentrations of nitrogen (150 mg L) and phosphorus (40 mg L).

In tannery effluent, *Scenedesmus* sp. was cultivated at different effluent concentrations (between 20% and 100%). The results showed that the adaptation to this source of nutrients was effective and that *Scenedesmus* sp. showed a higher biomass production at 88.4% of tannery effluent (da Fontoura *et al*. 2017). *Scenedesmus* sp. was tested at different nutrient concentrations (15%, 30%, 45% and 60%) of sewage treatment plants. There was a high tolerance to high nutrient conditions, where the highest biomass production was obtained at the concentration of 60% (Jebali *et al*. 2018). The biomass production of *Scenedesmus* sp. was obtained using wet market effluent. The biomass production of these organisms was tested at different proportions of the effluent (10, 25, 50, 75 and 100%). The highest biomass production occurred at 50% of effluent (Apandi *et al*. 2019).

Table 5: Biomass production of *Scenedesmus* sp. in different types of effluents compared to the literature.

Microalgae	**Medium**	**Biomass**	**References**
Scenedesmus sp.	Municipal wastewater	0.30 g L^{-1} d^{-1}	Dickinson *et al*. (2013)
Scenedesmus sp.	Domestic wastewater	20 g m^{-2} d^{-1}	Yin-Hu *et al*. (2013)
S. dimorphus	Domestic wastewater	0.24 g L^{-1}	Zhang *et al*. (2014)
Scenedesmus sp.	Bovine wastewater	0.12 g L^{-1} d^{-1}	Ledda *et al*. (2016)
Scenedesmus sp.	Tannery wastewater	0.90 g L^{-1}	Da Fontoura *et al*. (2017)
Scenedesmus sp.	Domestic wastewater	7.80 g m^2	Jebali *et al*. (2018)
Scenedesmus spp.	Wastewater from primary sewage treatment	0.44 ± 0.02 g L^{-1}	Khan *et al*. (2018)
Scenedesmus sp.	Domestic wastewater	1.22 g L^{-1}	Pandey *et al*. (2019)
Scenedesmus sp.	Wet market wastewater	0.098 g L d	Apandi *et al*. (2019)
S. obliquos	Municipal wastewater	0.42 g L^{-1}	Ansari *et al*. (2019)

S. oblíquos and C. Vulgaris	Bovine wastewater	1.7 g L	Hu *et al*. (2019)
S. abundans	Pharmaceutical wastewater	0.97 ± 0.01 g L	Nayak & Ghosh (2019)
Scenedesmus sp.	Industrial wastewater	1.75 ± 0.60 g L^{-1} d^{-1}	Mercado *et al*. (2020)
Scenedesmus sp.	Wet market wastewater	0.073 g L d	Apandi *et al*. (2021)
Scenedesmus sp.	abattoir efluente	19.24 g m^{-2} d^{-1}	Shayesteh *et al*. (2021)
Scenedesmus sp.	Domestic wastewater	0.062 g L d	Thangam *et al*. (2021)
Scenedesmus sp.	Swine wastewater	0.414 g/L	Zhao *et al*. (2022)
Scenedesmus sp.	Domestic wastewater	0.55 g/L	Wang *et al*. (2022)

Table 6 compares the biomass production of *C. vulgaris* grown in various types of effluents. We observe that the biomass production of *C. vulgaris* found in this study is similar as the biomass production of *C. vulgaris* cultivated in domestic effluent. Our results corroborate those of Miao *et al*. 2016 where the highest biomass production of *C. vulgaris* was obtained when cultivated in 100% domestic sewage. This confirms the potential use of this effluent as an alternative culture medium for growth and biomass yield for this species.

Table 6: Biomass production of *C. vulgaris* in different types of effluents compared to the literature.

Microalgae	**Medium**	**Biomass**	**References**
C. vulgaris	Swine wastewater	0.49 ± 0.26 g L	Abou-Shanab *et al*. (2013)
C. vulgaris	Municipal wastewater	0.195 g L^{-1}	Cabanelas *et al*. (2013)
C. vulgaris	Municipal wastewater	0.52 – 0.76 g L^{-1}	Li *et al*. (2013)
Chlorella sp.	Brewery wastewater	1.5 g L	Farooq *et al*. (2014)
C. vulgaris	Municipal wastewater	0.138 g L d	Ebrahimian *et al*. (2014)
C. vulgaris	Domestic wastewater	0.81 g L^{-1}	Trevisam *et al*. (2014)
C. vulgaris	Swine wastewater	3.96 g L	Wang *et al*. (2015)
C. vulgaris	Domestic wastewater	1.57 ± 0.43 g L	Fernández-Linares *et al*. (2017)
C. vulgaris	Municipal wastewater	0.05 g L d	Mujtaba & Lee (2017)

C. vulgaris	Pulp wastewater	1.31 g/L	Daneshvar *et al*. (2018)
C. vulgaris	Municipal wastewater	0.070 ± 0.001 g L d	Ge *et al*. (2018)
C. vulgaris	Municipal wastewater	1.6 g L^{-1}	Znad *et al*. (2018)
C. vulgaris	Fish wastewater	0.257 ± 0.001 g L^{-1} d^{-1}	Trivedi *et al*. (2019)
Chlorella sp.	Landfill wastewater	0.192 g L^{-1}	Silva *et al*. (2020)
Chlorella sp.	Textile wastewater	3.94 – 4.89 g cm^3	Wu *et al*. (2020)
C. vulgaris	Municipal wastewater	2.1 g L^{-1}	Carneiro *et al*. (2021)
C. vulgaris	Poultry slaughterhouse Wastewater	1.2 g L^{-1}	Hilares *et al*. (2021)
C. vulgaris	Industrial wastewater	2.82 g L^{-1}	Rodrigues-Sousa *et al*. (2021)
C. vulgaris	Municipal wastewater	0.67 g L	Pooja *et al*. (2022)

We note that, comparing the production of chlorophyte biomass between different types of effluents, we verify that depending on the type of effluent used, there is or not a need to dilute the nutrients found in effluents. In this case, the amounts of nutrients in the domestic effluent tested sufficient for an optimal growth performance and biomass production of *S. acuminatus* and *C. vulgaris*.

Alternative culture media have been proposed for the growth of freshwater microalgae. Among the commercial media most used for microalgae cultivation are BG-11, TAP, BBM, Chu-10, and D-Medium (Pandey *et al*. 2023). These commercial culture media are effective for the growth of freshwater microalgae, especially when it comes to cultivation for the isolation of new strains and on a laboratory scale. In this way, an alternative means has been sought to reduce the cost of production compared to commercial cultivation media. An example is NPK, an agricultural fertilizer that has stood out for being a low-cost fertilizer in relation to the commercial cultivation media and efficient for the growth of species of the genera *Scenedesmus*, *Chlorella*, *Pediastrum*, *Ankistrodesmus* and *Desmodesmus* in small-scale controlled cultivations

(Sipaúba-Tavares & Rocha 2003). However, for productions of microalgae on a large scale, the cost with NPK increases.

According to Pandey *et al*. (2023), *Scenedesmus* sp. can produce greater biomass in BG-11 culture medium than in wastewater, mainly in effluents from lactic acid industry. However, when we compare the biomass of *Scenedesmus* sp. between the domestic effluent and this culture medium, the biomass is higher in the effluent (Wang et al., 2016). Ansari *et al*. (2019) cultivated *Scenedesmus obliquus* in domestic effluent. The authors obtained higher yields of lipids and carbohydrates compared to those cultivated in BG-11. Regarding *C. vulgaris*, it had a higher biomass yield when cultivated in domestic effluent than in BG-11 medium (Miao *et al*. 2016). Thus, the use of effluents as an alternative medium can be a solution for large-scale microalgae production. Therefore, the use of different types of effluents rich in nitrogen and phosphates has been taken into account. In addition to promoting optimal growth and yield of chlorophyte biomass, they can be safely disposed of after remediation without harming aquatic ecosystems.

We conclude that water eutrophicated by undiluted domestic effluent is the best option, among those tested, as an alternative medium for growing planktonic green microalgae. The organisms of *C. vulgaris* are the best option for biomass usage, as they achieve the highest growth and biomass production in a shorter period.

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Credit Authorship Contribution Statement

Raize Castro-Mendes: Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Roles/Writing - original draft; Writing - review & editing.

Renan Gomes do Nascimento: Conceptualization, Methodology, Supervision, Writing - review & editing.

Edinaldo Nelson do Santos-Silva: Conceptualization, Methodology, Writing - review & editing, Project administration, Resources.

Conflict of Interest

The authors declare there no conflict of interest that could perceive to influence the results of the research.

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CAPÍTULO II

CASTRO-MENDES, R. C.; NASCIMENTO, R. G.; ARZABE, A. A. F.; PRIMEIRO, L. J. O. G.; BANDEIRA, M. G. S.; SANTOS-SILVA, E. N. Use of microalgae in the bioremediation of water eutrophicated by domestic effluent in an urban pond in the Amazon. Acta Amazonica. http://dx.doi.org/10.1590/1809-4392202303621

ENVIRONMENTAL SCIENCES | ORIGINAL ARTICLE

Use of microalgae in the bioremediation of water eutrophicated by domestic effluent in an urban pond in the Amazon

Raize CASTRO-MENDES*, Renan G. NASCIMENTO, Maiby G. S. BANDEIRA, Luis J. O. G. PRIMEIRO, Alexander F. ARZÁBE, Edinaldo N. dos SANTOS-SILVA
Instituto Nacional de Pesquisas da Amazônia - INPA, Laboratório de Plâncton, Av. André Araújo, 2936 - Petrópolis, 69067-375 Manaus - AM
*Corresponding author: raize.mendes@gmail.com

ABSTRACT

The disposal of domestic effluents without an adequate treatment may increase nitrogen and phosphorus levels in natural water bodies. Bioremediation using microalgae is one of the solutions for treating effluents before disposal. We tested the effect of *Scenedesmus acuminatus*, *Chlorella vulgaris* and *Planktothrix isothrix*, as well as the effect of dilutions, on the nutrient concentration in water eutrophicated by domestic effluent in an urban lake in the Brazilian Amazon. We inoculated the three species in monoculture in undiluted water (PW0), and 50% (PW50) and 90% (PW90) diluted water. The experiment lasted 10 days and every 24 hours we removed a bottle of each treatment for nutrient analysis. The three species were equally efficient in removing ammonia in PW0. Nitrate removal rate was highest for *Chlorella vulgaris* in PW0, and higher for *C. vulgaris* and *P. isothrix* in PW50 and PW90. Orthophosphate removal efficiency was higher for *S. acuminatus* and *C. vulgaris* in PW0, equally efficient for the three species in PW50, and higher for *C. vulgaris* and *P. isothrix* in PW90. We concluded that the three species of microalgae tested are efficient in removing ammonia. *Scenedesmus acuminatus* was not an ideal species for nitrate removal. *Planktothrix isothrix* was efficient in removing nutrients when domestic wastewater is diluted. *Chlorella vulgaris* was efficient in removing nutrients from domestic wastewater whether diluted or not.

KEYWORDS: cyanobacteria, chlorophytes, nutrient removal, phytoplankton, wastewater treatment

Utilização de microalgas na biorremediação de águas eutrofizadas por efluente doméstico em um lago urbano na Amazônia

RESUMO

O descarte de efluentes domésticos sem tratamento adequado pode elevar os níveis de nitrogênio e fósforo em corpos hídricos naturais. A biorremediação com o uso de microalgas é uma solução para o tratamento de efluentes antes do descarte. Nós testamos o efeito de *Scenedesmus acuminatus*, *Chlorella vulgaris* e *Planktothrix isothrix* e o efeito da diluição da água sobre a concentração de nutrientes da água eutrofizada por efluente doméstico de um lago urbano na Amazônia brasileira. Inoculamos as três espécies em monocultura em água não diluída (PW0) e diluída a 50% (PW50) e 90% (PW90). O experimento durou 10 dias e a cada 24 horas retiramos um recipiente de cada tratamento para análise de nutrientes. As três espécies foram igualmente eficientes na remoção de amônia em PW0. A eficiência de remoção de nitrato foi mais alta com *C. vulgaris* em PW0, e mais alta com *C. vulgaris* e *P. isothrix* em PW50 e PW90. A eficiência de remoção de ortofosfato foi mais alta com *S. acuminatus* e *C. vulgaris* em PW0, igualmente eficiente para as três espécies em PW50, e mais alta com *C. vulgaris* e *P. isothrix* em PW90. Concluímos que as três espécies de microalgas testadas são eficientes na remoção da amônia. *Scenedesmus acuminatus* não foi ideal para a remoção de nitrato. *Planktothrix isothrix* foi eficiente na remoção de nutrientes quando a água residual doméstica é diluída. *Chlorella vulgaris* foi eficiente na remoção de nutrientes de águas residuais domésticas, estando diluída ou não.

PALAVRAS-CHAVE: cianobactéria, clorofíceas, fitoplâncton, remoção de nutrientes, tratamento de águas residuais

INTRODUCTION

In Brazil, 55% of the population have sewage treatment, 18% have their sewage colleted, but not treated, and 27% have neither collection nor treatment of sewage (ANA 2022). This scenario is worse in the northern region of Brazil. According to the National Sanitation Information System (SNIS 2021), the state of Amazonas has 21.3% of sewage collection and 20.5% of treated sewage is from consumed domestic wastewater.

The capital city of Amazonas, Manaus, is among the 20 worst Brazilian cities as for sewage treatment (Instituto Trata Brasil 2024). This means that the majority of wastewater is directed in untreated state to streams that cross the city and which become so-called "open sewers" that flow into the Negro River. The four main river basins which are occupied by the urban area of Manaus (São Raimundo, Educandos, Tarumã-Açú and Puraquequara) are contaminated, mainly by domestic sewage, and present high levels of pollutants such as nitrogen, phosphorus, heavy metals, and pharmaceuticals (Pinto et al. 2009; Rico et al. 2021).

The disposal of sewage without adequate treatment changes natural concentrations (e.g. nitrogen and phosphorus) in water bodies that cause artificial eutrophication, and can result in the growth of cyanobacteria, which release cyanotoxins and prevent the growth of other organisms, loss of aquatic biodiversity, and poor water quality (Dokulil and Teubner 2011). To mitigate the problem of artificial eutrophication, it is necessary to treat sewage before disposal (Zhou et al. 2022). Among sewage treatments, the most common in Brazil uses anaerobic processes, by which organic matter is converted into carbon dioxide and methane (Cornelli et al. 2014). However, one of the main limitations to this treatment is its low effectiveness in reducing nitrogen and phosphorus levels (Cornelli et al. 2014).

In intensive sewage treatment systems, there are generally five stages, where removal of nitrogen and phosphorus occurs at the second stage (Oswald 1988). The simultaneous removal of these nutrients is crucial for improving the quality of secondary effluent from

sewage treatment stations (STSs) aiming to prevent eutrophication (Zhou et al. 2022). Due to public concern regarding environmental preservation and the health risks caused by pollution and water scarcity, wastewater disposal standards are becoming increasingly stringent, accelerating the need to modernize STSs (Zhou et al. 2022).

Advanced nitrogen and phosphorus removal for secondary effluents is not limited to a single process, as it requires a combination that includes bioremediation (Zhou et al. 2022). Some microorganisms, such as microalgae, have the ability to remove nutrients from the water during their growth (Lourenço 2006). The application of microalgae to wastewater has shown some desirable results in water purification and nutrient recovery (Vaz et al. 2023). For example, a reduction of 82.4% in ammonia concentration and of up to 90.6% in phosphorus concentration was observed in nutrient removal efficiency by *Chlorella* sp. (Wang et al. 2009). *Chlorella vulgaris* Beijerink 1890 was able to remove ammonia and phosphorus from effluents from secondary sewage treatment within 48 hours (Kim et al. 2013). In the study of Wong et al. (2015), *Scenedesmus quadricauda* (Turpin) Bréb was able to remove more than 95% of ammonia and 90% of phosphorus in secondary effluent treatment within five days (Wong et al. 2015).

Microalgae removal efficiency may depend on effluent filtration and dilution (Santos et al. 2021). For example, *C. vulgaris* was tested in domestic effluent at dilutions of 100%, 75%, 50%, and 25%. The highest efficiency in removing ammonia (98.6%) and total phosphorus (86%) was achieved at the 25% dilution (Miao et al., 2016). Therefore, effluent dilutions can be important, as the concentration of nutrients (e.g., ammonia, nitrate, and orthophosphate) can vary, influencing the ability of microalgae to remove nutrients.

In general, species of Chlorophyceae exhibit excellent results in the removal of nutrients (e.g., nitrogen and phosphorus) from wastewater. However, it is worth noting that some species of Cyanophyceae, such as *Anabaena* sp., *Spirulina* sp., *Oscillatoria* sp., *Synechococcus* sp., *Phormidium* sp., and *Aphanothece microscopica* Nägeli, have been efficient in

the removal of nitrogen and phosphates (Gupta et al. 2013). Whereas cyanobacteria are successful in inhabiting and forming blooms in eutrophic environments, it is interesting to investigate whether certain species also have the ability to remove nutrients, expanding the catalogue of known species in this regard, such as *Planktothrix isothrix* (Skuja) Komárek and Komárek, a species that forms blooms in urban aquatic environments of the Amazon region (Pascoaloto et al. 2015).

The treatment of domestic effluents plays a primary role in preserving water quality in and around Amazonian urban centers. It is important to test species of Chlorophyceae common in the Amazon region, such as *Scenedesmus acuminatus* (Lagerhein) Chodat and *C. vulgaris*, in addition to Cyanophyceae such as *P. isothrix,* often found in eutrophic environments in the region, to assess their efficiency in removing nutrients. Furthermore, it is important not only to determine if these species remove nutrients, but also to identify which one is more efficient in nutrient removal, considering the different dilutions of the effluent. Therefore, we tested the efficiency of two green microalgae, *Scenedesmus acuminatus* and *Chlorella vulgaris*, and the cyanobacterium *Planktothrix isothrix* in reducing dissolved nutrient concentrations in different dilutions of water eutrophicated by domestic effluent from an urban pond in Manaus.

MATERIAL AND METHODS

Study site and inoculum acquisition

We used water from an eutrophicated urban pond in the city of Manaus (Japiim Pond), Amazonas state (Brazil) as cultivation medium. The pond is 155 m long, 45 m wide and up to 4.8 m deep (Figure 1). It receives water from rainfall and domestic effluent from surrounding properties (e.g., households and commercial establishments). The water from the pond flows into a stream that originates in the area of the Federal University of Amazonas (UFAM) and is a tributary of the Quarenta Stream. Water for the experiment was collected in September 2021.

Green microalgae strains were obtained from the Plankton Laboratory at the National Institute for Research in the Amazon - INPA. *Planktothrix isothrix* samples were collected directly from the pond with a 20-µm mesh plankton net. In the laboratory, the samples were washed with distilled water, concentrated in a 20-µm mesh filter, measured under acommon microscope with a micrometric eyepiece and counted in a Sedgewick-rafter camera, before being inoculated in the experimental units.

Experimental design and protocol

The experiment was carried out over 10 days under controlled laboratory conditions at INPA and consisted in testing the differential effect of biomass growth of *S. acuminatus*, *C. vulgaris* and *P. isothrix* on the reduction of nutrient concentrations in the eutrophicated water of the target pond.

We considered two treatments: 1) species (three levels), and 2) pond water dilution, at three levels: (a) undiluted pond water (PW0); (b) pond water diluted to 50% with distilled water (PW50); and (c) pond water diluted to 90% with distilled water (PW90) (Table 1). Undiluted pond water without any inoculant was used as control. Ten replications were used for each combination of treatments and the control (n = 120 experimental units). Each experimental unit consisted of a 900-mL PET (polyethylene terephthalate) bottle. The ten experimental units for each treatment were placed in separate compartments on a shelf, with each compartment having the same light distribution (1900 lux) and temperature (28 ºC).

Figure 1. Panoramic view of the study site, Japiim pond, and its surroundings in the city of Manaus, Amazonas state, Brazil. Credit: Bruno Barreto.

Table 1. Initial volume of filtered pond water, distilled water and biovolume of inoculant (Scenedesmus acuminatus, Chlorella vulgaris and Planktothrix isothrix) used in each dilution treatment level. PW0 = undiluted pond water; PW50 = pond water diluted to 50% PW90 = pond water diluted to 90%; Control = filtered undiluted pond water without innoculant.

Diluition level	Filtered pond water (mL)	Distilled water (mL)	Inoculant concentrate (mL)
Scenedesmus acuminatus			
PW0	873	0	27
PW50	436.5	436.5	27
PW90	87.3	785.7	27
Chlorella vulgaris			
PW0	800	0	100
PW50	400	400	100
PW90	80	720	100
Planktothrix isothrix			
PW0	821	0	79
PW50	410.5	410.5	79
PW90	82.1	738.9	79
Control			
PW0	900	0	0
PW50	450	450	0
PW90	90	810	0

We collected 60 L of surface water using a PVC pipe (1 m long; 5 cm diameter) with a check valve attached to the extremity, which was inserted vertically into the water

column. The water was transported to the laboratory, where it was filtered in a manually constructed filter with a 20-L PET (polyethylene terephthalate) bottle (Figure 2; Santos et al. 2023). Before inserting the material into the bottle, we washed it with distilled water and sterilized it with 0.5 ml of sodium hypochlorite per liter. The bottle was filled (from top to bottom) with layers of 20 µm mesh net, wool-based fabric, coarse rolled pebbles (19-38 mm largest diameter), medium-sized pebbles (6.4-12.7 mm), fine pebbles (3.4-6.7 mm), a plastic screen to retain the pebbles (0.27 mm) and fine sand. After filtration, we stored the water in a bucket, added 0.5 ml of sodium hypochlorite per liter and kept in the dark for 24 hours.

Each replicate consisted of a 900-mL bottle container provided with constant aeration to aid in the determination of CO_2 and prevent the inoculant cells from settling on the bottom of the bottle (Sipaúba-Tavares and Rocha 2003). We used 250 ml of water from each dilution treatment level to analyze the initial nutrient concentration. Following the dilution and inoculation process in the bottles, we transported them to the cultivation room under the specified conditions. The photoperiod was 12 h light/12 h darkness at room temperature of 28 ºC. This temperature was chosen because it is the average water temperature in the Japiim pond. Every 24 hours we extracted 300 mL of water from one of the ten replicates in each treatment level to measure nutrient concentration

Inoculant biovolume

The three species used in this study have different cell size and shape, which is why we chose biovolume as a measure of inoculum. We used the BioCalc software to calculate the biovolume of each organism and the mean biovolume of the 15 organisms for each species (Santos-Silva et al. 2019). To achieve similar initial biovolume among inoculant species, we isolated 15 organisms of each species and measured the cell width and length of these organisms under an optical microscope equipped with a micrometered eyepiece using 40 x magnification. We counted the number of cells with a Sedgewick Rafter

camera. We estimated the biovolume of each species in each replicate by multiplying the mean cell volume by the number of organisms counted in 100 ml. The initial inoculum biovolume used for the three species was 0.74 mg L-1.

Nutrient concentration

The 300-mL water samples taken every 24 hours were filtered using glass fiber filters and a vacuum pump with a power of 0.17 kW. The concentrations of nitrate (NO_3) and orthophosphate (PO_4^3) was measured according to Golterman et al. (1978). Ammonia (NH_4^+) was measured using flow injection analysis (FIA) (Ruzicka and Hansen 1975; Stewart 1976). For both methods, calibration curves with specific standards were used and we used a spectrophotometry technique for reading.

Data treatment

Nutrient concentration curves over time were estimated for each treatment level. The removal efficiency (RE, %) was determined according to equation [1]

$$RE = \frac{S_0 - S_f}{S_0} \text{X } 100\% \qquad (1)$$

where: S_0 is the concentration of a given nutrient at the initial time t_0 and S_f is the concentration of that same nutrient at the final time t_f.

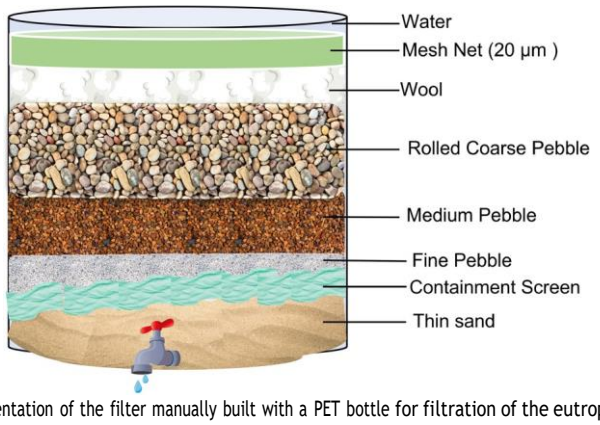

Figure 2. Schematic representation of the filter manually built with a PET bottle for filtration of the eutrophisized water from an urban pond used

as culture medium. See Material and Methods for specifications. Image adapted from https://sustentavel.com.br/filtro-de-agua-caseiro/. Credit: Raize Castro-Mendes.

To test the effect of the factors species (*S. acuminatus, C. vulgaris* and *P. isothrix*) and dilution (PW0, PW50 and PW90) on the concentration of nutrients (ammonia, nitrate, and orthophosphate) we used a multivariate analysis of variance (MANOVA). Subsequently, to highlight the significant difference in nutrient concentrations between treatments, we conducted a one-way analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. As an a posteriori test to compare means, we used the Tukey test with a significance level of level of $\alpha = 0.05$. All analyses were undertaken in the R 4.1 statistical platform (R Core Team 2021).

RESULTS

Evolution of nutrient concentration

In PW0 inoculated with *C. vulgaris* there was a reduction of ammonia on the third day (Figure 3a), and of nitrate and orthophosphate on the fourth day (Figure 3b,c). In PW50 inoculated with *C. vulgaris* and *P. isothrix* there was a reduction in ammonia on the second day (Figure 3d). *Planktothrix isothrix* and *C. vulgaris* reduced nitrate and orthophosphate on the second day, respectively (Figure 3e,f). In PW90, none of the three species reduced ammonia (Figure 3g), however, *C. vulgaris* reduced nitrate on the second day (Figure 3h), and both *C. vulgaris* and *P. isothrix* reduced orthophosphate on the sixth day (Figure 3i).

Removal efficiency

Nitrogenous compounds – In PW0, *S. acuminatus* and *C. vulgaris* had 100% removal efficiency (RE) for ammonia, while *P. isothrix* had 78.6% RE for this nutrient (Table 2). In PW50, *S. acuminatus* was not efficient in removing ammonia, while *C. vulgaris* and *P. isothrix* had 90% and 69.4% RE for ammonia, respectively. *Scenedesmus acuminatus*, *C. vulgaris*, and *P. isothrix* presented 18%, 98.7%, and 93.8% RE for nitrate, respectively. In PW90, *S. acuminatus* was not efficient in removing ammonia nor nitrate. *Chlorella vulgaris* and *P.*

isothrix had 91.4% and 98.4% RE for nitrate, respectively.

Orthophosphate – In PW0, the highest orthophosphate RE was 100% for *S. acuminatus* and *C. vulgaris. Planktothrix isothrix* had an RE of 12.9% at this dilution (Table 2). In PW50, RE for S. acuminatus, *C. vulgaris*, and *P. isothrix* was 100%, 99.3%, and 82.4%, respectively. In PW90, *S. acuminatus* had no RE for orthophosphate, while RE for *C. vulgaris* and *P. isothrix* was 100%.

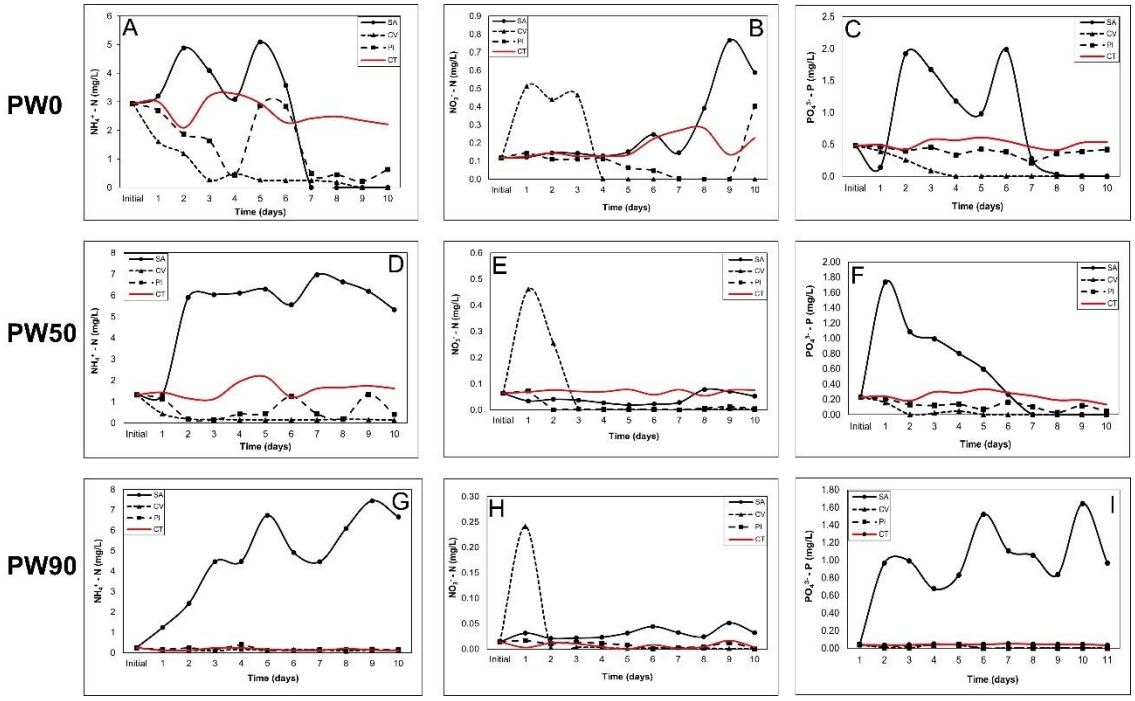

Figure 3. Evolution over 10 days of nutrient concentration in water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil) innoculated with microalgae (*Scenedesmus acuminatus* and *Chlorella vulgaris*) and a cyanobacterium (*Planktothrix isothrix*). PW0 = undiluted pond water (A, B, C); PW50 = 50% diluted pond water (D, E, F); PW90 = 90% diluted pond water (G, H, I). Nutrients: ammonia (A, D, G); nitrate (B, E, H); orthophosphate (C, F, I). SA = *S. acumintaus*, CV = *C. vulgaris*; PI = *P. isothrix*; CT = Control.

Treatment effect on nutrient concentration

The concentrations of ammonia, nitrate and orthophosphate varied significantly with species and water dilution (MANOVA, $p < 0.001$; Table 3). In PW0, average ammonia concentration throughout the 10 days was significantly lower with *C. vulgaris* than with *S. acuminatus*, *P. isothrix* and the control ($p < 0.001$; Figure 4a; Table 4). In PW50 and PW90, ammonia concentration with S. acuminatus was significantly higher than with *C. vulgaris*, *P. isothrix* and the control ($p < 0.001$; Figure 4b, c; Table 4). There was no

significant difference among treatment levels for nitrate concentration (Figure 4d-f; Table 4). In PW0, orthophosphate concentration was significantly lower with *C. vulgaris* than with *S. acuminatus*, *P. isothrix* and the control ($p < 0.05$; Figure 4g; Table 4). In PW50, orthophosphate was significantly lower with *C. vulgaris* and *P. isothrix* than with *S. acuminatus* and the control ($p < 0.05$; Figure 4h; Table 4), and in PW90, orthophosphate was significantly higher with S. acuminatus than with *C. vulgaris*, *P. isothrix* and the control ($p < 0.001$; Figure 4i; Table 4).

Table 2. Nutrient removal efficiency (RE) in eutrophic water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil) innoculated with microalgae (*Scenedesmus acuminatus* and *Chlorella vulgaris*) and a cyanobacterium (*Planktothrix isothrix*). PW0 = undiluted pond water; PW50 = 50% diluted pond water; PW90 = 90% diluted pond water. NH_4^+ - N = ammonia, NO_3^- N = nitrate, PO_4^{3-} - P = orthophosphate.

Wastewater	Nutrient	RE (%)		
		S. acuminatus	*C. vulgaris*	*P. isothrix*
PW0	NH_4^+ - N	100	100	78.6
	NO_3^- - N	0	100	0
	PO_4^{3-} - P	100	100	12.9
PW50	NH_4^+ - N	0	90	69.4
	NO_3^- - N	18	98.7	93.3
	PO_4^{3-} - P	100	99.3	82.4
PW90	NH_4^+ - N	0	63.5	43
	NO_3^- - N	0	91.4	98.4
	PO_4^{3-} - P	0	100	100

Table 3. MANOVA results for the effect of water dilution and innoculate species on nutrient concentration in water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil). PW0 = undiluted pond water; PW50 = 50% diluted pond water; PW90 = 90% diluted pond water. SA = *Scenedesmus acumintaus*, CV = *Chlorella vulgaris*; PI = *Planktothrix isothrix*.

Treatments	Pillai's trace	F	DF	P value
PW0 (SA, CV, PI)	0.606	3.37	9.120	<0.001
PW50 (SA, CV, PI)	0.949	6.17	9.120	<0.001
PW90 (SA, CV, PI)	0.855	5.32	9.120	<0.001

Table 4. Results of simple ANOVA followed by a Tukey *a posteriori* test for each evaluated nutrient concentration within each tested dilution level in water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil). PW0 = undiluted pond water; PW50 = 50% diluted pond water; PW90 = 90% diluted pond water. SA = *Scenedesmus acumintaus*, CV = *Chlorella vulgaris*; PI = *Planktothrix isothrix*; CT = control. P values in bold are significant at α = 0.05.

Treatments	Nutrients	Sum of squares	DF	Mean of squares	F	P	*Post hoc* Tukey test
PW0 (SA, CV, PI)	Ammonia	27.084	3	9.0281	5.57	**0.003**	CV < (SA = PI = CT)
	Nitrate	0.169	3	0.0562	2.00	0.130	
	Orthophosphate	2.595	3	0.8651	5.18	**0.004**	CV < (SA = PI= CT)
Residuals	Ammonia	64.884	40	1.6221			
	Nitrate	1.126	40	0.0282			
	Orthophosphate	6.679	40	0.1670			
PW50 (SA, CV, PI)	Ammonia	169.6273	3	56.54244	51.13	**<0.001**	SA > PI = (CV = CT)
	Nitrate	0.0232	3	0.00775	1.31	0.284	
	Orthophosphate	1.4328	3	0.47759	5.50	**0.003**	(SA = CT) > (CV = PI)
Residuals	Ammonia	44.2334	40	1.10584			
	Nitrate	0.2364	40	0.00591			
	Orthophosphate	3.4714	40	0.08678			
PW90 (SA, CV, PI)	Ammonia	152.95173	3	50.98391	37.64	**<0.001**	SA > (CV = PI = CT)
	Nitrate	0.00430	3	0.00143	1.08	0.369	
	Orthophosphate	7.35891	3	2.45297	55.20	**<0.001**	SA > (CV = PI= CT)
Residuals	Ammonia	54.18537	40	1.35463			
	Nitrate	0.05319	40	0.00133			
	Orthophosphate	1.77763	40	0.04444			

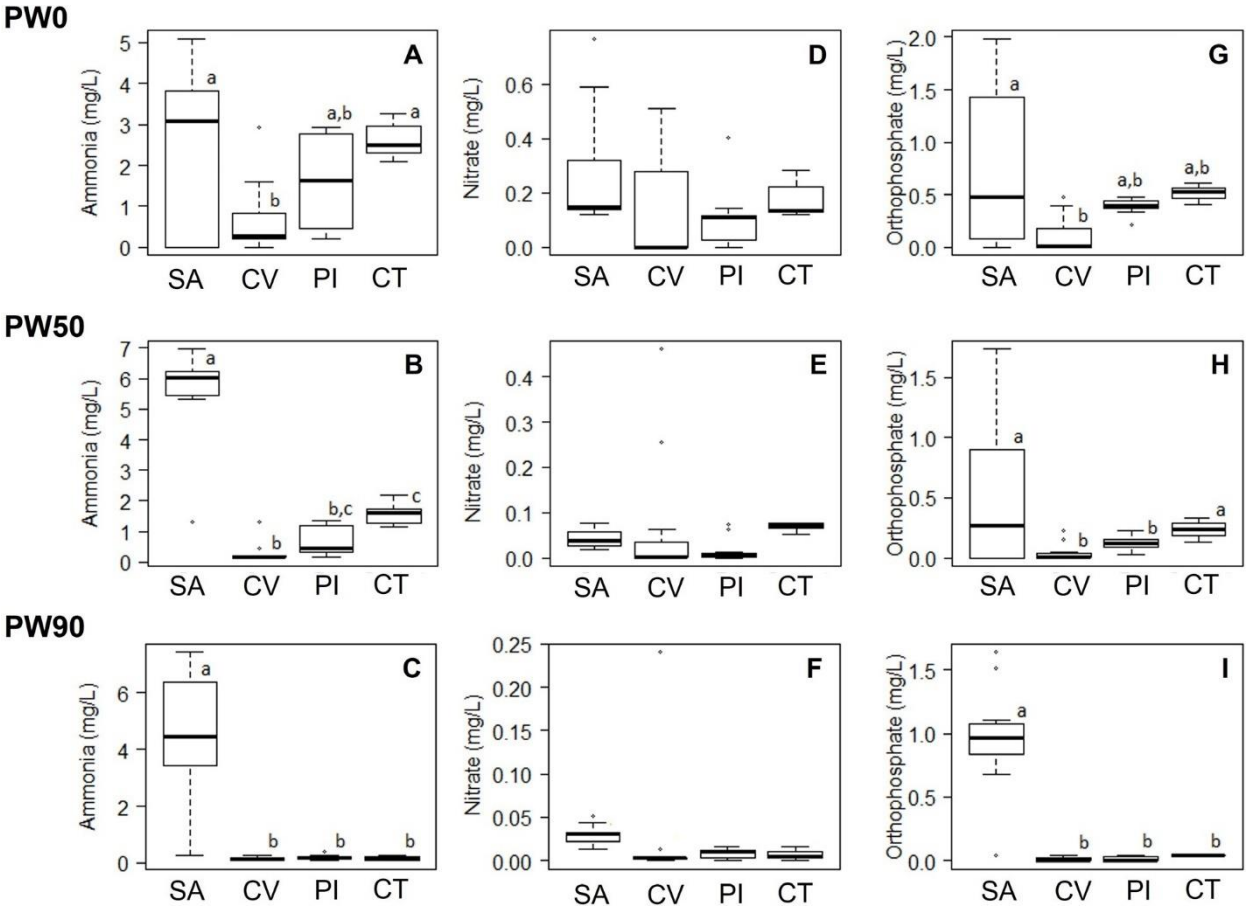

Figure 4. Comparison of the average nutrient concentration among dilution treatment level (PW0, PW50 and PW90) and innoculate species (*Scenedesmus acuminatus*, *Chlorella vulgaris* and *Planktothrix isothrix*) in water contaminated with domestic effluent from an urban pond in Manaus (Amazonas, Brazil). A-C – ammonia; D-F – nitrate; G-I – orthophosphate. PW0 = undiluted pond water; PW50 = pond water diluted by 50%; PW90 = pond water diluted by 90%. SA = *S. acumintaus*, CV = *C. vulgaris*; PI = *P. isothrix*; CT = control. Different lower-case letters above box-plots within each graph indicate significant differences according to a *post hoc* Tukey test.

DISCUSSION

Nutrient removal Microalgae play a crucial role in nitrogen cycling in aquatic environments, participating in biochemical processes such as amination, transamination, and deamination (Round 1983). These processes allow microalgae to regulate their nitrogen levels, synthesize amino acids, and eliminate excess nitrogen (Round 1983). Therefore, the excess of ammonia in PW50 and PW90 with *S. acuminatus* can be explained by the deamination process. In deamination, an amino group is removed from an amino acid, resulting in a keto acid and free ammonia, which is important for the catabolism of amino acids and the release of nitrogen in excretable forms (Round 1983). Some species

of microalgae of the genera *Scenedesmus*, *Haematococcus*, *Ankistrodesmus* and *Hormidium* have a high capacity for deamination, leading to the release of ammonia into the medium (Round 1983). Furthermore, under cultivation stress conditions such as low light intensity, low temperature, alkaline pH, or low nutrient concentrations, microalgae can release extracellular organic matter (EOMs), including carbohydrates, proteins, amino acids, lipids, and organic acids (Wu et al. 2016).

The three species efficiently removed ammonia in PW0. Although microalgae can assimilate other forms of nitrogen, such as nitrate and nitrite, these organisms tend to preferentially assimilate ammonia due to its lower energy cost. This preference arises because ammonia can be directly incorporated into amino acids, whereas nitrate must first be reduced to nitrite and then to ammonia before it can be utilized. This process of reducing nitrate to ammonia requires energy in the form of NADPH, making it a more complex and energetically costly process for the cell (Flores and Herrero 2005; Takabayashi et al. 2005; Glibert et al. 2015; Liu et al. 2017; Singh et al. 2019).

Our results indicate that, in general, *C. vulgaris* removes nutrients (e.g., ammonia, nitrate, and orthophosphate) more quickly than *S. acuminatus* and *P. isothrix*. These findings support those of Wang et al. (2009), who observed that *Chlorella* sp. removed ammonia from wastewater by day 2 and nitrate and orthophosphate by day 3 in a 10-day experiment at a sewage treatment plant in the USA. In contrast, our study showed that *S. acuminatus* required more time to remove nutrients, which is consistent with other studies on ammonia and orthophosphate removal using *Scenedesmus* sp. in domestic wastewater from a sewage treatment plant in Mexico (Oliveira et al. 2018) and *S. quadricauda* in wastewater from a sewage treatment plant in China (Wong et al. 2015).

According to the literature, the efficiency of ammonia removal by *Scenedesmus* sp. can vary between 70% and 98% (Table 5). In our study, *S. acuminatus* achieved a 100% efficiency in removing ammonia in undiluted water, confirming previous findings. This result

suggests that dilution is unnecessary to achieve effective ammonia removal for this species. On the other hand, the efficiency of ammonia removal by *Chlorella* sp. varies between 44.4% and 100%, as reported in the literature (Table 5). In our study, *C. vulgaris* showed a removal efficiency greater than 90% across all dilution treatments. This indicates that medium dilution does not significantly affect the ammonia removal efficiency for this species, which remains consistently high under all conditions.

Similarly, *P. isothrix* demonstrated higher removal efficiency in undiluted water, suggesting that, like *S. acuminatus*, dilution is not necessary for efficient ammonia removal. This observation aligns with findings by Silva-Benavides and Torzillo (2012), who observed the removal of ammonia (59 mg L^{-1}) by *Planktothrix* sp. in a secondary treatment plant in Italy over a 10-day period.

According to the literature, the efficiency of nitrate removal by *Scenedesmus* sp. can vary between 65% and 100% (Table 5). However, our results diverge, particularly for *S. acuminatus*, which was inefficient in removing this nutrient across all treatments. The key parameters influencing nitrate removal include nitrate concentration, photoperiod, pH, and temperature (Taziki et al. 2015). The low efficiency of nitrate removal by *S. acuminatus* may be attributed to these parameters, particularly because the nitrate concentrations in our treatments were lower than those typically reported in the literature (Taziki et al. 2015), ranging from 45 to 1914 mg L.

Certain microalgae species such as *C. vulgaris* and *Neochloris oleoabundans* S. Chantanachat & H. C. Bold have demonstrated higher removal efficiency with increased nitrate concentrations (Jeanfils et al. 1993; Wang and Lan 2011). Therefore, variation in nitrate concentrations can significantly influence the removal, assimilation, and growth efficiency specific to each taxon (Taziki et al. 2015). Despite using photoperiod, temperature, and pH levels within levels recommended by the literature (Taziki et al. 2015), nitrate concentrations across our treatments likely played a crucial role in the inefficient nitrate

removal of *S. acuminatus*. Future studies on *S. acuminatus* should explore different nitrate concentrations to address this knowledge gaps. In contrast, nitrate removal efficiency of *C. vulgaris* exceeded 90% across all treatments, in accordance with the literature (Table 5) and its known potential in nutrient bioremediation. *Planktothrix isothrix* had highest nitrate removal efficiency in PW50 and PW90, indicating it is efficient in removing nitrate even at low concentrations of this nutrient.

Regarding orthophosphate, removal efficiency of *Scenedesmus* sp. ranges from 4.7% to 90% in the literature (Table 5). In our study, removal efficiency of *S. acuminatus* was 100% in undiluted water and PW50, indicating effectiveness at higher orthophosphate concentrations. In contrast, *Chlorella* sp. typically displays removal efficiencies between 33% and 99% (Table 5), and had consistently high removal efficiency across all treatments in this study, suggesting that medium dilution does not significantly impact its ability to remove orthophosphate. *Planktothrix isothrix* also showed efficient orthophosphate removal in both PW50 and PW90, indicating potential for effective nutrient removal even at lower concentrations.

Table 5. Removal efficiency of Scenedesmus and Chlorella in different effluents. NH_4^+ = ammonia, NO_3^- = nitrate, PO_4^{3-} = orthophosphate; na = not applicable.

Nutrient	Removal efficiency (%)				Period (Days)	Wastewater type	Reference
	Scenedesmus sp.	*Chlorella* sp.	*Scenedesmus acuminatus*	*Chlorella vulgaris*			
NH_4^+	n.a	n.a	100	63.5 – 100	10	Domestic	This study
	n.a	82.4	n.a	n.a	10	Domestic	Wang et al. (2009)
	n.a	44.4 – 45.1	n.a	n.a	12	Industrial	Lim et al. (2010)
	n.a	98	n.a	n.a	24	Municipal	Li et al. (2013)
	n.a	100	n.a	n.a	10	Municipal	Ebrahimian et al. (2014)
	98	92.3	n.a	n.a	20	Domestic	Guerrero-Cabrera et al. (2014)
	95	n.a	n.a	n.a	16	Domestic	Wong et al. (2015)
	70-98	n.a	n.a	n.a	7	Domestic	Nayak et al. (2016)
	85.6	n.a	n.a	n.a	25	Tannery	Da Fontoura et al. (2017)
	>97	n.a	n.a	n.a	16	Domestic	Oliveira et al. (2018)
	81.9	n.a	n.a	n.a	14	Municipal	Ansari et al. (2019)
	n.a	93.6	n.a	n.a	10	Aquaculture	Hesnis et al. (2020)
	93.1	n.a	n.a	n.a	10	Domestic	Wang et al. (2022)
	n.a	>50	n.a	n.a	10	Textile	Wu et al. (2020)
	30	n.a	n.a	n.a	14	Domestic	Thangam et al. (2021)
	71.8	n.a	n.a	n.a	10	Swine	Zhao et al. (2022)
No_3^-	n.a	n.a	18	91.4 – 100	10	Domestic	This Study
	n.a	62.5	n.a	n.a	10	Domestic	Wang et al. (2009)
	n.a	82	n.a	n.a	10	Municipal	Ebrahimian et al. (2014)
	70-98	n.a	n.a	n.a	7	Domestic	Nayak et al. (2016)
	65	n.a	n.a	n.a	10	Industrial	Usha et al. (2016)
	100	n.a	n.a	n.a	14	Municipal	Ansari et al. (2019)
	n.a	92.2	n.a	n.a	10	Aquaculture	Hesnis et al. (2020)
	71.2	n.a	n.a	n.a	14	Domestic	Thangam et al. (2021)
	n.a	93	n.a	n.a	13	Municipal	Pooja et al. (2022)
PO_4^{3-}	n.a	n.a	100	99.3 – 100	10	Domestic	This Study
	n.a	90.6	n.a	n.a	10	Domestic	Wang et al. (2009)
	n.a	33.1 – 33.3	n.a	n.a	12	Industrial	Lim et al. (2010)
	90	80	n.a	n.a	20	Domestic	Guerrero-Cabrera et al. (2014)
	90	n.a	n.a	n.a	16	Domestic	Wong et al. (2015)
	70-98	n.a	n.a	n.a	7	Domestic	Nayak et al. (2016)
	71.2	n.a	n.a	n.a	10	Industrial	Usha et al. (2016)
	n.a	>99	n.a	n.a	10	Municipal	Ge et al. (2018)
	>97	n.a	n.a	n.a	16	Domestic	Oliveira et al. (2018)
	4.7	n.a	n.a	n.a	14	Municipal	Ansari et al. (2019)
	n.a	89.2	n.a	n.a	10	Aquaculture	Hesnis et al. (2020)
	89.6	n.a	n.a	n.a	14	Domestic	Thangam et al. (2021)

Which species is ideal for nutrient removal?

Our results showed that the three species exhibit unequal nutrient removal capabilities. Based on these findings, we can identify several potential approaches for applying these microalgae species in sewage treatment, particularly for domestic wastewater in the Amazon region. The first approach involves dilution, which needs additional water use, which increases the overall expense of the system and constitutes a drawback (Acién et al. 2017). Therefore, avoiding dilution would make more sense economically. One solution would be to use rainwater for dilution, a viable option in the Amazon region, especially during the rainy season from November to April. Thus, if dilution is to be avoided, it is

crucial to select species that demonstrate the highest removal efficiency in undiluted domestic wastewater. In our study, the Chlorophyceae *S. acuminatus* and *C. vulgaris* were the most effective in this regard. On the other hand, dilution can be advantageous for nutrient conservation. In such cases, if dilution is feasible, the recommended species are *C. vulgaris* and *P. isothrix.*

The second approach concerns the types of nutrients present in wastewater. The availability and high concentrations of ammonia, nitrate, and orthophosphate can be detrimental to certain organisms. For instance, in intensive fish farming ponds, elevated ammonia concentrations can reduce survival rates, inhibit growth, and cause various physiological dysfunctions in fish (Tomasso 1994). Therefore, understanding which nitrogen compounds or phosphates are removed by microalgae is important. In this context, if complete nutrient removal from wastewater is necessary,

C. vulgaris and *P. isothrix* are the recommended species. Conversely, if only ammonia removal is required, all three species are suitable. In this case, *C. vulgaris* can be used independently of dilution, while *S. acuminatus* should be used without dilution, and *P. isothrix* with dilution. However, if the specific goal is nitrate removal, *C. vulgaris* is the preferred choice, regardless of wastewater dilution. For specific orthophosphate removal, all three species are effective, with the same recommendations regarding dilution as for ammonia.

The third approach involves using the biomass of the species employed for nutrient removal from domestic wastewater. While this study does not primarily focus on biomass, considering the fate of the microalgae biomass after nutrient removal is pertinent, particularly because *P. isothrix* is the predominant species in our study pond, and is potentially neurotoxic and hepatotoxic (Sivonen and Jones 1999). One viable application of cyanobacterial biomass is in the production of biofertilizers, leveraging their ability to fix atmospheric nitrogen into forms absorbable by plants. This approach not only promotes sustainability by reducing reliance on chemical fertilizers, but also supports organic

farming practices (Singh et al. 2016). It is important to emphasize that the application of microalgae biomass must be carried out responsibly, considering the nature of the wastewater used.

Regarding the nutrient removal process, filtration and pre- treatment are already well established steps in sewage treatment processes (Cornelli et al. 2014). However, in secondary and tertiary treatment stages, the removal efficiency for ammonia, nitrate, and orthophosphate are often inadequate. Therefore, an effective alternative is to integrate microalgae into bioremediation processes targeting these nutrients during these treatment stages. In the case of our pond, which hosts a sewage treatment plant [Manaus municipal secretariat for the environment (SEMMAS), personal communication] it would be indicated to conduct large-scale trials, which are essential using the microalgae tested in here, integrated into the treatment plant's processes. It is crucial to emphasize that large-scale trials are essential for any application, whether for nutrient removal or biomass utilization.

CONCLUSIONS

The three species of microalgae tested were efficient in removing ammonia. Our results indicated that *Scenedesmus acuminatus* is not an ideal species for nitrate removal, that *Planktothrix isothrix* is efficient in removing nutrients when domestic wastewater is diluted, and that *Chlorella vulgaris* is efficient in removing nutrients from domestic wastewater independently of dilution. We suggest large-scale testing with these species for nutrient removal in Amazonian wastewaters, and their inclusion in secondary sewage treatments. Our results are promising for sewage treatment in the Amazon region, where nutrient management, which is essential for environmental preservation and public health, is still little implemented. Each species presented unique characteristics of nutrient removal, allowing flexibility in choosing the most suitable species according to specific treatment conditions and aims. This study reinforces the potential of microalgae as a viable and sustainable biotechnological solution for wastewater treatment, contributing to the development of more efficient and ecological

environmental sanitation practices in the Amazon.

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