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Instituto de Pesquisas Hidráulicas  
Programa de Pós-Graduação em Recursos Hídricos e Saneamento  
Ambiental

Elizandro Oliveira Silveira

**Tratamento de efluentes urbanos e recuperação de energia  
através de sistema integrado composto por reator anaeróbio,  
microalgas e *wetland* construído de fluxo vertical**

PORTO ALEGRE

2021

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Tese apresentada ao Programa de Pós-Graduação em Recursos Hídricos e Saneamento Ambiental da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do grau de doutor.

**Orientadora:** Dra. Lúcia Helena Ribeiro Rodrigues

**Co-orientador:** Dr. Ênio Leandro Machado

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Prof. Dr<sup>a</sup>. Lúcia Helena Ribeiro Rodrigues – UFRGS  
Orientadora

---

Prof. Dr. Ênio Leandro Machado – UNISC  
Co-orientador

---

Prof. Dr<sup>a</sup>. Maria Cristina de Almeida Silva – UFRGS  
Examinadora

---

Prof. Dr<sup>a</sup>. Cristina Calheiros - UNIVERSIDADE DO PORTO  
Examinadora

---

Prof. Dr. Odorico Konrad – UNIVATES  
Examinador

*A minha amada filha Elisa.*

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## RESUMO

O presente trabalho teve como objetivo propor um sistema integrado de tratamento de efluentes urbanos composto por reator anaeróbio, microalgas autóctones e *wetland* construído de fluxo vertical, com macrófita nativa do Sul do Brasil, além de recuperar energia através da biomassa gerada durante o processo. A pesquisa foi desenvolvida junto a estação de tratamento de águas residuais da Universidade de Santa Cruz do Sul (ETE/UNISC). Foi proposto um sistema integrado composto por reator anaeróbico (RA), microalgas (MA) e *wetland* construído de fluxo vertical (WCFV) para tratamento das águas residuais produzidas no campus da Universidade com tempo total de detenção hidráulica (TDH) de 17 dias. O sistema integrado foi configurado para proliferação de microalgas, a partir de efluente pós-tanque equalizador da ETE e com polimento final através de *wetland* construído, tendo como partida a configuração do fotobioreator sem inoculação de cepas selvagens ou comerciais de microalgas e com a utilização da espécie de macrófita emergente *Hymenachne grumosa* no WCFV. Os resultados obtidos demonstraram que o sistema integrado (RA, MA e WCFV) apresenta bom desempenho na redução de COD; DBO<sub>5</sub> e Fósforo Total (63,22%; 61,18% e 53,91%, respectivamente), além de taxas de remoção de Nitrogênio Amoniacal (N-NH<sub>3</sub>); Coliformes totais e *Escherichia coli* de 99,98%; 99,99% e 100%, respectivamente. A obtenção das amostras de biomassa algácea para produção de biogás foi realizada através de separação e coleta das microalgas com período de TDH de 14 dias, enquanto a biomassa referente às macrófitas utilizadas no *wetland* construído foi realizada a cada 6 meses. A conversão da biomassa das microalgas em biogás foi em média de 2322,51 NmL-gSV<sup>-1</sup> com CH<sub>4</sub> de 54,61% (inverno/2019); de 4491,47 NmL-gSV<sup>-1</sup> com CH<sub>4</sub> de 57,17% (primavera/2019) e com geração de biogás de 3826,70 NmL-gSV<sup>-1</sup> com CH<sub>4</sub> de 44,26% para biomassa *wetland* construído inverno e primavera/2019. Em relação à redução da genotoxicidade, observou-se que o sistema integrado (WCFV) foi eficiente, uma vez que apresentou uma redução significativa ( $p < 0,001$ ) na frequência de micronúcleos (MN) e aberrações cromossômicas (CA) quando comparado com o sistema convencional de tratamento (ETE). Ainda, foi desenvolvido um separador de biomassa microalgal de baixo custo, sem aporte energético com depósito de patente junto ao Instituto Nacional de propriedade Intelectual (INPI). Assim, os resultados do estudo destacam a relevância na proposição do sistema integrado como alternativa de tecnologia sustentável aplicada ao tratamento de águas residuais, uma vez que, além de tratar o efluente de modo eficiente, também demonstrou potencial geração de energia, reforçando o conceito de sustentabilidade no saneamento ambiental.

**Palavras chave:** Sistema integrado, efluentes urbanos, *wetland* construído, microalgas autóctones, testes toxicológicos, recuperação energética.

## ABSTRACT

The present work aimed at to propose an integrated system for the treatment of urban wastewaters besides recovering energy through the biomass generated during the process. The system was composed of an anaerobic reactor (AR), autochthonous microalgae (MA) and vertical flow constructed wetlands (VFCW) vegetated with native macrophyte of the South of Brazil with full hydraulic detention time (HDT) of 17 days. The research was carried out at the wastewater treatment plant (WWTP) of the University of Santa Cruz do Sul (ETE/UNISC). The integrated system was configured for MA proliferation, from wastewaters arising from the equalizer tank of WWTP and with final polishing by VFCW, starting with the configuration of the photobioreactor without inoculation of wild or commercial strains of MA and with the use of emerging macrophyte species *Hymenachne grumosa* in VFCW. The results obtained showed that the integrated system (RA, MA and WCFV) reduced COD, BOD<sub>5</sub> and Total Phosphorus by 63.22%; 61.18% and 53.91%, respectively, besides achieving good removal rates for Ammoniacal Nitrogen (N-NH<sub>3</sub>) Total coliforms and 99.98% *Escherichia coli*; 99.99% and 100%, respectively. The collection of algal biomass samples for biogas production was performed by separating and collecting the microalgae with a HDT of 14 days, while the biomass referring to the macrophytes used in the VFCW was carried out every 6 months. The conversion of MA biomass into biogas averaged 2322.51 NmL-gSV<sup>-1</sup> with 54.61% CH<sub>4</sub> (winter/2019); 4491.47 NmL-gSV<sup>-1</sup> with 57.17% CH<sub>4</sub> (spring/2019) and with biogas generation of 3826.70 NmL-gSV<sup>-1</sup> with 44.26% CH<sub>4</sub> for VFCW biomass winter and spring/ 2019. Regarding the reduction of genotoxicity, it was observed that the integrated system was efficient, as it presented a significant reduction ( $p < 0.001$ ) in the frequency of micronuclei (MN) and chromosomal aberrations (CA) when compared to the treatment of the WWTP. Furthermore, a low-cost, energy-free, microalgal biomass separator was developed with a patent deposit at the National Institute of Intellectual Property (INPI). Thus, the results obtained in the present study highlight the relevance of proposing the integrated system as an alternative of sustainable technology applied to the treatment of wastewaters, since, in addition to treating the wastewaters in an efficient manner, it also demonstrated potential for energy generation, reinforcing the concept of sustainability in environmental sanitation.

**Keywords:** Integrated system, urban wastewaters, constructed wetland, autochthonous microalgae, toxicological assays, energy recovery.



## LISTA DE ABREVIATURAS E SIGLAS

ABMS- *Automated biogas measurement system*  
ABNT- Associação Brasileira de Normas Técnicas  
AMPTS II- *Automatic methane potential test system II*  
ANOVA- *Analysis of Variance*  
AOAC- *Association of Official Analytical Chemists*  
AR- *Anaerobic Reactor*  
BBP- *Biochemical Biogas Potential*  
BMP- *Biochemical Methane Potential*  
BNC- *Binucleated Cells*  
BOD<sub>5</sub>- *Biochemical Oxygen Demand*  
CA- *Chromosome aberrations*  
CaCO<sub>3</sub> L<sup>-1</sup>- Carbonato de Cálcio  
CD- *Cells in division*  
CE(I)50- Concentração Efetiva Inicial Mediana  
CETER- Central de tratamento de resíduos  
CH<sub>4</sub> – Metano  
Cl- *Chlorine*  
CN- Controle Negativo  
CO- *Carbon monoxide*  
COD- Carbono Orgânico Dissolvido  
CONSEMA- Conselho Estadual do Meio Ambiente (Rio Grande do Sul)  
CO<sub>2</sub> - Dióxido de carbono  
CP- Controle Positivo  
CPETS - Centro de Pesquisa em Energias e Tecnologias Sustentáveis  
DBO<sub>5</sub> - Demanda Bioquímica de Oxigênio em 5 dias  
DMSO- Dimetilsulfóxido  
DNA- *Deoxyribonucleic acid*  
DQO- Demanda Química de Oxigênio  
EB- Efluente Bruto  
EDTA- Ácido Etileno Diamino Tetra-acético  
ETE- Estação de Tratamento de Esgoto  
FD- Frequência de Dano  
FISIBIO- Filtro Separador de Biomassa  
GEE- Gases do Efeito Estufa  
GI - *Germination Index*  
HCl- Cloreto de hidrogênio (ácido clorídrico)  
HDPE - *High-density polyethylene*  
HDT- *Hydraulic Detention Time*  
HP- Medida de potência  
HPTH -*High pressure thermal hydrolysis*  
HSSFCWs -*Horizontal subsurface flow constructed wetlands*  
H<sub>2</sub>O- Água  
H<sub>2</sub>S- *Hydrogen sulfide*  
ID- Índice de Dano  
INPI- Instituto Nacional de Propriedade industrial  
IPH- Instituto de Pesquisas Hidráulicas  
ISO- *International Organization for Standardization*  
K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> - *Potassium dichromate*  
LATTAE- Laboratório de Tecnologia de Tratamento de Águas e Efluentes  
MA- Microalgas  
mca- Metros de coluna de Água  
MI- *Mitotic Index*  
mg L<sup>-1</sup> – Miligramas por litro.

MNC- *Micronucleated cells*  
MOA- Microorganismos associados  
 $m^3 d^{-1}$ - Metros cúbicos por dia.  
NBR- Norma brasileira de regulamentação técnica  
NBS- *Nature-based solutions*  
NC- *Negative control*  
NmL-gSV<sup>-1</sup>-  
NO<sub>3</sub><sup>-</sup>- *Nitrate*  
NO<sub>2</sub><sup>-</sup>-*Nitrite*  
N-NH<sub>3</sub><sup>-</sup>- Nitrogênio Amoniacal  
NTK- Nitrogênio Total Kjeldahl  
NTU- *Nephelometric Turbidity Unit*  
N<sub>2</sub>- *Nitrogen*  
OD- Densidade Óptica  
OECD- *Organisation for Economic Co-operation and Development*  
O<sub>2</sub> - *Oxygen*  
PEAD- Polietileno de Alta Densidade  
PEE- *Percentage of epicotyl elongation*  
PGI- *Percentage of germination inhibition*  
pH- potencial de hidrogênio  
PLC - *Programmable logic controller*  
POA's- Processos Oxidativos Avançados  
PVC- Policloreto de Vinila  
P+L- Produção Mais Limpa  
Q- Vazão  
RA- Reator Anaeróbico  
RCEST- *Research Center in Energy and Sustainable Technologies*  
RE- *Root Elongation*  
RW- *Raw wastewaters*  
SAC's- Sistemas Alagados Construídos  
SD- Sistema de Distribuição  
SG - *Seed Germination*  
STD- Sólidos Totais Dissolvidos  
TCS - *Total cells per sample*  
TDH- Tempo de detenção hidráulica  
TKN- *Total Kjidal Nitrogen*  
TP- *Total Phosphorus*  
TS- *Total Solids*  
UA- Unidades arbitrárias  
UASB- *Upflow Anaerobic Sludge Blanket*  
UFC- Unidade formadora de colônia  
UFRGS- Universidade Federal do Rio Grande do Sul  
UNISC- Universidade de Santa Cruz do Sul  
UNIVATES- Universidade do Vale do Taquari de Ensino Superior  
USEPA- *United States Environmental Protection Agency*  
uT- Unidade de Turbidez  
UWWTP - *University wastewater treatment plant*  
UV- Ultravioleta  
WC- *Wetland* Construído  
WCFS- *Wetlands* Construídos de Fluxo Superficial  
WCFV- *Wetland* Construído de Fluxo Vertical  
VFWC- *Vertical Flow Constructed Wetland*  
VSI -*Volatile Solids of the inoculum*  
VSS- *Volatile Solids of the sample*

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## 1. INTRODUÇÃO

A contaminação dos recursos hídricos e a crescente escassez de água potável vêm se tornando um dos maiores problemas ambientais da era moderna. O crescimento populacional desordenado, combinado a falta de saneamento básico, principalmente em países em desenvolvimento, torna cada vez mais insustentável a situação (VYMAZAL, 2011; WU *et al.*, 2014 e SILVEIRA *et al.*, 2017).

Os *wetlands* construídos (WCs) como tecnologia baseada em bioprocessos aplicados ao tratamento de efluentes, normalmente integrados com processos de pré-tratamento anaeróbio, apresentam grande potencial de recuperação de energia e nutrientes à baixo custo, no processo de tratamento e recuperação de águas residuárias (LU *et al.*, 2015 e ABRAHAMS *et al.*, 2017). Diversos estudos apontam a utilização dos WCs como tecnologia de polimento final de águas residuárias (VON SPERLING, 2005; VYMAZAL, 2010 e ANDRADE, 2015). Outros pesquisadores têm utilizado processos combinados com WCs, minimizando os fatores de carga através de pré-tratamento (SILVEIRA *et al.*, 2019; CELENTE *et al.*, 2019 e SILVEIRA *et al.*, 2020). Experiências de sistemas integrados de tratamento de águas residuárias visando reuso podem ser citadas, como por exemplo, ROUSSEAU (2006); VON SPERLING (2005); RAI (2013); LU *et al.* (2015) e SILVEIRA (2018), demonstrando ser a combinação de tecnologias um caminho promissor na amplificação da eficiência operacional com custos reduzidos.

O avanço conceitual que os autores anteriormente citados apresentam, considera a Estação de Tratamento de Efluentes (ETE) como unidade produtiva sustentável, capaz de recuperar energia, água, nutrientes e contaminantes que possam configurar bioprodutos. Ou seja, impactos ambientais se tornam oportunidades de atividades econômicas ambientalmente sustentáveis.

Considerando aspectos de *eco-design* para remediação de águas residuárias, SILVEIRA *et al.* (2017) propuseram um sistema integrado, combinando o uso sequencial de microalgas (MA) e *wetlands* construídos de fluxo vertical (WCFV) para o tratamento de águas residuárias, associando a avaliação ecotoxicológica, fitotoxicológica e genotoxicológica destas águas. Neste sentido, a continuidade de pesquisas que integrem métodos biológicos com anaerobiose, uso de microalgas e sistemas de *wetlands* construídos poderá permitir recuperar energia e nutrientes das águas residuárias e outros compostos inorgânicos, reforçando o conceito de ETE como uma unidade produtiva, e não, apenas como solução final de processo.

Assim, o presente estudo propõe uma avaliação direcionada ao desenvolvimento de um sistema integrado de tratamento de efluentes urbanos sustentável, voltado para o tratamento e recuperação energética, através da integração de processos de fitorremediação, a partir do uso de microalgas; biorremediação, com utilização de microorganismos associados e fitorremediação, através do uso das macrófitas no *wetland* construído. Todos estes bioprocessos associados à utilização de ensaios toxicológicos como ferramenta de monitoramento, além da recuperação da energia com a biomassa gerada durante o processo, trazendo assim o conceito de *Eco Design* aplicado ao saneamento ambiental.

### **1.1. Justificativa**

A instituição de processos de tratamento de efluentes urbanos com apelo ambiental e voltado à recuperação energética faz-se cada vez mais necessária, uma vez que a gestão sustentável da água é uma questão fundamental para o estabelecimento do conceito de economia circular no tratamento de águas residuais. A gestão sustentável da águas urbanas exige o uso e o tratamento da água de maneira eficiente e sustentável. Assim, cresce a cada dia a urgência de concepção de novas tecnologias que possam, além de tratar efluentes urbanos, recuperar energia através do processo. Além de ser uma alternativa promissora no tratamento de águas residuais de forma descentralizada, um sistema integrado composto por reator anaeróbio, microalgas e *wetland* construído, surgem como um exemplo de soluções baseadas na natureza (*NBS-Nature based solutions*) que atuam como uma tecnologia de tratamento de água contaminada, com potencial de aplicação em escala real em um futuro próximo.

### **1.2. Hipótese central**

A presente tese está fundamentada na proposição de um sistema que integra processo anaeróbio, associado com microalgas e macrófitas em *wetland* construído de fluxo vertical no tratamento de efluente urbano universitário, como proposta de tecnologia de *eco design* aplicada ao saneamento ambiental. Alia ao tratamento, a avaliação eco/genotoxicológica e a recuperação energética através da biomassa gerada durante o processo de tratamento.

### 1.3. Objetivos

#### *Objetivo geral*

Desenvolver e avaliar um sistema integrado para tratamento de efluentes urbanos, composto por reator anaeróbio, microalgas e *wetland* construído de fluxo vertical como tecnologia de tratamento sustentável.

#### *Objetivos específicos*

- Avaliar o desempenho do sistema integrado de tratamento de efluentes, combinando microalgas, com pré-tratamento por anaerobiose e pós-tratamento com macrófitas em *wetland* construído de fluxo vertical.
- Avaliar índices de toxicidade do sistema integrado de tratamento de efluentes através de ensaios de fitotoxicidade, ecotoxicidade e genotoxicidade.
- Determinar a recuperação energética através da biomassa gerada por microalgas e macrófitas do *wetland* construído durante o processo de tratamento.

### 1.4. Estrutura da tese

A tese está estruturada com uma primeira parte introdutória, com breve revisão bibliográfica acerca dos temas propostos, seguida de três capítulos/manuscritos independentes, mas que no conjunto se complementam e abordam o objetivo geral desta tese, além da descrição de invenção (modelo de utilidade) submetida ao processo de solicitação de patente junto ao INPI. Diferentes abordagens são consideradas seguindo a concepção da proposta de configuração de um sistema que integre tecnologias baseadas em bioprocessos no tratamento de efluentes urbanos, bem como prospectam soluções energéticas à área de pesquisa. O primeiro artigo está diretamente relacionado ao objetivo específico 1 e é intitulado: “*Integrated system comprising an anaerobic reactor; microalgae and constructed wetlands as a proposal for the treatment of urban effluents: Eco design applied to environmental sanitation*”. O artigo tem como principal objetivo avaliar o desempenho do sistema integrado na remoção dos agentes eutrofizantes do efluente. Na sequência, o segundo artigo que está relacionado ao objetivo específico 2, intitulado: “*Biomonitoring of urban wastewaters treated by an integrated system combining microalgae and constructed wetlands*” que tem como foco principal a utilização de ensaios toxicológicos (*Allium cepa* e *Daphnia magna*) como ferramenta de monitoramento aplicada ao saneamento ambiental. O terceiro artigo,



alinhado ao objetivo específico 3, é intitulado “*Energy recovery from biomass biodigestion generated by an integrated system composed of microalgae and constructed wetland*”, e considera a utilização das biomassas algácea e macrofítica geradas durante o tratamento do efluente como potencial matéria prima na produção de energia. Em seguida, a descrição da invenção desenvolvida durante o desenvolvimento do projeto de tese, com o detalhamento do objeto criado e o protocolo de pedido de patente junto ao Instituto Nacional de Propriedade intelectual (INPI) sob o registro número BR 20 2021 011625 6. Por fim, os principais resultados são discutidos de forma integrada, retomando a hipótese central do trabalho e as conclusões e recomendações da tese são apresentadas.

## **2. REVISÃO BIBLIOGRÁFICA**

### **2.1. Tecnologias limpas aplicadas ao saneamento**

Dentre as subdivisões para o conceito de *Tecnologias Limpas* estão às ações para produção de Energias Limpas; Economia Circular; Transporte Sustentável; Uso Sustentável de Recursos e Materiais; Eficiência Energética e Gestão Sustentável das Águas. Neste último tópico englobam-se a preservação dos mananciais; recuperação de áreas com potencial ciclo hidrológico sustentável; captação; distribuição; uso e reuso com favoráveis condições de enquadramento à norma da legislação (AZIMI & PENDASHTEH, 2016).

A referência de processos sustentáveis é considerada uma alternativa tanto para o tratamento de água de captação quanto para a remediação de efluentes. Combinar etapas de pré-tratamento sem geração de resíduos, uso de energia renovável, insumos químicos de baixo impacto, processos de fitorremediação fixadores de CO<sub>2</sub> e recuperadores de nutrientes com redução do potencial eutrofizante, agregados às unidades descentralizadas, o quanto possível de menor porte (entre 20-200 m<sup>3</sup>dia<sup>-1</sup>) permitirão controle mais efetivo de economia circular para reuso de frações orgânicas como adubo, da água residuária sem gastos expressivos de energia para distribuição, bem como ter manutenção operacional de menores custos (SILVEIRA, 2018; LU *et al.*, 2015; FONDER & HEADLEY, 2013 ).

Dentre as experiências dos sistemas integrados de tratamento de águas residuárias visando reuso podem ser citados os trabalhos como ROUSSEAU (2006); VON SPERLING (2005); RAI (2013); LU *et al.* (2015) e SILVEIRA (2018), demonstrando ser a combinação de tecnologias um caminho promissor na amplificação da eficiência operacional com custos reduzidos.

AZIMI & PENDASHTEH (2016) referenciam o conceito de *Tecnologias Verdes* a partir da *Química Verde* para a remediação ambiental mais sustentável, especialmente com considerações para as estações de tratamento de águas residuárias. O avanço conceitual que os autores anteriormente citados apresentam, considera a *Engenharia Verde*, referenciando o aspecto operacional de ETE's (Estação de Tratamento de Esgotos) já existentes com Produção Mais Limpa (P+L), destacando eco-eficiência. Nesta abordagem se considera a ETE como unidade produtiva sustentável, capaz de recuperar energia, água, nutrientes, e contaminantes que possam configurar aditivos químicos. Ou seja, impactos ambientais se tornam oportunidades de atividades econômicas e ambientalmente sustentáveis. Sustentabilidade ambiental que será inventariada e avaliada considerando procedimentos como a Análise de Ciclo de Vida, por exemplo, (AZIMI & PENDASHTEH, 2016 e ABDEL FATTAH, 2018).

Considerando aspectos de *eco-design* para remediação de águas residuárias, SILVEIRA *et al.* (2017) propuseram um sistema integrado, combinando o uso sequencial de microalgas (MA) e *wetland* construídos de fluxo vertical (WCFV) para o tratamento de águas residuárias, associando a avaliação ecotoxicológica, fitotoxicológica e genotoxicológica destas águas. Os resultados revelaram que os principais impactos ambientais das águas residuárias estudadas estão associados ao elevado potencial de eutrofização, devido às concentrações de  $N-NH_3$  ( $68,8 \pm 25,7 \text{ mg L}^{-1}$ ), P total ( $7,71 \pm 2,5 \text{ mg L}^{-1}$ ), e  $DBO_5$  ( $526,4 \pm 177 \text{ mg L}^{-1}$ ), carga patogênica e genotoxicidade ( $p < 0,0001$ ). Os resultados também mostraram que o sistema integrado (MA + WCFV) não foi capaz de reduzir satisfatoriamente os valores de P total (apenas 4%). No entanto, o sistema proposto (MA + WCFV) evidenciou resultados muito promissores para a remoção de nitrogênio, com ênfase na remoção de nitrogênio amoniacal ( $N-NH_3 = 100\%$ ) e demanda bioquímica de oxigênio após cinco dias ( $DBO_5 = 57\%$ ). O sistema integrado eliminou completamente a ecotoxicidade e a genotoxicidade do efluente bruto.

Associado ao conceito de tecnologias limpas aplicadas ao saneamento, a abordagem conhecida por NBS (*Nature based solutions*), traz o mesmo pensamento de aplicação de tecnologias mais adequadas ambientalmente, baseadas na conservação dos ecossistemas e seus recursos (KABISCH *et al.*, 2016 e RAYMOND *et al.*, 2017). Estas tecnologias estão ligadas fortemente a ideia de melhorar os serviços ecossistêmicos, além disso, em casos onde há estágios de degradação avançada as NBS são importantes na remediação ou compensação dos impactos, por exemplo, elas podem ser aplicadas para compensar a falta de capacidade do solo de armazenar água, comprometimento de

recarga de aquíferos ou falta de mecanismos de regulação climática em áreas urbanas (KRAUZE; WAGNER, 2019).

Diante dos conceitos apresentados podemos destacar a aplicação de *wetlands* construídos como NBS, da mesma forma, sistemas que utilizam microalgas fotossintetizantes como polimento de águas residuárias podem ser enquadrados no mesmo conceito. Assim, sistemas que integram tecnologias com apelo ambiental (NBS) trazem benefícios, não apenas na recuperação de águas residuárias, mas também no entorno e no equilíbrio paisagístico do local onde são aplicadas (MUSSINELLI *et al.*, 2018; ALBERT *et al.*, 2019; CALHEIROS *et al.*, 2019 e SILVEIRA *et al.*, 2020).

## **2.2. Utilização de microalgas aplicadas ao saneamento**

Nos últimos anos surgiram tecnologias ambientais alternativas como, por exemplo, a utilização de microalgas na remediação de águas residuárias associada à produção de energia e tratamento biológico de efluentes urbanos. Alguns pesquisadores têm utilizado efluentes como substrato para desenvolver biomassa microalgal com a finalidade de produzir bioprodutos nas mais variadas áreas de produção, desde bioprodutos para o mercado de cosméticos até bioenergia (MEZZOMO *et al.*, 2010; PACHECO *et al.*, 2015 e YADALA & CREMASCHI, 2016). Outros estudos indicam efluentes ricos em matéria orgânica como fonte para produção de biomassa de microalgas, como a capacidade de biofixação, fonte de biomassa e produção de energia com baixo custo de aplicação e operação são alguns dos motivos pelo qual as microalgas têm sido utilizadas, principalmente na produção de bioenergia (CELENTE *et al.*, 2019 e SILVEIRA *et al.*, 2019).

Estudos referentes à utilização de microalgas como tecnologia de tratamento de efluentes, assim como a forma mais adequada da separação de fases de crescimento destes micro-organismos passa a ser investigada intensivamente a partir da década de 90. YADALA & CREMASCHI (2014) investigaram a maior geração de microalgas em reatores tipo coluna, placas e sistemas tubulares. Já DE-BASHAN & BASHAN (2010) investigaram microesferas e placas de materiais inertes de polímeros sintéticos, filtros de papel, flóculos de caseína e glutaraldeído, esponjas naturais, entre outros, para fixação e separação de fases das microalgas.

CELENTE *et al.* (2019) desenvolveram estudo que avaliou o uso combinado de depuradores com microalgas e zonas úmidas construídas verticais de fluxo descendente

para as instalações sanitárias. Após um tempo de detenção hidráulica de 21 dias, a unidade experimental alcançou a eficiência média na redução de 52% para condutividade, sólidos solúveis totais, fósforo solúvel e carbono orgânico total, enquanto para turbidez, N-NH<sub>3</sub>, *Escherichia coli* e coliformes totais a redução foi superior a 95%. Em acordo com a norma brasileira ABNT 13969/97, o efluente tratado é elegível para reutilização na lavagem de pisos e calçadas, irrigação e fins de paisagismo.

Apesar de promissora, há desvantagens para o emprego de microalgas no tratamento de efluentes. Algumas limitações da utilização de microalgas são a proporção relativamente baixa na remoção de contaminantes, fósforo e o tempo de retenção mais longo nas estações de tratamento (dias em comparação com horas). Na melhor das hipóteses, de acordo com procedimentos operacionais atuais, esta tecnologia pode ser complementar, podendo ser combinada com outras tecnologias biológicas, químicas e/ou físico-químicas (SANTIAGO, 2013).

### **2.3. Wetlands construídos (WCs) como tratamento de águas residuais**

A tecnologia de WCs é considerada uma solução baseada na natureza (NBS), em que os processos naturais são otimizados para melhorar a qualidade da água. Os WCs são caracterizados por custos de estabelecimento relativamente baixos, robustez, operação e manutenção fáceis e alto potencial para aplicação em países em desenvolvimento, particularmente por pequenas comunidades rurais (RODRIGUEZ-DOMINGUEZ *et al.*, 2020 e CALHEIROS, RANGEL & CASTRO, 2014).

É crucial que sistemas de tratamento de água residual cumpram inteiramente a sua função, removendo a carga orgânica e microrganismos patogênicos. As águas residuais, muitas vezes, caracterizam-se por grandes variações de quantidade e qualidade ao longo do ano, afetando negativamente a performance dos tratamentos convencionais (CALHEIROS *et al.*, 2019). Este cenário é particularmente potencializado em zonas rurais e remotas onde as águas residuais domésticas são parcialmente tratadas em fossas sépticas. As águas residuais podem apresentar variados microrganismos patogênicos (ou potencialmente) que dependendo da sua concentração, poderão constituir um risco para a saúde pública (VYMAZAL, 2005 e CALHEIROS *et al.*, 2015).

Alguns estudos com águas residuais provenientes de fossa séptica e posteriormente tratada por WCs foram desenvolvidos em escala real, pretendendo mimetizar as condições de tratamento que ocorrem nas zonas úmidas naturais, podendo ser usados para melhorar a qualidade da água de diversas origens (WU *et al.*, 2014;

CALHEIROS *et al.*, 2015 e SEZERINO *et al.*, 2018). Por se tratar de uma tecnologia simples, de custo reduzido e fácil operação, os WCs surgem como uma excelente alternativa para tratamento de águas residuais com possibilidade de reciclar e reutilizar as águas. Além disso, enquadram-se harmoniosamente na paisagem e potencializam a estética dos espaços abertos (RODRIGUEZ-DOMINGUEZ *et al.*, 2020; DELL'OSBEL *et al.*, 2020 e CALHEIROS *et al.*, 2019).

Os WCs são sistemas que integram vários intervenientes que interagem de forma a promover o tratamento da água (KADLEC & WALLACE, 2009). As plantas utilizadas no sistema são importantes para fornecer a estrutura que suporta muitos dos processos de remoção de poluentes e a utilização de plantas ornamentais para este fim tem ganhado interesse. Da mesma maneira, os microrganismos que atuam nestes sistemas constituem também uma extensa área de pesquisa, como bactérias, fungos e eucariotas (CALHEIROS *et al.*, 2019 e DELL'OSBEL *et al.*, 2020). O funcionamento destes sistemas ainda não é totalmente compreendido e são necessários mais dados sobre: i) processos físico-químicos, ii) dinâmica da macrofauna/macroinvertebrados, e iv) ciclo de vida do sistema, para uma aplicação mais ampla dos WCs (CALHEIROS *et al.*, 2015).

#### **2.4. Sistemas integrados de tratamento de águas residuais**

O lançamento de águas residuárias não tratadas está entre as principais causas de alteração na qualidade dos mananciais, devido a potencial ocorrência de eutrofização, em função da elevada carga de nutrientes. A contaminação dos corpos d'água representa uma ameaça grave à saúde pública e ao equilíbrio dos ecossistemas aquáticos, principalmente das águas superficiais (ABDEL-RAOUF *et al.*, 2012; SILVEIRA, 2018 e SEZERINO & PELISSARI *et al.*, 2021).

Diante deste cenário, há necessidade de desenvolver tecnologias capazes de recuperar a qualidade das águas residuárias com o intuito de minimizar a toxicidade dos efluentes, complementando o tratamento, antes da disposição final nos corpos hídricos receptores (HERNANDO *et al.*, 2005; CUNHA, 2006 e SILVEIRA *et al.*, 2017). As tecnologias convencionais disponíveis para tratamento de águas residuárias são baseadas em métodos químicos e físicos que ainda são alternativas de custo elevado e ambientalmente insustentáveis, já que requerem uso de produtos químicos, que além de emitirem CO<sub>2</sub>, removem ao invés de recuperar, Nitrogênio e Fósforo (ARBIB *et al.*, 2014 e CAI *et al.*, 2013).

Sistemas que integram tecnologias de tratamento de águas residuárias são estudados e aplicados há muitos anos, geralmente voltadas para a minimização de custos operacionais e promoção de remoção dos agentes eutrofizantes de forma mais sustentável através de tecnologias limpas. A utilização de *wetlands* construídos como polimento final após um pré-tratamento por anaerobiose é fortemente aplicado em diversos países, podendo ser considerado um exemplo de sucesso (ROSSEAU, 2006; VON SPERLING, 2005; RAI *et al.*, 2013; LUTTERBECK *et al.*, 2020).

Nesse sentido, diversos sistemas de tratamento de efluentes vêm sendo estudados, dentre eles os desenvolvidos por LU *et al.*, 2015; RODRIGUES *et al.*, 2016 e ABRAHAMS *et al.*, 2017, com o objetivo de recuperar a qualidade dos corpos d'água e a um baixo custo, reduzindo o tempo de detenção dos processos e produtos químicos utilizados usualmente. A integração de processos de tratamento visando à recuperação de nutrientes é uma tendência, já que o objetivo não é apenas reduzir a eutrofização dos corpos d'água para disposição final, mas, além disso, os esforços tendem a possibilitar o reuso dessas águas residuárias pós-tratamento (BADHE *et al.*, 2014 e SILVEIRA *et al.*, 2017).

Geralmente integrados com processos de pré-tratamento anaeróbio, os WCs têm grande potencial de recuperação de energia e nutrientes com baixo custo, reduzindo os índices de degradação ambiental no processo de recuperação de águas residuárias (LU *et al.*, 2015 e ABRAHAMS *et al.*, 2017). Diversos estudos apontam a utilização dos WCs com variadas configurações como polimento final de águas residuárias (VYMAZAL, 2010; VYMAZAL, 2011, ANDRADE, 2015 e SEZERINO & PELISSARI *et al.*, 2021). Os WCs podem ser configurados com fluxo horizontal ou vertical, contínuo, semi-contínuo ou em batelada (FONDER & HEADLEY, 2013 e VON SPERLING, M. & SEZERINO, P. H. 2018), e a escala de operação também é variada sendo utilizada desde fluxo contínuo em condomínios residenciais até escala de bancada em estudos experimentais (DUSFRENE *et al.*, 2015 e SILVEIRA, 2018).

A partir de bioprocessos, ASLAN & KAPDAN (2006) propuseram a utilização de microalgas com foco na recuperação energética e de nutrientes, além do reuso de efluentes tratados como estratégia de biorremediação. Um dos primeiros trabalhos que se têm registro combinando WCs como polimento final a partir de microalgas, foi o estudo conduzido por GSCHLÖBL *et al.* (1998) que pesquisaram a tendência de complemento de processos com potencial eutrofizante residual em lagoas de estabilização, que necessitavam de polimento final para descarte dos efluentes pós tratamento em corpos

receptores sensíveis. KONNERUP *et al.* (2011) aplicaram o mesmo princípio da pesquisa de GSCHLÖBEL *et al.* (1998) no tratamento de águas residuárias na piscicultura com resultados similares.

Nos últimos anos, as pesquisas com sistema combinando microalgas e *wetlands* construídos vêm sendo desenvolvidas e testadas com maior frequência, com a utilização de espécies nativas, tanto nos tanques de ploriferação de microalgas, quanto nas macrófitas utilizadas nos *wetlands* construídos, da mesma forma que as configurações dos fotobioreatores e dos *wetlands* foram variadas (SILVEIRA *et al.*, 2017; SILVEIRA, 2018; CELENTE *et al.*, 2019 e SILVEIRA *et al.*, 2020).

## **2.5. Recuperação energética aplicada ao tratamento de águas residuais**

A busca por alternativas de geração energética para atender a demanda global vem sendo cada vez mais fonte de pesquisa e debate no meio científico e tecnológico. Embora os combustíveis fósseis sejam responsáveis por atender a maior parte desta demanda, fatores como a degradação ambiental e a limitação de reservas tornam o uso desta matriz energética finita e potencialmente poluidora (HASAN *et al.* 2019).

A dependência de energia sempre será de extrema importância para a sociedade no desenvolvimento e manutenção de suas atividades básicas. Nos últimos anos a demanda por energia elétrica cresceu, aumentando a necessidade de diversificar as fontes de geração e fornecimento de energia. Neste contexto, a geração de biogás através de resíduos orgânicos urbanos e agroindustriais surge como alternativa de energia renovável na geração de bioenergia (DALPAZ, *et al.* 2020).

A proposição de alternativas que possam trazer soluções efetivas a este cenário passam, obrigatoriamente, por uma transição deste modelo energético para uma matriz expandida de fontes de energia renováveis. A medida que algumas das atuais alternativas de fontes energéticas mais utilizadas, como o vento (eólica) e solar, dependem das condições climáticas, a geração de biogás através da digestão anaeróbia de matéria orgânica não está associada diretamente a essas variáveis climatológicas. (ABAD, CHERRETT, & HOLDSWORTH, 2015).

Outra vantagem considerável da utilização de biogás é o fato deste poder ser armazenado, uma vantagem significativa que pode acomodar demandas energéticas variáveis, sendo assim, um componente essencial entre as opções de energia renovável (HAHN *et al.*, 2014 e WALL *et al.*, 2016).

O biogás é parte importante do ciclo do carbono, podendo ser utilizado como um combustível potencial para geração de energia, que, após purificado, pode atingir os mesmos padrões que o gás natural fóssil. Desta forma, pode ser usado como energia renovável substituindo o atualmente empregado. Como resultado da digestão anaeróbica, o conteúdo de carbono na biomassa é reduzido e o conteúdo de nitrogênio se concentra, o resíduo gerado por diferencial do digerido pode ser utilizado em solos como biofertilizante (RASLAVICIUS, GRZYBEK, & DUBROVIN, 2011; DA ROS *et al.*, 2014 e KARLSSON *et al.*, 2014).

A partir da digestão anaeróbia, o material orgânico contido na biomassa é convertido em biogás, onde seus principais constituintes são o gás metano (CH<sub>4</sub>) e o dióxido de carbono (CO<sub>2</sub>) (KONRAD *et al.*, 2016a e RIBEIRO *et al.*, 2016). Outros gases também são encontrados em menores proporções tais como: vapores de água (H<sub>2</sub>O); sulfeto de hidrogênio (H<sub>2</sub>S); hidrocarbonetos; amônia (NH<sub>3</sub>); oxigênio (O<sub>2</sub>); monóxido de carbono (CO) e nitrogênio (N<sub>2</sub>) (GHOUALI, SARI & HARMAND, 2015; RYCKEBOSC, DROUILLON & VERVAEREN, 2011 E COIMBRA-ARAÚJO *et al.*, 2014).

Estudos foram desenvolvidos com o objetivo de potencializar a produção de biogás através da combinação de substratos orgânicos em um processo denominado co-digestão (MATA-ALVAREZ *et al.*, 2014 e KARLSSON *et al.*, 2014). A co-digestão pode promover uma melhor relação carbono/nitrogênio (C/N), principais nutrientes utilizados por microorganismos na digestão anaeróbia, assegurando que todo o carbono disponível no meio possa ser consumido pelos microorganismos (KONRAD *et al.*, 2016a).

O estudo do biogás ganhou espaço internacionalmente nos últimos anos, uma vez que além de poder ser utilizado como fonte de energia renovável para geração de calor e energia, também pode gerar combustível (biometano), sendo assim uma alternativa sustentável aos combustíveis fósseis (LEÓN & MARTÍN, 2016; HASAN *et al.*, 2019; LANTZ, 2012).

No Brasil, o desenvolvimento estrutural da bioenergia surge como uma oportunidade estratégica, principalmente para as comunidades rurais que podem ter nesta tecnologia uma auto-provisão de energia, além de comercializar o excedente da produção no sistema nacional de abastecimento energético, uma vez que, atualmente, o setor elétrico permite injetar a produção excedente de energia na rede de distribuição



(ARAÚJO *et al.*, 2014 e DA SILVA, NETO & SEIFERT, 2016 e KONRAD *et al.*, 2016b).

Estudos apontam que a digestão de resíduos animais e/ou vegetais ricos em carbono, com resíduos que complementam estes substratos, têm sido amplamente utilizados pela indústria com resultados positivos na geração de biogás (HASAN *et al.*, 2019 e ZHANG *et al.*, 2013), além de demonstrarem que é possível usar a tecnologia de digestão anaeróbia na conversão de energia, tornando esta, uma tecnologia útil para a sociedade (LUMI *et al.*, 2015; KONRAD *et al.*, 2016b).

Assim cresce de forma significativa a importância de se propor alternativas de geração de energia de forma sustentável e a custos de operação e configuração relativamente baixos, uma vez que existem muitas formas de se obter energia limpa com a reutilização de diversos “resíduos” orgânicos. Um excelente exemplo de aplicação deste conceito pode ser observado nos chamados projetos NBS, uma vez que estes sistemas geralmente produzem biomassa durante a operação podendo ser esta utilizada na produção de biogás (KRAUZE; WAGNER, 2019 e KONRAD *et al.*, 2021).

### 3. INTEGRATED SYSTEM COMPRISING AN ANAEROBIC REACTOR; MICROALGAE AND CONSTRUCTED WETLANDS AS A PROPOSAL FOR THE TREATMENT OF URBAN EFFLUENTS: ECO DESIGN APPLIED TO ENVIRONMENTAL SANITATION

Este manuscrito é a primeira versão do artigo que será submetido para publicação em periódico de livre acesso. Artigo com enfoque analítico experimental do desempenho do sistema integrado proposto como tecnologia de tratamento de efluentes.

#### Abstract

The development of sustainable urban effluent treatment processes reinforces the importance of creating Eco Design alternatives applied to environmental sanitation. The present work aimed to develop and operate an integrated system combining microalgae, with pre-treatment by anaerobiosis and post-treatment with vertical flow constructed wetland, as an alternative for urban effluent treatment and biomass generation with energy potential during the process. The research was carried out with the wastewater treatment plant at the University of Santa Cruz do Sul (WWTPU). An integrated system consisting of an anaerobic reactor (AR), microalgae (MA) and vertical flow constructed wetland (VFCW) for the treatment of wastewater produced on the University campus was proposed. The integrated system was configured with hydraulic detention time (HDT), aiming at the proliferation of microalgae, from post-tank effluent equalizer from the WWTPU and with final polishing through VFCW, starting with the configuration of the photobioreactor without inoculation of wild strains or commercial microalgae and with the use of the native macrophyte species *Hymenachne grumosa* (VFCW). The results obtained demonstrated that the integrated system (AR, MA and VFCW) presents good performance in COD and Total Phosphorus reductions (43% and 61.31%, respectively), in addition to removal rates of Ammoniacal Nitrogen (N-NH<sub>3</sub>) of approximately 98%. Thus, these results highlight the relevance of the proposition of the integrated system as an alternative of sustainable technology applied to the treatment of wastewater, since, in addition to treating the effluent, it also showed potential energy generation, reinforcing the proposed concept.

**Key words:** Integrated system, urban effluents, vertical flow constructed wetland, microalgae, biomass, energy potential.

### 3.1. Introduction

One of the biggest challenges faced by society today is directly related to the pollution of water resources, degradation of water sources and water scarcity. These aspects, together with the lack of sanitation, can be indicated as the most relevant and worrying environmental problems of the modern era. The situation becomes increasingly unsustainable due to the combined effects of disorderly population growth and environmentally hostile activity (VYMAZAL, 2011; WU *et al.*, 2014; SILVEIRA, 2018; JABRI *et al.*, 2019).

Added to the pollution and degradation of water resources is the growing global concern about water scarcity, not only in the scientific community, but also among the general public. It is estimated that global water demand is expected to continue increasing at a rate of 1% per year until 2050 (COLARES *et al.*, 2019; SILVEIRA *et al.*, 2020).

The main technologies used for the treatment of effluents and wastewater are based on chemical and physical methods, which are still costly and environmentally unsustainable alternatives, as they require the use of chemical products, which, in addition to emitting CO<sub>2</sub>, remove nutrients from the process instead to retrieve them (ARBIB *et al.*, 2014; CAI *et al.*, 2013; SILVEIRA, 2018).

In this scenario, the need for an adequate management of water resources grows through the proposition of technologies that can bring the concept of circular economy closer to the treatment of effluents. Thus, studies must increasingly investigate the applications of sustainable sanitation systems that can, in addition to effluent treatment, enable the recovery of degraded areas with landscape integration, energy generation and allow the reuse of wastewater (CALHEIROS *et al.*, 2015; MACHADO *et al.*, 2015; SEZERINO *et al.*, 2018; DELL'OSBEL *et al.*, 2020).

The integration of treatment processes aimed at nutrient recovery is a trend, as the objective is not only to reduce the eutrophication of water bodies for final disposal, in addition, efforts tend to enable the reuse of these post-treatment wastewaters (SILVEIRA *et al.*, 2020; CALHEIROS *et al.*, 2019; DELL'OSBEL *et al.*, 2020).

The so-called clean technologies have emerged in recent years as an alternative for the remediation of wastewater associated with the production of clean energy. An example of this concept is the use of microalgae as a biological treatment of urban effluents, in which researchers have used effluents as a substrate to develop microalgal biomass in order to produce bioproducts in the most varied areas of production, from the

cosmetics market to bioenergy (PACHECO *et al.*, 2015, YADALA & CREMASCHI, 2016; MILLEDGE *et al.*, 2019). Studies have been carried out using effluents rich in organic matter as a source for the production of microalgae biomass. The biosorption capacity, biomass source and energy production with low application and operation cost are some of the reasons why microalgae have been used, mainly in the production of bioenergy (MEZZOMO *et al.*, 2010; SILVEIRA, 2018; SILVEIRA *et al.*, 2019).

Another example of “clean technologies” in sanitation are the constructed Wetlands (CWs), considered a promising alternative for the decentralized treatment of wastewater, due to their simple construction and maintenance (WU *et al.* 2014; SILVEIRA, 2018). Generally integrated with pre-treatment processes, CWs have great potential for energy and nutrient recovery at a low cost, reducing environmental pressure indices in the wastewater recovery process (LU *et al.*, 2015; ABRAHAMS *et al.*, 2017; SILVEIRA *et al.*, 2019). In addition, CWs systems combined with other technologies are emerging as an alternative to the lack of basic sanitation in developing countries, especially when applied after a primary treatment such as septic tanks, instead of collecting and transporting sewage to a central station treatment, which is very common for rural households.

The CWs technology is considered a nature-based solution (NBS), where natural processes are optimized to improve water quality. CWs are characterized by relatively low establishment costs, robustness, easy operation and maintenance, and high potential for application in developing countries, particularly for small rural communities (RODRIGUEZ-DOMINGUEZ *et al.*, 2020; CALHEIROS, RANGEL & CASTRO, 2014).

Although promising, the technologies described above have limitations when applied in isolation. The use of microalgae in effluent treatment, for example, has a relatively low proportion of phosphorus (P) removal and a longer retention time in treatment plants (days compared to hours). Effluent treatment systems with CWs have several environmental gains, however, there are factors that restrict their use. For example, the need for larger surface areas to remove nitrogen and total phosphorus (LU *et al.*, 2015; SILVEIRA *et al.*, 2017, SILVEIRA, 2018).

In this way, it is justified to propose a system that integrates the technologies described above, which can significantly contribute to the improvement and reduction of operating costs, amplifying the efficiency of the treatment system, in addition to recovering energy through the biomass generated during the process, thus bringing the

concept of sustainable Sewage treatment plant and Eco Design applied to environmental sanitation.

Thus, the objective of this work was to develop and evaluate an integrated system combining microalgae, with anaerobic pre-treatment and post-treatment with vertical flow constructed wetland, aiming at the final recovery of urban effluents, with a focus on effluent treatment and regeneration of the biomass produced during the process with energy potential.

## **3.2. Materials and methods**

### **3.2.1. Characterization of the study site and experimental design**

The project was developed at the Institute of Hydraulic Research of the Federal University of Rio Grande do Sul (IPH/UFRGS) in partnership with UNISC, together with the wastewater treatment plant at the University of Santa Cruz do Sul/RS (WWTPU) campus. The effluent drained for treatment at the WWTPU comes from the urinals and toilets, since the restaurants have grease and oil separator boxes that are later sent to other treatment centers outside the university. Other residues such as those used in the laboratories are collected and segregated for subsequent referral to the Waste Treatment Center (CETER).

The treatment plant has seven treatment stages (Fig. 1) consisting of: grating; desander; equalizer tank; anaerobic sludge blanket upflow reactor (UASB); aerated biofilter; decanter and sludge drying bed. The average operating flow of the station was estimated at 103.2 m<sup>3</sup> day<sup>-1</sup> after treatment, the effluent is sent to the municipal collection network, and ends up being indirectly discarded by galleries and the municipal rain channel, in the Stream Lajeado, which has a average flow of 588.2 m<sup>3</sup> day<sup>-1</sup> (SILVEIRA, 2018).

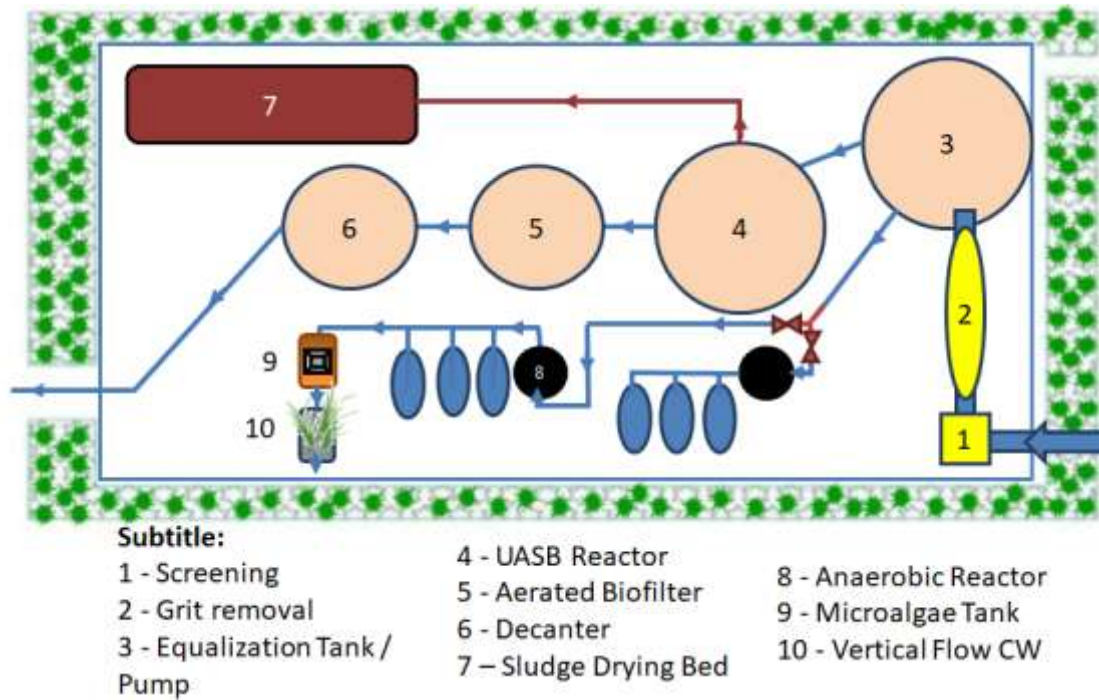


Figure 1: General configuration of the integrated system (8) Anaerobic Reactor (RA), (configuration and operating data), (9) Microalgae (MA) and (10) Vertical Flow Constructed Wetland (VFCW).

The integrated system composed of a commercial anaerobic reactor (AR) BAKOFTEC, microalgae (MA) and vertical flow constructed wetland (VFCW) was developed and built for the proliferation of MA from effluent pre-treated by RA, post-tank equalizer from the wastewater treatment plant at the University (WWTPU) Unisc. The system for the proliferation of microalgae biomass was designed with a box of 200 liters of volume, pyramid-shaped acrylic cone with 20L of useful volume. At the apex of the cone a structure of PVC pipes integrated with a hose connected to a submerged pump with an effluent recirculation rate of 400L h<sup>-1</sup>, once connected to the recirculation pump the system started without inoculation of wild or commercial strains of microalgae. The configuration of the integrated system was developed using the concept of low energy and operating cost, for this it was organized with the boxes in a slope with gravity flow, without energy input (Fig. 2).

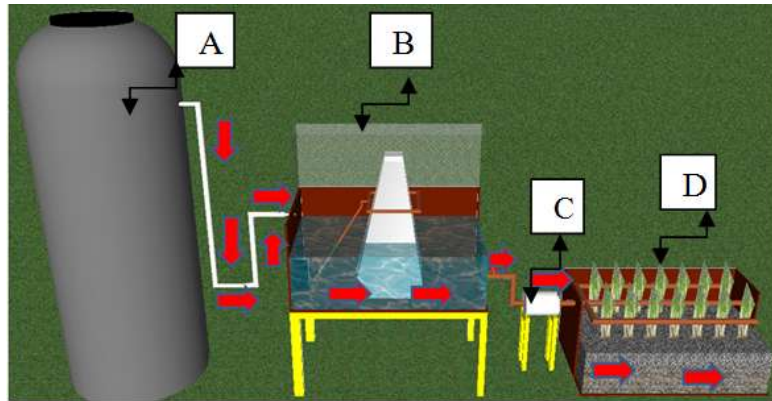


Figure 2: Integrated university effluent treatment system: (A) Anaerobic reactor; (B) Microalgal proliferation photobioreactor; (C) Biomass separator filter and (D) vertical flow constructed wetland.

The system was operated in a batch regime considering the flow of the university affluent as described in figure 2, with a total HDT of 17 days, of which 3 days in the AR; 7 days in the microalgae photobioreactor (MA) and 7 days in the VFCW. The MA photobioreactor was fed with 90L every 7 days from the AR, while the VFCW compartment received effluent pre-treated with the MA after 7 days of recirculation, after passing through the biomass separator filter (Fig. 4 A), also with a volume of 90L.

For the VFCW, a 200L working volume box with a rectangular compartment (92 cm long; 55 cm wide; 58.5 cm in height) (high density polyethylene (HDPE) box) was used. The substrate was composed of a layer of approximately 10 cm of crushed stone N<sup>o</sup>. 4 (64-100 mm), placed on the bottom of the boxes, a layer of approximately 5 cm of gravel (Basalt Stones) N<sup>o</sup>. 1 (20-40 mm) which was added to the first layer and then the emerging macrophytes of the species *Hymenachne grumosa* were planted (Fig.3).



Figure 3: Construction of subsurface vertical flow vertical constructed wetland biomass (VFCW) and production of *Hymenachne grumosa* biomass.

The species *Hymenachne grumosa* was selected as the macrophyte for the vertical flow constructed wetland (VFCW) tank configuration due to its adaptive characteristics, such as easy acclimatization to the study site, supporting total phosphorus (TP) load factors of at least  $0.3 \text{ gm}^{-1}\text{day}^{-1}$ ; for having a root system of at least 40 cm in length; allow pruning every 3 or 4 months (MACHADO *et al.*, 2015; SILVEIRA *et al.*, 2020), as well as for the good performance already evidenced in other works using the studied university effluent (HORN *et al.*, 2014; SILVEIRA *et al.*, 2017).

The load factors applied to the different stages of the proposed integrated system, as well as the treatment unit on the UNISC university campus, were configured in order to enhance each stage of the treatment. In this way, the loading of the systems was purposely differentiated from each other to maximize their action. The AR had its supply carried out every 3 days for 1 hour with effluent post-tank equalizer from WWTPU UNISC, totaling 900 L per batch. Then, right after the anaerobic treatment for 3 days, 90 L of effluent from the AR were administered in the microalgae plowing tank (MA), with a corresponding application rate, with a HDT of 7 days. The effluent rates applied to the VFCW compartment, from the microalgae proliferation tank (MA), received the same 90L, but at a rate of  $20 \text{ cm day}^{-1}$  according to the data described by GSCHLÖBL *et al.* (1998). To this end, the configuration of the effluent application mechanism was developed with the intention of supplying it homogeneously, so that percolation could occur on the entire surface of the compartment (Fig. 4). To promote percolation on the surface, a distribution system (DS) was developed with fork-shaped PVC pipes, with 1 mm holes in the lower base of the pipes (Fig. 4B).

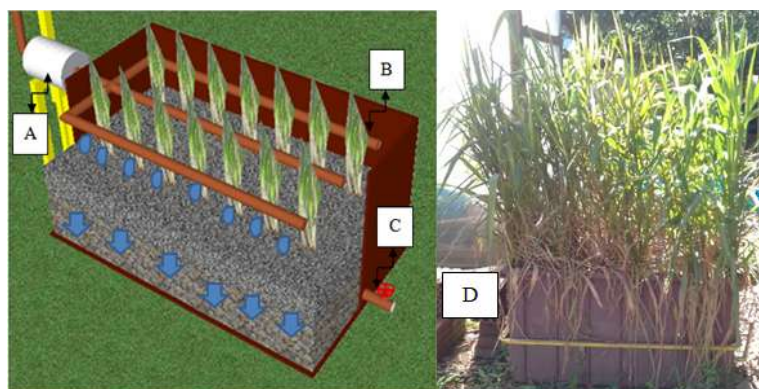


Figure 4: Urban tributary flow in the Wetland Constructed of Vertical Flow (WCFV); Biomass Separator Filter - FISIBIO (A); Distribution System-SD (B) post-treatment effluent output (C) and WCFV (D).

The integrated system was operated for 1 year and 9 months, between the period of August 2018 and May 2020. During this period, physicochemical and biological



analyzes were performed. Samples were obtained weekly, with analyzes performed following international protocols as described in table 1.

The analyzes carried out according to a pre-determined schedule, which separated the analyzes according to their frequency, being distributed as follows: monitoring analyzes (bench tests); performance analysis; disinfection analyzes and toxicological analyzes.

Thus, the weekly frequency was adopted for the analysis of the total dissolved solids parameters (TSD); conductivity; apparent color; pH and turbidity (monitoring analyses). The parameters total phosphorus; COD; BOD5 and total nitrogen were monitored fortnightly (performance analyses), while the other analyzes were carried out monthly (disinfection analysis: total Coliforms and *Escherichia coli*). For comparative effect of performance, the results obtained by the treatment plant of the WWPTU of the campus-UNISC were used as a parameter.

Table 1: Analytical methods used for the characterization of the raw and treated wastewaters in this study.

Parameters	Method	Reference
Total phosphorus (IP)	Colorimetric/ammonium molybdate	APHA/AWWA, 2012
COD	Colorimetric/titration	APHA/AWWA, 2012
BOD <sub>5</sub>	Winkler method/ titration	APHA/AWWA, 2012
Ammonia nitrogen (N-H <sub>3</sub> )	Distillation/titration	APHA/AWWA, 2012
TSD	Optic method	APHA/AWWA, 2012
Turbidity (NTU)	Optic method	APHA/AWWA, 2012
pH	Potentiometric	APHA/AWWA, 2012
Conductivity	Electrochemical	APHA/AWWA, 2012
Absortimetric color (420mm)	Colorimetric	APHA/AWWA, 2012
Thermotolerant coliforms (CFU100mL <sup>-1</sup> )	Petri 3M™ Petrifilm™ E. coli/Coliform Count Plates	AOAC, 2000
<i>E. coli</i> (CFU100mL <sup>-1</sup> )	Petri 3M™ Petrifilm™ E. coli/Coliform Count Plates	AOAC, 2000
Phytotoxicity <i>L. sativa</i> *	Test organism exposure/ <i>Lactuca sativa</i>	SOBRERO & RONCO, 2004

\*Adapted from cited literature.

Biomass (microalgae and macrophytes) produced during the effluent treatment process were removed from the integrated system and sent to the bioenergy generation process (Biogas) at the Sustainable Energy and Technology Research Center (CPETS) of the University of Vale do Taquari-UNIVATES. Due to the differences in the development cycle, the biomass collections referring to MA were carried out in

accordance with the 14-day HDT, while the biomasses referring to the macrophytes used in the vertical flow constructed wetland (*H. grumosa*) were obtained after 6 months of operation.

### 3.2.2. Phytotoxicity Test

Phytotoxicity tests using *Lactuca sativa* as test organism were carried out monthly, using as reference adaptations of the methods proposed by SOBRERO & RONCO (2004). For the purposes of presenting the results obtained, the mean and standard deviation statistics were used for both germination and growth indices.

## 3.3. Results and discussion

### 3.3.1. Analytical characterization of the influent

UNISC's WWPTU was developed to meet a demand of around 18,000 people, including employees, visitors and students. The average flow of the WWPTU is approximately  $103 \text{ m}^3 \text{ d}^{-1}$ , with records ranging from  $57.6 \text{ m}^3 \text{ d}^{-1}$  to  $129.6 \text{ m}^3 \text{ d}^{-1}$ . The classification of effluents used during the research can be considered as urban domestic effluents as they originate from the toilets on the university campus, called gray and yellow water (SILVEIRA *et al.*, 2020). Regarding the application rates of the parameters, load factors were used considering the recommendations published by VON SPERLING & SEZERINO (2018) for wetlands built with vertical flow. The general parameters for the characterization of the affluent applied to the different treatment units of the integrated system are presented in table 2.

Table 2: Load application variables in the different treatment stages of the integrated system and UNISC treatment plant.

Variables raw effluent	RA	MA	VFCW (Final integrated system)	UNISC Treatment Plant (WWPTU)
Afluent Volume (L)	900	90	90	103,200
HDT (days)	3	7	7	1
*COD $\text{mg L}^{-1}$	319.36±75.24	246.38±113.49	201.80± 92.06	319.36±75.24
*N-NH3 $\text{mg L}^{-1}$	55.37±37.22	48.91±25.94	7.95±15.43	55.37±37.22
*Total P $\text{mg L}^{-1}$	1.15±0.87	1.02±0.35	0.81±0.28	1.15±0.87
*DBO5 $\text{mg L}^{-1}$	235.37±78.74	205.03±106.99	170.44±117.52	235.37±78.74

\* Affluent Concentration. AR: Anaerobic reactor; MA: Microalgae; VFWC: vertical flow constructed wetland; UNISC Treatment Plant.

The load indices applied to the integrated system and to the WWPTU were similar to those described by SILVEIRA *et al.* (2020), in a study with effluent of the same origin with load application rates of 345.37 mg L<sup>-1</sup> for COD; 234.5 mg L<sup>-1</sup> of BOD<sub>5</sub>; 28.88 mg L<sup>-1</sup> for N-NH<sub>3</sub> and 1.68 mg L<sup>-1</sup> for Total P. These results represent corresponding periods, as they were produced between 2018 and 2020.

When we analyze the results obtained by SILVEIRA *et al.* (2017), in a study also using effluent from the same origin, we can observe a relative difference in the concentration of the affluent load parameters described by them, with results of 7.71 mg L<sup>-1</sup> of Total P; 526.4 mg L<sup>-1</sup> of BOD<sub>5</sub> and 68.8 mg L<sup>-1</sup> of N-NH<sub>3</sub> for example.

This difference may be related to the period in which the studies were conducted and the number of people who attended the university campus, since in 2015, the year represented in the study by SILVEIRA *et al.* (2017), the number of people who used the university campus facilities was around 12,000 among students, visitors and employees. While in the period of development of this current study (2018 to 2020) the number of people, among all groups, does not reach 6,000 campus users.

The characteristics of the influent loading to the treatment systems described in table 2 demonstrate a lower concentration of organic matter compared to studies carried out with university effluent in other periods. Data recorded by DUPONT & LOBO (2012) and HORN *et al.* (2014), using the WWPTU UNISC in their studies found concentrations above that described in this study, especially for total phosphorus. This fact may be directly related to the current situation on the university campus, with a significant drop in students, due to recurring budget cuts in student funding and also, even if for a shorter period (2020), the global pandemic of Covid-19.

The different ways of loading the AR, MA and VFCW integrated system were purposely configured to enhance the effects between stages, in addition, the longer HDT in each unit compared to other studies previously developed with similar systems was implemented with two main objectives: adopt a standard for the production and collection of microalgal biomass and propose an alternative to increase total phosphorus adsorption by the integrated system.

Nutrient removal results through the integrated system can be seen in table 3.

Table 3. Characterization of the raw and treated wastewaters by the integrated system (AR; MA and FVCW) and by the university treatment plants and comparative analysis with the Brazilian and international resolutions.

Parameters	Raw wastewater	Integrated System (AR; MA and VFCW)	WWTPU (Wastewater treatment plant at the University)	CONSEMA/RS Resolution-355/17 $100 \leq Q < 200 \text{ m}^3\text{d}^{-1}$	UWTD 91/271/EEC
Total phosphorus ( $\text{mg L}^{-1}$ )	$1.15 \pm 0.87$	$0.62 \pm 0.21$	$0.85 \pm 0.34$	4	1
COD ( $\text{mg L}^{-1}$ )	$319.36 \pm 75.24$	$117.49 \pm 74.58$	$139.12 \pm 86.86$	$\leq 330$	125
BOD <sub>5</sub> ( $\text{mg L}^{-1}$ )	$235.37 \pm 78.74$	$144.01 \pm 112.91$	$162.93 \pm 106.26$	$\leq 120$	25
N-NH <sub>3</sub> ( $\text{mg L}^{-1}$ )	$55.37 \pm 37.22$	$0.01 \pm 0.03$	$49.93 \pm 30.30$	$\leq 20$	10
TSD ( $\text{mg L}^{-1}$ )	$466.31 \pm 161.61$	$262.87 \pm 89.48$	$519.49 \pm 159.85$	-	-
Turbidity (NTU)	$152.40 \pm 104.89$	$5.62 \pm 5.14$	$15.73 \pm 7.27$	-	-
pH	$7.15 \pm 0.45$	$7.13 \pm 0.51$	$7.37 \pm 0.46$	Between 6.0 and 9.0	Between 6.0 and 9.0
Conductivity ( $\mu\text{Scm}^{-1}$ )	$912.34 \pm 365.06$	$554.79 \pm 222.56$	$1014.61 \pm 283.95$	-	-
Absortimetric Color ( $\lambda=420 \text{ nm}$ )	$0.6 \pm 0.47$	$0.14 \pm 0.34$	$0.47 \pm 2.16$	No change the color of the receiving body	
*Total coliforms (CFU/100 mL)	$313 \times 10^6 \pm 2 \times 10^5$	$313 \times 10^2 \pm 4.17 \times 10^2$	$4.16 \times 10^6 \pm 4.35 \times 10^5$	-	-
* <i>E.coli</i> (CFU/100mL <sup>-1</sup> )	$1.9 \times 10^6 \pm 4.35 \times 10^5$	$0.53 \times 10^2 \pm 0.76 \times 10^2$	$4.16 \times 10^6 \pm 4.35 \times 10^5$	-	-

\* There are no basic limits established by state law to a maximum release flow up to  $200 \text{ m}^3\text{day}^{-1}$ . CONSEMA: Resolutions 355/17.

\* Number of samples for COD; BOD<sub>5</sub>; N-NH<sub>3</sub>= 24 and other parameters with number of samples= 96.

Since the resolution of CONSEMA 355/17 does not stipulate maximum values for the flow rate for the study under discussion ( $103.2 \text{ m}^3 \text{ day}^{-1}$ ), the flow  $200 \leq Q < 500 \text{ m}^3 \text{ day}^{-1}$  (CONSEMA 355/17).

Considering the Brazilian legislation, from CONSEMA Resolution 355/2017 it was possible to observe that the integrated system meets the specifications of the legislation properly, with the exception of parameter DBO5. The same performance can be verified in relation to values consistent with the emission requirements referring to European Standards.

Through the results obtained during the monitoring period with the integrated system (AR, MA and VFCW combined) it was possible to observe that the nutrient removal values were higher, compared to the WWPTU/UNISC. The performance of the integrated system achieved a removal of 53.91% of total P; 61.18% of BOD5 and 63.22% of COD, with emphasis on data referring to the removal of N-NH<sub>3</sub>; total coliforms and *Escherichia coli* by the integrated system with 99.98%; 99.99% and 99.99% respectively. These results are far above the results obtained by WWPTU/UNISC, proving to be a promising alternative for environmental sanitation, although there is a difference in scale between the treatments compared in this study.

Study by SILVEIRA *et al.* (2020) used an anaerobic process (Anaerobic reactor) and microalgae as a preliminary treatment to vertical flow constructed wetland in an integrated system, achieving an average removal of 58% for BOD5, 63% for total P and 100% for N-NH, results very close to those obtained in this study.

Study by ELFANSSI *et al.* (2017), using different configurations of constructed wetlands with vertical flow vegetated with *Phragmites australis*, obtained mean values of turbidity reduction (99.7%); total coliforms (99.9%) and *E. coli* (100%) considered extremely satisfactory. These values are very similar to those found in the present study, with a reduction in turbidity of 96.31%; total coliforms, 99.99% and *E. Coli*, 99.99%.

Other studies using constructed wetlands as a treatment system obtained results very similar to those observed in this study using the integrated system as an alternative to treatment. CALHEIROS *et al.* (2015) obtained in a study developed with a horizontal flow constructed wetland vegetated with different species of ornamental plants in a tourism unit in Portugal, total coliform disinfection values with an average of 99% efficiency.

Study by COLARES *et al.* (2019) used anaerobic reactors followed by hybrid wetlands and ozonation reaching average reduction efficiencies of 78.9% for COD, 91%

for nitrogen, 99.1% for turbidity and 75% for phosphorus in the first 8 months.

DELL'OSBEL *et al.* (2020) using different configurations of wetlands with ornamental plants, obtained reduction results for COD; BOD5; Total P and N-NH<sub>3</sub> 76.53%; 83.64%; 94.03% and 93.84% respectively.

The results obtained by the authors described above, with HDT equal to those applied in this study, using constructed wetlands, present better total P removal efficiency; COD and BOD5 when compared to the integrated system AR, MA and VFCW, on the other hand, the integrated system presents N-NH<sub>3</sub> removal rates higher than these systems. Still, the performance in relation to the other study parameters, such as disinfection of the treated effluent, for example, are very similar, the main differential and purpose of the integrated system is the reduction of built-up area in relation to the technology only using wetlands. Since the sizing and removal of Nitrogen are the main limitations of this technology, the configuration of the integrated system was developed to solve this difficulty.

The results obtained show the generation of biomass from microalgae through the proliferation of autochthonous species from the southern region of Brazil, where the project was developed. Once the integrated system was configured to operate in an open way, that is, exposed to natural weathering, the photobioreactor, after the acclimation period, developed a green color, indicating the presence of microalgae mostly of the *Chlorophyceae* class (Figure 5 : A and B).

The hydraulic detention time (HDT) of 7 days of the effluent in the microalgal proliferation tank and the oxygen supply caused by the recirculation of the liquid through the recirculation pump, proved to be a gain in the production of microalgal biomass and an improvement in the removal of nutrients such as phosphorus total (Table 3). This longer hydraulic detention time was based on the studies by PACHECO *et al.* (2015) who describe 14 days as the average “optimal” period for microalgal proliferation before the onset of decline and subsequent death.

As the filling of the microalgal proliferation tank (photobioreactor) was carried out every 7 days, there was a need to find a way to remove the microalgal biomass diluted in the effluent. To this end, a low-cost, energy-free biomass separator filter was created that removed the biomass from the microalgae, which were then reintroduced into the photobioreactor. And only after the 14 day hydraulic detention period were they removed from the integrated system and refrigerated for anaerobic digestion tests and subsequent conversion to energy.

The biomass separator filter (Fig. 2 C), proposed during the execution of the pilot project, obtained its patent application filed under the registration number BR 20 2021 011625 6 with the National Institute of Industrial Property (INPI).

The macrophyte used in VFCW, *Hymenacne grumosa*, is a native species in the region, abundant in the southern fields, widely used as animal food (Figure 5: C and D). The option for this native species is due to its high biomass production and its history of good results in reducing physicochemical parameters as described by SILVEIRA *et al.* (2019).



Figure 5: Biomass generation and removal during the urban effluent treatment process through the integrated MA (A and B) and WCFV (C and D) systems.

The proposal to combine treatments using microalgae (MA) and macrophytes in VFCW was based on the background described by GSCHLÖBL *et al.* (1998), pioneers in the use of constructed wetlands as a final polish in lagoons with microalgae. The results can be seen in figure 6 where it is possible to verify an efficiency of 95% of MA removal through VFCW. With the results shown in figure 6, we can see that VFCW was effective in retaining the diluted microalgal cells in the effluent, reducing their presence at the end of the treatment process, a fact that confirms the idea cited by GSCHLÖBL *et al.* (1998) in pond polishing.

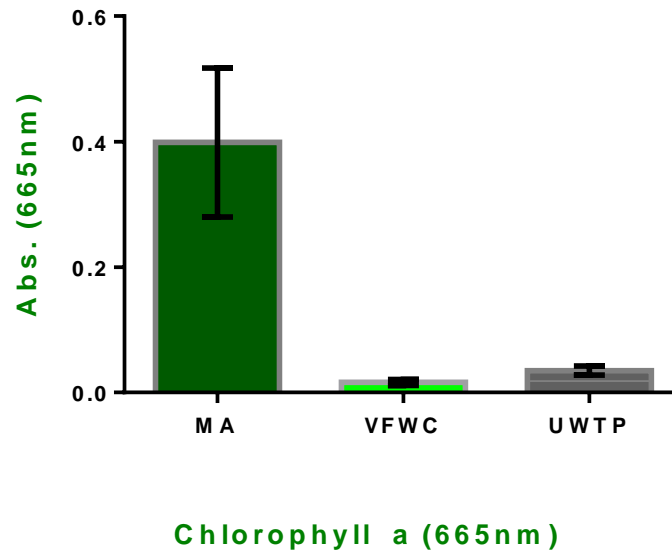


Figure 6: Removal of microalgae (MA) through final polishing with vertical flow constructed wetland (VFWC) and concentration of microalgae in UWTP.

Furthermore, it was possible to observe that even without the contribution of a system with microalgae in the treatment platform of the university's (WWPTU), which is composed of several stages, there was presence of microalgae in the final treatment process, about 7.5%, even with a higher incidence when compared to the 5% integrated system, a fact that reinforces the idea that microalgae are present in the local environment.

Although physicochemical analyzes are of fundamental importance and widely used in studies involving the treatment of urban effluents, these alone are not able to assess the levels of toxicity present in these waters, for this, it is necessary to use specialized tests for determine the effluent toxicity levels as a complementary approach.

Thus, as a tool for investigating the different urban effluent treatment processes, the results of the experiments carried out using *Lactuca sativa* as a test organism in phytotoxicity tests are shown in figure 7.



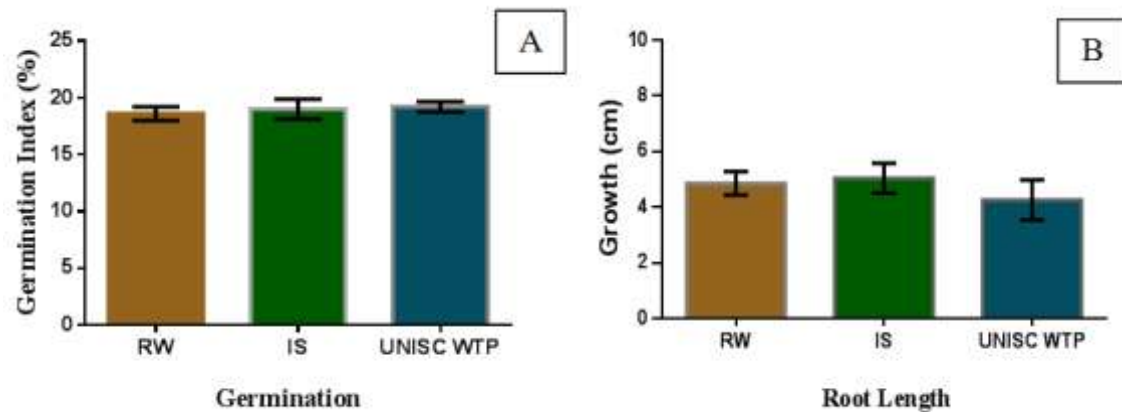


Figure 7: Phytotoxicity test with test organism *Lactuca sativa*, germination (A) and growth (B) indices.

As seen in figure 7, the results of both germination and growth of *Lactuca sativa* showed a homogeneity with little data dispersion between the raw effluent and the studied treatments. Therefore, the effluent does not show toxicity in the analyzes performed. SILVEIRA (2018) obtained results similar to those observed in this study, with the application of tests with the same test organism in the period from October 2014 to December 2015.

In a study by RODRIGUES *et al.* (2013), the authors highlight the use of *Lactuca sativa* as a test organism for toxicity monitoring tests for urban and industrial effluents. However, they attribute the increase in salinity and consequently the high conductivity as germination inhibiting factors. This fact is not observed in this study. Although the results of this test do not demonstrate toxicity in the studied effluent, it is important to emphasize that other toxicological tests may present toxicity levels hidden by the phytotoxicity analysis system.

SILVEIRA *et al.* (2020) found significant toxicity levels through phytotoxicity, cytotoxicity and genotoxicity assays using *Alhium cepa* as test organism for the same type of effluent used in this study. In general, the results of this study showed an adequate performance of the integrated system in relation to the results presented by the WWPTU/UNISC, with better efficiency in all analyzed parameters. Saved the proper dimensioning proportions, it can be verified with the obtained results that the proposition of the integrated system as an alternative of environmental sanitation has potential for wide use as a technology for the treatment of urban effluents. However, it is important to emphasize that the proposition of an integrated system as an alternative to conventional technologies requires studies on a real scale and continuous flow for a better basis for evaluation and comparison.

Finally, we can show that the design of the integrated system consisting of an anaerobic reactor, microalgae and vertical flow constructed wetland as an alternative treatment technology based on bioprocesses presented good performance and promising application to the treatment of urban effluents, positioning itself as a technology alternative focusing on environmental sanitation and energy recovery.

### **3.4. Conclusions**

The results obtained were promising and indicate that the proposed integrated system has great potential for application to the decentralized treatment of urban and/or rural wastewater.

The use of native species in the development of the integrated RA, MA and VFCW system reinforces the importance of evaluating alternatives for wastewater treatment with Eco Design applied to environmental sanitation.

The possibility of producing energy from biomass generated by the integrated system during the process in which water quality is recovered proposes a really promising alternative for sustainable technology.

Thus, the integrated system appears as an alternative for environmental sanitation with great potential for application in the treatment of urban effluents, in addition to recovering energy, it may, with some adjustments, come to remedy the deficiencies presented by the conventional technologies currently used.

#### 4. BIOMONITORING OF URBAN WASTEWATERS TREATED BY AN INTEGRATED SYSTEM COMBINING MICROALGAE AND CONSTRUCTED WETLANDS

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##### Abstract

The objectives of the present study were to apply different toxicological assays for monitoring the toxicity of treated and untreated university campus urban effluents. The research was conducted at the Wastewater Treatment Plant of the University of Santa Cruz do Sul (UNISC), from October 2018 to April 2019. An integrated system with Anaerobic Reactor, Microalgae (MA) and Constructed Wetlands (CWs) was proposed for detoxification of urban wastewaters produced at a university campus with hydraulic detention time of 17 days. *Daphnia magna* (Ecotoxicity) and *Allium cepa* (Phytotoxicity, Cytotoxicity and Genotoxicity) were used as tools to monitor the efficiency of the integrated system. Obtained results showed that the integrated system (MA + CWs) was able to meet the emission standards of Resolution CONSEMA 355/17 in relation to COD and BOD<sub>5</sub>, besides achieving removal rates higher than 98% and 97% for N-NH<sub>3</sub> and TKN, being much more efficient than the campus wastewater treatment plant (UWWTP). The results of Ecotoxicity presented the raw wastewaters (RW) as slightly toxic and absence of ectotoxicity in all the treatments steps. Regarding phytotoxicity, the results showed no significant differences between treatments. The Citogenetic assays indicated a significant increase in MI (mitotic index), ( $p < 0.001$ ) after treatment by CWs compared to the final treatment UWWTP while the results regarding BNC (binucleated cells) did not present significant differences among the treatments. Micronucleus (MN) indexes were significantly different between the UWWPT and the integrated system ( $p < 0.01$ ). In relation to chromosome aberrations (CA) the results indicate a significant difference between the CWs and UWWTP treatments ( $p < 0.01$ ) and RW and CWs ( $p < 0.001$ ), confirming the detoxifying potential of the integrated system when compared to UWWPT. Thus, the results of the present research highlight the relevance in the proposition of the integrated system as an alternative of cleaner technology to the detriment of conventional technologies applied in wastewater treatment.

Keywords: Urban Wastewater; Microalgae; Constructed Wetland; Integrated System; Detoxification.

#### 4.1. Introduction

It has become a common understanding that water shortage will increasingly affect people worldwide. According to the UNESCO's report, due to a combination of population growth, socio-economic development and changing consumption patterns, global water demand is expected to continue increasing at a rate of 1% a year until 2050. According to them, over 2 billion people live in countries experiencing high water stress, and about 4 billion people experience severe water scarcity during at least one month of the year, so that three out of ten people do not have access to safe drinking water while six out of ten people do not have access to safely managed sanitation services. Therefore the sustainable use of the water resources is a crucial issue that has been worldwide discussed over the last decades (LUTTERBECK *et al.*, 2018)

In this sense, reclaimed water constitutes an interesting alternative to supplement water resources, alleviate environmental loads and address the imbalance between water demand and water supply (RUSSO *et al.*, 2019). Nevertheless, due to the presence of a great variety of emerging contaminants (ECs), which can result in toxic and adverse effects on aquatic organisms and consequently on humans (RIZZO *et al.*, 2019), the reuse of urban wastewaters for some purposes is still a challenging task. As urban wastewaters are composed of a complex matrix of environmental contaminants which might be toxic and only partially or even not eliminated by wastewater treatment systems (RADIĆ *et al.*, 2010; HEMACHANDRA and PATHIRATNE, 2017), they are among the major point sources of surface water contamination (RIZZO *et al.*, 2015).

Biological systems, such as Constructed Wetlands (CWs) and Microalgae (MA) have been frequently considered as interesting alternatives for the treatment of urban wastewaters since they can be used in integrated systems as pre or post-treatments (SILVEIRA *et al.*, 2017; LUTTERBECK *et al.*, 2017). CWs besides being low-cost alternatives, might offer other advantages like as dispense the use of chemicals products, provide satisfactory treatment of pollutants, such as organic matter and recycling nutrients and can be used in small and medium communities with low maintenance and operation requirements (VYMAZAL and KRÖPFELOVÁ, 2009; MARZO *et al.*, 2018; LUTTERBECK *et al.*, 2017). Moreover, CWs have a lower visual impact and lead to the production of smaller quantities of sewage sludge when compared to traditional WWTPs and to the production of biomass that can be used in animal feed (VYMAZAL and KRÖPFELOVÁ, 2008; SEZERINO *et al.*, 2003). The application MA for the treatment of different wastewaters have been increasingly investigated over the last years, especially

considering that these systems allow for the recovery of energy and nutrients as well as provide natural effluent oxygenation (SHANKAR, 2011), preventing the need of mechanical aeration that accounts for up to 50% of the total treatment plant cost (REARDON, 1995).

Despite the benefits of the application of biological systems in the treatment of urban wastewaters, mostly the evaluation of the efficiency of these systems is based on the chemical/physicochemical analysis. So, taking into account the great diversity of chemical pollutants and pathogens present in urban wastewaters, the poor removal rates for some contaminants and the potential adverse effects that wastewaters might inflict on wildlife and humans because of the additive, synergistic, or antagonistic interactions between the chemicals present in wastewaters, traditional physicochemical analyses should be complemented by bioassays using different species as test organisms respond to all the compounds in wastewaters (PRASSE *et al.*, 2015).

Because of its high sensitivity against different wastewaters and capacity to detect toxic effects at several end points, plant bioassays have been extensively used during the last years in the environmental monitoring (LEME and MARIN-MORALES, 2009; LEME, ANGELIS and MARIN-MORALES, 2008). Among them, the *Allium cepa* test system has received special attention and has been frequently used to detect the cytotoxic, genotoxic and mutagenic potential of wastewaters containing different environmental contaminants (LUTTERBECK *et al.*, 2015; LUTTERBECK *et al.*, 2018). Besides be an easy handling assay and don't require previous treatments of tested samples, as well as the addition of exogenous metabolic system, as in the Ames test, the *A. cepa* bioassays present a good correlation with other test systems, e.g. mammals (RANK and NIELSEN, 1994) and allows the assessment of different endpoints (LEME and MARIN-MORALES, 2009).

The biomonitoring through aquatic ecotoxicology assays can also provide valuable insights about the potential toxic effects of raw and treated wastewaters. *Daphnia magna* is a microcrustacean with worldwide distribution in freshwater that has been widely used as a test organism in bioassays involving several chemical compounds present in aquatic ecosystems. Because of its importance in the food chain, sensitivity to toxic agents, and easy handling in the laboratory, standard methods with *D. magna* have been recommended by international environmental agencies such as the Organisation for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (USEPA) (KERN *et al.*, 2015)

So, considering the scarce number of studies dealing with the potential of biological systems to reduce/mitigate the possible toxic effects of urban wastewaters, the present paper aims to assess the efficiency of an integrated system with microalgae and constructed wetlands using a battery of different bioassays.

## 4.2. Materials and Methods

### 4.2.1. Description of Integrated System

The research was carried out at the wastewater treatment plant (WWTP) of the University of Santa Cruz do Sul (UNISC), from October 2018 to April 2019. The wastewaters are drained from the urinals and toilets and treated at the WWTP, which comprises seven treatment steps: grating; sand trap; equalizer tank; anaerobic upflow sludge blanket (UASB); aerated biofilter; decanter; and sludge drying bed.

The system was developed and built for the proliferation of microalgae (MA) from the wastewaters of the equalizer tank and was composed of a 200L working volume box, with a pyramid-shaped acrylic cone (Fig 1). At the apex of the cone a PVC pipe structure integrated with a hose connected to a submerged recirculation pump with flow rate (Q) of 400/1000 L h<sup>-1</sup> 13 W-P: 2.0 (Meters of Water Column). Once connected to the recirculation pump the system started without inoculation of wild or commercial strains of microalgae.



Figure 1: Schematic representation of the integrated wastewater treatment system. A: Microalgae unit. B: Vertical Sub superficial Constructed Wetlands (CW).

After one week of activity the contents of the box were green, indicating the presence of MA. Thus, after the acclimation period, the system was loaded every seven days. For the CWs, a box of 200L working volume with rectangular compartment (92 cm in length; 55 cm in width; 58.5 cm in height) (plastic box made of high-density polyethylene (HDPE) was used. The substrate was composed of a layer of

approximately 10 cm of crushed stone no. 4 (64–100 mm) placed at the bottom of the boxes while a layer of approximately 5 cm of crushed stone no. 1 (20–40mm) was added onto the first layer. Basalt crushed stones were used. *Hymenachne grumosa* was selected as the macrophyte in our study because of its desirable characteristics such as easy acclimatization to the study site, supporting load factors of total P of at least  $0.3 \text{ g m}^{-1} \text{ day}^{-1}$ , to have a root system of at least 40 cm in length and allow the pruning every 3 or 4 months (MACHADO *et al.*, 2015).

The system operated in a batch modus considering the 3-day Hydraulic Detention Time (HDT) for Anaerobic Reactor (AR), 7 days for MA and 7 days for CWs after the treatment by MA. A 2-inch polyethylene hose and a 1 HP submersed centrifugal pump were used to drain the wastewaters from the equalizer tank to the anaerobic reactor (AR). The AR was fed every three days. 90L of this effluent were then drained to the MA system every 7 days. After the MA treatment, wastewaters were filtrated and thereafter driven to the CWs. In a final step, CWs were also fed with 90L MA pre-treatment effluent.

Samples were obtained after a TDH of 17 days, collected before loading the systems and analyzed in sequence, thus ensuring the sameness of the samplings. The collection points were divided into: Raw wastewaters (RW) collected at the output of equalizer tank; output of the Anaerobic Reactor (AR), output of the Microalgae (MA) system, and output of the Constructed Wetlands (CWs) box. For comparison purposes, post-treatment samples from the university's WWTP (UWWTP) were also regularly collected.

#### **4.2.2. Wastewater analytical characterization**

The characterization of the raw and treated wastewaters included analysis of total phosphorus (P), chemical oxygen demand (COD), biochemical oxygen demand (BOD<sub>5</sub>), ammonia nitrogen (N-NH<sub>3</sub>), nitrate, nitrite, total dissolved solids (TDS) conductivity, pH, turbidity (NTU), total Kjeldahl nitrogen (NTK), absorptiometric color (420 nm), and thermotolerant coliforms. Table 1 summarizes the analysis as well as the methods and references adopted. Samples were taken weekly and analyzed thereupon the collection.

Table 1: Analytical methods used for the characterization of the raw and treated wastewaters.

Parameters	Method	Reference
COD	Colorimetric/titration	APHA/AWWA, 2012
BOD <sub>5</sub>	Winkler method/ titration	APHA/AWWA, 2012
Conductivity	Electrochemical	APHA/AWWA, 2012
pH	Potentiometric	APHA/AWWA, 2012
Turbidity (NTU)	Optic method	APHA/AWWA, 2012
Total phosphorus (P)	Colorimetric/ammonium molybdate	APHA/AWWA, 2012
Ammonia nitrogen	Distillation/titration	APHA/AWWA, 2012
Total Kjeldahl nitrogen (NTK)	Acid digestion	APHA/AWWA, 2012
Nitrate	Colorimetric/Phenyldisulfonic acid	APHA/AWWA, 2012
Nitrite	Spectrophotometric	APHA/AWWA, 2012
Absortiometric color (420 nm)	Colorimetric	APHA/AWWA, 2012
Thermotolerant coliforms	Petri 3M™ Petrifilm™ E. coli/Coliform Count Plates	AOAC, 2000

### 4.3. Toxicity studies

#### 4.3.1. Ecotoxicity assays

Acute ecotoxicity assays tests involving the microcrustacean *Daphnia magna* Straus 1820 were performed. The methodological procedures followed the Brazilian Association of Technical Standards (ABNT (2003) 12.713 standard). A more detailed description of the assays can be found in the elsewhere (LUTTERBECK *et al.*, 2014). Raw effluents and treated samples collected at different treatments stages of the integrated system (anaerobic unit/SSFCWs/UV) were tested and prepared with volumetric precision at a geometric progression ratio of ½. Due to the variation in the composition of the raw effluents, 5 different concentrations were tested (100–6.25%). To estimate EC<sub>50</sub>, the non-parametric statistical method (Trimmed Spearman-Kärber Method) proposed by HAMILTON *et al.* (1979) was used. A relative toxicity scale proposed by LOBO *et al.* (2006), with classification criteria of 25th, 50th (median), and 75th percentiles, was used to classify the results of the 48-h EC<sub>50</sub> values.



### 4.3.2. *Allium cepa* assays

Phytotoxicity assays were carried out by adapting the method of SOBRERO and RONCO (2004) while the cytogenetic evaluation considered methodological adaptations proposed in FISKESJO (1985), FISKESJÖ (1995) for the mitotic index; GRANT (1982), for chromosome aberrations and MA *et al.* (1995) for the analysis of micronuclei. 20 seeds of *A. cepa* of the same variety (Periform Bay, ISLA PRO) and without any chemical treatment were germinated in Petri dishes and incubated at  $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 5 days under visible and UV light; each dish was covered with filter paper and individually poured with 5 ml of raw and treated samples. Distilled water was used as negative control and cupric sulfate (3 mg/L) as positive control.

After the germination period, the seeds were measured and quantified for the germination index and root length. Subsequently, 20 rootlets per sample were selected, separated from whole seeds and placed directly into tubes containing TRIS (physiological pH) for 24 hours. After the 24 hour period, the radicles were washed with distilled water and placed in 70% alcohol tubes. To prepare the slides, the radicles were withdrawn from the alcohol and washed with distilled water, then placed in 1% HCl solution to perform cell lysis, after placing the radicles under immersion, the microtube was placed in the germinator at  $25^{\circ}\text{C}$  per 30 minutes. After the lysis period, the 1% HCL solution was discarded and the radicles again washed with distilled water. Then, with the aid of a scalpel, the radicles were cut and macerated with Pistil; stained with (name of the dye), placed on the Blade and covered with cover slip. The slides were analyzed using conventional optical microscopy ( $\times 400$  magnification). A more detailed description of the test procedures can be found elsewhere (LUTTERBECK *et al.*, 2015)

The percentages of relative Seed Germination (SG), Root Elongation (RE) were calculated based on eqs. (1) and (2):

$$\text{GI} = \frac{\text{seeds germinated}}{\text{seed germinated in control}} \times 100 \quad (1)$$

$$\text{RE} = \frac{\text{mean root length}}{\text{mean root length in control}} \times 100 \quad (2)$$

The cytotoxicity was evaluated based on the analysis of the Mitotic Index (MI) which corresponds to the percentage of cells divided by treatment, according to Eq. (3).

$$\text{MI} = \frac{\text{CD}}{\text{TCS}} \times 100 \quad (3)$$

where MI = Mitotic index; CD = Cells in division; TCS = Total cells per sample.

Chromosome aberrations (CA) found in the different phases of the cell cycle (metaphase, anaphase, and telophase) were considered to assess the genotoxicity and their quantification was restricted to the presence of changes such as losses, fragments, delays, bridges, adhesions, viscosity, among others. The mutagenicity of the wastewaters was determined by the presence of micronucleated cells (MNC). CA and MCN were determined by their frequency in the total number of cells counted and compared to the negative control.

#### **4.3.3. Data analysis of the toxicity assays**

The statistical analysis of the toxicity assays was performed using analysis of variance (ANOVA) followed by Dunn's test for multiple comparisons in relation to the negative control (NC), considering the significance level of 5% ( $p < 0.05$ ). The statistical program used was software GraphPad Prism 6.0.

### **4.4. Results and Discussion**

#### **4.4.1. Wastewater analytical characterization**

The WWTP of the University of Santa Cruz do Sul was configured to meet the demands of the wastewaters produced by a population equivalent of 18,000. The treatment plant operates with a mean flow of approximately  $103 \text{ m}^3 \text{ day}^{-1}$ , with variations ranging from  $129.6$  to  $57.6 \text{ m}^3 \text{ day}^{-1}$ , and flow peaks occurring between 12 and 17 h.

The wastewaters produced at the campus have characteristics of typical urban wastewaters, since were generated in urinals and toilets and may be, therefore, regarded as black and yellow waters (PACHECO *et al.*, 2015). General characterization parameters of the raw wastewaters are presented in Table 2. As one can observe, several parameters are over the limits established by national and international resolutions. BOD<sub>5</sub> and COD values are, respectively, 2.2 and 1.1 times over the maximal emission limits allowed by the Resolution 355/17 established by the Rio Grande do Sul State Council on the Environment (CONSEMA), the state council for effluent disposal in Rio Grande do Sul while for European guideline for urban wastewater treatment (DIRECTIVE 1991) this values are almost 5 and 3 times above the permitted limits. However, it must be highlighted that the effluent presents good biodegradability condition, with a COD/BOD<sub>5</sub> ratio of 1.4 (JARDIM and CANELA, 2004)

Table 2: Characterization of the raw and treated wastewaters by the integrated system (MA + CWs) and by the university treatment plants and comparative analysis with the Brazilian and international resolutions.

Parameters	Raw wastewater	Integrated System (MA + CWs)	UNISC Treatment Plant	CONSEMA/RS	
				Resolution-355/17 $100 \leq Q < 200 \text{ m}^3 \text{d}^{-1}$	UWTD 91/271/EEC
Total P ( $\text{mg L}^{-1}$ )	0.838	0.406	0.803	4	1
COD ( $\text{mg L}^{-1}$ )	366.31	217.02	215.13	$\leq 330$	125
BOD <sub>5</sub> ( $\text{mg L}^{-1}$ )	262.5	112.5	87.15	$\leq 120$	25
N-NH <sub>3</sub> ( $\text{mg L}^{-1}$ )	31.7	<0.5	22.6	$\leq 20$	10
Nitrate ( $\text{mg L}^{-1}$ )	0.32	<0.1	<0.1	-	-
Nitrite ( $\text{mg L}^{-1}$ )	<0.1	<0.1	<0.1	-	-
TKN ( $\text{mg L}^{-1}$ )	44.09	1.21	23.55	-	-
TSD ( $\text{mg L}^{-1}$ )	419	221	402	-	-
Turbidity (NTU)	64	2.56	12.48	-	-
pH	7.32	7.03	6.9	Between 6.0 e 9.0	Between 6.0 e 9.0
Conductivity ( $\mu\text{Scm}^{-1}$ )	822	441	803	-	-
Color ( $\lambda = 420 \text{ nm}$ )	0.26	0.05	0.08	No change the color of the receiving body	-
EC <sub>50</sub> (I) 48h	77.1%	100%	100%	TF=1 for acute toxicity* EC <sub>50</sub> = 100%	-
Total coliforms (CFU/100 mL)	$>1.6 \times 10^6$	110	$>1.6 \times 10^6$	-	-
* <i>E.coli</i> CFU/100 mL)	$>1.6 \times 10^6$	<18	$>1.6 \times 10^6$	-	-
Copper	<0.02	<0.02	<0.02	0.5 mg/L	-
Total Chromium	<0.05	<0.05	<0.05	0.5 mg/L	-
Iron	0.08	0.05	0.04	10 mg/L	-
Manganese	0.06	0.08	0.05	1.0 mg/L	-
Zinc	0.1	<0.02	0.11	2.0 mg/L	-

\* There are no basic limits established by state law to a maximum release flow up to  $200 \text{ m}^3 \text{day}^{-1}$ . CONSEMA Resolutions 355/17.

The investigated wastewaters also present strong eutrophying load, especially due to presence high levels of nitrogen compounds. The obtained results are in line with the study conducted by HORN *et al.* (2014). Table 2 also shows the high pathogen load of the raw wastewaters, with total coliforms (UFC/100 mL) and *Escherichia coli* (UFC/100 mL)  $> 1.6 \times 10^6$ .

Considering the performance of the treatments it is possible to note that integrated system (MA + CWs) was able to meet the emission standards of the CONSEMA 355/17 Resolution regarding COD and BOD<sub>5</sub>. Nevertheless, despite the reductions of both parameters, it failed to attend the more strict values of the UWTD 91/271/EEC –European urban wastewater treatment directive. On the other hand the MA + CWs system presented removals rates higher than 98 % and 97 % for N-NH<sub>3</sub> and TKN, been much more efficient than the campus wastewater treatment plant (Table 2). It must be pointed out that studies using CWs as the main phytoremediation agents have, in general, presented difficulties for removing loading pollutants such as ammonia nitrogen, needing to increase the required area to achieve removal rates that may be under emission ceilings legislations (ÁLVAREZ, RUÍZ and SOTO, 2008; HORN *et al.*, 2014). Thus, the good removal rates attained in this study demonstrated that the MA + CWs system might be a promising possibility for the treatment of effluents with high levels of nitrogen compounds.

Finally, the disinfection potential of the integrated system is also noteworthy. After the treatment the pathogen loads of the untreated effluents were reduced by five orders of magnitude and so fully comply with the emission standards of the CONSEMA 355/17 resolution.

#### **4.4.2. Toxicity experiments**

#### **4.4.3. Ecotoxicity assays**

Table 3 presents the results of the acute ecotoxicity tests. Based on the classification proposed by LOBO *et al.* (2006), the raw wastewaters can be classified as slightly toxic. Results of researches carried out by SILVEIRA *et al.* (2017) and HORN *et al.* (2014) indicated that untreated effluents also produced at a university campus were “moderately toxic”. This less toxic condition might be attributed to a lower eutrophying load of the wastewaters investigated here when compared to the above mentioned studies, mainly nitrogen compounds, which have already been reported to attribute toxicity to wastewaters (DÜPONT and LOBO, 2012).

As can be observed all the treatments steps fully removed the acute toxicity against *D. magna* and in this way fully attend the CONSEMA resolution 355/17 (Table 3). However it must be pointed out that acute toxicity tests may fail to detect the toxic effects of some compounds. So, complementary chronic ecotoxicity assays should be carried out in future studies to confirm the absence of toxicity.

Over the past years several studies have investigated the detoxification potential of CWs and MA. Results of acute ecotoxicity experiments carried with *D. magna* showed that raw wastewaters produced at a university campus passed from a condition of “moderately toxic” to “slightly toxic” after the treatments with sequential CWs (HORN *et al.*, 2014). Nevertheless, authors of the same study verified that the treatment was ineffective to remove the chronic toxicity against *Ceriodaphnia dubia*. A research carried out with wastewaters produced at a rural property showed complete absence of acute toxicity towards *D. magna* after the treatment with four sequential CWs (LUTTERBECK *et al.* 2018).

Table 3: Results of the ecotoxicity assays carried out with *D. magna* and classification based on according to Lobo *et al.* (2006).

<b>SAMPLES</b>	<b>EC (I) 50% 48h</b>	<b>RELATIVE TOXICITY%</b>
<b>RW</b>	77,11%±1,41	Slightly toxic
<b>AR</b>	100	No Toxic
<b>MA</b>	100	No toxic
<b>CWs</b>	100	No toxic
<b>UWWTP</b>	100	No toxic

A study carried out by FRANCHINO *et al.* (2016) investigated the ecotoxicity of diluted piggery digestate before and after the treatment with *Chlorella vulgaris*. The

obtained results showed the detoxification potential of the MA, which fully eliminated the acute and chronic toxicity against *D. magna*.

#### 4.4.4. Phytotoxicity Experiments

As one can observe in Fig. 2A, wastewaters treated by MA presented significant higher germination index (GI) ( $p < 0.05$ ) when compared to the ones treated by the CWs. This result can be attributed to the presence of higher organic matter values as well as to a more alkaline pH, characteristic of this phase of the integrated system treatment (SILVEIRA, 2018). Moreover, the reduction of the conductivity by MA treatment ( $357 \mu\text{S}/\text{cm}$ ) in relation to the CWs ( $441 \mu\text{S}/\text{cm}$ ) might also be related to the significant increase of the GI. According to (RODRIGUES *et al.*, 2013), high electrical conductivity inhibits germination because it affects osmoregulation, leading to organelle failure responsible for this function. No significant differences were observed among the other steps of the integrated system or in relation to the university treatment plant.

Regarding root elongation (RE) (Fig. 2B), it is noteworthy that RW presented significant higher growth in comparison to AR ( $p < 0.01$ ), MA ( $p < 0.001$ ) and UWWTP ( $p < 0.01$ )(Data not shown). Higher organic load present in the RW may have favored the RE of the analyzed samples. No significant differences were registered between RW and CWs samples.

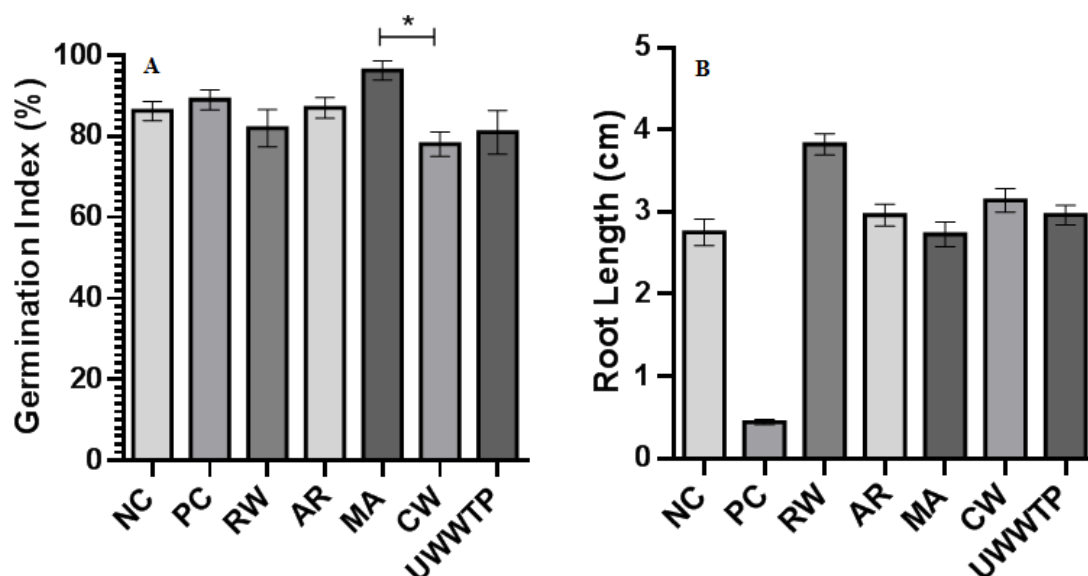


Figure 2: Germination Index (A) and Root Length (B) of the samples submitted to different treatments steps. Abbreviations: Negative Control (NC), Positive Control (PC), Raw Wastewater (RW), Anaerobic Reactor (AR), Microalgae (MA), Constructed Wetland (CW) UNISC Wastewater Treatment Plant (UWWTP). The groups are represented by the median and the minimum and maximum variation. For

each group 500 cells were counted with 10 replicates totaling 5000 cells. Only significant differences regarding the comparison of CW vs MA and CW vs UWWTP were pointed out. The other significant differences between the groups tested are presented in table S2. Kruskal-Wallis test followed by Dunn's post-test for multiple comparisons; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

LEIVA *et al.* (2019) evaluated the phytotoxicity of wastewater treated with horizontal subsurface flow constructed wetlands (HSSFCWs) combined with Chlorine (Cl) and UV using seeds of *Raphanus sativus* (*R. sativus*) and *Triticum aestivum* (*T. aestivum*) and considering three different endpoints: percentage of germination inhibition (PGI), percentage of epicotyl elongation (PEE) and germination index (GI). According to the authors positive effects (PGI and PEE  $< 0\%$  and GI  $> 80\%$ ) on *R. sativus* and *T. aestivum* were observed in Petri dishes bioassays using effluents treated by HSSF-Cl, HSSF-UV. However, toxic effects of HSSFCWs-Cl and HSSFCWs-UV on seeds germination and epicotyl elongation of both plant species were detected in soil samples (PGI and PEE  $> 0\%$  and GI  $< 80\%$ ). According to LEIVA *et al.* (2019), these negative effects on the three endpoints evaluated in this study might be relate to the presence of free Chlorine (FCl) in the case of HSSFCWs-Cl, since it can react with the organic matter of the soil, leading to the formation of organo-halogenated byproducts (OX) that may affect physiological plant parameters such as photosynthesis and plant growth. Moreover, the authors attributed the phytotoxicity of both systems (HSSFCWs-Cl, HSSFCWs-UV) on soil bioassays to the high EC (1,275– 1,278  $\mu\text{S}/\text{cm}$ ) values and  $\text{NH}_4^+-\text{N}$  concentrations ( $47.5 \pm 37.6$  and  $75.8 \pm 13.3$ ).

MELO *et al.* (2018) investigated the toxicity of untreated and treated wastewaters of three types of agro-industrial by-products using algal treatment with *C. vulgaris*. Authors observed that untreated corn steep liquor inhibited the roots development process, and consequently led to a significant lower GI ( $p < 0.05$ ) in comparison to the negative control. MELO *et al.* (2018) attributed this result higher nutrients and organic matter loads, especially Nitrate ( $\text{NO}_3^-$ ) which is considered a low toxicity substance, but as the final product of nitrification, it can accumulate in large quantities, especially in closed culture systems. Results of the corn steep liquor treated by MA, showed a complete elimination of  $\text{NO}_2^-$ ,  $\text{NO}_3^-$  and P, and removed 65.16% of COD.

#### 4.4.5. Cytogenetic assays

As already mentioned above, the *A. cepa* test system enables the evaluation of different endpoints. In the present study the cytotoxicity of wastewaters before and after the treatments was determined by the alterations of the Mitotic Index (MI), characterized by the total number of dividing cells in cell cycle. According to LEME and MARIN-MORALES (2009) significant reduction of the MI in comparison to the negative control may be due to a chemical action in the growth and development of exposed organisms. On the other hand, MIs higher than the negative control are results of an increase in cell division, which can be harmful to the cells, leading to a disordered cell proliferation and even to the formation of tumor tissues.

The results obtained in the present study (Fig. 3A) indicated statistically significant increase of MI (214.3 %) after the treatment by CWs in comparison to the negative control ( $p < 0.001$ ). Effluents treated by CWs also presented significant higher MI when compared to MA (44.4%) ( $p < 0.05$ ) and UWWTP (54.9%) ( $p < 0.001$ ). Some authors tried to establish concentration ranges for the determination of cytotoxic conditions (sub-lethal and lethal levels) related to the responses presented by the meristematic cells of *A. cepa*. A decrease of approximately 22% in the MI compared to the negative control causes sublethal effects (ANTOSIEWICZ, 1990), whereas a reduction greater than 50% (cytotoxic threshold) often has a lethal effect (SHARMA, 1983; PANDA and SAHU, 1985).

Regarding the frequency of Binucleated Cells (BNC) no significant differences were verified between CWs and UWWTP (Fig. 3B), suggesting that both systems are efficient in removing this type of cytotoxicity, since RW showed a significant increase in cell type (Table S2). Thus, the obtained results indicated a possible capacity of the integrated system (MA + CWs) to remove the cytotoxicity of the investigated wastewaters.



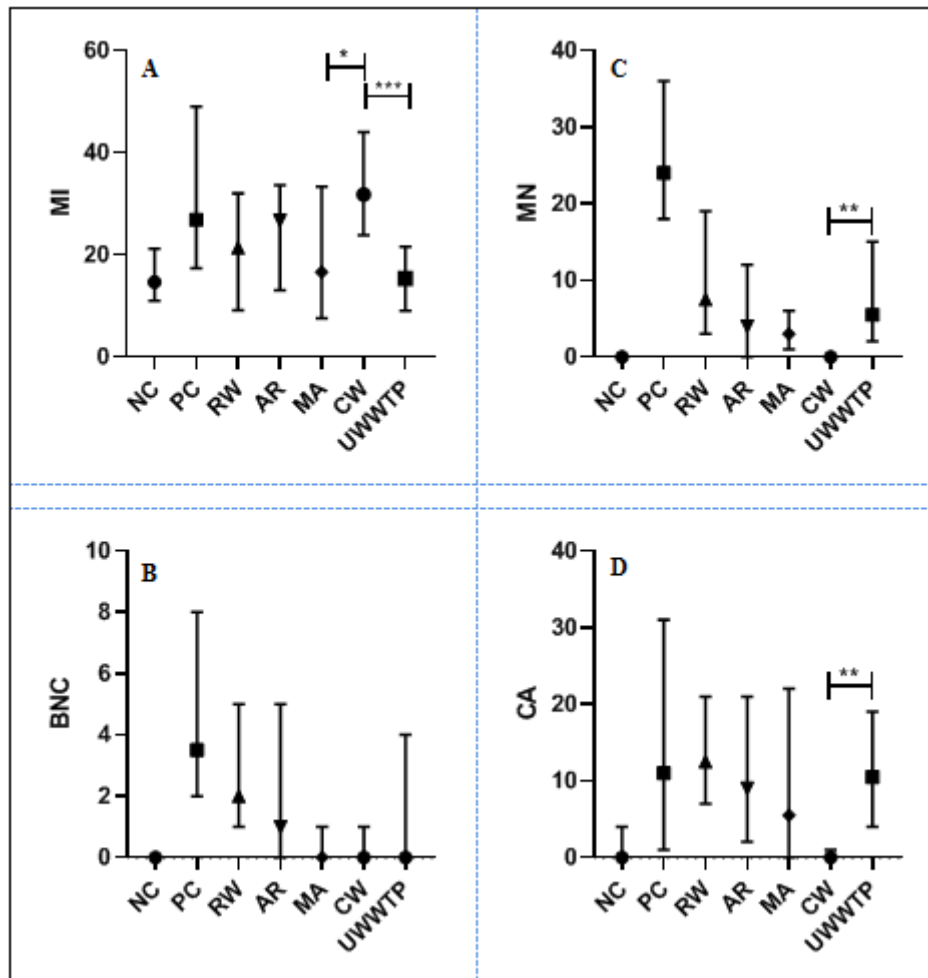


Figure 3: Evaluation of cytotoxicity through the *Allium cepa* test. A: Mitotic Index; B: Micronucleus; C: Binucleated Cells; D: Chromosome Aberrations: Abbreviations: Negative Control (NC), Positive Control (PC), Raw Wastewater (RW), Anaerobic Reactor (AR), Microalgae (MA), Constructed Wetland (CW) UNISC Wastewater Treatment Plant (UWWTP). The groups are represented by the median and the minimum and maximum variation. For each group 500 cells were counted with 10 replicates totaling 5000 cells. Only significant differences regarding the comparison of CW vs MA and CW vs UWWTP were pointed out. The other significant differences between the groups tested are presented in table S2. Kruskal-Wallis test followed by Dunn's post-test for multiple comparisons; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

MELO *et al.* (2018) observed a significant reduction ( $p < 0.05$ ) of the rates of cell division of untreated corn steep liquor when compared to the negative control, confirming the cytotoxic potential of this effluent. The authors verified that the treatment with *C. vulgaris* besides reducing the values of nutrients and the organic load increased the MI, being a good alternative for the reduction of the cytotoxicity. On the other hand, both untreated sugarcane vinasse and treated sugarcane vinasse wastewaters showed significant reduction ( $p < 0.05$ ) of the MI, indicating that the MA treatment was not

effective to reduce cytotoxic effects of this wastewaters. MELO *et al.* (2018) attributed these results to the presence of some heavy metals.

Micronuclei (MN) are considered by many authors, traditional and reliable indicators of mutagenicity potential of compounds present in waters and wastewaters. According to (Fenech, 2000), MN results from damages wrongly repaired (or not repaired) in the parental cells being easily measured in daughter cells as a structure similar to the main nucleus, but in a reduced size. Therefore, MN arise from the development of some chromosome aberrations (CA), such as, chromosome breaks and losses or may still derive from other processes as polyploidization, in which they originate from the elimination of exceeding DNA of the main nucleus in an attempt to restore the normal conditions of ploidy (FERNANDES *et al.*, 2007; LEME and MARIN-MORALES, 2009).

In the present study the results pointed towards a significant difference between RW and CWS ( $p < 0.001$ ), demonstrating that the treatment proposed by the integrated system (MA + CWs) was efficient, completely removing the mutagenicity of the investigated wastewaters. The conventional system (UWWPT) did not present a significant difference in relation to RW. Besides, the data presented in the Fig. 3C, show a significant differences ( $p < 0.01$ ) of the MN indexes of CWs when compared to UWWPT, indicating that the integrated system also presented better performance concerning the removal of the mutagenic effects of the wastewaters when compared to conventional treatment. So, the results discussed above highlight the inefficiency of the conventional effluent treatment system to remove mutagenic agents from wastewater which can lead to important genetic alterations in different organisms present the receptor bodies, as mentioned by FERNANDES *et al.* (2007) and LEME and MARIN-MORALES (2009).

Chromosome aberrations (CA) presented in the samples were used in the *A. cepa* test as a parameter to detect potentially genotoxic agents. Structural chromosomal alterations may be induced by several factors, such as DNA breaks, inhibition of DNA synthesis and replication of altered DNA. Results of our research showed that RW presented strong genotoxic potential in comparison to NC ( $p < 0.001$ ) (Fig 3D). When compared to NC, AR and UWWTP also presented significant induction of chromosome aberrations ( $p < 0.01$ ). On the one hand, CW achieved a very significant reduction of the genotoxic activity in comparison to RW ( $p < 0.001$ ). On the other hand, no statistical significant differences were observed between RW and UWWTP (Table S2),

demonstrating that the conventional treatment system was also inefficient regarding this endpoint.

The study carried out by MELO *et al.* (2019) verified that *C. vulgaris* treatment could reduce genotoxicity from corn steep liquor (TC) and vinasse (TV), reflecting the efficiency of both treatments for this parameter. However, non-treated groups UW, UC, UV and TW presented values of genotoxic effects ( $p < 0.05$ ) significantly higher when compared to negative control but significantly lower ( $p < 0.05$ ) when compared to positive controls (Trifluralin and Methyl-methanesulfonate).

So, as reported by MELO *et al.* (2019) the application of MA treatment can reduce genotoxicity rates; however, as shown in the present study, an integration of technologies can significantly improve the performance of the treatment of urban effluents. In summary, the results of cytogenetic evaluation presented in this section demonstrated the efficiency of the MA + CW system for reducing/removing the toxicity of four different endpoints.

#### **4.5. Conclusions**

The results obtained through this research presented data of relevance for the proposal of integrated systems as alternative of cleaner technologies in detriment to the conventional technologies applied in wastewater treatment. Through the study it was possible to observe that although phytotoxicity and acute ecotoxicity assays are analyzes of relative importance, this tests alone are not able to make precise inferences as to the toxicological potential of treated or untreated effluents. Therefore, it is becoming increasingly necessary to apply cytotoxicity/genotoxicity tests as a tool to monitor the efficiency of urban effluent treatment.

Based on the cytogenetic evaluation, it was possible to verify the efficiency of the MA + CW system, since it presented a much better performance than the conventional UWWTP, completely removing the genotoxicity and mutagenicity and significantly reduced the cytotoxicity of the investigated wastewaters. Furthermore, it also achieved higher removals of organic loads. Thus, the proposed integrated system proved that this technology is a low-cost alternative and promising application in the treatment of wastewater, even on a large scale.

## 5. ENERGY RECOVERY FROM BIOMASS BIODIGESTION GENERATED BY AN INTEGRATED SYSTEM COMPOSED OF MICROALGAE AND CONSTRUCTED WETLAND

Este manuscrito foi submetido ao periódico Sustainable Energy Technologies and Assessments.

### Abstract

The present study evaluated the potential for biogas generation from microalgae (MA) biomass and macrophytes used in vertical flow constructed wetlands (VFCW). The integrated system was developed for the treatment of university wastewaters and was composed of sequential anaerobic reactor (AR), MA and VFCW. Samples were obtained by separation and collection of MA after a hydraulic retention time of 14 days, frozen and taken to the laboratory, while the macrophytes of VFCW were obtained, by pruning, every six months. The average biogas generation was 2322.51 NmL-gSV<sup>-1</sup> with 54.61% CH<sub>4</sub> (winter/2019); 4491.47 NmL-gSV<sup>-1</sup> with 57.17% CH<sub>4</sub> (spring/2019); 680.78 NmL-gSV<sup>-1</sup> with 16.04% CH<sub>4</sub> (summer/2020) and 681.0 NmL-gSV<sup>-1</sup> with 19.86% CH<sub>4</sub> (autumn/2020) for MA biomass and generation of biogas of 3826.70 NmL-gSV<sup>-1</sup> with 44.26% CH<sub>4</sub> for VFCW biomass in winter and spring/2019 and of 829.68 NmL-gSV<sup>-1</sup> with 17.06% CH<sub>4</sub> in summer and autumn /2020.

**Keywords:** Microalgae; Treatment; Biogas; Biomass; Constructed wetland.

### 5.1. Introduction

The growing search for energy generation alternatives to meet the global demand has increasingly raised the number of researches and debates in the scientific and technological spheres. Although fossil fuels are responsible for meeting most of this demand, factors such as environmental degradation and limited reserves make the use of this energy matrix finite and potentially polluting (PACHECO *et al.*, 2015; HASAN *et al.*, 2019 and MARTENS *et al.*, 2021).

Dependence on energy will always be an extremely important point for society regarding the development and maintenance of its basic activities. Over the last years the demand for electricity has grown, increasing the need to diversify the sources of energy

generation and supply. Therefore, the generation of biogas through urban and agro-industrial organic waste emerges as a renewable energy alternative in the context bioenergy generation (DALPAZ, *et al.*, 2020).

The proposition of alternatives that may bring effective solutions to this scenario necessarily involves a transition from the current energy model to an expanded matrix of renewable energy sources. As some of the current alternatives for the most used energy sources, such as wind and solar, depend on climatic conditions, the generation of biogas through anaerobic digestion of organic matter is not directly associated with these climatological variables. (ABAD, CHERRETT, & HOLDSWORTH, 2015).

Another considerable benefit of using biogas is the fact that it can be stored, a significant advantage that can accommodate variable energy demands, making it an essential component among the available renewable energy options (HAHN *et al.*, 2014 and WALL *et al.*, 2016).

The generation of biogas is an important part of the carbon cycle and can be used as a potential fuel for energy production. After being purified, biogas can reach the same standards as fossil natural gas, replacing it, and providing so an alternative of renewable energy. As a result of the anaerobic digestion, the carbon content in the biomass is reduced and the nitrogen content is concentrated. Thus, the waste generated by the digestate differential can be used, for example, in soils as a biofertilizer. (RASLAVICIUS, GRZYBEK, & DUBROVIN, 2011; DA ROS *et al.*, 2014 and KARLSSON *et al.*, 2014).

From the anaerobic digestion, the organic material contained in the biomass is converted into biogas, where its main constituents are methane gas (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>) (KONRAD *et al.*, 2016a and RIBEIRO *et al.*, 2016). Other gases also present, although in smaller proportions, are: water vapors (H<sub>2</sub>O); hydrogen sulfide (H<sub>2</sub>S); Hydrocarbons; ammonia (NH<sub>3</sub>); oxygen (O<sub>2</sub>); carbon monoxide (CO) and nitrogen (N<sub>2</sub>) (GHOUALI, SARI & HARMAND, 2015; RYCKEBOSC, DROUILLON & VERVAEREN, 2011 and COIMBRA-ARAÚJO *et al.*, 2014).

Studies have been developed aiming to enhance the production of biogas through the combination of organic substrates in a process called co-digestion (MATA-ALVAREZ *et al.*, 2014 and KARLSSON *et al.*, 2014). Co-digestion can promote a better carbon/nitrogen (C/N) ratio, the main nutrients used by microorganisms in anaerobic digestion, ensuring that all the carbon available in the medium may be consumed by microorganisms (KONRAD *et al.*, 2016a).

Researches on the generation of biogas have received growing attention in recent years, since besides being used as a renewable energy source for heat and energy generation, it can also generate fuel (biomethane), thus constituting a sustainable alternative to fossil fuels (LEÓN & MARTÍN, 2016; HASAN *et al.*, 2019; LANTZ, 2012).

In Brazil, the structural development of bioenergy emerges as a strategic opportunity, mainly for rural communities that can have a self-supply of energy in this technology. Additionally, the surplus production may be commercialized in the national energy supply system, since currently; the electricity sector allows injecting excess energy production into the distribution network (ARAÚJO *et al.*, 2014 and DA SILVA, NETO & SEIFERT, 2016 and KONRAD *et al.*, 2016b).

Studies show that the digestion of carbon-rich animal and/or vegetable residues, with residues that complement these substrates, have been widely used by industry with positive results in the generation of biogas (HASAN *et al.*, 2019 and ZHANG *et al.*, 2013). Besides, some of the obtained results demonstrated that it is possible to use anaerobic digestion technology in energy conversion, making this a useful technology for society (LUMI *et al.*, 2015 and KONRAD *et al.*, 2016b).

Other studies analyzed the proposal to generate biogas as an alternative for energy recovery in consolidated phytoremediation systems, such as wastewater treatment through the use of macrophytes used in constructed wetlands, recovering energy by the anaerobic digestion of plants (ROJ-ROJEWSKI *et al.*, 2019; PROCHNOW *et al.*, 2005 and WÖHLER-GESKE *et al.*, 2016).

Moreover, some researchers used algal biomass from microalgae applied in different processes of the development of bioproducts and in the treatment of wastewater as an alternative source of biogas generation offering possibilities of reuse and energy recovery (GONZÁLEZ-FERNANDEZ *et al.*, 2012; PASSOS *et al.*, 2014 and GUTIÉRREZ *et al.*, 2015).

So, the search for environmentally viable solutions for the wastewater recovery process lead many researchers towards combining treatment systems, with the idea of minimizing costs and enhancing performance, in addition to recovering energy during the process. Since the energy issue also corresponds to an urgent demand and requires the attention of researchers, the proposition of integrated systems with energy recovery becomes a trend in the near future.

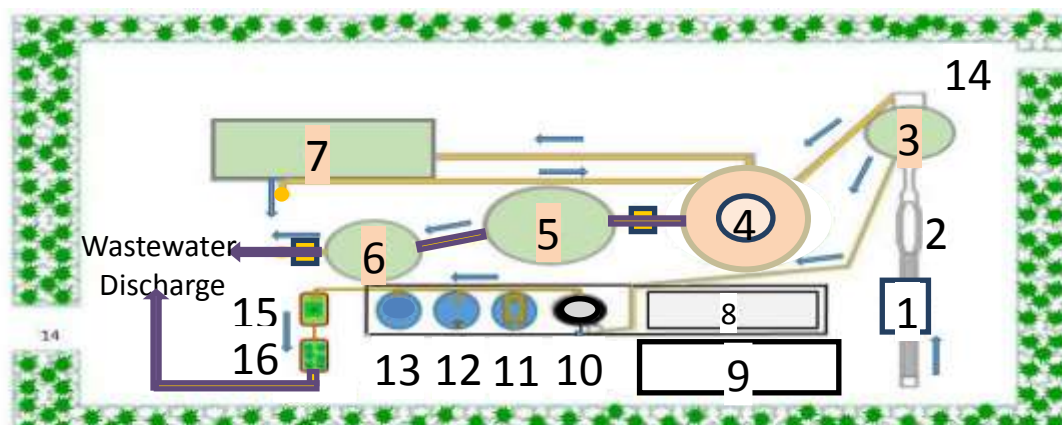
Therefore, this study evaluated the potential for biogas generation from microalgae (MA) biomass and macrophytes used in constructed wetland (CW) arising from the urban wastewater treatment process, as well as from the biogas generated in the production of electric energy as an alternative for environmental sanitation and energy recovery.

## 5.2. Materials and methods

### 5.2.1. Characterization of the study area

Biomass generation took place during the treatment process of the urban wastewaters generated at a university campus. The integrated system was composed of an anaerobic reactor, microalgae (MA) and vertical flow constructed wetland (VFCW). The pilot project was conceived and operated at the wastewater treatment plant (WWTP) of the University of Santa Cruz do Sul/RS, (UNISC) Brazil, (Figure: 1).

The WWTP was configured to meet the demands of wastewater produced by a population equivalent to 18,000 people, operating with an average flow of approximately  $103 \text{ m}^3 \cdot \text{day}^{-1}$ . Fluctuations range from  $129.6$  to  $57.6 \text{ m}^3 \text{ day}^{-1}$  and flow peaks occur between 12 and 17 h (SILVEIRA *et al.*, 2017). For this experiment, six stages of analyses were carried out at the Research Center in Energy and Sustainable Technologies (RCEST) of the University of Vale do Taquari - UNIVATES, located at the campus of Lajeado/RS, Brazil.



#### Legend

- |                                   |                                       |                 |
|-----------------------------------|---------------------------------------|-----------------|
| 1 – Grit chamber                  | 6- Secondary Decanter                 | 12 - SSHFCW     |
| 2- Sand box                       | 7- Sludge Drying Bed                  | 13 - CWFFF      |
| 3 - Equalization and pumping tank | 8 and 9 - For other research with CWs | 14- Access ways |
| 4- UASB                           | 10- Pilot UASB                        | 15- MA          |
| 5 - Aerated Biofilter             | 11 - VFCW                             | 16 – VFCW       |

Figure1: Representative scheme of the UNISC's WWTP (1,2,3,4,5,6 and 7) and pilot research projects with different configurations of CW systems (10, 11, 12 and 13) and integrated system composed of an anaerobic reactor, MA and VFCW (10, 15 and 16).

The integrated system was designed for the treatment of urban wastewaters (Figure: 2). Biomass generated during the treatment process were used as raw material in the conversion to biogas.

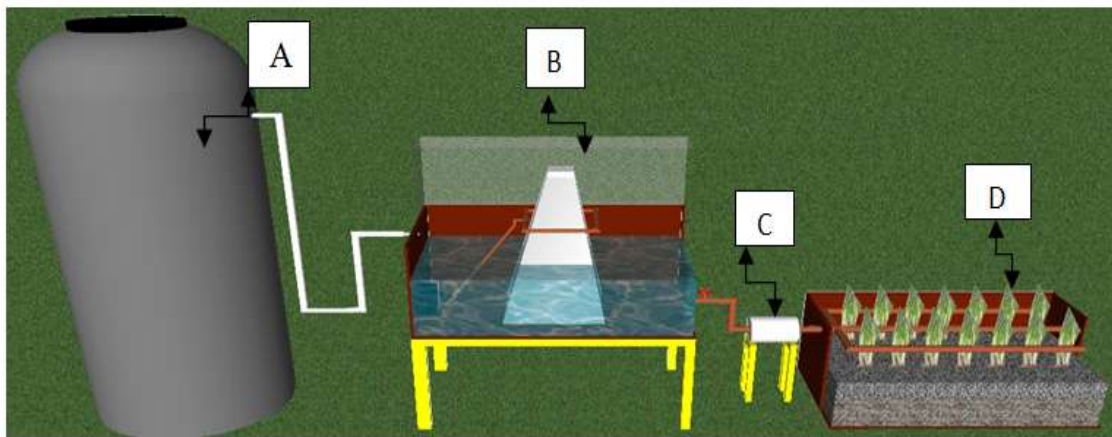


Figure 2: Integrated treatment system: anaerobic reactor (A); microalgae photobioreactor (B); biomass separator filter (C) and vertical flow constructed wetlands (D).

### 5.2.2. Sampling

The project was developed and monitored from August/2018 to May/2020. As of August 2018, the prototype of the integrated system was configured and, after a period of 15 days of acclimatization, it began to be regularly monitored by physicochemical analyses. After a monitoring period of six months, including chemical, physical and biological parameters, the stages of collection and analyses of the respective biomass generated during the treatment process of the evaluated effluent were programmed.

As described above, the production of biomass used as raw material for the generation of biogas was obtained from the treatment of university campus wastewaters treated by the integrated system during the study period. Table 1 summarizes the physicochemical and biological analyses and the respective methods used to assess the efficiency of the treatment system.



Table 1: Analytical methods used for the characterization of the raw and treated wastewaters.

Parameters	Method	Reference
Total phosphorus (TP)	Colorimetric/ammonium molybdate	APHA/AWWA, 2012
COD	Colorimetric/titration	APHA/AWWA, 2012
BOD <sub>5</sub>	Winkler method/ titration	APHA/AWWA, 2012
Ammonia nitrogen (N-H <sub>3</sub> )	Distillation/titration	APHA/AWWA, 2012
TSD	Optic method	APHA/AWWA, 2012
Turbidity (NTU)	Optic method	APHA/AWWA, 2012
pH	Potentiometric	APHA/AWWA, 2012
Conductivity	Electrochemical	APHA/AWWA, 2012
Absortimetric color (420nm)	Colorimetric	APHA/AWWA, 2012
Thermotolerant coliforms (CFU100mL <sup>-1</sup> )	Petri 3M™ Petrifilm™ E. coli/Coliform Count Plates	AOAC, 2000
<i>E.coli</i> (CFU100mL <sup>-1</sup> )	Petri 3M™ Petrifilm™ E. coli/Coliform Count Plates	AOAC, 2000

The analyses of the generated biomass were divided into seasonal periods, thus establishing four stages of collection and analyses related to microalgal biomass: Winter (2019); spring (2019); summer (2019/2020) and autumn (2020). Regarding the biomass from plants used in the VFCW, collections and analyses were carried out in two stages, corresponding to the seasonal periods referring to winter and spring 2019; summer and fall 2020.



Figure 3: Period of biomass generation during the treatment using the integrated system: microalgae (A) and plants used in the VFCW (B).

In the first stage, microalgae biomass samples, collected with hydraulic detention time (HDT) of 14 days and during the months of June, July and August 2019 (winter), were evaluated. The procedure was repeated for each corresponding period, September, October and November 2019 (spring), December 2019, January and February 2020

(summer) and finally, the period corresponding to autumn 2020, March, April and May (Figure: 3A). The samples were preserved at frozen, being taken to the Laboratory of Bioreactors at the University of Vale do Taquari- UNIVATES, to assess the potential for energy generation after the end of each seasonal period. The samples from the tank containing the macrophytes used in the VFCW were collected after pruning, carried out in two periods: one after the winter and spring 2019, and another after the summer and autumn 2020, making a six-month interval between the samplings (Figure: 3B).

The methodological difference between the collection of microalgae biomass and the macrophyte biomass of the VFCW is mainly due to the developmental characteristics of plants (slower growth cycle) and microalgae (faster growth cycle). The collection of microalgal biomass was carried out in two stages: in the first, after 7 days of HDT, the biomass was removed after passing the pre-treated wastewaters through the microalgae by the biomass separating filter (Figure: 2C) and reintroduced into the photobioreactor (Figure: 2B). In the second, the biomass was again removed after passing through the separator filter and, together with the biomass adhered to the photobioreactor and the box, collected, preserved and refrigerated.

### **5.2.3. Experiment characterization**

The analyses were performed by an automated biogas flow measurement system through a programmable logic controller (PLC) system called automated biogas measurement system (ABMS) and with the automatic methane potential test system (AMPTS II) as described by KONRAD *et al.* (2021).

### **5.2.4. Inoculum characterization**

The inoculum used for anaerobic digestion of the respective biomass was provided by the Research Center in Energy and Sustainable Technologies (CPETS) of UNIVATES, which comes from other experiments already carried out, thus containing microorganisms fully adapted to mesophilic anaerobic digestion (37°C). The inoculum used presented basic characteristics with precision and efficiency, among them the pH >7.0 and <8.5; Ammonia < 2.5 g of N-NH<sub>4</sub> L<sup>-1</sup>; Alkalinity >3 g CaCO<sub>3</sub> L<sup>-1</sup>.

The inoculum/sample ratio was defined according to the protocol applied in the experiment, based on the calculation of Total Solids (TS) and Volatile Solids of the sample (VSS), as described in the STANDARD METHODS (2017), and Volatile Solids of the inoculum (VSI) using a 2x1 ratio (VDI 4630, 2006).

### 5.2.5. Anaerobic digestion assay

The VDI 4630 (2006) method was used to carry out the anaerobic digestion in this experiment. The method primarily consists of determining the inoculum solids and the biomass to be tested, as described above. pH analyses were also performed. For this purpose, homogenized portions of these two materials were separated, proceeding with the analyses and storing the remainder for the time of testing effectively, with the inoculum being kept in the incubator and the biomass in the refrigerator.

The biomass inoculated in the reactor was weighed (Figure: 4 A) and then the inoculum/sample ratio was defined. In the reactors, previously defined sample and inoculum were added. The exact amount added to each reactor, both inoculum and sample, was duly registered through a written note (Figure: 4 B and 4 C). Thereafter, the material was homogenized and the pH analyses of each reactor were performed.

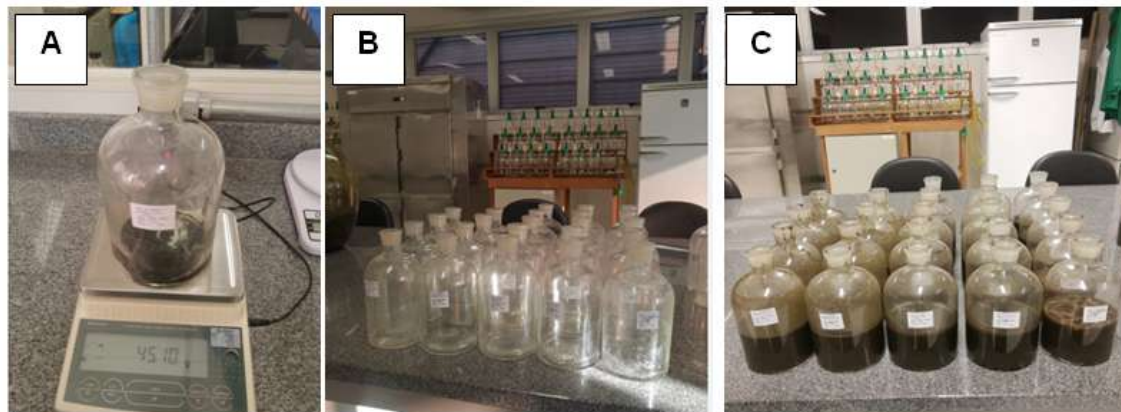


Figure 4: Anaerobic digestion assay steps: weighed biomass (A); reactors prepared to receive the samples (B) and reactors ready to start the experiment.

### 5.2.6. Methane content evaluation

The methodology adopted for monitoring and measuring the generated biogas was similar to the described by KONRAD *et al.* (2016b). The proposed system uses liquid displacement as the measurement principle. Methane was analyzed by the advanced Gasterter specific sensor, produced by PRONOVA Analysentechnik GmbH & Co.

## 5.3. Results and discussion

The results obtained in the generation of biomass from microalgae were produced through the proliferation of native species from the southern region of Brazil, where the pilot project was developed and operated. As it is an open system, after the

acclimatization period, the photobioreactor developed a green color indicating the presence of microalgae mostly of the *Chlorophyceae* class (Figure: 5A). The plant used in VFCW was the emerging macrophyte *Hymenacne grumosa*, a native species of the study region, abundant in the southern fields and widely used as animal food. The option for this native species is due to its high biomass production and its capability of achieve good results regarding the reduction of physicochemical parameters as described by SILVEIRA *et al.* (2019).



Figure 5: Biomass generation during the treatment process of the evaluated urban effluent: microalgae (*Chlorophyceae*) (A) and *Hymenacne grumosa* (used in the constructed wetland) (B).

The biomass production related to the macrophyte used in the VFCW was similar to that described by SILVEIRA (2018), who carried out a study using the species *H. grumosa* as a phytoremediator of university campus wastewaters. The biomass generated arose from the treatment of university campus wastewaters treated during the study period. The results referring to physicochemical monitoring show a reduction of 61% for Total Phosphorus; 43.25% for COD; 45.81% for BOD<sub>5</sub>; 98% for Ammoniacal Nitrogen; 97.35% for turbidity and 90% for apparent color removal compared to the data recorded for the raw wastewaters (Table: 2). In addition, the integrated system achieved disinfection rates of 99.99% for Thermotolerant Coliforms and *Escherichia coli* (Table: 2), an index similar to those described by Silveira *et al.* (2020), who developed a study with an integrated system composed of sequential microalgae and CWs and focused on evaluating the detoxification potential by comparing raw and treated wastewaters.

Table 2: Characterization of the raw and treated wastewaters by the integrated system (AR; MA and FVCWs) and by the university treatment plants and comparative analysis with the Brazilian and international resolutions.

Parameters	Raw wastewater	Integrated System (AR; MA and VFCW)	UNISC Treatment Plant	CONSEMA/RS Resolution 355/17: $100 \leq Q < 200 \text{ m}^3\text{d}^{-1}$	UWTD 91/271/EEC
Total phosphorus ( $\text{mg L}^{-1}$ )	1.68±1.39	0.65±0.08	0.86±0.43	4	1
COD ( $\text{mg L}^{-1}$ )	345.37±139	196.01±79.47	215.8±95.61	≤330	125
BOD <sub>5</sub> ( $\text{mg L}^{-1}$ )	234.5±114.48	127.08±158.54	112.5±134.8	≤120	25
N-H <sub>3</sub> ( $\text{mg L}^{-1}$ )	28.88±20.27	0.64±0.75	16±1.39	≤20	10
TSD ( $\text{mg L}^{-1}$ )	452.83±124.86	291.63±151.44	513.83±136.6	-	-
Turbidity (NTU)	212.33±42.66	5.64±4,52	16.86±8.49	-	-
pH	7.08±0.33	7.3±0.3	7.4±0.4	Between 6.0 and 9.0	Between 6.0 and 9.0
Conductivity ( $\mu\text{Scm}^{-1}$ )	902±248.61	583.35±102.89	1029.5±268.59	-	-
Absortimetric Color ( $\lambda=420 \text{ nm}$ )	0.56±0.28	0.06±0.01	0.09±0.04	No change the color of the receiving body	-
*Total coliforms (CFU/100 mL)	$313 \times 10^6 \pm 2 \times 10^5$	$313 \times 10^2 \pm 4.17 \times 10^2$	$4.16 \times 10^6 \pm 4.35 \times 10^5$	-	-
* <i>E. coli</i> (CFU100mL <sup>-1</sup> )	$1.9 \times 10^6 \pm 4.35 \times 10^5$	$0.53 \times 10^2 \pm 0.76 \times 10^2$	$4.16 \times 10^6 \pm 4.35 \times 10^5$	-	-

\* There are no basic limits established by state law to a maximum release flow up to  $200 \text{ m}^3\text{day}^{-1}$ . CONSEMA/Resolutions 355/17.

\* Number of samples for COD; BOD<sub>5</sub>; N-NH<sub>3</sub>= 18 and other parameters with number of samples= 72.

In relation to the results of the biogas production from the analyzed biomass, it was possible to observe a difference between the analyzed stages (Table: 3). Although these differences were already expected, due to the structure of the experiment, which was configured to operate in an “open” way, that is, fully exposed to natural weather and without control of environmental variables.

These differences were enhanced between the experiments carried out in 2019 (Winter and Spring) and 2020 (Summer and Autumn), probably because they were extremely affected by two main factors: the dry period that severely hit the study region (December/2019 to May/2020) and the drastic reduction in the generation of university wastewaters because of the university recess period (holidays) and the COVID-19 global pandemic. These factors hampered the supply of the integrated system, since, in addition to suffering from the drought, the wastewaters generated during the period were far below standards, both in terms of quantity and organic load. So, the performance and energy generation of the integrated system was affected, consequently interfering in the energy production of biomass generated in the period.

Table 3: Mean concentrations and standard deviations ( $\pm$  Sd) of biogas and methane (CH<sub>4</sub>) production in the analyzed steps.

Season period	Microalgae <i>Chlorophycea</i> Inflow Mean $\pm$ SD		Constructed wetland <i>Hymenacne grumosa</i> Inflow Mean $\pm$ SD	
	Biogas production NmL- gSV <sup>-1</sup>	CH <sub>4</sub> %	Biogas production NmL- gSV <sup>-1</sup>	CH <sub>4</sub> %
Winter 2019	2322.51 $\pm$ 625.10	54.61	–	–
Spring 2019	4491.47 $\pm$ 168.95	57.17	–	–
Summer 2020	680.78 $\pm$ 132.65	16.04	–	–
Autumn 2020	681.00 $\pm$ 72.89	19.86	–	–
Winter and Spring 2019	–	–	3826.70 $\pm$ 277.77	44.26
Summer and Autumn 2020	–	–	829.68 $\pm$ 31.05	17.06

During the collection and analyses stages of samples corresponding to winter 2019 and spring 2019 (Table: 3) it was possible to observe that the averages of CH<sub>4</sub> generation in this study obtained similar results to those found in other bioenergy

production studies with different substrates. As, for example, the results described by SILVA *et al.* (2020) that obtained CH<sub>4</sub> generation of 60% on average using municipal solid waste as biomass.

In the studies carried out by LIN *et al.* (2011), using fruit residues and food waste, and ZHANG *et al.* (2013) who used food waste and cattle manure, respectively, the concentration of CH<sub>4</sub> presented an average of 58.75% and 41%, respectively. The means obtained in the present study in the winter and spring stages registered CH<sub>4</sub> values for microalgal biomass, equivalent to those found by LIN *et al.* (2011); while the results obtained with biomass from plants in the VFCW (*H. grumosa*) were similar to those found by ZHANG *et al.* (2013).

ALZATE *et al.* (2012) researched the potential for biogas and methane generation of different genera of algae and microalgae, with results of Biochemical Biogas Potential (BBP) and Biochemical Methane Potential (BMP) for *Chlorophyceae* microalgae of 581.08 NmL-gSV<sup>-1</sup> and 398 Nml-gSV<sup>-1</sup>, respectively. Values very similar to those described in this study, where data of 502.70 NmL-gSV<sup>-1</sup> and 312.43 NmL-gSV<sup>-1</sup> were recorded (Figure 6: A and B), during the spring period, the main period of microalgae development.

KEYMER *et al.* (2013) when using high pressure thermal hydrolysis (HPTH) to verify the methane yield potential during the digestion of microalgal biomass in the biogas generation process, found BBP and BMP values for *Chlorophyceae* of 798 NmL-gSV<sup>-1</sup> and 307 Nml-gSV<sup>-1</sup>, similar to those showed in Figure 6: A.

Studies carried out by WÖHLER-GESKE *et al.* (2016), using the VDI 4630 (2006) method as a reference, analyzed different reed species obtained from sugarcane cutters in Germany, the Netherlands and other reed exporting countries around the world associated with the generation of biogas. The results indicated values with BBP of 551.5 NmL-gSV<sup>-1</sup> and BMP of 311.4 NmL-gSV<sup>-1</sup>, very close to the values obtained in the present research, mainly in step I (First pruning- Macrofite), where the observed BBP and BMP values were of 554.49 Nml-gSV<sup>-1</sup> and 312.26 Nml-gSV<sup>-1</sup> respectively.

ROJ-ROJEWSKI *et al.* (2019) examined the biogas and methane production of reed species of the genera *Phragmites*, *Glyceria* and *Carex*, harvested in the Narew River valley in northeastern Poland. The authors found levels of BMP 102-221 NmL.gvs<sup>-1</sup>, similar to those observed for grasses collected in fields and natural wetlands by other researchers. However, these values were much lower when compared to species obtained from pastures or CWs.

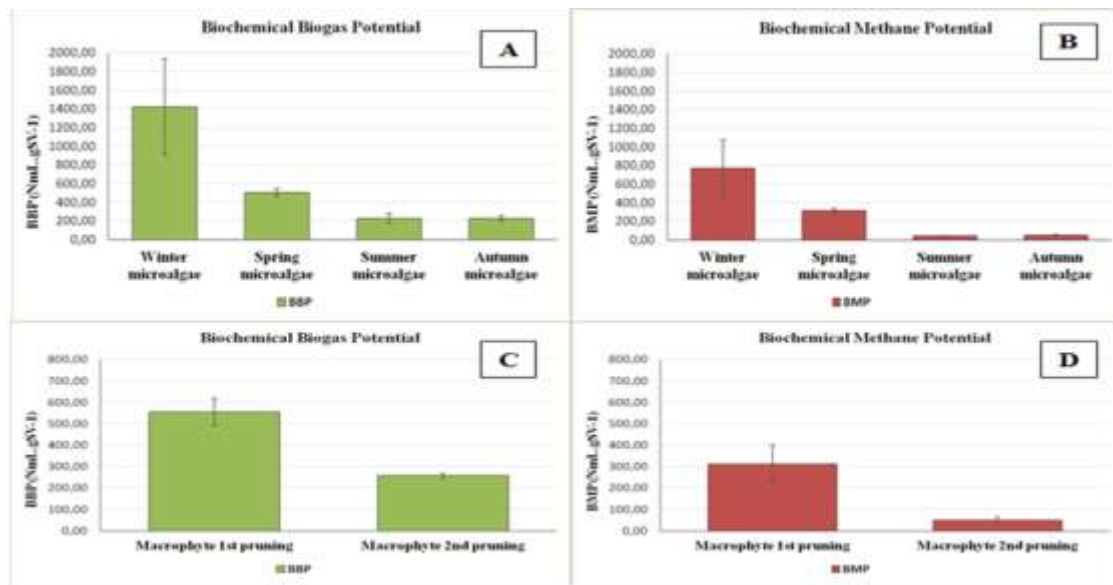


Figure 6: Biochemical Biogas Potential (A) and Biochemical Methane Potential (B) of the microalgal biomass accumulated during the analyses steps; and biomass of macrophytes used in the VFCW (C) and (D).

The results described by ROJ-ROJEWSKI *et al.* (2019) point out as a reason for this difference in energy capacity, the suboptimal condition of the C: N ratio. This fact may explain the results obtained in the experiment represented in Figure 6 C and 6 D (second stage) in which the data presented 258.1 Nml. gVS<sup>-1</sup> e 50.50 Nml. gVS<sup>-1</sup> of BBP and BMP, respectively. Although both, species of plants and the environmental conditions were different, there is a possibility that the remarks described by ROJ-ROJEWSKI *et al.* (2019) might elucidate the cause related to low yield in biogas generation during step 2 of the analyses of this study.

In the case of the aforementioned authors, the plants used were obtained from a preserved environment, since the Narew River valley is located in a national reserve in Poland. This fact initially, refers to a place without pollution; therefore, without an abundance of nutrients dissolved in water, place where plants grow. In the present study, the step of biomass obtainment for the plants used in the VFCW (Second Pruning-Macrophyte) was carried out during the pandemic period, therefore, without activity on the university campus and, consequently, without the generation of wastewaters at the WWTP.

It is possible that the results referring to analyses equivalent to the periods of summer and autumn/2020 (Figure 6: A and B) were affected by the same factor described by ROJ-ROJEWSKI *et al.* (2019), since the microalgae used, despite having a



less complex cellular composition than the macrophytes, also suffered from the lack of nutrients contained in the university effluent due to the COVID-19 pandemic, unbalancing the C:N ratio.

The results described in this study reinforce the idea of integrating actions aimed at the application of nature-based solutions (NBS), from phytoremediation processes, since the conversion of biomass into energy was part of a process created to detoxify urban wastewaters, thus being an initiative to develop treatments based on self-sustainable cycles applied to environmental sanitation.

#### **5.4. Conclusions**

The levels of energy generation obtained in this study showed encouraging results, especially considering the analysis stage referring to the spring period, where the most favorable climatic conditions for microalgae development and proliferation occur. The results obtained demonstrated that both microalgal biomass and macrophyte biomass can be used as raw material for the production of bioenergy, being an excellent environmental alternative. Thus, the conversion of biomass, which would be a potential waste, into thermal energy, demonstrates that it is possible to develop ideas that can bring combined environmental solutions, enhancing environmental sanitation projects as applied sustainable alternatives.

**6. FILTRO SEPARADOR DE BIOMASSA MICROALGAL (Depósito de patente Modelo de Utilidade, número do registro: BR20202101162, Instituição de registro: INPI - Instituto Nacional da Propriedade Industrial. Depósito PCT: 15/06/2021)**

**Resumo**

A presente invenção é destinada à filtração física de microalgas utilizada para o pré-tratamento de efluentes integrado a um sistema de tratamento de tecnologia de saneamento ambiental, ou ainda, em bioprocessos de cultivo de microalgas. Trata-se de um equipamento de baixo custo e capaz de reduzir o impacto ambiental dos efluentes. O filtro separador de biomassa microalgal promove a separação das microalgas através de filtração por gravidade e vazão do afluente, podendo ser utilizado em saídas de tratamento de efluentes urbanos, tanques de águas residuárias, sistemas de tratamento à base de microalgas, entre outros.

**6.1. Campo da invenção**

A invenção objeto encontra-se vinculada ao setor da engenharia ambiental, mais especificadamente à aplicação sanitária, associada a equipamentos destinados a redução de impactos ambientais aplicados ao tratamento de efluentes.

**6.2. Fundamentos da invenção**

As tecnologias convencionais disponíveis são, em sua maioria, baseadas em métodos químicos e físicos que ainda são alternativas de custo elevado e ambientalmente insustentáveis, já que requerem uso de produtos químicos, que além de emitirem CO<sub>2</sub>, removem nitrogênio e fósforo, ao invés de recuperá-los.

Alguns estudos apontam a utilização de microalgas como alternativa de remediação de águas residuárias, outros indicam efluentes ricos em matéria orgânica como fonte para a produção de biomassa de microalgas. Características como capacidade de biossorção, fonte de biomassa e produção de energia com baixo custo de aplicação e operação são alguns dos motivos pelos quais as microalgas têm sido utilizadas, especialmente na produção de bioenergia.

O presente equipamento foi desenvolvido como uma alternativa de baixo custo energético e econômico para separação de biomassa microalgal em sistemas potencialmente suscetíveis à proliferação de microalgas. Verificado o estado da técnica existente, foram localizadas algumas informações sobre processos de patentes, tais como

*“Method and apparatus for wastewater treatment using external selection”* (WO 2016/004082), que apresenta similaridade com o invento objeto desta redação, no entanto destinado a um sistema de tratamento de águas residuais por meio de lodo ativado. No equipamento WO 2016/004082 a separação da biomassa se dá através de processos já conhecidos, como flotação e sedimentação, por exemplo.

O processo intitulado “Sistema de tratamento de efluentes através de biomassa de plantas aquáticas, aplicado na separação e remoção de óleos, graxas, metais e correlatos” (PI 0605558-3) trata de uma tecnologia de tratamento de efluentes com enfoque no processo de separação de graxas e óleos através da biomassa vegetal. Diferindo-se da invenção apresentada, visto que o objetivo do filtro proposto consiste na remoção da biomassa microalgal por gravidade e granulometria.

No mesmo sentido, o processo denominado “Filtro de biomassa em camadas para remoção de corante e processo de descolorimento de efluentes utilizando filtro de biomassa em camadas” (BR 202012001921-9), utiliza-se de materiais como areia e serragem no processo de filtração, além de reguladores de vazão e outros aportes energéticos, enquanto o filtro proposto utiliza filtração por granulometria, por gravidade e sem aporte energético no processo.

A mesma diferenciação ocorre ao avaliar o processo PI 1005516-9 (“Aparelho de decomposição de biomassa”), que propõe um aparelho que decompõe um material de biomassa contendo celulose, hemicelulose e lignina, utilizando-se ainda de aporte energético e químico durante o processo de decomposição da biomassa e na separação sólido/líquido.

Ainda, a existência de outros processos já conhecidos, apontados na literatura, e, inclusive, alguns citados nos processos acima, podem ser mencionados. Entre eles destaca-se a floculação química, eficiente na remoção de pequenas algas, mas que devido a aplicação podem proporcionar reações químicas altamente sensíveis ao pH; também o processo de centrifugação, porém este exige grande quantidade de energia.

### **6.3. Descrição da invenção**

Observado o estado da técnica e a necessidade de produtos ambientalmente sustentáveis, surge a invenção do filtro separador de biomassa. A extração, ou seja, a remoção da biomassa microalgal de forma eficiente é essencial para que o tratamento de águas residuais atendam padrões de qualidade estabelecidos na legislação ambiental vigente.

Visando preencher as lacunas apresentadas no estado da técnica atual, o filtro separador de biomassa microalgal é um equipamento físico, que possui como vantagens com relação às soluções já conhecidas o fato de não ser necessário o aporte de energia, apresentar um baixo custo para produção, bem como não utilizar componentes químicos em seu processo.

O invento proposto proporciona a separação das microalgas através da filtração por gravidade, por meio da diferença de dimensão celular e os orifícios do filtro. Seu funcionamento para a separação de biomassa baseia-se na diferença de granulometria, através da gravidade e vazão promovida pela pressão na coluna d'água ao qual o efluente é submetido. A biomassa fica retida à medida que passa pelos discos dentro do filtro. Assim, devido à diferença de granulometria das microalgas, algumas ficam no início do filtro, no primeiro disco, outras no segundo disco, outras no terceiro, e assim sucessivamente, até o último disco. Após a operação, o filtro deve ser higienizado para posterior utilização.

O uso do filtro tem como finalidade separar a biomassa de microalga gerada a partir do pré-tratamento de efluentes, permitindo assim que o efluente gerado seja submetido ao tratamento convencional de resíduo sem a presença desta biomassa de microalga. A construção do filtro se dá a partir de materiais resistentes à água, como é o caso do ácido polilático, sendo introduzido em saídas de tratamento de efluentes; tanques de água residuárias; sistemas para tratamento à base de microalgas; entre outros. Seu funcionamento independe de um sistema energético, pois ocorre a partir da gravidade e pressão da água ao passar pelo tubo, promovendo a vazão necessária para a separação da biomassa.

O filtro é constituído por uma estrutura tubular formada pelos tubos superior (3) e inferior (6), fixados através de duas conexões idênticas, sendo uma conexão frontal (1) e posterior (4). Os tubos superior e inferior apresentam cavidades internas (5), onde o conjunto de discos de filtros individuais (2) é fixado (Figura: 18). O tamanho do filtro varia conforme a sua aplicação, devendo seguir a mesma proporção em todas as suas partes.

Os discos do filtro (2) possuem diâmetro variável de acordo com o tamanho do filtro, com espessura de 1,5mm a 2 mm, apresentando perfurações de 2,0mm a 2,5 mm (Figura: 18 (2)). A disposição de tais filtros deve ocorrer de forma que a ordenação dos mesmos se dê no sentido da maior perfuração para as menores perfurações, sendo que a

cada nível dos discos de filtro haverá a retenção da biomassa microalgal à medida que ocorre o decrescimento da granulometria.

#### **6.4. Exemplos de concretizações da invenção**

O filtro separador de biomassa microalgal é um equipamento produzido em material resistente à água, tal como o ácido poliláctico, que pode ser integrado a diversos sistemas de tratamentos, entre os quais se destacam as saídas de tratamento de efluentes urbanos, tanques de águas residuárias e sistemas de tratamento à base de microalgas.

Sua produção é de baixo custo e de baixo impacto ambiental, uma vez que a remoção da biomassa no pré-tratamento proporciona diversos benefícios, tais como a recuperação energética através da utilização da biomassa gerada em bioprodutos e/ou bioenergia. Ainda, o uso do filtro evita que os nutrientes removidos do efluente, como Nitrogênio e Fósforo principalmente, sejam reintroduzidos no processo após a morte das microalgas, reeutrofizando o sistema de tratamento, além de evitar que as microalgas cheguem aos corpos hídricos receptores do efluente tratado.

O uso do filtro mostra-se eficiente ao que se propõe como avanço tecnológico, apresentando a separação da biomassa microalgal com eficácia superior a 80%, excelente resultado considerando a ausência de custos energéticos associados.

#### **6.5. Reivindicações**

1. FILTRO SEPARADOR DE BIOMASSA MICROALGAL, caracterizado por ser constituído de estrutura tubular formada pelos tubos superior (3) e inferior (6), fixados através de duas conexões idênticas, sendo uma conexão frontal (1) e posterior (4), possuindo internamente discos de filtros individuais (2) (Figura: 2).
2. FILTRO SEPARADOR DE BIOMASSA MICROALGAL, de acordo com a reivindicação 1, caracterizado pelo fato dos tubos superior e inferior apresentam cavidades internas (5), onde o conjunto de discos de filtros individuais (2) é fixado (Figura: 2).
3. FILTRO SEPARADOR DE BIOMASSA MICROALGAL, de acordo com a reivindicação 1, caracterizada por apresentar discos de filtros internos com perfurações variadas, dispostos da abertura maior para a menor.
4. FILTRO SEPARADOR DE BIOMASSA MICROALGAL, de acordo com a reivindicação 1, caracterizada por não fazer o uso de aporte energético, tendo seu funcionamento a partir da gravidade e pressão natural da água.

5. FILTRO SEPARADOR DE BIOMASSA MICROALGAL, de acordo com a reivindicação 1, caracterizada pela remoção da biomassa microalgal no pré-tratamento de efluentes.

6. FILTRO SEPARADOR DE BIOMASSA MICROALGAL, de acordo com a reivindicação 1, caracterizada por ser construído em material resistente à água.

### 6.6. Breve descrição dos desenhos

A estrutura do filtro separador de biomassa (FISEBIO) está assim representada: A Figura 1 apresenta a vista geral do equipamento. A Figura 2 ilustra a vista expandida do equipamento.



Figura 1: Desenho geral do Filtro Separador de biomassa (FISEBIO).

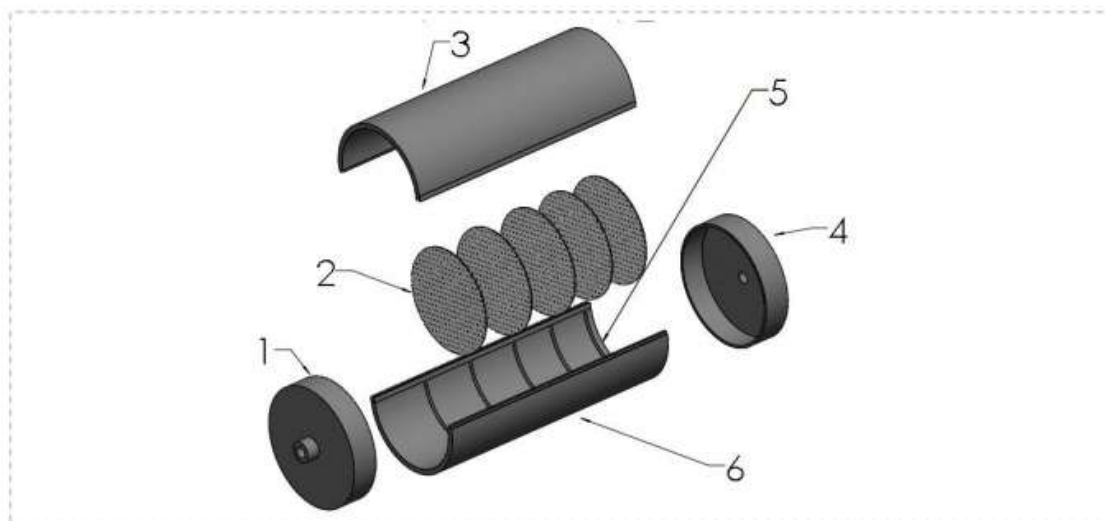


Figura 2: Desenho esquemático estendido: tubos superior (3) e inferior (6); conexão frontal (1) e posterior (4); discos de filtros individuais (2) e cavidades internas de encaixe dos discos (5).

## 7. CONSIDERAÇÕES FINAIS

A proposição de sistemas que utilizem recursos naturais como alternativa de remediação de poluentes, vem obtendo cada dia mais espaço nas tecnologias aplicadas, principalmente quando trazemos para discussão a questão do saneamento e /ou a falta dele. A importância do saneamento básico é fundamental para o desenvolvimento social, visto que, muitas são as doenças associadas à falta de saneamento, além disso, os ecossistemas associados também sofrem com a disposição de águas residuárias nos corpos hídricos.

Como as tecnologias convencionais em geral, são ambientalmente impactantes e possuem um custo elevado, em comparação aos sistemas baseados em processos naturais, muitos estudos e pesquisas foram desenvolvidos com o objetivo de desenvolver alternativas de saneamento ambiental com enfoque no tratamento de águas residuárias. Dentre tantos exemplos, podemos destacar os *wetlands* construídos, uma tecnologia que é amplamente difundida e aplicada no mundo inteiro, principalmente como sistemas de polimento final de efluentes urbanos. Também têm destaque na aplicação em áreas descentralizadas, como exemplo de saneamento ambiental.

O uso de microalgas, como exemplo bioprocessos aplicados ao saneamento, surge com destaque, mesmo que em menor proporção, já que geralmente o cultivo de microalgas está associado à produção de bioprodutos, e não necessariamente ao tratamento de águas residuárias, mesmo assim, é possível encontrar diversos estudos com microalgas aplicadas ao tratamento de efluentes, principalmente na última década.

Desta forma, ao desenvolver este projeto de tese procurou-se não apenas criar um sistema que integrasse as tecnologias descritas anteriormente, mas sim, ao combiná-las, potencializar suas qualidades individuais para que fosse possível obter maior rendimento e eficiência no tratamento do efluente estudado. Além disso, todos os detalhes que envolveram o desenvolvimento do sistema integrado foram projetados para que ao tratar o efluente pudessemos obter bioenergia renovável e limpa (Biogás), como bioproduto com aplicação futura na própria estação de tratamento (ETE), ou em qualquer outro local.

Também foram realizados ensaios toxicológicos com organismos-testes amplamente aplicados nas mais diversas áreas de pesquisa ao redor do mundo, como ferramenta de monitoramento da efetividade de descontaminação do efluente pós-tratamento.

É fundamental ressaltar que todo o sistema proposto e avaliado neste estudo foi configurado com espécies nativas da região onde o experimento foi desenvolvido e operado, demonstrando assim mais um ponto positivo da tecnologia proposta, já que este o sistema apresenta potencial para ser configurado em qualquer parte do globo com as espécies da região em que estiver. Entretanto, novas pesquisas devem ser realizadas para que se possa afirmar isso, porém, guardadas as devidas proporções, é bem provável que o sistema integrado com microalgas e macrófitas aquáticas (WC's) possa apresentar resultados similares aos encontrados neste estudo.

Os resultados obtidos neste estudo apresentam dados que fomentam a ideia de integrar sistemas de tratamento de efluentes utilizando bioprocessos, com espécies nativas de microalgas e macrófitas, com recuperação energética eficiente durante o processo, ou seja, é possível promover a recuperação de águas residuárias para reuso e ao mesmo tempo gerar energia limpa no processo.

Apesar de ter aqui representado resultados promissores, é necessário ressaltar a importância de se efetuar novos estudos, tendo como base o sistema integrado RA, MA e WCs, principalmente, com aplicação em escalas maiores e fluxo semi-contínuo e/ou contínuo, para podermos, efetivamente, recomendar esta tecnologia como tratamento de efluentes urbanos em larga escala.

## **7.1. RECOMENDAÇÕES**

Baseado nos resultados obtidos a partir dos experimentos realizados neste estudo é possível sugerir novos estudos experimentais e recomendações práticas para auxiliar a aplicação desse tipo de sistema tais como:

- Avaliar o desempenho do sistema integrado com outras configurações de *wetlands* construídos, por exemplo: *wetlands* de fluxo horizontal;
- Avaliar o desempenho do sistema integrado com outras configurações de reatores de proliferação de microalgas;
- Avaliar o desempenho do sistema integrado em fluxo contínuo;
- Avaliar o desempenho do sistema integrado em escala real, como tratamento descentralizado;
- Investigar de modo detalhado a fixação de carbono e a remoção de nutrientes em um maior período de monitoramento;



- Utilizar combinações de espécies diferentes de microalgas e macrófitas no sistema integrado para verificar aspectos como fixação de carbono e geração de biomassa;
- Estudar as interações dos microorganismos associados ao sistema integrado, tanto na etapa com as microalgas quanto com as macrófitas;
- Propor variações de configuração para potencializar a melhor configuração do sistema integrado como foco no equilíbrio entre o tratamento do efluente e a geração de energia durante o processo;
- Avaliar diferentes formas de utilizar a biomassa produzida durante o processo de tratamento, tanto em bioprodutos quanto da bioenergia;
- Analisar de forma mais aprofundada as interações microbianas que ocorrem durante o processo de tratamento nas diferentes etapas do sistema integrado;
- Verificar o potencial de geração de nutrientes através da compostagem dos resíduos finais pós-conversão da biomassa em bioenergia;
- Desenvolver estudos direcionados ao potencial de aplicação do sistema integrado como alternativa de saneamento ambiental;
- Realizar um estudo de Avaliação de Ciclo de Vida (ACV) comparando o sistema integrado de tratamento de efluentes com outros sistemas;

## **8. APÊNDICES**

### **8.1. Material complementar 1 - Toxicological tests using *Daphnia magna* as a monitoring tool applied to the environmental sanitation.**

#### **Text S1**

Cultivation of *D. magna* organisms was performed in 10 lots using 2L beaker glass, each containing 1.5 L of culture medium prepared with distilled/deionized water and nutrient and with a pH ranging from 7 to 8. The reconstituted water was aerated for at least 12 h to ensure complete solubilization of the salts, dissolved oxygen saturation, and pH stabilization prior to its use. Each batch of organisms contained approximately 30 individuals, and they were exclusively female because females are genetically identical. The organisms were maintained in a temperature-controlled environment at 20

$\pm 2$  °C and photoperiods of 16 h. The sensitivity of the *D. magna* cultures was evaluated monthly using potassium dichromate ( $K_2Cr_2O_7$ ).

The method consists of exposure of individuals at 2–26 h of life to different sample concentrations for 48 h in the dark at a controlled temperature ( $20 \pm 1$  °C). Samples were prepared with volumetric accuracy and geometric progression at a ratio of one half. Five concentrations ranging between 6.25 and 100 % were tested in 50-mL beakers containing 25 mL of the samples. The different concentrations were tested in duplicate, so 10 individuals were placed in each beaker for a total of 20 individuals/concentration.

Tabela complementar 1: Relative toxicity scale for 48 h EC50 determinations with *Daphnia magna*.

Percentile	EC <sub>50</sub>	Toxicity
25%	<25%	Extremely toxic
50%	25–50%	Highly toxic
75%	50–75%	Moderately toxic
	>75%	Slightly toxic

## 8.2. Material complementar 2 – Toxicological tests using *Allium cepa* as a monitoring tool applied to the environmental sanitation.

Complementary table 2: Evaluation of cytotoxotoxicity of effluents using the *Allium cepa* test.

	NC	PC	RW	AR	MA	CW	UWWTP	<i>p</i>
<b>MI</b>	15.1±3.3 14.7 (11.0-21.2) ab	30.6±11.8 26.9 (17.4 - 49.0) ac	22.3±7.6 21.4 (9.2-32.0)	24.2±8.1 26.7 (13.0-33.6)	18.0±7.6 16.6 (7.6-33.4)	32.3±6.4 31.8 (23.8-44.0) bcd	14.6±5.0 15.4 (9.0-21.6) d	<0.0001
<b>MN</b>	0.0 0.0 abc	24.9±5.6 18.0 (24.0-18.0) adef	8.3±5.2 7.5 (3.0-19) g	4.3±3.6 4.0 (0.0-12.0) d	3.1±1.5 3.0 (1.0-6.0) e	0.0 0.0 fgh	6.5±4.6 5.5 (2.0-15.0) bch	<0.0001
<b>BNC</b>	0.0 0.0 ab	4.0±2.3 3.5 (2.0-8.0) acef	2.2±1.4 2.0 (1.0-5.0) bcdgh	1.3±1.6 1.0 (0.0-5.0)	0.2±0.4 0.0 (0.0-1.0) dg	0.1±0.3 0.0 (0.0-1.0) eh	0.8±1.3 0.0 (0.0-4.0) f	<0.0001
<b>CA</b>	0.5±1.3 0.0 (0.0-4.0) abcd	12.4±8.8 11.0 (1.0-31.0) a	12.6±4.8 12.5 (7.0-21.0) be	10.8±5.9 9.0 (2.0-21.0) c	6.8±6.0 5.5 (0.0-22.0)	0.2±0.4 0.0 (0.0-1.0) ef	10.4±6.5 10.5 (4.0-19.0) df	<0.0001

Abbreviations: Negative Control (NC), Positive Control (PC), Raw Wastewater (RW), Anaerobic Reactor (AR), Microalgae (MA), Constructed Wetland (CW) UNISC Wastewater Treatment Plant (UWWTP)

MI: Mitotic Index; MN: Micronucleus; BNC: Binucleated cells; Chromosomics Aberrations

Data are presented as mean ± standard deviation and median (minimum-maximum);

Equivalent letters in the comparison between groups represent significantly different medians ( $p < 0.05$ ) compared by the Kruskal-Wallis test followed by Dunn's post-test for multiple comparisons.

## 9. REFERÊNCIAS

### 9.1. Revisão bibliográfica

ABAD, A. V., CHERRETT, T., & HOLDSWORTH P. Waste-to-fuel opportunities for British quick service restaurants: A case study. **Resources Conservation and Recycling**, 104(A), 239–253. 2015.

ABDEL-RAOUF, N., AL-HOMAIDAN, A.A., IBRAHEEM, I.B.M. **Microalgae and wastewater treatment**. Saudi Journal of Biological Sciences, 19(3), 257-275, 2012.

ABDELFATTAH, I. Treatment of Highly Polluted Industrial Wastewater Utilizing lean and Low Cost Technologies: Review Article. Ibrahim Abdelfattah/**Journal of Environmental Accounting and Management** 6(2) 167–185. 2018.

ABNT. NBR 13969 - Septic tank - Units for treatment and disposal of liquid effluents - Project, construction and operation Descriptor: Septic tank. Válida a partir de 30.10.1997. 1997.

ABRAHAMS, J. C. *et al.* The Brookside Farm Wetland Ecosystem Treatment (WET) System: A Low-Energy Methodology for Sewage Purification, Biomass Production (Yield), Flood Resilience and Biodiversity Enhancement. **Sustainability**. 2017.

ALBERT, C. *et al.* Addressing societal challenges through nature-based solutions: How can landscape planning and governance research contribute? **Landscape and Urban Planning**, v. 182, p. 12–21, 2019.

ANDRADE, H. H. B. Avaliação de um Sistema de Tratamento de Águas Residuárias por *Wetland* Construído com Etapa Aerada. **CLEANER PRODUCTION TOWARDS A SUSTAINABLE TRANSITION**. São Paulo – Brazil – May 20th to 22 nd- 2015.

ARBIB, Z.; *et al.* 2014. Capability of different microalgae species for phytoremediation processes: Wastewater tertiary treatment, CO<sub>2</sub> bio-fixation and low cost biofuels production. **Water Research**, 49(0), 465-474.

AZIMI, SC; PENDASHTEH, A. **Green Technologies for Wastewater Treatment**. Second International Conference in New Research on Chemistry & Chemical Engineering, pg. 1-16. 2016.

BADHE, N. *et al.* Role of algal biofilm in improving the performance of free surface, up-flow constructed Wetland. **Bioresour Technology** 169, 596-604. 2014.

BUNDHOO, Z.M. Renewable energy exploitation in the small island developing state of Mauritius: Current practice and future potential. **Renewable and Sustainable Energy Reviews**, 2017.

CAI, T., PARK, S.Y., LI, Y. Nutrient recovery from wastewater streams by microalgae: Status and prospects. **Renewable and Sustainable Energy Reviews**, 19(0), p. 360-369. 2013.

- CALHEIROS, C.S.C. *et al.* 2015. Constructed wetland with a polyculture of ornamental plants for wastewater treatment at a rural tourism facility. **Ecol. Eng.** 79, 1–7. <https://doi.org/10.1016/j.ecoleng.2015.03.001>. 2015.
- CALHEIROS, C.S.C. *et al.* 2019. Toxicity Abatement of Wastewaters from Tourism Units by Constructed Wetlands. **Water**, 11:2623. <https://doi.org/10.3390/w11122623>.
- CALHEIROS, C. S. C.; RANGEL, A. O. S. S. & CASTRO, P. M. L. (2014). Constructed wetlands for tannery wastewater treatment in Portugal: ten years of experience. **International Journal of Phytoremediation**. 16(9):859-870.
- CELENTE, GS. *et al.* Algae turf scrubber and vertical constructed wetlands combined system for decentralized secondary wastewater treatment. **Environmental Science and Pollution Research** 26:9931–9937. 2019.
- COIMBRA-ARAÚJO, CH. *et al.* Brazilian case study for biogas energy: production of electric power, heat and automotive energy in condominiums of agroenergy. **Renew Sustain Energy Rev**; 40:826–39. . 2014.
- DALPAZ, R. *et al.* Using biogas for energy cogeneration: An analysis of electric and thermal energy generation from agro-industrial waste. **Sustainable Energy Technologies and Assessments** 40, 100774. 2020.
- DA ROS, C. *et al.* Winery waste recycling through anaerobic co-digestion with waste activated sludge. **Waste Manag**, 34:2028–35. 2014.
- DA SILVA, RG; NETO, I M; SEIFERT, S.S. Electric supply security and the future role of renewable energy sources in Brazil. **Renewable and Sustainable Energy Reviews**, Amsterdam, v. 59, p. 328-341, 2016.
- DE-BASHAN, L. E. AND BASHAN, Y. Immobilized microalgae for removing pollutants: Review of practical aspects. **Bioresource Technology**, 101, p. 1611–1627, 2010.
- DELL’OSBEL, N., *et al.* Bibliometric Analysis of Phosphorous Removal Through Constructed Wetlands. **Water Air Soil Poll.** 231(3), 1-18.[doi.org/10.1007/s11270-020-04513-1](https://doi.org/10.1007/s11270-020-04513-1). 2020.
- DUFRESNE, K., *et al.* Metal Retention Mechanisms in Pilot-Scale Constructed Wetlands Receiving Acid Mine Drainage, 10 th International Conference on Acid Rock Drainage and IMWA Annual Conference, 1-10, 2015
- FONDER, N; HEADLEY, T. The taxonomy of treatment wetlands: A proposed classification and nomenclature system. **Ecological Engineering**.v.51, p. 203-211, 2013.
- GHOUALI, A. SARI, T. HARMAND, J. Maximizing biogas production from the anaerobic digestion. **J Process Control** 2015; 36:79–88.
- GSCHLOBL, T. *et al.* Constructed wetlands for effluent polishing of lagoons. **Water Resource**. Vol. 32, No. 9, p. 2639-2645, 1998.

HAHN, H., *et al.* Review of concepts for a demand-driven biogas supply for flexible power generation. **Renewable and Sustainable Energy Reviews**, 29, 383–393. 2014.

HAMAWAND, I. *et al.* Bioenergy from cotton industry wastes: A review potential. **Renewable and Sustainable Energy Reviews** v. 66, p. 435-448, 2016.

HERNANDO, M. D. *et al.* Toxicity assays applied to wastewater treatment. **Talanta**, n. 65, p. 358-366, 2005.

HASAN, C. *et al.* Biogas generation related to carbon removal from anaerobic co-digestion of sludge, blood, and swine manure combined in different proportions: Production of biogas by anaerobic co-digestion. **Environ Qual Manage**; 28:115–122. 2019.

KABISCH, N. *et al.* Nature-based solutions to climate change mitigation and adaptation in urban areas: perspectives on indicators, knowledge gaps, barriers, and opportunities for action. **Ecology and Society**, v. 21, n. 2, art.39, 2016.

KADLEC, R.H., WALLACE, S., (2009). Treatment Wetlands. 2nd Edition. **CRC Press. Taylor & Francis Group**. Boca Raton. Florida. 2009.

KARLSSON, T. Manual básico de biogás / Tommy Karlsson [*et al.*]. - Lajeado: **Ed. da Univates**, 69 p. 2014.

KONNERUP, D. *et al.* Treatment of fishpond water by recirculating horizontal and vertical flow constructed wetlands in the tropics. **Aquaculture**, 313 57–64, 2011.

KONRAD, O. *et al.* Comparison of two gas volume measurement systems by evaluating biochemical methane potential. **Environ Qual Manage**; 1–7. 2021.

KONRAD, O. *et al.* Enhancing the analytical capacity for biogas development in Brazil: assessment of an original measurement system for low biogas flow rates out of agricultural biomass residues. **Eng. Agríc.** vol.36 no.5.p. 792-798. 2016b.

KONRAD, O. *et al.* Quantification and characterization of the production of biogas from blends of agro-industrial wastes in a largescale demonstration plant. **Acta Sci**; 38:415–21. 2016a.

KRAUZE, K.; WAGNER, I. From classical water-ecosystem theories to nature-based solutions — Contextualizing nature-based solutions for sustainable city. **Science of the Total Environment**, v. 655, p. 697–706, 2019.

LANTZ, M. The economic performance of combined heat and power from biogas produced from manure in Sweden—A comparison of different CHP technologies. **Applied Energy, Amsterdam**, v. 98, p. 502-511, 2012.

LEÓN, E.; MARTÍN, M. Optimal production of power in a combined cycle from manure-based biogas. **Energy Conversion and Management**, Oxford, v.114, p. 89-99, 2016.

LU, S., PEI, L., BAI, X. Study on method of domestic wastewater treatment through new-type multi-layer artificial wetland. **International journal of hydrogen energy** 40, 11207 and 11214, 2015.

LUMI, M.; *et al.* Potencial de geração de biogás a partir da suplementação de óleo de babaçu em dejetos de animais. **Ciência e Natura, Santa Maria**, v.37, n.42, p. 756-766, set./dez. 2015.

LUTTERBECK, C. *et al.* 2020. Hospital laundry wastewaters: a review on treatment alternatives, life cycle assessment and prognosis scenarios. **J. Clean. Prod.** JCLP\_122851 <https://doi.org/10.1016/j.jclepro.2020.122851>.

MEZZOMO, N. *et al.* Cultivation of microalgae *Spirulina platensis* (*Arthrospira platensis*) from biological treatment of swine wastewater. **Ciência e Tecnologia de Alimentos**, v. 30, n. 1, p. 173-178, 2010.

MUSSINELLI, A. *et al.* Il ruolo delle Nature Based Solutions nel progetto architettonico e urbano. **Techne**, v. 15, p. 116-123, 2018.

PACHECO, M.M.; HOELTZ M.; MORAES M.S.; SCHNEIDER R.C. Microalgae: cultivation techniques and wastewater phycoremediation. **Journal of A.Environmental Science and Health**, v. 50, n. 6, p. 585-601.2015.

RAI, *et al.* Constructed wetland as an ecotechnological tool for pollution treatment for conservation of Ganga river. **Bioresour. Technol.** 148, 535-541. 2013.

RASLAVICIUS, L., GRZYBEK, A., & DUBROVIN, V. Bioenergy in Ukraine possibilities of rural development and opportunities for local communities. **Energy Policy**, 39(6), 3370–3379. 2011.

RAYMOND, C. M. *et al.* A framework for assessing and implementing the co-benefits of nature-based solutions in urban areas. **Environmental Science and Policy**, v. 77, p. 15-24, 2017.

RIBEIRO, EM. *et al.* Power generation potential in posture aviaries in Brazil in the context of a circular economy. **Sustain Energy Technol Assessments**; 18:153–63. 2016.

RODRIGUES, A.C.D. *et al.* Mecanismos de Respostas das Plantas à Poluição por Metais Pesados: Possibilidade de Uso de Macrófitas para Remediação de Ambientes Aquáticos Contaminados. **Rev. Virtual Quim.** 8 (1), 262-276, 2016.

RODRIGUEZ-DOMINGUEZ, M. A.; Konnerup D.; Brix H.; Arias; C. A. Constructed Wetlands in Latin America and the Caribbean: A Review of Experiences during the Last Decade. **Water**, 12, 1744; 2020. Doi: 10.3390/w12061744.

RODRIGUEZ, L. C. de A. *et al.* Phytotoxicity and cytogenotoxicity of water and sediment of urban stream in bioassay with *Lactuca sativa*. **Rev. bras. eng. agríc. ambient.** vol.17 n.10, p. 1099 -1108, 2013.

ROUSSEAU, D. Performance of Constructed Treatment Wetlands: Model-Based Evaluation and Impact of Operation and Maintenance Working Van Aangelegde Zuiveringsmoerassen: **Modelgebaseerde Evaluatie En Impact Van.** [s.l.] Ghent University, 2006.

RYCKEBOSCH, E. DROUILLON, M. VERVAEREN, H. Techniques for transformation of biogás to biomethane. **Biomass Bioenergy**; 35:1633–45. 2011.

SANTIAGO, A. F. Avaliação do desempenho de lagoas de alta taxa no tratamento de esgoto pré-desinfectado submetidas a diferentes níveis de radiação solar/Anibal da Fonseca Santiago.-Viçosa, MG, 2013.

SEZERINO, P.H., *et al.* 2018. Wetlands Construídos aplicados no Tratamento de Esgoto Sanitário. (Recomendações para implantação e boas práticas de operação e manutenção. **GESAD – UFSC/FUNASA**).

SEZERINO, P.H. & PELISSARI, C. *et al.* Wetlands construídos como ecotecnologia para o W539 tratamento de águas residuárias: experiências brasileiras [recurso eletrônico] / organização de Pablo Heleno Sezerino, Catiane Pelissari – 1.ed. - Curitiba: **Brazil Publishing**, 2021.

SILVEIRA, E.O., *et al.* 2020. Biomonitoring of urban wastewaters treated by an integrated system combining microalgae and constructed wetlands. **Sci. Total Environ.** 705, 135864. <https://doi.org/10.1016/j.scitotenv.2019.135864>. 2020.

SILVEIRA, E. O. *et al.* 2017. Performance of an integrated system combining microalgae and vertical flow constructed wetlands for urban wastewater treatment. **Environmental Science Pollution Research**, V.24. P. 20469-20478, 2017.

SILVEIRA, E. O. Fitorremediação de efluentes urbanos microalgas e *wetlands* construídos: saneamento ambiental como tecnologia mais limpa./Elizandro Oliveira Silveira e Ênio Leandro Machado. -1.ed. Rio de Janeiro: **Autografia**, 140p. 2018.

SILVEIRA, E. O. *et al.* Integrated system with microalgae and constructed wetland in vertical flow for urban wastewater treatment. **Eng. Sanit. Ambient.** Rio de Janeiro Mai/Jun ,Vol. 24, n°4. 2019.

VON SPERLING, M. Princípio do tratamento biológico de águas residuárias – introdução à qualidade das águas e ao tratamento de esgotos. Belo Horizonte: **DESA**. v. 1. 2005.

VON SPERLING, M. & SEZERINO, P. H. 2018. Dimensionamento de wetlands construídos no Brasil. Documento de consenso entre pesquisadores e praticantes. **Edição especial Wetlands Brasil**. 2018.



VYMAZAL, J. (2005) Removal of Enteric Bacteria in constructed treatment wetlands with emergent macrophytes: a review. **Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering**. 40(6-7):1355-1367.

VYMAZAL, J. Constructed Wetlands for Wastewater Treatment. Review. **Water**. 2, p. 530 -549. 2010.

VYMAZAL, J. Plants used in constructed wetlands with horizontal subsurface flow: a review. **Hidrobiologia** 674, 133-156. 2011.

WALL, D. M., *et al.* Investigating two-phase digestion of grass silage for demand-driven biogas applications: Effect of particle size and rumen fluid addition. **Renewable Energy**, 86, 1215–1223. 2016.

WU, S. *et al.* Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. **Water research**, v.57, p. 40–55, 15 jun. 2014.

YADALA, S.; CREMASCHI, S. Design and optimization of artificial cultivation units for algae production. **Energy**, 78, 23–39. 2014.

YADALA, S.; CREMASCHI, S. A Dynamic Optimization Model for Designing Open-Channel Raceway Ponds for Batch Production of Algal Biomass. **Tusla-USA. Processes**, 4, 10. 2016.

ZHANG, C. *et al.* The anaerobic codigestion of food waste and cattle manure. **Bioresource Technology**, 129, 170–176. 2013.

## **9.2. Integrated system comprising an anaerobic reactor; microalgae and constructed wetlands as a proposal for the treatment of urban effluents: eco design applied to environmental sanitation**

ABRAHAMAS, J. C. *et al.* The Brookside Farm Wetland Ecosystem Treatment (WET) System: A Low-Energy Methodology for Sewage Purification, Biomass Production (Yield), Flood Resilience and Biodiversity Enhancement. **Sustainability**. 2017.

AOAC – Association of Official analytical Chemists. Method 990. n. 12 Official methods of analysis of AOAC International. 17. ed. Gaithersburg: **AOAC International**, p. 22-23. 2000.

APHA/AWWA – American Public Health Association. Standard Methods for the Examination of Water and Wastewater. 21. ed. Washington: **APHA/AWWA/WEF**, 2012.

ARBIB, Z.; *et al.* 2014. Capability of different microalgae species for phytoremediation processes: Wastewater tertiary treatment, CO<sub>2</sub> bio-fixation and low cost biofuels production. **Water Research**, 49(0), 465-474.

CAI, T., Park, S.Y., Li, Y. Nutrient recovery from wastewater streams by microalgae: Status and prospects. **Renewable and Sustainable Energy Reviews**, 19(0), p. 360-369. 2013.

CALHEIROS, C.S.C. *et al.* Constructed wetland with a polyculture of ornamental plants for wastewater treatment at a rural tourism facility. **Ecol. Eng.** 79, 1–7. <https://doi.org/10.1016/j.ecoleng.2015.03.001>. 2015.

CALHEIROS, C.S.C. *et al.* 2019. Toxicity Abatement of Wastewaters from Tourism Units by Constructed Wetlands. **Water**, 11:2623. <https://doi.org/10.3390/w11122623>.

CALHEIROS, C. S. C.; RANGEL, A. O. S. S. & CASTRO, P. M. L. (2014). Constructed wetlands for tannery wastewater treatment in Portugal: ten years of experience. **International Journal of Phytoremediation**. 16(9):859-870.

COLARES, G.S. *et al.* Combined system for the treatment and reuse of urban wastewater: the efficiency of anaerobic reactors + hybrid constructed wetlands + ozonation. **Water Sci. Technol.** <https://doi.org/10.2166/wst.2019.270>. 2019.

CONSEMA Resolution, 2017. CONSEMA Resolution 355, 2017. Criteria on the criteria and standards for the emission of liquid effluents for generating sources that discharge their effluents into surface waters in the State of Rio Grande do Sul. 2017.

DELL'OSBEL, N., *et al.* Bibliometric Analysis of Phosphorous Removal Through Constructed Wetlands. **Water Air Soil Poll.** 231(3),1-18.[doi.org/10.1007/s11270-020-04513-1](https://doi.org/10.1007/s11270-020-04513-1). 2020.

DÜPONT, A., LOBO, E.A., 2012. Evaluation of the efficiency of the sewage treatment plant from the University of Santa Cruz do Sul (UNISC), RS, Brasil. **Acta Limnol. Bras.** 24, 119–126.

ELFANSSI, S., *et al.* 2017. Phytoremediation of domestic wastewater using a hybrid constructed wetland in mountainous rural area. **Int. J. Phytorem.** 20 (1), 75–87. <https://doi.org/10.1080/15226514.2017.1337067>.

HORN, T. B. *et al.* Constructed wetland and photocatalytic ozonation for university sewage treatment. **Ecological Engineering** v. 63, p. 134-141, 2014.

JABRI, K. M., *et al.* Steadystate modeling of the biodegradation performance of a multistage moving bed biofilm reactor (MBBR) used for on-site greywater treatment. **Environ. Sci. Pollut. R**, 26(19), 19047-19062. <https://doi.org/10.1007/s11356-018-3984-9>. 2019.

GSCHLOBL, T. *et al.* Constructed wetlands for effluent polishing of lagoons. **Water. Resource.** Vol. 32, No. 9, p. 2639-2645, 1998.

LU, S., PEI, L., BAI, X. Study on method of domestic wastewater treatment through new-type multi-layer artificial wetland. **International journal of hydrogen energy** 40, 11207 and 11214, 2015.

LUTTERBECK, C. *et al.* 2020. Hospital laundry wastewaters: a review on treatment alternatives, life cycle assessment and prognosis scenarios. **J. Clean. Prod.** JCLP\_122851 <https://doi.org/10.1016/j.jclepro.2020.122851>.

MACHADO, Ê. L., *et al.* Constructed Wetlands Integrated with Advanced Oxidation Processes in Wastewater Treatment for Reuse. **The Handbook of Environmental Chemistry**: Springer Berlin Heidelberg, pp. 1-26.0. 2015.

MILLEDGE, J.J. *et al.* A Brief Review of Anaerobic Digestion of Algae for Bioenergy. **Energies** 2019, 12, 1166; doi: 10.3390/en12061166.

PACHECO, M.M.; HOELTZ M.; MORAES M.S.; SCHNEIDER R.C. Microalgae: cultivation techniques and wastewater phycoremediation. **Journal of A.Environmental Science and Health**, v. 50, n. 6, p. 585-601.2015.

RODRIGUEZ-DOMINGUEZ, M. A.; Konnerup D.; Brix H.; Arias; C. A. Constructed Wetlands in Latin America and the Caribbean: A Review of Experiences during the Last Decade. **Water**, 12, 1744; 2020. doi:10.3390/w12061744.

RODRIGUEZ, L. C. de A. *et al.* Phytotoxicity and cytogenotoxicity of water and sediment of urban stream in bioassay with *Lactuca sativa*. **Rev. bras. eng. agríc. ambient.** vol.17 n.10, p. 1099 -1108, 2013.

SEZERINO, P.H., *et al.* Wetlands Construídos aplicados no Tratamento de Esgoto Sanitário. (Recomendações para implantação e boas práticas de operação e manutenção. **GESAD – UFSC/FUNASA**). 2018.

SILVEIRA, E.O., *et al.* 2020. Biomonitoring of urban wastewaters treated by an integrated system combining microalgae and constructed wetlands. **Sci. Total Environ.** 705, 135864. <https://doi.org/10.1016/j.scitotenv.2019.135864>. 2020.

SILVEIRA, E. O. Fitorremediação de efluentes urbanos, microalgas e wetlands construídos: Saneamento ambiental como tecnologia mais limpa/ElizandroSilveira, Ênio Leandro Machado. -1 ed. Rio de Janeiro: **Autografia**, 140p. 2018.

SILVEIRA, E.O., *et al.* Performance of an integrated system combining microalgae and verticalflow constructed wetlands for urban wastewater treatment. **Environmental Science and Pollution Research** 24 (25), 20469–20478. 2017.

SILVEIRA, E. O. *et al.* Sistema integrado com microalgas e Wetland construído de fluxo vertical no tratamento de efluentes urbanos. **Eng. Sanit. Ambient.** Rio de Janeiro. Mai/Jun, Vol. 24, n°4. 2019.

SOBRERO, M. C.; RONCO, A. Ensayo de toxicidad aguda con semillas de lechuga (*Lactuca sativa* L). In:\_\_\_ MORALES, G. C. Ensayos toxicológicos y métodos de evaluación de calidad de aguas estandarización, intercalibración, resultados y aplicaciones. Centro internacional de investigaciones para el desarrollo: **Instituto Mexicano de Tecnología del Agua** (IMTA), México, 189p. 2004.

VON SPERLING, M. & SEZERINO, P. H. 2018. Dimensionamento de wetlands construídos no Brasil. Documento de consenso entre pesquisadores e praticantes. **Edição especial Wetlands Brasil**. 2018.

VYMAZAL, J. Plants used in constructed wetlands with horizontal subsurface flow: a review. **Hidrobiologia** 674, 133-156. 2011.

WU, S. *et al.* Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. **Water research**, v.57, p. 40–55, 15 jun. 2014.

### 9.3. Biomonitoring of urban wastewaters treated by an integrated system combining microalgae and constructed wetlands

ÁLVAREZ, J.A., RUÍZ, I., SOTO, M., 2008. Anaerobic digesters as a pretreatment for constructed wetlands. **Ecol. Eng.** 33 (1), 54–67. 2008.

ANTOSIEWICZ, D. 1990. Analysis of the cell cycle in the root meristem of *Allium cepa* under the influence of ledakrin. *Folia Histochem.* **Cytobiol.** 28 (1–2), 79–95. 1990.

AOAC–ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS, 2000. Method 990. n. 12 Official methods of analysis of AOAC International. 17. ed. **AOAC International**, Gaithersburg, pp. 22–23.

APHA/AWWA/WPCF, 2012. Standard Methods for the Examination of Water and Wastewater. 22nd ed.

DÜPONT, A., LOBO, E.A., 2012. Evaluation of the efficiency of the sewage treatment plant from the University of Santa Cruz do Sul (UNISC), RS, Brazil. **Acta Limnologica Brasiliensi** 24, 119–126.

FENECH, M., 2000 .The in vitro micronucleus technique. **Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis** 455 (1), 81–95.

FERNANDES, T.C.C., MAZZEO, D.E.C., MARIN-MORALES, M.A., 2007. Mechanism of micronuclei formation in polyploidized cells of *Allium cepa* exposed to trifluralin herbicide. **Pest. Biochem. Physiol.** 88 (3), 252–259.

FISKESJÖ, G., 1985. The *Allium* test as a standard in environmental monitoring. **Hereditas** 102 (1), 99–112.

FISKESJÖ, G., 1995. The *allium* test in wastewater monitoring. **Environmental Toxicology and Water Quality** 8 (3), 291–298.

FRANCHINO, M., TIGINI, V., VARESE, G.C., MUSSAT SARTOR, R., BONA, F., 2016. Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggyery digestate. **Science of The Total Environment** 569–570, 40–45.

GRANT, W.F., 1982. Chromosome aberration assays in *Allium*. A report of the U.S. Environmental Protection Agency Gene-Tox Program. **Mutat Res** 99 (3), 273–291.

HAMILTON, M.A., RUSSO, R.C., THURSTON, R.V., 1979. Trimmed Spearman-Kärber method for calculation of EC50 and LC values in bioassays. **Burlingt. Res.** 7, 114–119.

HEMACHANDRA, C.K., PATHIRATNE, A., 2017. Cytogenotoxicity screening of source water, wastewater and treated water of drinking water treatment plants using two in vivo test systems: *Allium cepa* root based and Nile tilapia erythrocyte based tests. **Water Research** 108, 320–329.

HORN, T.B., ZERWES, F.V., KIST, L.T., MACHADO, Ê. 2014. Constructed wetland and photocatalytic ozonation for university sewage treatment. **Ecological Engineering** 63, 134–141.

JARDIM, W. F.; CANELA, M. C. Fundamentos da Oxidação Química no Tratamento de Efluentes e Remediação de Solos. **Caderno Temático Volume 01**; Campinas, 2004.

KERN, D.I., DE OLIVEIRA SCHWAICKHARDT, R., LUTTERBECK, C.A., KIST, L.T., ALCAYAGA, E.A.L., MACHADO, Ê., 2015. Ecotoxicological and genotoxic assessment of hospital laundry wastewaters. **Archives of Environmental Contamination and Toxicology** 68 (1), 64–73.

LEIVA, A.M., ALBARRÁN, A., LÓPEZ, D., VIDAL, G., 2019. Evaluation of phytotoxicity of effluents from activated sludge and constructed wetland system for wastewater reuse. **Water Science and Technology** 79 (4), 656–667.

LEME, D.M., ANGELIS, D. D. F. D. AND MARIN-MORALES, M. A., 2008. Action mechanisms of petroleum hydrocarbons present in waters impacted by an oil spill on the genetic material of *Allium cepa* root cells. **Aquatic Toxicology** 88 (4), 214–219.

LEME, D.M., MARIN-MORALES, M.A., 2009. *Allium cepa* test in environmental monitoring: a review on its application. **Mutation Research/Reviews in Mutation Research** 682 (1), 71–81.

LOBO, E.A., RATHKE, F.S., BRENTANO, D.M., 2006. Ecotoxicologia aplicada: O caso dos produtores de tabaco na bacia hidrográfica do Rio Pardo RS, Brasil. pp. 41–68.

LUTTERBECK, C.A., DE OLIVEIRA SCHWAICKHARDT, R., STRAATMANN, A., KIST, L.T., LOBO, E.A., MACHADO, Ê., 2014. Electrooxidation combined with ozonation in hospital laundry effluents treatment. **CLEAN–Soil, Air, Water** 42 (5), 601–608.

LUTTERBECK, C.A., KERN, D.I., MACHADO, Ê., KÜMMERER, K., 2015. Evaluation of the toxic effects of four anti-cancer drugs in plant bioassays and its potency for screening in the context of waste water reuse for irrigation. **Chemosphere** 135, 403–410.

LUTTERBECK, C.A., KIST, L.T., LOPEZ, D.R., ZERWES, F.V., MACHADO, Ê. 2017. Life cycle assessment of integrated wastewater treatment systems with constructed wetlands in rural areas. **Journal of Cleaner Production** 148, 527–536.

LUTTERBECK, C.A., ZERWES, F.V., RADTKE, J.F., KÖHLER, A., KIST, L.T., MACHADO, Ê., 2018. Integrated system with constructed wetlands for the treatment of domestic wastewaters generated at a rural property—Evaluation of general parameters ecotoxicity and cytogenetics. **Ecological Engineering** 115, 1–8.

MA, T.-H., XU, Z., XU, C., MCCONNELL, H., VALTIERRA RABAGO, E., ADRIANA ARREOLA, G., ZHANG, H., 1995. The improved *Allium/Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants. **Mutation Research/Environmental Mutagenesis and Related Subjects** 334 (2), 185–195.

MACHADO, Ê., LOURENÇO, A.M., KIST, L.T., SCHNEIDER, R.C.S., KERN, D., LOBO, E.A.A., LUTTERBECK, C.A., SILVEIRA, D.D., HORN, T.B., ZERWES, F.V., 2015. Constructed wetlands integrated with advanced oxidation processes in wastewater treatment for reuse. **The Handbook of Environmental Chemistry**. Springer Berlin Heidelberg, pp. 1–26.

MARZO, A., VENTURA, D., CIRELLI, G.L., AIELLO, R., VANELLA, D., RAPISARDA, R., BARBAGALLO, S., CONSOLI, S., 2018. Hydraulic reliability of a horizontal wetland for wastewater treatment in Sicily. **Science of The Total Environment** 636, 94–106.

MELLO, M.L.S., VIDAL, B.C., 1978. A reação de Feulgen. *Ciênc. Cult.* 30, 665–676.  
MELO, R.G., ANDRADE, A.F., BEZERRA, R.P., CORREIA, D.S., SOUZA, V.C., BRASILEIRO-VIDAL, A.C., VIANA MARQUES, D., PORTO, A.L.F., 2018. *Chlorella vulgaris* mixotrophic growth enhanced biomass productivity and reduced toxicity from agro-industrial by-products. **Chemosphere** 204, 344–350.

PACHECO, M.M., HOELTZ, M., MORAES, M.S.A., SCHNEIDER, R.C.S., 2015. Microalgae: Cultivation techniques and wastewater phycoremediation. **Journal of Environmental Science and Health Part A** 50 (6), 585–601.

PANDA, B.B., SAHU, U.K., 1985. Induction of abnormal spindle function and cytokinesis inhibition in mitotic cells of *Allium cepa* by the organophosphorus insecticide Fensulfothion. **Cytobios** 42, 167/168.

PRASSE, C., STALTER, D., SCHULTE-OEHLMANN, U., OEHLMANN, J., TERNES, T.A., 2015. Spoilt for choice: a critical review on the chemical and biological assessment of current wastewater treatment technologies. **Water Research** 87, 237–270.

RADIĆ, S., STIPANIČEV, D., VUJČIĆ, V., RAJČIĆ, M.M., ŠIRAC, S., PEVALEK-KOZLINA, B., 2010. The evaluation of surface and wastewater genotoxicity using the *Allium cepa* test. **Science of The Total Environment** 408 (5), 1228–1233.

RANK, J., NIELSEN, M.H., 1994. Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. **Mutation Research/Environmental Mutagenesis and Related Subjects** 312 (1), 17–24.

REARDON, D.J., 1995. Turning down the power. **Civil Engineering—ASCE** 65 (8), 54–56.

RIZZO, L., FIORENTINO, A., GRASSI, M., ATTANASIO, D., GUIDA, M., 2015. Advanced treatment of urban wastewater by sandfiltration and graphene adsorption for wastewater reuse: effect on a mixture of pharmaceuticals and toxicity. **Journal of Environmental Chemical Engineering** 3 (1), 122–128.

RIZZO, L., MALATO, S., ANTAKYALI, D., BERETSOU, V.G., ĐOLIĆ, M.B., GERNJAK, W., HEATH, E., IVANCEV-TUMBAS, I., KARAOLIA, P., LADO RIBEIRO, A.R., MASCOLO, G., MCARDELL, C.S., SCHAAR, H., SILVA, A.M.T., FATTA-KASSINOS, D., 2019. Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. **Science of the Total Environment** 655, 986–1008.

RODRIGUES, L. C. D. A., BARBOSA, S., PAZIN, M., MASELLI, B. D. S., BEIJO, L. A. and KUMMROW, F. (2013) Phytotoxicity and cytogenotoxicity of water and sediment of urban stream in bioassay with *Lactuca sativa*/Fitotoxicidade e citogenotoxicidade da água e sedimento de correço urbano em bioensaio com *Lactuca sativa*, **Revista Brasileira de Engenharia Agrícola e Ambiental**, 17, pp. 1099+.

RUSSO, N., MARZO, A., RANDAZZO, C., CAGGIA, C., TOSCANO, A., CIRELLI, G.L., 2019. Constructed wetlands combined with disinfection systems for removal of urban wastewater contaminants. **Science of the Total Environment** 656, 558–566.

SEZERINO, P.H., REGINATTO, V., SANTOS, M.A., KAYSER, K., KUNST, S., PHILIPPI, L.S., SOARES, H.M., 2003. Nutrient removal from piggery effluent using verticalflow constructed wetlands in southern Brazil. **Water Sci Technol** 48 (2), 129–135.

SHANKAR, B. A. (2011) Low-cost treatment for attenuation of nitrate from groundwater. **Journal of Engineering and Technology Research** 3(1), pp. 16–21, January 2011.

SHARMA, C., 1983. Plant meristems as monitors of genetic toxicity of environmental chemicals. **Curr Sci** 52.

SILVEIRA, E. O. Fitorremediação de efluentes urbanos microalgas e wetlands construídos: saneamento ambiental como tecnologia mais limpa./Elizandro Oliveira Silveira e Ênio Leandro Machado. -1.ed. Rio de Janeiro: **Autografia**, 140p. 2018.

SILVEIRA, E.O., MOURA, D., RIEGER, A., MACHADO, Ê., LUTTERBECK, C.A., 2017. Performance of an integrated system combining microalgae and verticalflow constructed wetlands for urban wastewater treatment. **Environmental Science and Pollution Research** 24 (25), 20469–20478.

SOBRERO, M. C. A. R. (2004) ‘Ensayo de toxicidad aguda con semillas de lechuga (*Lactuca sativa* L)’, Ensayos Toxicológicos Y Métodos De Evaluación De Calidad De Aguas. Mexico: **Instituto Mexicano de Tecnología del Agua (IMTA)**, pp. 202.

VYMAZAL, J., KRÖPFELOVÁ, L., 2008. Wastewater Treatment in Constructed Wetlands with Horizontal Sub-Surface Flow. **Springer**, Netherlands.

VYMAZAL, J., KRÖPFELOVÁ, L., 2009. Removal of organics in constructed wetlands with horizontal sub-surface flow: a review of the field experience. **Science of the Total Environment** 407 (13), 3911–3922.

#### **9.4. Energy recovery from biomass biodigestion generated by an integrated system composed of microalgae and constructed wetland**

ABAD, A. V., CHERRETT, T., & HOLDSWORTH P. Waste-to-fuel opportunities for British quick service restaurants: A case study. **Resources Conservation and Recycling**, 104(A), 239–253. 2015.

ALZATE, M. E. *et al.* Biochemical methane potential of microalgae: Influence of substrate to inoculum ratio, biomass concentration and pretreatment. **Bioresource Technology** 123 (2012) 488–494.

AOAC – ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Method 990. n. 12 Official methods of analysis of AOAC International. 17. ed. Gaithersburg: **AOAC International**, p. 22–23. 2000.

APHA/AWWA – AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Water and Wastewater. 21. ed. Washington: **APHA/AWWA/WEF**, 2005.

APHA/AWWA – AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods for the Examination of Water and Wastewater. 21. ed. Washington: **APHA/AWWA/WEF**, 2012.

ARAÚJO, CHC. *et al.* Brazilian case study for biogas energy: Production of electric power, heat and automotive energy in condominiums of agroenergy. **Renewable and Sustainable Energy Reviews**, Amsterdam, v. 40, 826–839, 2014.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS (ABNT). NBR 12713 - Ecotoxicologia aquática - Toxicidade aguda - Método de ensaio com *Daphnia spp.* (Cladóceras, Crustácea). **Associação Brasileira de Normas técnicas**. 3<sup>a</sup> ed. 2009.

CALHEIROS, C.S.C., *et al.* Constructed wetland with a polyculture of ornamental plants for wastewater treatment at a rural tourism facility. **Ecol. Eng.** 79, 1–7. <https://doi.org/10.1016/j.ecoleng.2015.03.001>. 2015.

COLARES, G.S. *et al.* Combined system for the treatment and reuse of urban wastewater: the efficiency of anaerobic reactors + hybrid constructed wetlands + ozonation. **Water Sci. Technol.** <https://doi.org/10.2166/wst.2019.270>. 2019.

DALPAZ, R. *et al.* Using biogas for energy cogeneration: An analysis of electric and thermal energy generation from agro-industrial waste. **Sustainable Energy Technologies and Assessments** 40 (2020) 100774.



DA ROS, C. *et al.* Winery waste recycling through anaerobic co-digestion with waste activated sludge. **Waste Manag**, 34:2028–35. 2014.

DA SILVA, RG; NETO, I M; SEIFERT, S.S. Electric supply security and the future role of renewable energy sources in Brazil. **Renewable and Sustainable Energy Reviews**, Amsterdam, v. 59, p. 328-341, 2016.

DELL'OSBEL, N., *et al.* Bibliometric Analysis of Phosphorous Removal Through Constructed Wetlands. **Water Air Soil Poll.** 231(3), 1-18.[doi.org/10.1007/s11270-020-04513-1](https://doi.org/10.1007/s11270-020-04513-1). 2020.

GONZÁLEZ-FERNANDEZ, C. *et al.* Thermal pretreatment to improve methane production of Scenedesmus biomass. **Biomass and Bioenergy** 40 (2012)105-111. <http://dx.doi.org/10.1016/j.biombioe.2012.02.008>.

GUTIÉRREZ, R. *et al.* Harvesting microalgae from wastewater treatment systems with natural flocculants: Effect on biomass settling and biogas production. **Algal Research** 9 (2015) 204–211.

HAHN, H., *et al.* Review of concepts for a demand-driven biogas supply for flexible power generation. **Renewable and Sustainable Energy Reviews**, 29, 383–393. 2014.

HASAN, C. *et al.* Biogas generation related to carbon removal from anaerobic co-digestion of sludge, blood, and swine manure combined in different proportions: Production of biogas by anaerobic co-digestion. **Environ Qual Manage**; 28:115–122. 2019.

JABRI, K. M., *et al.* Steadystate modeling of the biodegradation performance of a multistage moving bed biofilm reactor (MBBR) used for on-site greywater treatment. **Environ. Sci. Pollut. R**, 26(19), 19047-19062. <https://doi.org/10.1007/s11356-018-3984-9>. 2019.

KARLSSON, T. Manual básico de biogás / Tommy Karlsson [*et al.*]. - Lajeado: **Ed. da Univates**, 69 p. 2014.

KEYMER, P. *et al.* High pressure thermal hydrolysis as pre-treatment to increase the methane yield during anaerobic digestion of microalgae. **Bioresource Technology** 131 (2013) 128–133.

KONRAD, O. *et al.* Comparison of two gas volume measurement systems by evaluating biochemical methane potential. **Environ Qual Manage**; 1–7. 2021.

KONRAD, O. *et al.* Enhancing the analytical capacity for biogas development in Brazil: assessment of an original measurement system for low biogas flow rates out of agricultural biomass residues. **Eng. Agríc.** vol.36 no.5.p. 792-798. 2016b.

KONRAD, O. *et al.* Quantification and characterization of the production of biogas from blends of agro-industrial wastes in a largescale demonstration plant. **Acta Sci**; 38:415–21. 2016a.

LANTZ, M. The economic performance of combined heat and power from biogas produced from manure in Sweden—A comparison of different CHP technologies. **Applied Energy**, Amsterdam, v. 98, p. 502-511, 2012.

LEÓN, E.; MARTÍN, M. Optimal production of power in a combined cycle from manure-based biogas. **Energy Conversion and Management**, Oxford, v.114, p. 89-99, 2016.

LIN, J.; *et al.* Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of China. **Journal of Environmental Sciences**, v.23, p.1403-1408, 2011. DOI: [https://doi.org/10.1016/S1001-0742\(10\)60572-4](https://doi.org/10.1016/S1001-0742(10)60572-4).

LUMI, M.; *et al.* Potencial de geração de biogás a partir da suplementação de óleo de babaçu em dejetos de animais. **Ciência e Natura, Santa Maria**, v.37, n.42, p. 756-766, set/dez. 2015.

MACHADO, Ê. L., *et al.* Constructed Wetlands Integrated with Advanced Oxidation Processes in Wastewater Treatment for Reuse. **The Handbook of Environmental Chemistry**: Springer Berlin Heidelberg, pp. 1-26.0. 2015.

MARTENS, M. *et al.* The greenhouse gas emission effects of rewetting drained peatlands and growing wetland plants for biogas fuel production. **Journal of Environmental Management** 277 (2021) 111391.

PACHECO, M.M.; HOELTZ M.; MORAES M.S.; SCHNEIDER R.C. Microalgae: cultivation techniques and wastewater phycoremediation. **Journal of A.Environmental Science and Health**, v. 50, n. 6, p. 585-601.2015.

PASSOS, F., *et al.* Anaerobic digestion of microalgal biomass after ultrasound pretreatment. **Waste Management** 34 (2014) 2098–2103.

PROCHNOW, A. *et al.* Seasonal pattern of biomethanisation of grass from landscape management. **Agric. Eng. Int. CIGR Ejournal** 7 (2005), <http://www.cigrjournal.org/index.php/Ejournal/article/view/606/600>.

RASLAVICIUS, L., GRZYBEK, A., & DUBROVIN, V. Bioenergy in Ukraine possibilities of rural development and opportunities for local communities. **Energy Policy**, 39(6), 3370–3379. 2011.

RIBEIRO, EM. *et al.* Power generation potential in posture aviaries in Brazil in the context of a circular economy. **Sustain Energy Technol Assessments**; 18:153–63. 2016.

ROJ-ROJEWSKI, S. *et al.* Anaerobic digestion of wetland biomass from conservation management for biogas production. **Biomass and Bioenergy** 122 (2019) 126– 132.

RYCKEBOSCH, E. DROUILLON, M. VERVAEREN, H. Techniques for transformation of biogás to biomethane. **Biomass Bioenergy**; 35:1633–45. 2011.

SEZERINO, P.H., *et al.* Wetlands Construídos aplicados no Tratamento de Esgoto Sanitário. (Recomendações para implantação e boas práticas de operação e manutenção. **GESAD – UFSC/FUNASA**). 2018.

SILVA, C. O.; *et al.* Resíduos sólidos orgânicos domésticos como substrato potencial para produção de biogás. **Revista Ibero-Americana de Ciências Ambientais**. Fev a Mar 2020 - v.11 - n.2.

SILVEIRA, E.O., *et al.* Biomonitoring of urban wastewaters treated by an integrated system combining microalgae and constructed wetlands. **Sci. Total Environ.** 705, 135864. <https://doi.org/10.1016/j.scitotenv.2019.135864>. 2020.

SILVEIRA, E.O., *et al.* Performance of an integrated system combining microalgae and verticalflow constructed wetlands for urban wastewater treatment. **Environmental Science and Pollution Research** 24 (25), 20469–20478. 2017.

SILVEIRA, E. O. *et al.* Sistema integrado com microalgas e Wetland construído de fluxo vertical no tratamento de efluentes urbanos. **Eng. Sanit. Ambient.** Rio de Janeiro. Mai/Jun, Vol. 24, n°4. 2019.

SILVEIRA, E O. Fitorremediação de efluentes urbanos, microalgas e wetlands construídos: Saneamento ambiental como tecnologia mais limpa/Elizandro Silveira, Ênio Leandro Machado. 1ed. Rio de Janeiro: **Autografia**, 140p. 2018.

SOBRERO, M. C.; RONCO, A. Ensayo de toxicidad aguda con semillas de lechuga (*Lactuca sativa L.*). In:\_\_\_ MORALES, G. C. Ensayos toxicológicos y métodos de evaluación de calidad de aguas estandarización, intercalibración, resultados y aplicaciones. Centro internacional de investigaciones para el desarrollo: **Instituto Mexicano de Tecnología del Agua (IMTA)**, México, 189p. 2004.

STANDARD METHODS. Standard methods for the examination of water and wastewater, 2540 Solids- 2540 G.Total, Fixed, and Volatile Solids in Solid and Semisolid Samples 23th Edition, 2017.

VDI-VEREIN DEUTSCHER INGENIEURE. 4630: Vergärung organischer Stoffe Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche, **ICS** 13.030.30; 27.190, 2006.

VYMAZAL, J. Plants used in constructed wetlands with horizontal subsurface flow: a review. **Hidrobiologia** 674, 133-156. 2011.

WALL, D. M., *et al.* Investigating two-phase digestion of grass silage for demand-driven biogas applications: Effect of particle size and rumen fluid addition. **Renewable Energy**, 86, 1215–1223. 2016.

WÖHLER-GESKE, A. *et al.* Yield, fermentation kinetics and the role of quality properties of thatching reed (*Phragmites australis*) during discontinuous anaerobic fermentation. **Ind. Crop Prod.** 83 (2016) 701709, <https://doi.org/10.1016/j.indcrop.2015.12.041>.

WU, S. *et al.* Development of constructed wetlands in performance intensifications for wastewater treatment: a nitrogen and organic matter targeted review. **Water research**, v.57, p. 40–55, 15 jun. 2014.

ZHANG, C. *et al.* The anaerobic codigestion of food waste and cattle manure. **Bioresource Technology**, 129, 170–176. 2013.