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HIGH RATE ALGAL PONDS FOR POST-TREATING SEWAGE FROM UASB REACTORS: TREATMENT EFFICIENCY, ANAEROBIC CO-DIGESTION AND SUSTAINABILITY

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Thesis submitted to the post-graduation course in Sanitation, Environment and Water Resources at the Federal University of Minas Gerais and Environmental Engineering of Universitat Politècnica de Catalunya as a partial requirement for obtaining a Doctorate in co-tutela degree in Sanitation, Environment and Water Resources by UFMG and Environmental Engineering by Universitat Politècnica de Catalunya.

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“A vida das gentes neste mundo, senhor Sabugo, é isso. Um rosário de piscados. Cada pisco é um dia. Pisca e mama, pisca e brinca, pisca e estuda, pisca e ama, pisca e cria filhos, pisca e geme os reumatismos, e por fim pisca pela última vez e morre.

– E depois que morre?, perguntou o Visconde.

– Depois que morre, vira hipótese. É ou não é?”

Monteiro Lobato

“O correr da vida embrulha tudo. A vida é assim: esquenta e esfria, aperta e daí afrouxa, sossega e depois desinquieta. O que ela quer da gente é coragem”

Guimarães Rosa

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ABSTRACT

This PhD thesis aimed at evaluating the performance of high rate algal ponds (HRAP) to post-treat the anaerobic effluent from an upflow anaerobic sludge blanket (UASB) reactor fed with sewage. The work analysed the system accomplishment in terms of treatment efficiency, micropollutants removal and biogas production, through the anaerobic co-digestion of raw sewage and microalgal biomass (with and without solar thermal pre-treatment), in a demonstration-scale system. In addition, the research intended at assessing the sustainability of the UASB+HRAP system using life cycle assessment (LCA), in terms of its environmental impact in comparison with other UASB post-treatment technologies.

Sewage treatment efficiency was analysed through BIO_ALGAE 2 mathematical model, which enabled the understanding and optimisation of the symbiotic relation between microalgae and bacteria. To this, experimental data from demonstration-scale systems in tropical climate conditions were used for model calibration. In addition, different scenarios were considered by varying HRAP hydraulic retention time (HRT) (4, 6 and 8 days). Results obtained showed an efficient removal of COD (70%), TSS (42%), N-NH_4 (57%) and P-PO_4 (30%) in the UASB+HRAP system. From the evaluated scenarios, the operation of HRAP at 4 days of HRT showed to be optimal in terms of sewage treatment and energy production, with lower area requirement.

Regarding the removal of micropollutants (pharmaceuticals and endocrine disruptors), samples were collected periodically from raw sewage, UASB reactor and HRAP effluents. All monitored compounds were found in raw sewage, with occurrence rates ranging from 70 to 100%. Micropollutant removal in the UASB reactor ranged from none (-25.12% for the hormone EE2-ethinylestradiol) to 85% removal (E2-estradiol), due to the incapacity of anaerobic processes. However, the overall UASB+HRAP system was highly efficient for removing most compounds, with removal rates ranging between 65% (ibuprofen) to 95% (estrone).

To evaluate the co-digestion of raw sewage and microalgal biomass in UASB reactors two phases were considered: without and after thermal pre-treatment using solar heating. In both cases, an UASB reactor fed with only raw sewage was used as control. During the first phase, the results showed a methane yield increase of 35% after anaerobic co-digestion with microalgae, from 156 to 211 NL CH_4 kg^{-1} VS. An energy assessment showed a positive energy balance, with an annual average net ratio of 2.11 in the UASB+HRAP system. Regarding the results after microalgal biomass pre-treatment, organic matter solubilization reached 32% increase in terms of total COD. Furthermore, methane yield was increased by 45% compared to mono-digestion with raw sewage, from 81 to 117 NL CH_4 kg^{-1} COD. The energy assessment showed a positive energy balance, with an annual average net ratio of 2.52 in the sewage treatment system.

Finally, the environmental impact of HRAP as post-treatment technology following UASB reactors was carried out using LCA for comparison with other post-treatments: trickling filters, polishing ponds and constructed wetlands. The results showed that among the 8 categories evaluated, HRAP showed better performance in 4 of them. The study concluded that HRAP may be considered a potential technology following UASB reactors and its environmental impacts can be further improved by using appropriate materials and construction techniques.

RESUMO

Esta tese teve como objetivo avaliar o desempenho de lagoas de algas de alta taxa (LAT) como pós-tratamento do efluente anaeróbio de um reator UASB alimentado com esgoto doméstico. O trabalho analisou o desempenho desse sistema em termos de eficiência de tratamento, remoção de micropoluentes e produção de biogás, através da co-digestão anaeróbia de esgoto bruto e biomassa microalgal (com e sem pré-tratamento solar térmico), em escala de demonstração. Além disso, essa pesquisa também a avaliou o impacto ambiental do fluxo de tratamento UASB+LAT, através da ferramenta de avaliação do ciclo de vida (ACV), comparando com outros fluxos de tratamento já consolidados para a realidade brasileira.

A eficiência do tratamento de esgoto foi analisada por meio do modelo matemático BIO_ALGAE 2, que possibilitou o entendimento e otimização da relação simbiótica entre microalgas e bactérias. Para isso, dados experimentais do sistema em escala de demonstração e em condições de clima tropical foram utilizados para calibração do modelo. Ademais, a partir do modelo calibrado, diferentes cenários foram simulados variando o tempo de detenção hidráulica (TDH) das LAT (4, 6 e 8 dias). Os resultados obtidos mostraram uma remoção eficiente de DQO (70%), SST (42%), $N-NH_4$ (57%) e $P-PO_4$ (30%) no sistema UASB + LAT. Dos cenários avaliados, a operação das LAT com 4 dias de TDH mostrou-se ótima em termos de tratamento de esgoto e potencial de produção de energia, com menor necessidade de área.

Em relação à remoção dos micropoluentes (fármacos e desreguladores endócrinos), foram coletadas periodicamente amostras de esgoto bruto, reator UASB e efluentes das Lagoas. Todos os compostos monitorados foram encontrados no esgoto bruto, com taxas de ocorrência variando de 70 a 100%. A remoção do micropoluente no reator UASB variou de nenhum (-25,12% para o hormônio EE2-etinilestradiol) a 85% de remoção (E2-estradiol), devido à incapacidade dos processos anaeróbicos. No entanto, o sistema UASB + LAT em geral foi altamente eficiente para remover a maioria dos compostos, com taxas de remoção variando entre 65% (ibuprofeno) a 95% (estrona).

Para avaliar a co-digestão de esgoto bruto e biomassa microalgal em reatores UASB foram consideradas duas fases: sem e após pré-tratamento térmico com aquecimento solar. Em ambos os casos, um reator UASB alimentado apenas com esgoto bruto foi usado como controle. Durante a primeira fase, os resultados mostraram um aumento no rendimento de metano de 35% após a co-digestão anaeróbia com microalgas, de 156 NL CH_4 kg^{-1} SV para

211 NL CH_4 kg^{-1} SV. Uma avaliação energética mostrou um balanço energético positivo, com uma relação média anual entre energia produzida e consumida de 2,11 no sistema UASB+LAT. Em relação aos resultados após o pré-tratamento da biomassa microalgal, a solubilização da matéria orgânica atingiu uma eficiência de 32% em termos de DQO total. Além disso, o rendimento de metano aumentou em 45% em comparação com a mono-digestão com esgoto bruto, de 81 NL CH_4 kg^{-1} DQO para 117 NL CH_4 kg^{-1} DQO. A avaliação energética apresentou balanço positivo, com relação média anual entre energia produzida e consumida de 2,52 para o sistema avaliado.

Finalmente, o impacto ambiental das LAT como pós-tratamento de efluente de reatores UASB foi realizado usando ACV para comparação com outras tecnologias já consolidadas para o pós-tratamento de reator UASB no Brasil: filtro biológico percolador, lagoas de polimento e *wetland* construído. Os resultados mostraram que dentre as 8 categorias avaliadas, o sistema de LAT apresentou melhor desempenho em 4. O estudo concluiu que as LAT podem ser considerado uma tecnologia potencial e sustentável para pós tratar efluente de reatores UASB e seus impactos ambientais podem ser melhorados usando materiais e técnicas de construção apropriados.

RESUMEN

Esta tesis tuvo como objetivo evaluar el desempeño de lagunas de alta tasa (LAT) como postratamiento del efluente anaeróbico de un reactor UASB depurando aguas residuales domésticas. El trabajo analizó el desempeño de este sistema evaluando la eficiencia del tratamiento, remoción de microcontaminantes y producción de biogás, mediante la codigestión anaeróbica de aguas residuales brutas y biomasa de microalgas (con y sin pre-tratamiento térmico solar), en escala de demonstración. Además, esta investigación también evaluó el impacto ambiental del flujo de tratamiento UASB + LAT, utilizando de la herramienta de evaluación del ciclo de vida (ACV), comparándolo con otros flujos de tratamiento ya consolidados para la realidad brasileña.

La eficiencia de la depuración de las aguas residuales se analizó por el modelo matemático BIO_ALGAE 2, que permitió comprender y optimizar la relación simbiótica entre microalgas y bacterias. Para ello, se utilizaron datos experimentales del sistema en escala de demostración y en condiciones climáticas tropicales para calibrar el modelo. Además, a partir del modelo calibrado, se simularon diferentes escenarios variando el tiempo de detención hidráulico (TDH) de las lagunas (4, 6 y 8 días). Los resultados mostraron una eficiente remoción de DQO (70%), SST (42%), N-NH_4 (57%) y P-PO_4 (30%) en el sistema UASB + LAT. De los escenarios evaluados, la operación de las LAT con 4 días de TDH resultó excelente para la depuración de aguas residuales y potencial de producción de energía, con menor necesidad de área.

Acerca de la remoción de microcontaminantes (fármacos y disruptores endocrinos), periódicamente se recolectaron muestras de las aguas residuales sin tratar, reactor UASB y efluentes de las lagunas. Todos los compuestos monitoreados se encontraron en las aguas residuales sin tratar, con tasas de ocurrencia que oscilan entre el 70 y el 100%. La eliminación de los microcontaminantes en el reactor UASB osciló entre nada (-25,12% para la hormona EE2-etinilestradiol) y 85% de eliminación (E2-estradiol), debido a la incapacidad de los procesos anaeróbicos. Sin embargo, el sistema UASB + LAT en general fue muy eficaz en la eliminación de la mayoría de los compuestos, con tasas de eliminación que desde el 65% (ibuprofeno) al 95% (estrona).

Para evaluar la codigestión de aguas residuales sin tratamiento y biomasa de microalgas en reactores UASB, se consideraron dos fases: sin y después del pretratamiento térmico con calentamiento solar. En ambos casos, se utilizó como control un reactor UASB alimentado solo con aguas residuales sin tratar. Durante la primera fase, los resultados mostraron un aumento del 35% en el rendimiento de metano después de la co-digestión anaeróbica con microalgas, de 156 NL CH_4 kg^{-1} SV a 211 NL CH_4 kg^{-1} SV. Una evaluación energética demostró un balance positivo, con un ratio medio anual entre energía producida y consumida de 2,11 en el sistema UASB + LAT. En cuanto a los resultados tras el pretratamiento de la biomasa de microalgas, la solubilización de la materia orgánica alcanzó una eficiencia del 32% en términos de DQO total. Además, el rendimiento de metano aumentó en un 45% en comparación con la mono-digestión con aguas residuales sin tratar, de 81 NL CH_4 kg^{-1} DQO a 117 NL CH_4 kg^{-1} DQO. La valoración energética arrojó un saldo positivo, con una ratio medio anual entre energía producida y consumida de 2,52 para el sistema evaluado.

Finalmente, el impacto ambiental del LAT como postratamiento de efluentes de reactores UASB se llevó a cabo utilizando LCA como comparación con otras tecnologías ya consolidadas para el postratamiento del reactor UASB en Brasil: filtro biológico percolador, lagunas de pulimiento y humedal construido. Los resultados mostraron que, entre las 8 categorías evaluadas, el sistema LAT se desempeñó mejor en 4. El estudio concluyó que LAT puede considerarse una tecnología potencial y sostenible para el postratamiento de efluentes de reactores UASB y sus impactos ambientales pueden mejorarse utilizando materiales y técnicas de construcción adecuada.

SUMMARY

FIGURE LIST

TABLE LIST

LIST OF ABBREVIATIONS, SIGNS AND SYMBOLS

ACMP - Aerated complete mixing ponds

AMO -Ammonium monooxygenase

AOP – Advanced oxidation process

APS - Advanced Pond System

BOD_5 - biochemical oxygen demand

CAPEX – Capital expenditure

CH4 – Methane

CO_2 – carbon dioxide

COD – Chemical oxygen demand

CW - Constructed wetlands

DEHP - Di-2-(2-etil-hexil) phthalate

DNA - Deoxyribonucleic acid

DO – Dissolved oxygen

E2 - 17β-estradiol

E3 -Estriol

EC – Europe constitution

ED -Endocrine disruptors

EDTA - Ethylenediamine tetraacetic acid

EE2- 17α-ethinylestradiol

FAP – Facultative aerated ponds

FP - Facultative ponds

GC – MS - Gas chromatography coupled to mass spectrometry

H2 – Hydrogen

H2S – Sulphur

HRAP – High rate algal pond

HRT – Hydraulic retention time

K_a - Dissociation or acidity constant

K_{bio} - Biodegradation coeficiente

K_d - Distribution coeficiente

Kg – kilogram

LCA – Life Cycle assessment

LCI - Life cycle inventory

LCIA - Life Cycle Impact Assessment

LOD - Limit of detection

LOQ - Limit of quantification

m – meters

MP -Maturation ponds

N-NH4 – Ammonium

O&M - Operation and maintenance

°C - Celsius degrees

OPEX - Operational expenditure

P - Phosphorus

PhACs - Pharmaceuticals

PP - Polishing ponds

PVC - Polyvinyl chloride

RNA - Ribonucleic acid

s – seconds

SPE - Solid phase extraction

STP - Sewage treatment plant

TKF - Trickling filters

TKN - Total kjeldahl nitrogen

TSS - Total suspended solids

UASB - Upflow anaerobic sludge blanket

UK – United Kingdom

VS -Volatile solids

1 INTRODUCTION

In Brazil, anaerobic reactors are widely used for domestic sewage treatment, in particular upflow anaerobic sludge blanket (UASB) reactors. The main advantage of UASB reactors for sewage treatment is its low operating cost, associated with the capacity of treating a high flow rate in a low surface area. Moreover, generated biogas has a high energetic potential use in the sewage treatment plant (STP) or for the surrounding communities (CHERNICHARO, C. A., 2007). However, the effluent from UASB reactors may not meet some legislation standards and, therefore, usually require post-treatment step. Numerous technologies have already been studied and proven efficient for post-treating UASB effluent, for example trickling filters, polishing ponds and constructed wetlands (MUNGRAY; MURTHY; TIRPUDE, 2012). However, selecting the most appropriate treatment units for particular sanitary, geographic, socioeconomic and cultural scenarios is a complex process (BRESSANI-RIBEIRO, THIAGO *et al.*, 2019). Recent investigation evaluating 1667 full-scale STPs in several regions of Brazil showed that 667 of them applied UASB reactors followed by post-treatment. Among the adopted post-treatment technologies, 64% of them were trickling filters (TKF) or polishing ponds (PP) (CHERNICHARO, C. A. DE L.; RIBEIRO; GARCIA; *et al.*, 2018). Constructed wetlands (CW) have only recently been applied as an option for treating UASB effluent (SEZERINO *et al.*, 2015) and are still not widely used.

In this scenario, another possible option to enhance sanitation in STPs using UASB reactors may be through high rate algal ponds (HRAP). These systems are alternatives to polishing ponds, with lower height, thus increasing and potentializing microalgal biomass production. Advantages of HRAP systems include: lower construction and operation costs, compared to activated sludge systems; negligible demand for electricity; efficient removal of nutrients, pathogens and micropollutants and; possibility to produce and harvest microalgal biomass. However, up to date little is known regarding their behaviour of HRAP treating UASB reactors effluent. Regarding the final effluent quality from HRAP, previous studies attained 94% removal of BOD and 60-85% of COD and 91% removal of N-NH_4 (DE GODOS, I. *et al.*, 2016; VASSALLE; SUNYER CALDÚ; *et al.*, 2020; VILLAR-NAVARRO *et al.*, 2018). Specifically in other pollutants like micropollutants, few studies evaluated the removal mechanisms of these compounds by HRAP and even less by the UASB + HRAP system. Therefore, the presence of these contaminants in the effluent from UASB reactors and their removal in HRAP should be investigated.

Regarding the microalgae grown in HRAP, biomass may be harvested and valorized into biofuels, fertilizers or animal feed (ARASHIRO *et al.*, 2018). Among the downstream processes, microalgae conversion to biogas through anaerobic digestion seems to be the most straightforward alternative since it does not require dehydration, pretreatment for compound separation and may use existing reactors and infrastructure (PASSOS *et al.*, 2017). More specifically, when an anaerobic reactor is the first sewage treatment unit, an alternative could be to recirculate harvested microalgal biomass to the UASB reactor for co-digestion with raw sewage. This process may be beneficial as it makes profit of existing infrastructure and since the synergistic effect of both substrates (sewage and microalgae) may increase biogas production and energy outcome. The mentioned flowchart alternative (UASB+HRAP with biomass recirculation) has still not been proposed and investigated in previous work. Anaerobic co-digestion of microalgal biomass has been extensively investigated, however with other feedstock, as sewage sludge, agricultural wastes, manure (SOLÉ-BUNDÓ *et al.*, 2019). Finally, since sewage treatment technology solutions should be an equilibrium balance between economic, environmental and technical aspects (VON SPERLING, MARCOS; CHERNICHARO, 2005a), the selection and analysis of them require the integration of these multiple. The selection of inadequate sewage treatment technologies may result in low treatment performance and operational and maintenance difficulties (VAN LIER; LETTINGA, 1999). In this way, an analysis of the most used and emerging technologies for post treatment of effluents from UASB reactors should be considered.

Based on the described challenges and the literature gaps to be filled, the current research aimed to investigate and evaluate a STP composed of a UASB reactor followed by a HRAP. The goal was to evaluate the system based on its treatment efficiency, anaerobic co-digestion by microalgal biomass recirculation and environmental assessment. For this, two years of experimental data collection was carried out in a demonstration scale facility treating real raw sewage. Data was calibrated and validated using a mathematical model in order to evaluate the optimal operation conditions of the UASB + HRAP system. Anaerobic digestion was assessed through biogas production in UASB reactors only fed with raw sewage and also after recirculation with harvested microalgal biomass. Moreover, biomass valorization was studied in two different phases, with and without solar thermal pretreatment to enhance hydrolysis and consequently, biogas production. Finally, for evaluating environmental aspects, life cycle assessment (LCA) was carried out to compare different UASB post-treatment solutions for evaluating the more sustainable approach and verify HRAP viability.

1.1 Thesis structure

This thesis was divided in 10 chapters. The initial part corresponding introduction and contextualization: i) Chapter 1***, Introduction and Thesis structure; ii)*** Chapter 2, ***Literature review*** and; iii) Chapter 3, ***Hypotheses and objectives***. The subsequent five chapters were based on published or prepared/submitted scientific papers, as described following. Chapter 4 is entitled ***Deciphering microalgae-bacteria interactions in raceways ponds treating anaerobic effluent in tropical conditions through biokinetic modelling.*** This chapter was based on the validation of the mathematical model Bio Algae 2 using experimental data of the demonstration-scale UASB+HRAP system. The work was developed in partnership with the GEMMA group at the Polytechnic University of Catalonia. This chapter will give place to a paper that is in its final preparation phase. Chapter 5 is entitled ***Can high-rate algal ponds be used as post-treatment of UASB reactors to remove micropollutants?*** The chapter shows and discusses the results of the experimental campaign carried out in the demonstration-scale system to evaluate micropollutants removal. This chapter was based on the article of the same name, published in Chemosphere Journal. Chapter 6 is entitled ***Upflow anaerobic sludge blanket in microalgae-based sewage treatment: co-digestion for improving biogas production.*** This chapter contains the results of the experimental investigation on anaerobic co-digestion of the microalgae produced in HRAP, without any pre-treatment. This chapter was based on the article of the same name, published in Bioresource Technology Journal. Chapter 7 is entitled ***Solar thermal pre-treatment to improve the anaerobic biodegradability of microalgal biomass in sewage treatment.*** This chapter contains the results of the second phase of the experimental investigation on anaerobic co-digestion of thermally pre-treated microalgae in a solar unit, with raw sewage. This chapter was based on the article of the same name, under review in Algal Research Journal. Chapter 8 is entitled ***Comparative life cycle assessment of UASB reactors coupled with low cost technologies for sewage treatment.*** This chapter is composed of results concerning the life cycle assessment comparison of different technologies used to post-treat anaerobic effluent from UASB reactors. The scientific paper based on this chapter is in its final step of preparation.

Finally, the closing chapters of this PhD thesis are: Chapter 9, ***Conclusions*** and, Chapter 10 ***Recommendations for future research,*** where potentialities and weaknesses of the proposed UASB+HRAP system were shown, as well as further investigation suggestions. At last, all references used in this thesis are listed, and other documents presented in supplementary materials.

2 LITERATURE REVIEW

2.1 Upflow anaerobic sludge blanket (UASB) reactors

UASB reactors are already consolidated for sewage treatment in Latin America, particularly in Brazil. This technology has expanded mainly in places with low availability of resources (financial, surface area, and skilled workers). The main advantage of UASB reactors when compared to conventional anaerobic reactors, is its high rate, with low hydraulic retention times (HRT) and high solid retention times (SRT). For liquid substrates (as sewage) it is preferable, while more concentrated substrates (as sludge) are used to be treated in continuous stirred tank reactors (CSTR). The uncoupling of HRT and SRT allows treatment of sewage in relatively small surface areas, with low energy consumption and low sludge generation (CHERNICHARO, C. A., 2007; DAUD *et al.*, 2018; VAN HAANDEL; LETTINGA, 1994).

UASB reactors remove suspended organic matter through conventional anaerobic pathways, as outlined following. Initially, hydrolytic bacteria use exoenzymes to hydrolyze complex and/or particulate substrates into simpler and dissolved substrates, which can be internally assimilated by the cells. Acidogenic bacteria degrade sugars, amino acids and fatty acids resulting from hydrolysis to several simpler compounds, such as organic acids. Acetogenic bacteria use organic intermediates to produce acetate, hydrogen, and carbon dioxide. In the last step, archaea produce methane from acetic acid (acetotrophic organisms) fermentation and/or from carbon dioxide using hydrogen (hydrogenotrophic organisms) reduction, while sulfate reducing bacteria uses sulfur derivatives as final electron acceptors to convert organic acids and hydrogen (CHERNICHARO, C. A., 2007; VAN HAANDEL; LETTINGA, 1994).

2.1.1 Biogas Recovery Potential from UASB reactors

Sewage conversion in UASB reactors generates three by-products: treated effluent (liquid phase), biological sludge (solid phase) and biogas (gas phase). For what concerns this PhD, biogas production, characteristics and downstream conversion will be highlighted following.

Biogas from anaerobic digestion is composed basically of methane, carbon dioxide and hydrogen sulfide. The biogas generated in UASB reactors treating sewage have normally the following composition: 60-85% methane (CH_4); 5-15% dioxide carbon (CO_2); 2-25% nitrogen (N_2); 0-0.3% carbon monoxide (CO); 0-3% hydrogen (H_2); 0-2% oxygen (O_2); and 1,000-2,000 ppmv (parts per million by volume) of hydrogen sulphide (H_2S) (NOYOLA et al, 2006; SILVEIRA, 2015). The emission of biogas to the environment contributes to the intensification of global warming, since

CH_4 has a strong greenhouse effect and its global warming potential is 28 times greater than that attributed to CO_2 over a 100-year horizon (WHO/UNICEF, 2018).

Currently, in Brazil, most of the STPs using UASB reactors adopt open flares for gas treatment through combustion. However, during the process exhaust gases are not confined and combustion is not controlled, consequently, temperature and residence time are difficult to specify. Additionally, the determination of biogas combustion conversion efficiency is not trivial, despite being an important parameter for the elaboration of greenhouse gas inventories (ROSA *et al.*, 2018). In addition, simple biogas flaring should be an avoided practice, since it is against the STP sustainability, while recovery and energy conversion pathways should be prioritised.

Analyzing from a thermal perspective, biogas from anaerobic digestion is classified as medium calorific potential (PERSSON; et al, 2007). However, after purification techniques, as degassing membranes and desorption columns, biogas can reach high calorific potential (up to 90% methane) (WIJFFELS; et al, 2003). Another concern, specifically from UASB reactors treating sewage, is the loss of dissolved methane to the liquid phase. Indications of up to 40% loss of dissolved methane have been reported (LOBATO; CHERNICHARO; SOUZA, 2012). Research carried out on demonstration scale showed dissolved methane recovery rates of 75%, which can represent an increase of up to 50% in the energy potential of UASB reactors (POSSETTI *et al.*, 2018; ROSA *et al.*, 2018).

Regarding biogas recovery and energy conversion, the main conversion techniques are summarised in Figure 2.1. Generally, in Brazil, there are two main straightforward pathways in STPs using UASB reactors: direct burning for heat recovery and conversion of into electricity and heat through combined heat and power plants (CHP) (PASSOS *et al.*, 2020; ROSA *et al.*, 2018). The first situation is mostly indicated for small STPs, where biogas production varies between 1 and 5 $Nm^3\ d^{-1}$. In this case, after H_2S removal, biogas is converted into thermal energy that can be used for domestic applications, such as cooking and water heating. In rural areas, with agricultural potential, recovered thermal energy from biogas could also be used for sludge sanitization for pathogen removal and soil application (PASSOS *et al.*, 2020). The second scenario is mainly indicated for medium and larger STPs (over 200,000 inhabitants). In this case, electricity produced from biogas may allow a reduction in energy costs in the same treatment plant, by means of an exchange / sale to local stakeholder. Moreover, heat produced in the same cogeneration process may be applied to dehydrate and reduce the volume of sludge produced, which allows saving on disposal and avoiding the transportation of a large volume of this by-product. Previous studies have shown that thermal application to sludge reduces its volume by 46% and pathogens content by up to 99.9% (CARTES *et al.*, 2018; KACPRZAK *et al.*, 2017; ROSA *et al.*, 2018). In addition, this practice reduces the emission of greenhouse gases by avoiding the emission of biogas to the atmosphere and by emissions from vehicles responsible for transportation. More advanced option for converting biogas is through its upgrading into biomethane. In this case, purification and removal of CO_2 is necessary to reach acceptable values for injection into the distribution grid (> 95%) (MUÑOZ, RAÚL *et al.*, 2015) and for the use as car engines (96%) (PAPACZ, 2011). The injection of biomethane into the grid may be used exploited by the nearby population or for the STP car fleet. This scenario would enable a decrease in the use of fossil fuels and, consequently, a decrease in the carbon footprint. However, this option is hampered due to the high concentration of nitrogen in the biogas from the UASB reactors.

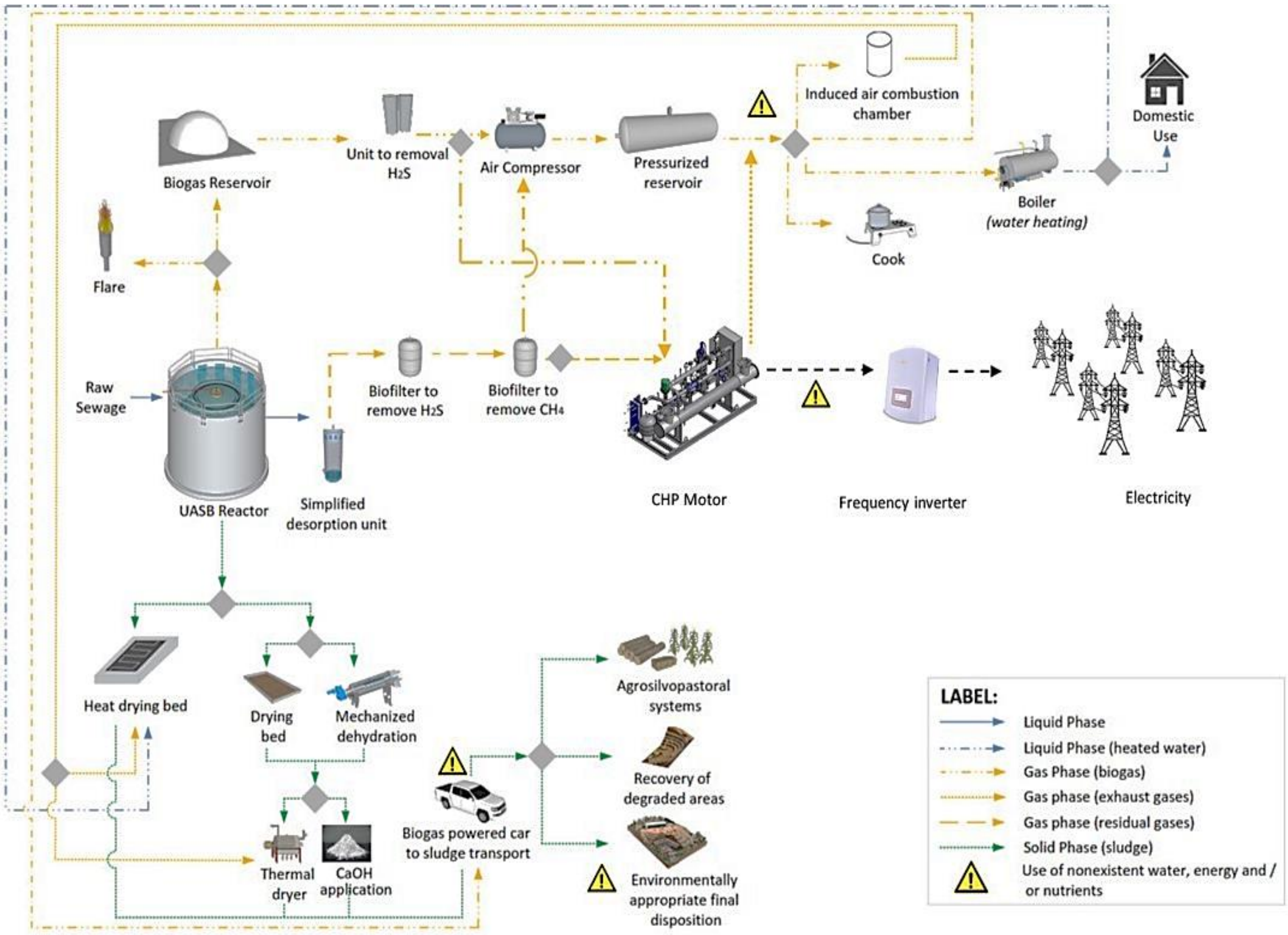

Figure 2.1. Flowsheet of biogas conversion techniques in STPs using UASB reactors

2.1.2 Post-treatment and effluent quality

Effluent from UASB reactors does not always meet discharge standards legislation regarding organic matter, nitrogen, phosphorus and pathogens. Therefore, it is normally required to implement an aerobic post-treatment unit in order to protect the environment and comply with legislation. The combined systems (anaerobic/aerobic), using UASB reactors as the first biological treatment stage, reaches necessary efficiencies to comply with the discharge standards of most Latin American countries. Thus, many options can be used to post-treat UASB effluent, combining performance and operational simplicity, necessary for the reality of developing countries such in Latin America. Among post-treatment technologies, the most commonly applied are maturation, stabilization or polishing ponds, constructed wetlands and trickling filters (BRESSANI-RIBEIRO et al., 2018; DOTRO et al., 2017; VERBYLA et al, 2017).

Previous study evaluating 2,734 decentralised and small-scale STPs in Latin America revealed a preference for a decentralized design for sewage treatment systems. This showed that most systems comprised: stabilization ponds (38%), activated sludge (26%) and UASB reactor + post-treatment (17%) (NOYOLA *et al.*, 2012). As can be seen, UASB reactor was the third technology most used, even though they were implemented first time in full scale, explained from its lower capital and operational expenditure (CAPEX and OPEX) compared to activated sludge (CHERNICHARO *et al.*, 2018).

A study dealing with data from 3,668 Brazilian STPs showed that 37% of them used UASB reactors followed by a post-treatment, of which 55% were trickling filters or polishing ponds (ANA, 2020). The results from Latin America, Brazil and the state of Minas Gerais are shown in Figure 2.2. As can be seen, UASB reactors are particularly applied in Brazil and even more in Minas Gerais (78% among 271 studied STPs), the state where this research was partly conducted (CHERNICHARO et al., 2018). Moreover, the performance of sewage treatment techniques and economic costs for the most common combined systems (UASB + post-treatment) are shown in Table 2.1. In this table the values of construction and maintenance were based on population ranges typical of the Brazilian sewage treatment scenario. It is observed that in terms of efficiency and costs the systems of ponds and wetlands were the most attractive compared to trickling filters and actived sludge. However, the need for an area for implantation often makes these technologies unfeasible for places with little land availability.

Table 2.1. Comparison of the main technologies used for UASB reactors post-treatment (Adapted from VON SPERLING; CHERNICHARO, 2005)

Parameters	UASB	UASB+Actived Sludge	UASB+Trickling Filters	UASB+Polish Pond	UASB+ Wetlands
COD (mg L^{-1})	180 to 270	60 to 150	70 to 180	100 to 180	50 to 180
Efficiency Removal (%)	55 to 70	75 to 88	73 to 88	70 to 83	70 to 85
TSS (mg L^{-1})	60 to 100	20 to 40	20 to 40	50 to 80	20 to 60
Efficiency Removal (%)	65 to 80	87 to 93	83 to 93	73 to 83	80 to 93
$N\text{-}NH_4^+$ (mg L^{-1})	>15	5 to 15	>15	10 to 15	10 to 20
Efficiency Removal (%)	<50	50 to 85	<50	50 to 65	35 to 65
Area Requirement (m^2 hab^{-1})	0.03 to 0.10	0.08 to 0.2	0.1 to 0.2	1.5 to 2.5	1.5 to 3.0
Construction Costs (€ inhab $year^{-1}$) *	12.3 to 20.3	28.5 to 44.9	24.3 to 36.7	16.3 to 28.5	20.3 to 36.7
Operational and maintenance costs (€ $inhab^{-1}$) *	0.8 to 1.2	2.4 to 4.1	1.7 to 2.6	1.5 to 2.4	1.6 to 2.3

Note: ***The published values (in reais – R$) for the year 2005 were adjusted to present values (2021) The costs in € were converted from the costs in Brazilian reais (R$) using the exchange rate of € 1.00 = R$ 6.76 (01/May/2021, Central Bank of Brazil).**

Figure 2.2. Comparison of sewage treatment technologies in Latin America, Brazil and the state of Minas Gerais

2.2 High Rate Algal Ponds (HRAP)

High rate algal ponds (HRAPs) were firstly developed by Professor William J. Oswald in the late 1950s at the University of California, Berkeley, (OSWALD, *et al.*, 1957; OSWALD; GOLUEKE, 1960). More recently, studies involving HRAPs have increased exponentially. In the mid-1990s, the average number of indexed publications was approximately 160 per year, while during the year 2020, 1400 publications were identified, showing the importance given by the academic community.

HRAPs have some improvements if compared to conventional stabilization ponds, such as an improved performance at lower area requirement, due to the incorporation of a simple mixing system to improve the hydraulic flow optimizing the incorporation of oxygen produced by photosynthesis from microalgae (FALLOWFIELD; CROMAR; EVISON, 1996) . In general terms, the system consists of open channels of 2 to 3 meters, where there is continuous movement in a closed circuit where velocity varies between 0.12 and 0.15 m s^{-1} ^{1} (MUÑOZ; GUIEYSSE, 2006; OSWALD *et al.*, 1957). It may be considered a low-cost treatment process due to the use of solar energy for increasing nutrients and organic matter removals through microalgae photosynthesis in shallow ponds (0.2 to 0.7 m) (CROMAR *et al.*, 1992). The shallow depth of HRAP enhances the rate of sunlight inactivation of thermotolerant coliforms, and promotes photo-oxidation of dissolved organic contaminants (COLLEY; HICKEY; QUINN, 2010). Operational HRTs depend on solar radiation and climatic conditions, varying between 3 and 8 days (CRAGGS, R. J. *et al.*, 2003).

If compared to conventional treatment, HRAPs offer several advantages, including lower footprint due the lower use of energy, absence of odour, tertiary level of treatment, ammonia uptake and phosphate precipitation (AZOV; SHELEF; MORAINE, 1982). The great performance of HRAP is possible due to the high levels of pH and dissolved oxygen reached in the reactor during photosynthesis, microalgae biological uptake and the constant movement of the liquid. In addition, microalgae produced and harvested from HRAPs can be converted into valuable products, as biofuels, fertilizers, bioplastics and pigments (ARASHIRO *et al.*, 2020; PASSOS *et al.*, 2014; RUEDA *et al.*, 2020).

However, as in any natural system, HRAPs are susceptible to environmental variations that can affect the whole treatment process. Among the most important parameters that are susceptible to natural variations are pH, temperature, light availability, dissolved oxygen concentration and the presence of predators and grazers (MUÑOZ; GUIEYSSE, 2006). The efficiency of HRAPs normally decreases at locals with low temperatures and low solar irradiation (RICHMOND, 1986). Muñoz *et al* (2004) observed that organic matter and nutrient removal efficiencies doubled when the temperature increased from 25 to 30 ºC. However, Chevalier *et al.* (2000) demonstrated that a cold-adapted cyanobacteria strain was suitable for nutrient removal at an average temperature of 15ºC. Sunlight intensity greatly varies during the day and during the year. Microalgae activity seem to increase with light intensity at 200-400 uE m^{-2}s^{-1}, where photosynthesis becomes saturated, while activity decreases at higher light intensities (OGBONNA; TANAKA, 2000). In fact, photoinhibition has been observed during the central hours of a sunny day when irradiance can reach up to 4000 uE m^{-2}s^{-1} (FUENTES-GRÜNEWALD *et al.*, 2013).

2.2.1 HRAP for sewage treatment

Since HRAP performance requires an effluent with low turbidity to allow solar radiation in microalgae, it is normally combined to a previous treatment unit (GARCÍA *et al.*, 2006). In most studies dealing with sewage treatment, the previous treatment consisted in a primary settler (ARASHIRO *et al.*, 2019; CRAGGS *et al.*, 2012; PICOT *et al.*, 1991; SOLIMENO *et al.*, 2019). However, other technologies can be used in place of a primary settler. This is the case of UASB reactors, which have the capacity to remove solids and organic matter from raw sewage, allowing the entry of an effluent with low turbidity, but still subject to treatment to comply the environmental standard. Microalgae separation and concentration is generally carried out in secondary settlers. This unit has particular importance since it allows biomass harvesting for downstream processing, but also it prevents microalgae from escaping in treated effluent.

In general, the mechanisms for removing pollutants (organic matter, nutrients and pathogens) from an HRAP are enabled from the high rate of oxygen production from microalgae photosynthesis. In terms of soluble and particulate organic matter, removal occurs through oxidation by heterotrophic bacteria. The oxygen supplied by the photosynthetic activity of the microalgae is the main source of this process. Specifically, in the case of particulate organic matter, the main removal route is associated with biological sorption by microalgae/bacteria floc, which is later sedimented in a settler (DE GODOS *et al.*, 2016). In addition, degradation of organic matter depends on the effluent content characteristics and the eventual control of pH through CO_2 in the HRAP, since efficiency is higher at pH below 9. Previous studies attained 94% removal of BOD and 60-85% of COD when CO_2 was added to maintain pH below 9 (CRAGGS *et al.*, 2003; VASSALLE *et al.*, 2020; VILLAR-NAVARRO *et al.*, 2018).

Regarding nitrogen compounds, the main removal routes in HRAP are assimilation by microalgae and volatilization. Previous study showed 91% removal of $N\text{-}NH_4$ via volatilization and/or assimilation (NASCIMENTO, 2001). An additional study highlighted volatilization of ammonia as the main mechanism by measuring day and night $N\text{-}NH_4$ concentrations in HRAP. The results showed that at night, when the pH decreased, there was an increase in $N\text{-}NH_4$, with lower rates of removal through nitrification and microalgae absorption. Considering that the transformation of forms of nitrogen to nitrate does not mean the effective removal of nitrogen, in this case, the absorption by biomass becomes the main way of removing this nutrient. Removal mechanisms depend on factors as effluent characteristics, microalgae growth and local climatic conditions (GARCÍA *et al.*, 2006).

For phosphorus, pH-dependent precipitation and microalgae assimilation are the main removal routes in HRAPs (CROMAR *et al.*, 1992; PICOT *et al.*, 1991). pH values between 8 and 10 and intense photosynthetic activity promote ammonia volatilization and chemical phosphate precipitation. In fact, literature demonstrated how phosphorus precipitation increased with each pH unit increase exceeding 8.2 (CROMAR *et al.*, 1992). Previous work showed results of phosphate removal efficiencies from 0 to 65% (GONÇALVES *et al.*, 2020; YANG *et al.*, 2020). According to the literature, polyphosphates and organic phosphorus are removed through adsorption to $CaCO_3$ formed when high pH values are reached. Moreover, the solubility of hydroxyapatite (Ca_5 (OH) $(PO_4)_3{}^{-)}$ is very low even at pH below 9.0 and, therefore, a portion of the orthophosphate can be removed if there is enough calcium in the solution (NURDOGAN; OSWALD, 1995). Additionally, Polyphosphate Accumulating Organisms (PAO) can play an important role in phosphate removal when the system design consists in an anaerobic followed by aerobic process, as for UASB+HRAP configuration. The alternation in respect to input carbon can induce PAOs growth. In anaerobic environment, volatile fatty acids produced by fermentative bacteria from sugars and low molecular organic compounds are converted and stored as polyhydroxyalkanoates (PHA), which may be recovered as valuable bioplastics precursors (PETERSEN *et al.*, 1998; SANTOS *et al.*, 1999; UBUKATA; TAKII, 1994).

Regarding pathogen removal, the decay of indicator organisms, such as *E. coli*, is generally associated with radiation UVB (280-320 nm) and UVA (320-400nm) from solar irradiation in open ponds (AZEVEDO; NOZAKI, 2008; BUCHANAN *et al.*, 2013). This range of radiation causes damage to the DNA or RNA of the microorganism genome. The direct germicidal action of solar radiation is not the unique responsible for the decay of microorganisms in these systems, it seems necessary to consider the synergistic interaction between radiation with factors such as pH, DO and endogenous sensitizing photos (BUCHANAN *et al.*, 2013). For instance, the high pH values reached during the day are also a relevant factor for the decay of pathogens in HRAP (NURDOGAN; OSWALD, 1995). In addition, high light intensities promote, through photosynthetic activity, environments rich in dissolved oxygen, with formation of singlet and superoxide oxygen, reactive oxygen compounds that cause damage to the microorganisms' DNA. Studies of such systems with an emphasis on the removal of pathogenic organisms are still needed (EL HAMOURI *et al.*, 1994).

Specifically for HRAPs, literature indicated that pH increase (above 9.2) promotes 100% of *E.coli* decay and probably other pathogenic organisms. Moreover, HRTs of 3 to 5 days, with continuous sewage inlet and complete mixing flow are enough to promote disinfection (OSWALD, 1991). Recent studies evaluated the removal of pathogens in HRAP operated at 5- and 7-day HRT and addition of CO_2. For 5-day HRT, the removal efficiencies in log units were 3.4 for *P. aeruginosa*, 2.5 for *E. coli*, 2.6 *Enterococcus sp*., 2.2 *C. perfringens* and 1.3 *Staphylococcus sp*., while for the 7 day HRT, the removal efficiencies were 3.8 for *P. aeruginosa*, 3.7 for *E. coli*, 3.1 *Enterococcus sp*., 2.6 *C. perfringens* and 1.7 *Staphylococcus sp*. The authors concluded that the addition of CO_2 and HRT values had no significant influence on the results (RUAS *et al*., 2020).

2.2.2 HRAP as post-treatment of UASB reactors

Sewage treatment system associating UASB reactors with HRAPs is still scarcely explored in scientific literature. In fact, when searching for articles indexed on the Scopus Platform using the terms "High rate algal ponds" AND "UASB reactors" OR "UASB" OR "Upflow Anaerobic Sludge Blanket" AND "SEWAGE" OR "WASTEWATER", only 14 articles were found from the 2000s, as shown in Figure 2.3.

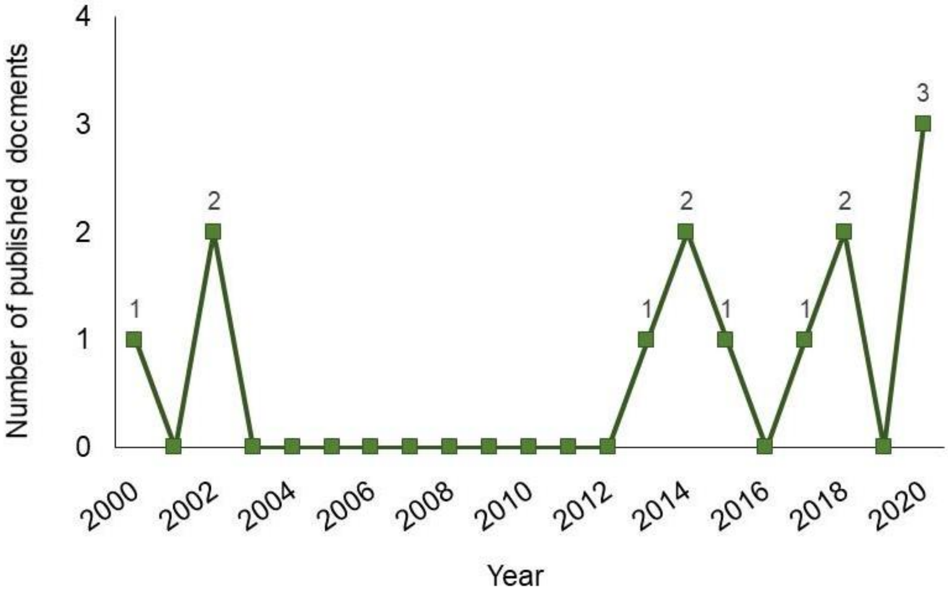

Figure 2.3. Publications on UASB reactor and HRAP for sewage treatment

Specifically in tropical countries, where UASB reactors are consolidated for sewage treatment, HRAPs have also great potential, due to climatic conditions. Previous study has justified the association of these treatment units with advantages, as an increase in the final effluent quality and a decrease in the required area, if compared to polishing ponds and constructed wetlands (SANTIAGO *et al.*, 2017). In fact, regarding the effluent quality, UASB+HRAP system configuration showed efficient removals of organic matter (COD and TSS) and nutrients (Table 2.2). As can be seen in Table 2.2, organic matter removal varied from 15% to 90% in published studies. This variation is mainly due to differences in the analytical procedure, if samples were or not filtered. It seems standardized that for analyzing organic matter and nutrient removal in HRAPs, samples should be filtered to eliminate microalgae, since biomass should be further separated from the effluent and recovered.

As already mentioned, removal of most contaminants is related to biological assimilation by heterotrophic bacteria and microalgae, which is essential for attaining a high quality effluent (SOLIMENO; GÓMEZ-SERRANO; ACIÉN, 2019). For nitrogen, it is important to highlight that in anaerobic reactor there is ammonia mineralization, although subsequent HRAP is able to assimilate or convert ammonium into nitrite or nitrate. Finally, as already discussed, association of UASB and HRAP may also stimulate the metabolism of PAOs, enabling PHA production (CROMAR *et al.*, 1992; GENTILI; FICK, 2017; SANTIAGO *et al.*, 2017).

Table 2.2. Summary of operational conditions and results of studies evaluating combined UASB+HRAP system for sewage treatment

	Reference						
Parameters	**Nascimento (2001)**	**De Godos (2015)**	**Santiago (2017)**	**Chatterjee (2018)**	**Villar-Navarro (2018)**	**Gonçalves (2020)**	**Espinosa (2021)**
UASB (HRT- hour)	9	8 to 16	7	8	15	8.8	7
HRAP (HRT-days)	9	3 to 7	4	5	6	4	8
COD Removal (%)	60	55 – 65*	30	50	82*	15	71
TSS Removal (%)	-116	48 -61*	-51	70**	53*	-144	40
N-NH4$^+$ Removal (%)	72*	26 – 73*	74*	85*	72*	80*	-
P-PO_4^{3-} Removal (%)	62	50	15	91	92	20	-

Filtered Sample** * After settler**

Regarding sewage disinfection, a previous study evaluated the global reduction and the concentrations of somatic coliphages, F-specific coliphages and *E. coli* in a UASB reactor followed by HRAP. The results showed e global removals of 4.28-log10 for *E. coli,* while for viral indicators the average values were 1.58-log10 for somatic coliphages and 2.14-log10 for F-specific coliphages. In this same study, a mass balance of pathogen concentrations indicated that only 14% of coliphages and 19% of *E. coli* concentrations were adsorbed on microalgal biomass (ESPINOSA *et al.*, 2021).

In relation to economical parameters, natural systems, costs associated with HRAP are mainly concerning area requirement, waterproofing and mechanical components installation. Study in HRAP pilot units showed implementation costs of € 40 m^2 for systems up to 150 m^2. In larger systems, there was estimated a reduction of at least 30% in these costs. Regarding the energy used to operate the HRAP, studies have shown a variation between 0.19 and 0.4 kWh m^{-3} (MARCIN;

MUCHA, 2015; SARPONG; GUDE, 2020). Slightly higher values (0.5 kWh m^{-3}) have been reported in HRAPs with biomass recirculation and 10 day HRT (TORRES-FRANCO *et al.*, 2021). When compared to the costs (CAPEX and OPEX) of other options for UASB reactor effluent post-treatment, the HRAP showed comparable results with other disseminated options as polishing ponds (Figure 2.4). The CAPEX, OPEX and area requirement for establish UASB reactors systems are low. However, as already mentioned, the need for a post treatment for these reactors is usual. Among the post-treatment shown in Figure 2.4 (most common in Brazil), the association UASB + HRAP, is able to significantly reduce the need for area compared to natural treatment systems (wetlands and polish ponds). In addition, CAPEX is also reduced, when compared to other technologies presented, as well as OPEX. Even though it is not yet widespread, the association UASB + HRAP shows to be an interesting option for the treatment of sewage, mainly in scenarios where the UASB are a principal sewage treatment technology, like in Brazil.

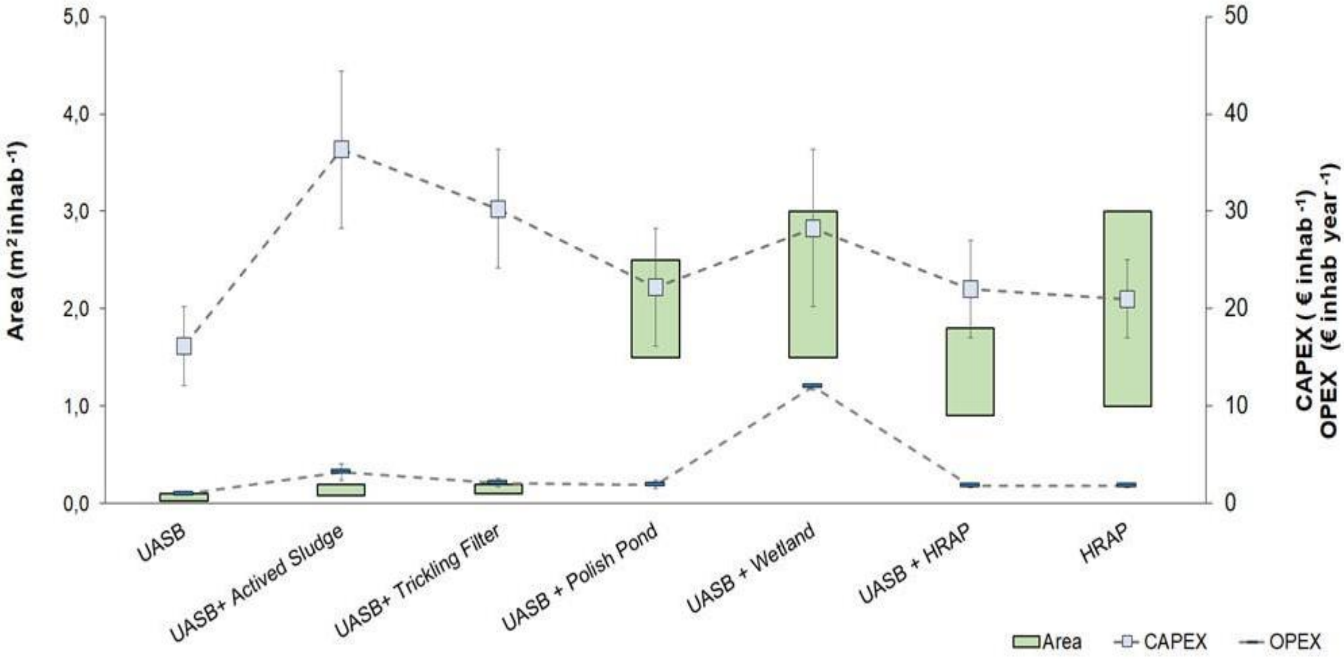

Figure 2.4. Area demand, CAPEX and OPEX for UASB+HRAP compared to other system configurations. Note: Estimates for an equivalent consumption of 150 L inhab-1 d-1. (Adapted from Von Sperling; Chernicharo (2005))

2.3 Micropollutants removal in UASB and HRAP systems

During the last decades, the presence of micropollutants in the aquatic environment has become a worldwide concern (LUO; GUO; NGO; *et al.*, 2014), due to the huge development of industries and technologies that resulted in the production of complex chemical compounds (BOLONG *et al.*, 2009). According to the literature, micropollutants are divided into six broad categories:

pharmaceuticals, personal products care, steroid hormones, surfactants, industrial chemicals and pesticides (LUO; GUO; NGO; *et al.*, 2014). Within these categories there are numerous subclasses that make up a range of existing micropollutants. These compounds are present in domestic and industrial effluents, surface and groundwater, sediments and even drinking water, in a lower concentration (BENFENATI *et al.*, 2003; DURHAN *et al.*, 2006; FERNANDEZ et al., 2007; PETROVIĆ et al, 2003; TERNES;et al 1999).

Micropollutants have numerous pathways into the environment, one of the main being the discharge of sanitary effluents, even if treated at a secondary level, since STPs are generally not designed to remove these compounds (KOLPIN *et al.*, 2002; NAKADA *et al.*, 2006; SNYDER *et al.*, 2003; TAN *et al.*, 2007)**.** One of the current concerns is related to their toxicity, which can bring adverse effects on organisms exposed to trace concentrations (LUO; GUO; HAO; *et al.*, 2014). The occurrence of micropollutants in the aquatic environment has been associated with increased cancer in the world population (associated with numerous other factors), endocrine disruption and resistance of microorganisms to antibiotics (FENT *et al*., 2006; PRUDEN *et al.*, 2006;COLOMER-LLUCH; JOFRE; MUNIESA, 2011)

Regarding world legislation, it can be confirmed that there are no directives or norms that regulate the disposal of most micropollutants (LUO et al., 2014). Some countries adopt regulations for a small portion of these compounds. As an example, Canada (Canadian Environmental Protection Act, 1999) recognized nonylphenol and nonylphenol ethoxylates as toxic compounds, among others. Another example is the European Union (Directive 2013/39 / EC), that adopted environmental quality standards to be followed for compounds such as octylphenol, nonylphenol, bisphenol A, DEHP and diuron. The UK has already limited emissions of 0.4 ng L^{-1}, 0.035 ng L^{-1} and 100 ng L^{-1} for micropollutants such as E2 (17β-estradiol), EE2 (17α-ethinylestradiol) and diclofenac respectively. In Brazil, the current legislation applicable to drinking water does not refer to guideline values when it refers to drugs and endocrine disruptors, since these compounds are not included in the parameters to be monitored in STPs.

Among the subclasses of micropollutants, those considered as endocrine disruptors (ED) and anti-inflammatories were considered as important substances to be investigated in greater depth, mainly due to the adversities these products can cause in the environment (BILA & DEZOTTI, 2007). To these compounds may be added antibiotics, which may lead to the creation of super-resistant bacteria caused by their indiscriminate use (KÜMMERER, 2001). Concerning the degradation of micropollutants, literature indicates that the main removal mechanisms are volatilization, oxidation, photodegradation, biological degradation and sorption (adsorption or absorption) (CIRJA *et al.*, 2008). Commonly, sewage treatment technologies apply the same mechanisms to remove conventional contaminants. Therefore, a small part of the micropollutants is often removed (LUO *et al.*, 2014). Among the aforementioned mechanisms, sorption and biodegradation have greater importance.

Based on the different routes, numerous studies have been developed to verify the performance of different sewage treatment technologies to remove these compounds. However, not all are efficient and economically viable, as advanced oxidative processes and nanofiltration (BRANDT *et al.*, 2013). In general terms, technologies with high biological activity, mainly the aerobic technologies, provide satisfactory degradation of micropollutants at a low implantation cost and simple operation, as HRAPs (CASTRO *et al.*, 2018; GARCÍA-GALÁN *et al.*, 2020; PAREDES *et al.*, 2016; VASSALLE; *et al.*, 2020 a-b).

In relation to removal in anaerobic technologies, like UASB reactors, studies have indicated inefficiency in removing a range of micropollutants (STASINAKIS *et al.*, 2013). Previous work showed that some drugs and endocrine disruptors were only considerably eliminated under aerobic conditions (SUAREZ et al., 2010). Other researches have confirmed low or even negative removals (synthesis) of contaminants in the UASB reactors (AQUINO; BRANDT; CHERNICHARO, 2013; IFELEBUEGU, 2011). The explanation is that under anaerobic conditions, even though there is a high affinity to sorption, micropollutants may be deprotonated (due to pH range), generating a repulsion of the solid phase (also negatively charged) and, therefore, remaining in the liquid phase (SUÁREZ *et al.*, 2008). Likewise, many factors may influence micropollutant removal in UASB reactors, as operational conditions (mainly low HRT), chemical composition of the compound, environmental conditions, sludge concentration and metabolite formation (ALVARINO *et al.*, 2014; CARBALLA *et al.*, 2007; DE GRAAFF *et al.*, 2011). Table 2.3 summarises the main results on micropoullutants removal in UASB reactors and/or HRAPs or similar microalgae-based system. It is important to highlight that the results, presented in Table 2.3 regard the class of micropollutants on this thesis investigated.

Table 2.3. Removal of micropollutants in UASB reactors and HRAPs

Micropollutants	Treatment	Removal Mean	Reference
Anti-inflammatory	HRAP	>90%	MATAMOROS *et al.*, (2015a)
	Tubular Photobioreactor	95%	VASSALLE *et al.*, (2020a)
	HRAP	45%	VASSALLE *et al.*, (2020b)
	HRAP	50%	GARCÍA-GALÁN, *et al.*, (2020)
	UASB + Trickling Filter	11%	BRANDT *et al.*,(2013)
	UASB + Wetlands	-20%	BRANDT *et al.*,(2013)
	UASB+ Polish Ponds	66%	BRANDT *et al.*,(2013)
	UASB+HRAP	>80%	VILLAR-NAVARRO *et al.*, (2018)
Natural Hormones	HRAP	>85%	HOM-DIAZ *et al.*, (2015)
	Photobioreactor	>90%	PARLADÉ *et al.*, (2018)
	Photobioreactor	90%	KIM, SAEWON *et al.*, (2017)
	Activated Sludge	80%	KOMOLAFE *et al.*, (2021)
	UASB + Trickling Filter	90%	KOMOLAFE *et al.*, (2021)
	Anaerobic Pond + Polish Pond	99%	KOMOLAFE *et al.*, (2021)
	UASB	49%	LOUROS *et al.*, (2021)
Artificial Hormones	HRAP	>90%	HOM-DIAZ *et al.*, (2015)
	Photobioreactor	90%	SOGANI *et al.*, (2020)
	Photobioreactor	80%	KIM, SAEWON *et al.*, (2017)
	UASB	39%	LOUROS *et al.*, (2021)

Another important concern regarding micropollutants in UASB reactors is the production and mainly the composition of the biogas. Literature has suggested that the presence of micropollutants can affect methanogenesis, mainly due to the presence of antibiotics. Previous studies have already been carried out indicating a decrease in biogas production and methane content due to the increased concentration of antibiotics in the domestic effluent (MITCHELL *et al.*, 2013). However, studies on larger scales are necessary. The scientific literature is still incipient in the identification of mechanisms that lead to the degradation of antibiotics in biological treatment systems. The interconnection between the operational parameters of the biological reactors and the processes of the microbial metabolism that lead to the degradation of drugs constitutes a great challenge. This understanding would allow the engineering control over the process and optimize removal conditions and biogas production (LUO *et al.*, 2014; SANZ *et al,* 1996).

Regarding HRAPs, removal mechanisms of micropollutants are dissociation by photolysis, sorption and biodegradation by microalgae intra and extracellularly (XIONG; KURADE; JEON, 2018). Most published studies on micropollutants are carried out in closed photobioreactors, with very few on open HRAPs. Even more restricted are the studies that evaluate the removal of micropollutants in the UASB reactor followed by HRAP combined system.

Complementing the results summarised in Table 2.3, with other micropollutants class, study presented 69 % in removal of tetracycline antibiotic at a lab scale HRAP (24 L) (DE GODOS *et al.*, 2012). In addition, great removal of Carbepenzine was reported with photo and biodegradation pathways (HOM-DIAZ; JAÉN-GIL; *et al.*, 2017). A more complete analysis of 26 contaminants, including pesticides, pharmaceuticals and plasticizers, in a pilot scale HRAP (470L) achieved removal efficiencies ranging from 0 to 99% depending on climatic conditions and HRT. The same work also conducted an ecotoxicological risk assessment, which demonstrated that the remaining concentration of chemicals had no acute toxicity risk (MATAMOROS *et al.*, 2015). Another study focusing on removal efficiencies of 12 pharmaceuticals and 26 of their corresponding main metabolites was carried out comparing HRAP with and without a previous primary treatment step. Reported results showed moderate (40-60%) to high (>60%) removals for most micropollutants, with exception to psychiatric drugs: carbamazepine, metoprolol and its metabolite, metoprolol acid (GARCÍA-GALÁN *et al.*, 2020).

These studies agreed that the main removal route of micropollutants by HRAP were sorption, photodegradation and biodegradation as corroborated by Norvill *et al.*, 2017 and Xiong *et al.*, 2016. On a whole, more research on UASB and HRAP on larger scales are necessary to fill scientific gaps. In addition, the interaction between UASB reactors and HRAPs must also be further investigated in order to evaluate the removal routes, including analyzes in the solid phases (anaerobic sludge and microalgal biomass).

2.4 Microalgal biomass recovery

Microalgae are single or multicellular organisms with microscopic dimensions (1-10µm) and capable of photosynthesis, due to the presence of chlorophyll. It is estimated that there are more than 800,000 different species of microalgae in different environments (XIA; MURPHY, 2016). Microalgae composition varies depending on species and environmental factors (as temperature, lighting, photoperiod, pH and mineral nutrients) (BECKER, 1994). When grown in open ponds used to treat sewage, as HRAPs, it also depends on affluent characteristics and operational parameters (as HRT, inoculation, biomass recirculation). Generally, microalgae are composed of carbohydrates, proteins and lipids, with varied proportions in dry mass varying between 4-64%, 6-61% and 2-40%, respectively (DEMIRBAS, 2010). Figure 2.5 shows a schematic image of microalgae content. In terms of energy production, the lipids are responsible for the highest theoretical methane yield (1.014 L CH4 g VS^{-1}), followed by proteins (0.851 L CH4 g VS^{-1}) and, finally, carbohydrates (0.415 L CH4 g VS^{-1}) (SIALVE *et al.*, 2009; ZABED *et al.*, 2020).

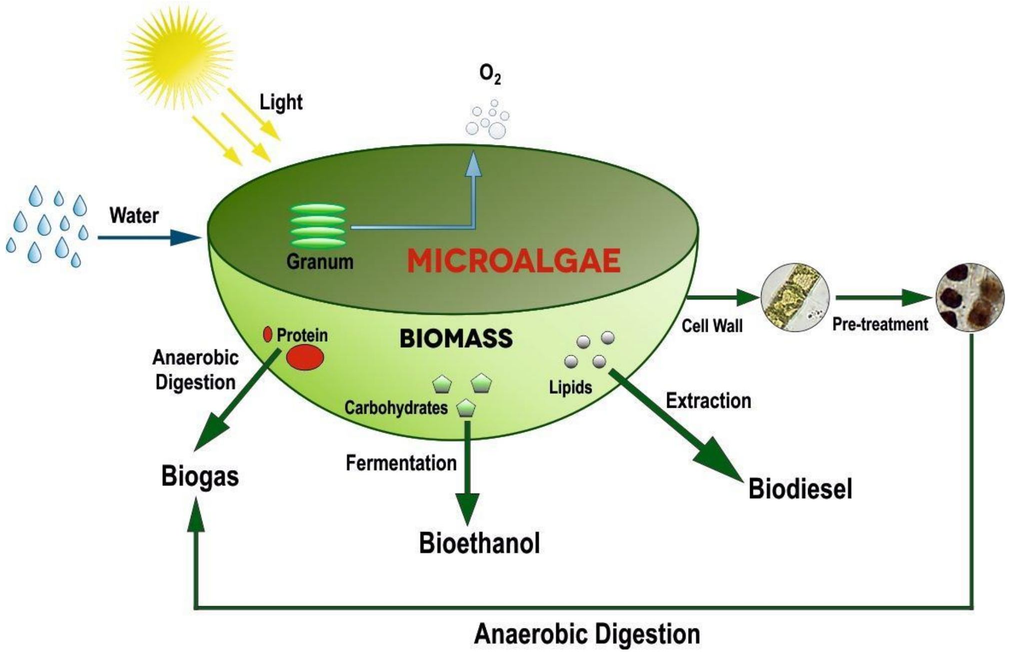

Figure 2.5. Biofuel production from microalgae Adapted from WANG & YIN (2018)

A beneficial characteristic of microalgal biomass grown in sewage treatment systems in association with bacteria, is its tendency to form flakes and aggregates, which eases separation mechanisms through sedimentation. In addition, these systems have a high productivity, since it is a mixotrophic environment, in which microalgae use both organic and inorganic carbon sources, as well as obtain energy through sunlight, which reduces the loss of biomass in periods of too little or too much lighting (photoinhibition) (PARK; CRAGGS; SHILTON, 2011; PASSOS; FERRER, 2014; XIA; MURPHY, 2016).

Microalgal biomass should be efficiently separated for promoting a clarified final effluent, but also for recovering and processing the biomass into valuable products. Many studies have suggested microalgal biomass harvesting and conversion into biofuels (biogas, bioethanol, biomethane and biodiesel), animal feed, bioplastics, pigments and biofertilizers (ARASHIRO *et al.*, 2020; ARASHIRO *et al.*, 2018; DÍEZ-MONTERO *et al.*, 2020; MARÍN *et al.*, 2019; PASSOS *et al.*, 2017; RUEDA *et al.*, 2020). Most researchers indicated that anaerobic degradation of microalgae for biogas production is the most straightforward downstream process, since it employs the entire biomass, without requiring complex separation procedures (GANESH SARATALE *et al.*, 2018; MONTINGELLI *et al.*, 2015; ZABED *et al.*, 2020).

2.4.1 Microalgal biomass anaerobic co-digestion

In respect to the UASB+HRAP system, a possible route for microalgal biomass valorization is through its recirculation and co-digestion in the UASB reactor with sewage. Indeed, anaerobic co-digestion of microalgae with other co-substrates have shown improvement in process rate and methane yield (GONÇALVES et al., 2020; SOLÉ-BUNDÓ et al., 2018) (Table 2.4). For this, the knowledge of the composition of microalgal biomass is important since in terms of energy production, the lipids from microalgae have a higher theoretical methane yield, followed by proteins and, finally, of carbohydrates (ZABED et al., 2020).

Table 2.4. Methane yield after microalgae co-digestion with different organic residues

Reactor	Microalgae Species	Co-substrate	Ratio MA:co-S[1]	Methane Yield (CH_4 mL·g VS^{-1})		Reference
				Microalgae	Co-digestion	
Batch	*Chlorella* sp.	Secondary sludge	0.41:0.59	124	295	(WANG, M. *et al.*, 2013)
Batch	*S. capricornutum*	Secondary sludge	0.25:0.75	271 ± 6	394 ± 14	(CAPORGNO; TROBAJO; *et al.*, 2015)
Batch	Mixed Culture	Secondary sludge	0.2:0.8	82	187 ± 9	(ARIAS *et al.*, 2018)
Semi-continuous	*Chlorella* sp.	Secondary sludge	0.21:0.79	230	253	(WANG, M.; PARK, 2015a)
	Micractinium sp.			209	236	
Batch	Mixed Culture	Secondary sludge	0.25:0.75	106.3 ± 0.2	258.3 ± 3.9	(ARASHIRO, LARISSA T. *et al.*, 2019)
			0.50:0.50	106.3 ± 0.2	237.6 ± 1.7	
Batch	*Chlorella* sp.	Primary Sludge	0.5:0.5	24.8 ± 2.0	31.1 ± 7.5	(KIM, J.; KANG, 2015)
Batch	Mixed Culture	Secondary sludge	0.25:0.75	160	460	(SOLÉ-BUNDÓ *et al.*, 2018)

[1] **Microalgae: co-substrate in volatile solids basis**

Among the use of different co-substrates, co-digestion with other by-products from STP is an interesting alternative to increase biogas production, while promoting a circular resource recovery approach (GONZÁLEZ-FERNÁNDEZ et al., 2011; SOLÉ-BUNDÓ; PASSOS; et al., 2019; ZHEN et al., 2016). Although some results have shown values of methane yield for mono-digestion (only one substrate) close to or even higher compared to co-digestion with microalgae, there are many other advantages, as reduction of volatile solids, increase in the hydrolysis rate and improvement in microalgae mono-digestion (CAPORGNO *et al.*, 2015; WANG *et al.*, 2013; WANG; PARK, 2015). For instance, a previous study attained 243 CH_4 mL.g VS^{-1} for mono-digestion of activated sludge, while the value was quite similar when co-digested *Micractinium sp* (236 CH_4 mL.g VS^{-1}) (WANG; PARK, 2015). However, the authors reported a 47.5% reduction in VS after co-digestion with secondary sludge, in addition to an increase in the biodegradability of microalgal biomass due to the increased hydrolysis caused by microorganisms present in the sludge.

Up to date, there was only one study found evaluated the co-digestion of microalgae in UASB reactors. In this study, on a pilot scale and continuous flow, methane yields were compared between mono sludge digestion in the UASB reactor and a mixture between that sludge and mixed microalgae culture. The results showed a worsening of the methane yield when the co-digestion practice was used (0.18 $Nm^3CH_4{\cdot}kgCOD_{removed}^{-1}$ x 0.12 $Nm^3CH_4{\cdot}kgCOD_{removed}^{-1}$). The decrease in methane production was associated by the authors with the use of a flocculator based on organic, cationic polymers to improve the sedimentability of microalgae. Due to the formation of resistant flakes and a possible inhibition of anaerobic digestion in the UASB reactor, these were the results obtained (GONÇALVES *et al.*, 2020).

2.4.2 Microalgal biomass pre-treatment

Most microalgae species grown in HRAPs to treat sewage contain a resistant cell wall that acts as a mechanical barrier to protect them against predatory microorganisms. This cell wall varies from species to species and depending on numerous factors (sewage characteristics, environmental factors), but it is basically composed of cellulose, hemicellulose and pectin. An issue concerning the anaerobic digestion process is that this barrier hinders its accessibility and biodegradability (DAY; SLOCOMBE; STANLEY, 2012; SIALVE; BERNET; BERNARD, 2009). According to the literature, due to the complex cell wall structure, anaerobic digestion is limited by the hydrolysis step, when bacteria solubilise proteins, lipids and carbohydrates. Hydrolytic bacteria use exoenzymes to access the macromolecules present in the cell wall to access the intracellular material (Figure 2.6).

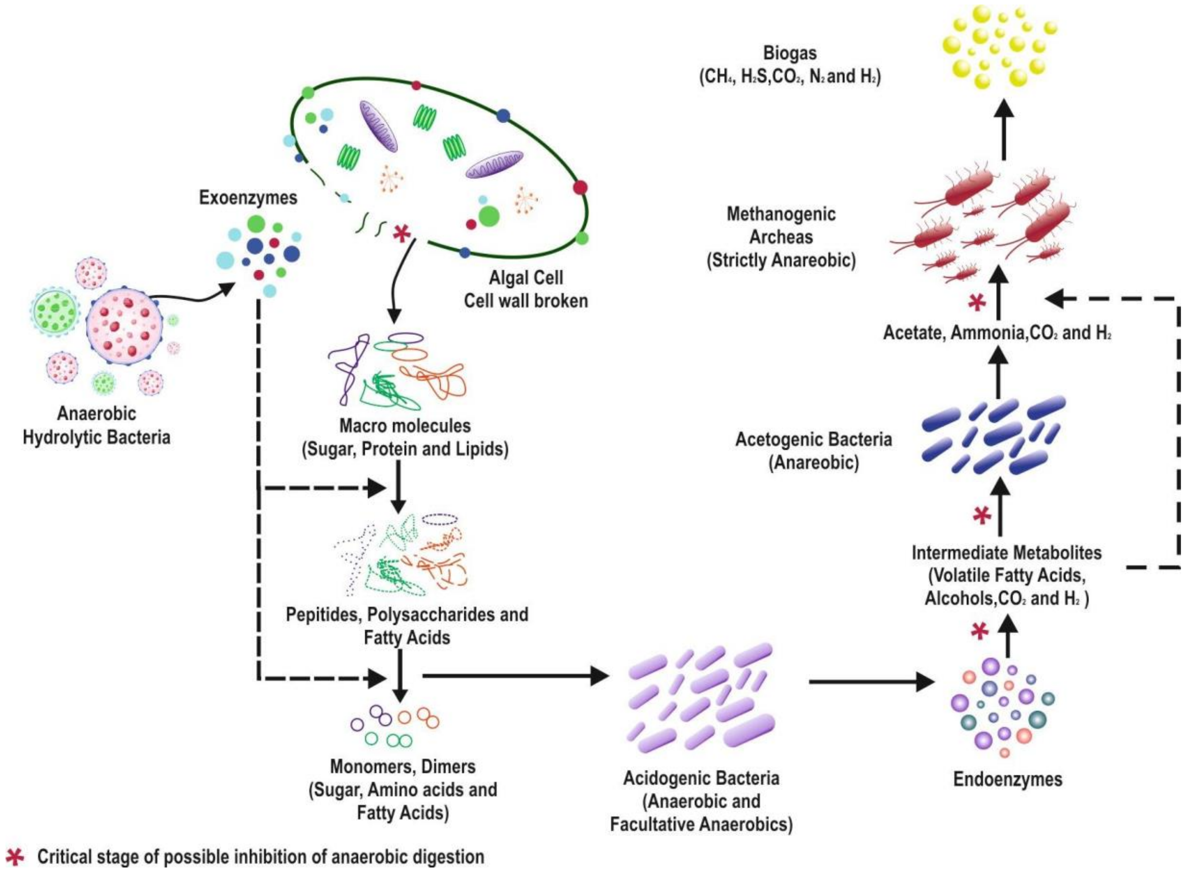

Figure 2.6. Schematic drawing of anaerobic digestion of microalgal biomass

The theoretical yield of methane obtained from microalgae is estimated between 0.47-0.80 L CH_4. gVS^{-1} (SIALVE *et al.*, 2009) however, experimental values are less due to the structure of the cell wall (CARRERE *et al.*, 2016; CARRILLO-REYES; BARRAGÁN-TRINIDAD; BUITRÓN, 2016; GONZÁLEZ-FERNÁNDEZ *et al.*, 2011; PASSOS; UGGETTI; *et al.*, 2014). In this scenario, the increase in methane yield may be carried out through microalgae co-digestion, but also by means of a pre-treatment step prior to anaerobic digestion of microalgae. This would disrupt the cell wall and increase the availability of intracellular macromolecules. Pre-treatment techniques for organic waste have been extensively investigated to improve the hydrolysis step of biomass and increase the biogas production (CARRERE et al., 2016). Several pre-treatment methods have been investigated for microalgae, including thermal, mechanical, biological (enzymatic) and chemical pre-treatment (CARRERE *et al.*, 2016; GONZÁLEZ-FERNÁNDEZ *et al.*, 2011; SARATALE *et al.*, 2018).

Among the studied techniques, thermal pre-treatment has been the most explored to increase methane yield from anaerobic digestion of microalgae, with application in full-scale systems. Results from previous studies are summarised in Table 2.5.

Table 2.5. Methane yield results after thermal pre-treatment prior to anaerobic digestion of microalgae

Microalgae	**Pre-treatment**	**Anaerobic Digestion**	**Results***	**Reference**
Mixed culture from STP	100°C 8 hour.	Continuous, 36ºC	Increase of 44%	(CHEN; OSWALD, 1998)
Scenedesmus	70, 90 °C; 3 hour	Batch, 35°C	Increase from 12% to 200% (different temperature)	(GONZÁLEZ-FERNÁNDEZ; SIALVE; BERNET; *et al.*, 2012b)
Chlorella *sp.* *and* Scenedesmus *sp*	50, 80 °C; 30 min	Batch, 37°C	Increase from 2% to 17% (different temperature)	(CHO *et al.*, 2013)
Chlorella vulgaris and *Scenedesmus obliquus*	165°C 30 min.	Batch, 38°C	Increase of 44%	(OMETTO *et al.*, 2014)
Mixed culture from STP	95°C 10 hours	Continuous, 37°C	Increase of 20%	(PASSOS; FERRER, 2014)
Mixed culture from STP	95°C 10 hours	Continuous, 37°C	Increase of 72%	(PASSOS; FERRER, 2014)
Mixed culture from STP	75°C 10 hours	Continuous, 37°C	Increase of 67%	(PASSOS; FERRER, 2014)
Scenedesmus obliquus	120°C 1,5 hours	Batch, 38°C	Increase of 37%	(CAMPO *et al.*, 2018)
Scenedesmus obtusiusculus	98°C 6 hours	Batch, 37°C	Increase of 76%	(CORTÉS-CARMONA *et al.*, 2018)
Mixed culture from STP	120°C 1 hours	Batch, 35°C	Increase of 18%	(PASSOS *et al.*, 2018)
Mixed culture from STP	180°C 15 min	Batch, 35°C	Increase of 18%	(PASSOS *et al.*, 2018)
Scenedesmus sp	100-120°C 2 hours	Continuous 35°C	Increase of 108%	(SCHWEDE *et al.*, 2013)
Mixed culture from STP	75°C 10 hours	Batch, 35°C	Increase of 62%	(SOLÉ-BUNDÓ *et al.*, 2018)
Mixed culture from STP	75°C 10 hours	Batch, 35°C	Increase from 30% to 67% (different ratio)	(ARASHIRO, *et al.*, 2019)

*** Compare with control**

According to the literature, thermal pre-treatment temperatures applied range between 55 and 170 °C and, therefore, may be. subdivided into three groups: low temperature (<100 ° C), hydrothermal (> 100 ° C) and steam explosion or thermal hydrolysis (140-170 ° C, 4-6 bar). In this PhD research, low temperature thermal pretreatment will be assessed. The main advantage of low temperature thermal pretreatment is its lower energy demand, associated with an improvement in microalgae methane yield. Previous study showed how this technique increased organic matter solubilization and increased anaerobic biodegradability, allowing a positive return in energy balance of the treatment system (PASSOS; UGGETTI; *et al.*, 2014). Moreover, other authors have mentioned that the thermal pre-treatment, when followed by anaerobic digestion at ambient temperature, can be used to reduce the energy requirement (JANKOWSKA; SAHU; OLESKOWICZ-POPIEL, 2017).

During thermal pretreatment, microalgal biomass is affected by applied temperature and exposure time (PASSOS *et al.*, 2013). Previous study, have suggested that temperature is the most influencing parameter rather than exposure time (ALZATE *et al.*, 2012). The authors tested different temperatures between 110 and 170 ° C and observed that regardless of the exposure time, the bioavailability and biodegradability of microalgal biomass organic compounds increased concomitantly with the increase in temperature. On the other hand, a disadvantage of thermal pre-treatment seems to be the high exposure time required when low temperatures are applied, which requires optimization (CARRERE *et al.*, 2016). Published results vary depending on microalgae used for each study. For instance, if pure microalgae cultures or mixed cultures grown in sewage treatment HRAPs. Literature has shown that mixed microalgal biomass forming flocs with extracellular polymers may hinder biomass hydrolysis (PASSOS; FERRER, 2014). Previous study obtained results of 70% in methane yield increase after microalgae thermal pre-treatment at 75 and 95 °C, but with exposure times, of 10 hours.

In general, most studies showed an increase in methane yield after thermal pre-treatment microalgae (Table 2.4). However, some results have shown a decrease from 3% to 13% of methane yield after applying o temperatures of 55 ºC for 24 hours (ALZATE *et al.*, 2012). Probably the extensive exposure time for pre-treatment may have caused microalgal biomass degradation during already during that step (AVILA *et al.*, 2020; CARVAJAL; PEÑA; PÉREZ-ELVIRA, 2013).

2.5 Applied mathematical models for microalgae-based system to treat sewage

Mathematical models are simplified and generalized representations of one system, taking into account the main characteristics that it represents (REICHERT *et al.*, 2001). They are composed of: (i) mathematical balancing equations, which describes concentrations in the reactor resulting from biological and chemical conversions and transport phenomena; (ii) parameters or coefficients that can be stoichiometric (balance of equations) or reaction rates and; (iii) data from experimental observations (HENZE *et al.*, 2006).

Commonly, biological processes in sewage treatment reactors are modelled using Jacques Monod kinetic rate of growth equation, which considers the availability of substrates and limiting factors (SOLIMENO *et al.*, 2017; SOLIMENO; GARCÍA, 2019). Modelling process in this case uses coefficients related to the specific maximum growth rate of the microorganism (µAm), halfsaturation constant or affinity coefficient (Kn) and the yield coefficient (YA), which should be applied only for balanced growth and not in the latency phase (HENZE *et al.*, 2008).

To enable the use of a reliable model, calibration steps must be performed. During this process, specific coefficients are adjusted in order to approximate estimated data to those measured, followed by verification, based on the analysis of residues and, in more specific cases, validation (HENZE *et al.*, 2008; VON SPERLING, 2007). In addition, sensitivity analysis can be used in order to understand the impact of each component and process on the modelled system.

The mathematical modeling of sewage treatment systems has been explored for decades. The first mathematical model focused on the simultaneous growth of microalgae and heterotrophic bacteria was developed in 1983 (BUHR; MILLER, 1983). This model was expanded in 1989, incorporating microalgae growth limitation parameters, as solar radiation (KROON *et al.*, 1989). However, it was still basic models with low interaction capacity, mainly due to the technological limitations. Recently, more complex models have been developed to represent the simultaneous growth and synergies, many of them based on models developed by the International Water Association (IWA), specifically the Activated Sludge Model 3, (ASM3) and River Model Quality Model 1 (RWQM1) (HENZE *et al.*, 2006, 2008; REICHERT, PETER *et al.*, 2001; SOLIMENO; GARCÍA, 2019).

Among those, RWQM1 was modified, calibrated and validated for HRAPs, however results were unable to reproduce the real system (BROEKHUIZEN *et al.*, 2012). The authors concluded that a deeper adaptation of the proposed base equation in the model was needed. Another study adapted the Activated Sludge Model 3 (ASM3) to describe a photobioreactor with constant lighting. This study concluded that, although the model was able to represent the growth of microalgae, the concentrations of biomass and the effluent ammonia, it failed to represent ammonia-oxidizing bacteria (VAN DER STEEN *et al.*, 2015). For the interaction of microalgae and bacteria, there are few models that are suitable for combining biochemical processes and the simultaneous effects of light intensity, temperature, pH, carbon limitation and high dissolved oxygen rates, and its effect on biomass growth (SOLIMENO et al., 2017). A comprehensive 3D model was implemented in Delft3D, based on ASM3, for facultative ponds (SAH *et al.*, 2011). Similarly, another study proposed using AQUASIM 2.1 software for including the effect of the wind (HO *et al.*, 2019). Although in both proposed models the results were promising, their applications to HRAP were restricted due to differences in biochemical processes and mixing conditions in tested ponds. The main mathematical models that have been developed to simulate biokinetic processes of autotrophic organism and bacteria are summarised in Table 2.6. It may be noticed how all models were based on the equations of Monod, Lambert and Beer's Law, and Arrhenius equation to explain the interactions along with nutrient availability, light and temperature respectively.

Table 2.6. Comparison of the general features of integrated mechanistic models for microalgae and bacteria interactions

Model	Based Model	Platform	Main process considered	Most relevant features
RWQM1 (Reichert *et al.*, 2001)	Authors	Conceptual Model	- Light limitation - Temperature dependence - Chemical equilibrium	-pH dynamics - Water quality - Mass balance equations in terms of BOD.
PHOBIA (Wolf *et al.*, 2007)	Wanner and Gujer (1986) and Wanner and Reichert (1996)	AQUASIM	- Biological conversion processes - Chemical conversion processes	- First approach in the mathematical modeling of the dynamics of phototrophic biofilms.
Sah et al. (2011)	ASM2, CWM1, RWQM1	Delft3D	- Light limitation and attenuation - Temperature dependence	- Anaerobic processes - CFD solution of a coarse 3D facultative pond model.
Broekhuizen et al. (2012)	RWQM1	Conceptual Model	- Light limitation - Temperature dependence - Chemical equilibrium	-Increase the respiratory losses by the heterotrophic osmotrophs
Van der Steen *et al.*, (2015)	ASM3	AQUASIM	- Light limitation - Photorespiration - Chemical equilibrium	- Failed in ASM-3 to represent ammonia-oxidizing bacteria.
BIO_ALGAE (Solimeno *et al.*, 2015)	ASM3, RWQM1	COMSOL MultiphysicsTM	- Light limitation and attenuation - Temperature dependence - Photorespiration - Chemical equilibrium	- Carbon limitation - Photorespiration - Transfer of gasses to atmosphere - Light intensity.
ASM-A (Wágner *et al*. 2016)	ASM-2d and their own	Conceptual Model	- Light limitation	
BIO_ALGAE 2 (Solimeno *et al.*, 2019)	ASM3, RWQM1, BIO_ALGAE	COMSOL MultiphysicsTM	- Light limitation and attenuation - Temperature dependence - Photorespiration - Chemical equilibrium	- Implementation of on-demand dioxide carbon injection for pH control.
Ho *et al.* (2019)	ASM2, RWQM1	AQUASIM	- Light limitation and attenuation - Temperature dependence	-Effect of wind
Yang et al. (2020)	ASM3, AMS-A	AQUASIM 2.0	- Light limitation - Photorespiration - Chemical equilibrium based on dissolve oxygen (DO) concentration.	- Inclusion of phosphate accumulating in microalgae-bacteria consortia.
ALBA (Casagli *et al.*, 2021)	RWQM1, BIO_ALGAE 2	AQUASIM	- Light limitation - Temperature dependence - Photorespiration - Chemical equilibrium	- Detailed model for pH computation.
ABACO (Sánchez-Zurano *et al.*, 2021)	RWQM1, BIO_ALGAE 2	MATLAB	- Light dependence -Endogenous respiration. - Growth and nutrient consumption as a function of nutrient availability.	- Features of heterotrophic and nitrifying bacteria.

A literature review on the models developed specifically for HRAP using Scopus database (Elsevier) and the key parameters "high AND rate AND algal AND pond AND modeling OR modeling OR model" was limited to 127 publications during 10 years (2011-2021). Some of the mentioned studies showed differences in the techniques and methodologies used for investigating advanced modeling strategies for microalgae-based systems. For example, a study used artificial neural networks to determine the production of microalgae, being able to estimate growth. However, the authors did not differentiate microalgae from other microorganisms, such as bacteria, generating uncertainties in the results (SUPRIYANTO *et al.*, 2019). There is still insufficient scientific information on advanced models considering the interaction of microalgae and bacteria and its influence on physical-chemical parameters.

More advanced model was developed simulating microalgae growth mechanistics inspired in the RWQM1 (SOLIMENO *et al.*, 2015). This mathematical model was built using COMSOL Multiphysics^{TM} platform and included microalgae growth under carbon limitation, gas transfers to the atmosphere and photorespiration (considering the kinetics of photosynthesis and photoinhibition).For this, microalgae growth was described as a function of light intensity, temperature and availability of nutrients. This model was further improved, based on the ASM3 model, and was named BIO_ALGAE, with particular application in HRAPs (SOLIMENO *et al.*, 2017). In BIO_ALGAE, the growth processes of autotrophic and heterotrophic bacteria and hydrolysis were added. The model was successfully validated for HRAP treating sewage in Barcelona, Spain at a one-year period and with systems operating at 4-day and 8-day HRT (SOLIMENO; GARCÍA, 2019). BIO_ALGAE was improved once more, transforming in BIO_ALGAE 2, where CO_2 injection and pH control were added. Another gain in this model was the insertion of the correlation between mass transfer coefficient and the reactor hydrodynamics (SOLIMENO; GÓMEZ-SERRANO; ACIÉN, 2019). In BIO_ALGAE 2, the variation of the biomass performance was treated as a function of pH, temperature and dissolved oxygen, continuously in daily and seasonal cycles, being calibrated with full scale reactors. The latest model published for HRAP was the ALBA (CASAGLI *et al.*, 2021). This model was based in BIO_ALGAE 2 and calibrated for a HRAP located in France, while it was implemented in AQUASIM platform. The model was able to predict the behaviour of HRAP in short and long terms, with the novelty of choosing the kinetic approach to describe the nutritional limitation and also in the level of pH representation. Furthermore, this study was focused on HRAP paddle-wheel velocity and its influence on oxygen consumption and production.

It is important to note that most models were developed and validated in Europe's temperate climate. A previous study at Australia evaluated the interaction between microalgae and bacteria, based on chemical equilibria, implemented in MATLAB (BELLO *et al,* 2017). The model compared with data output from BIO_ALGAE 2 and also from results obtained in tropical conditions. Although the authors affirmed the potential for using the model to optimize microalgae growth, it showed some deficiencies, as no differentiation between autotrophic and heterotrophic bacteria. A South African mathematical modeling that included microalgae, heterotrophic and autotrophic ammonia oxidizing bacteria and their interaction with organic matter and nutrients (N and P) was developed (VAN DER MERWE; BRINK, 2018). The researchers also found deficiencies, mainly in relation to the prediction of COD and volatile suspended solids (VSS). In this context, there seems to be a scientific gap for mathematical models in tropical climates, as Brazil, specifically for HRAPs used for UASB effluent post-treatment.

2.6 Life cycle assessment (LCA)

As previously discussed, the decision on selecting a particular technology for sewage treatment may be a challenging decision, that involves aspects, as implementation cost and environmental sustainability. For Brazilian reality, UASB reactor is a consolidated technology, often requiring a post-treatment step for increasing effluent quality and water reuse. In this way, life cycle assessment (LCA) may be considered an important tool to ease the decision-making on STPs based on UASB reactors.

Life cycle assessment may be defined as an analytical technique of quantitative and qualitative assessment of several environmental impacts of a product or process throughout its life cycle. It is composed of four phases: objective definition and scope, Life Cycle Inventory (LCI), Life Cycle Impact Assessment (LCIA) and Results interpretation. Life Cycle Impact Assessment (LCIA) procedures can be distinguished between "single phase" procedures and "multiphase" procedures. The main reason for a single-phase approach is its simplicity. In this procedure, the output of the Life Cycle Inventory phase is directly related to reference values (such as environmental quality standards or emission reduction costs), followed by inclusion of the resulting values. Examples of this procedure are the "Critical Volume" and "Ecopoints" methods. Due to its greater transparency, the multiphase procedure has been mostly applied by researchers (FERREIRA, 2004). Figure 2.7 shows the flowchart of the phases and their interconnections that compound a Life Cycle Assessment study.

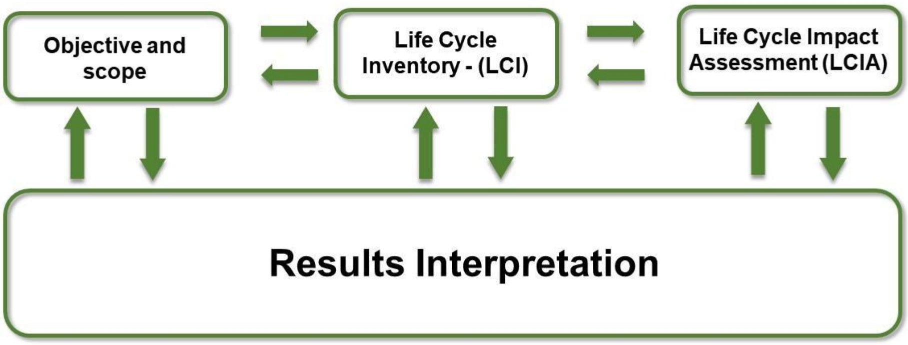

Figure 2.7. Life Cycle Assessment phases and interconnections (Adapted from NBR14004/2009)

The LCIA phase aims to provide additional information to assist the evaluation of the life cycle inventory (LCI), aiming to better understand its environmental significance (ABNT, 2014). LCIA associates the results of the LCI with its impact categories. For each impact category, an indicator is selected and the results provide information on the environmental issues associated with the inputs and outputs of the product system (VALDIVIA *et al.*, 2013). The selection and definition of the impact categories are carried out based on knowledge of the environmental processes and mechanisms. This phase includes mandatory and optional elements. The mandatory elements convert the results of the LCI into category indicators (environmental profile) for the different impact categories and the optional elements serve to normalize, group, the weight of the indicator results and data quality analysis techniques (NBR ISO 14044/2009).

In this phase specific softwares can be used, such as openLCA, SimaPro, GaBi, Umberto and Quantis. The most used is SimaPro, which was developed by the Dutch company Pre-Sustainability (JUNQUEIRA, 2016). With regard to the evaluation of impacts, there are numerous evaluation methods, such as CML, TRACI, Eco-Indicator 99, IMPACT 2002+, EPS 2000, ReCiPe and others. ReCiPe is an evolution of the CML method. The CML method, developed by scientists at the Center for the Environmental Science of Leiden University, has been the method mostly used in LCA work because of the broad categories it considered and its "problem-oriented approach to the environment." However, in 2008, the ReCiPe method was presented, which combined CML (problem oriented) and Eco-Indicator 99 (environmental damage oriented) methods. Consequently, it has become the most qualified method and used for new studies, even in those that consider single, isolated or reduced impact categories (GALLEGO *et al.*, 2008).

Even with different characteristics, these methods are basically shown in two points: *midpoint* and *endpoint*. At the midpoint, all flows listed in the LCI are aggregated into impact categories, according to a common characteristic in the cause-and-effect chain of the environmental mechanism. These elements are indicators of potential impact. The endpoint consists of characterizing the consequences of *midpoint* categories in protected areas at the *endpoint* of the environmental path, connecting the results of the inventory with the respective impact on the protected areas. Figure 2.8 represents the relationship between the results of the LCI and the *midpoint* and *endpoint* impact categories, based on ReCiPe method.

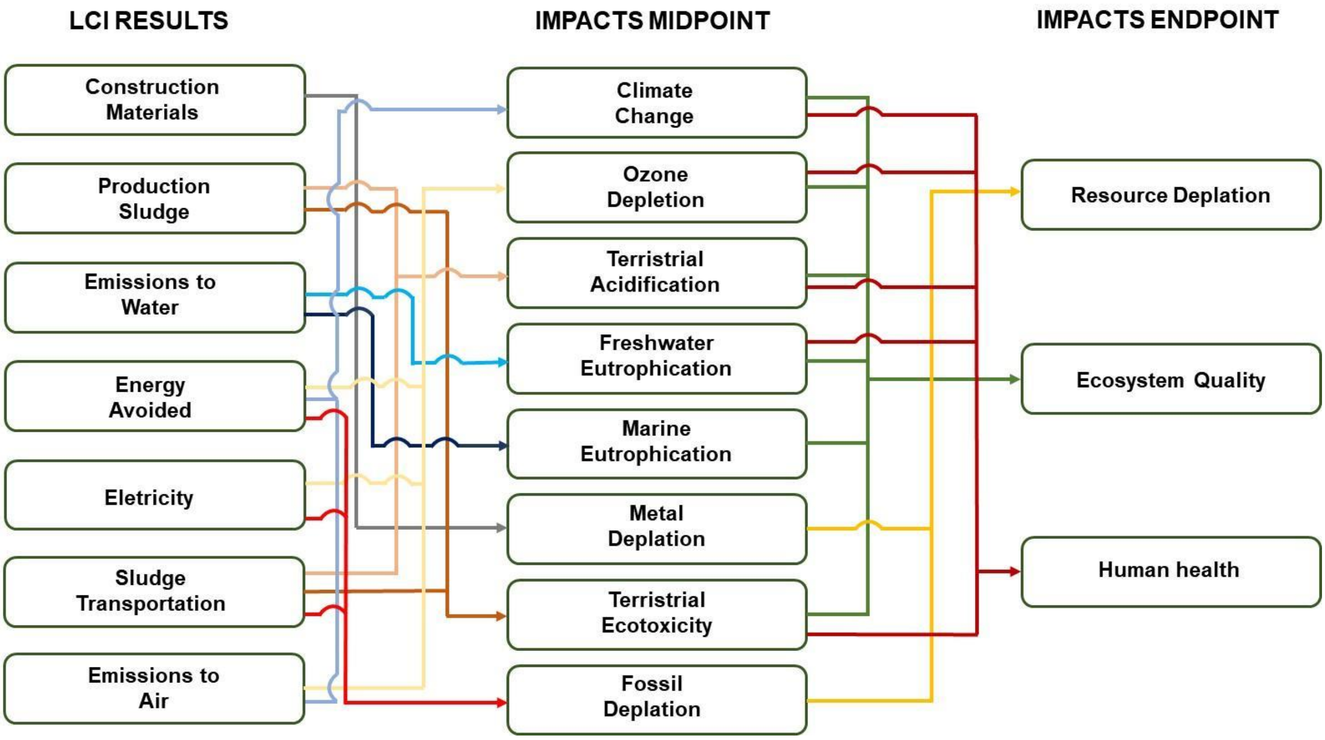

Figure 2.8. Relationship between the results of the LCI and the midpoint and endpoint impact categories (Adapted from NBR14004/2009)

Finally, in the interpretation phase, the results obtained are combined, analysed and identified according to the objective, providing information on emissions to the environment and the life cycle phases that contribute to certain environmental impacts. The results of the interpretation serve as conclusions and recommendations for improvements to reduce environmental impacts and assist in decision (SETAC, 1993).

For the analysis of uncertainty in LCA, the Pedigree Matrix is used. The matrix was introduced into the LCA field by WEIDEMA; WESNAS, (1996) and applied to the Ecoinvent database since 2005. The matrix considers two types of uncertainty parameters: intrinsic variability and uncertainty due to imperfect data without temporal and spatial verification (MULLER *et al.*, 2016). The LCA applied to the sewage treatment may be an important tool for improving decision making For developing sustainable STPs in Brazil, LCA may enhance circular economy approach for recovering by-products considering solid, liquid and gaseous phases.

2.6.1 LCA for UASB reactor STPs

The first studies using LCA for sewage treatment date from the 1990s (COROMINAS *et al.*, 2013). Searching for articles published on the Scopus platform, which included the keywords: “Life Cycle Assessment” AND “Wastewater Treatment” OR “Sewage”, only over 1,800 publications have been found dated from the 1990s until present date. Specifically, when the search included UASB reactors, publications dropped to approximate 20. LCA studies have not been found for HRAP systems post-treating sewage from UASB reactors. Table 2.7 summarises the main articles using LCA for evaluating STPs with UASB reactors or HRAPs. From the data, it is possible to observe how LCA has supported decision-making on treatment technologies considering different situations. Moreover, it is possible to identify gaps to improve its use, in order to amplify the accuracy and applicability.

Table 2.7. Comparison of the general features of integrated mechanistic models

Reference (Local)	Objectives	Method/ Software	Impact Categories	Main results
Tillman et al., (1998) -Sweden	Compare two alternative scenarios to collection, transport and treatment sewage.	Just LCIA	Just LCIA	Both scenarios with lower impact than the existing.
Lundin et al., (2000)- Sweden	Compare conventional treatment in different scale, with alternative separating resources.	Just LCIA	Just LCIA	Large-scale system with resources separation was more advantageous.
Dixon et al., (2003) -UK	Assess and compare the environmental impact of wetland to a conventional technology.	ReCiPe, /SimaPro	CO_2 emission and solid production.	The impact is reduced if the soil excavated be used in the bed.
Hospido et al., (2007)-Spain	Evaluate 4 STP (125,000 inhab.) with different technologies (primary and secondary treatment).	CML / SimaPro	EU, OZD, GW, AC, PO, AD, HT, ME, FE, TE.	High energy consumption in secondary treatment. Anaerobic Digestion reduces the impact of sludge in the soil.
Gallego et al., (2008)-Spain	Analyze different STP for small system.	CML / SimaPro	AD, GW, OZD, TE, PO, AC, EU.	Steps with the biggest contribution to the impact: water discharge, operation and, implementation.
Renou et al., (2008)-France	Evaluate methods of LCIA for anaerobic tank, activated sludge and clarification.	CML; Eco Indicator / SimaPro	AC, EU, RD, GW, HT.	Special attention for human toxicity due the difference between evaluated methods.
Weiss et al., (2008)-Sweden	Compare impacts and use of natural resources for: infiltration, chemical precipitation and filters for phosphorus removal.	Just LCIA	AD, GW, EU.	Chemical precipitation was more favorable to environmental conservation.
Foley et al., (2010)-	LCIA of anaerobic reactor, activated sludge, biological removal of nutrients and stabilization pond.	Just LCIA	Just LCIA	The better the quality of the final effluent, the greater the impacts.
Lopsik, (2013) Estonia	LCA to evaluate constructed wetlands and active sludge technologies.	ReCiPE / SimaPro	All off midpoint and endpoint categories.	The main impact of the wetland was on the use of clay expanded. Sludge impacts activated were associated with energy use and quality of final effluent.

Table 2.7 (continuation). Comparison of the general features of integrated mechanistic models

Reference (Local)	Objectives	Method/ Software	Impact Categories	Main results
Gutierrez, (2014) Brazil	LCA of the operational phase of facultative ponds, anaerobic ponds, maturation ponds, upflow anaerobic sludge blanket (UASB) and constructed wetlands system	ReCiPE / SimaPro	All off midpoint and endpoint categories	Major differences among the technologies evaluated were related to sludge production and atmospheric emissions.
Amaral et al (2019)-Brazil	Complete LCA for full scale plants, using UASB reactor, to evaluate different options for final disposal of biological sludge and biogas	ReCiPE / SimaPro	GW, OZD, TE, FWE,	The use of biogas for drying sludge and destined for agriculture was the best option.
Garfi el al (2017) Spain	LCA to compare: Actived sludge; constructed wetlands and HRAP	ReCiPE / SimaPro	RD, HT, EU, TE	Nature-based solutions were the most environmentally friendly alternatives.
Arashiro et al (2018)-Spain	LCA to evaluate HRAP system for wastewater treatment where microalgal biomass is valorised for biogas production and biofertilizer and compare with actived sludge.	ReCiPE / SimaPro	RD, HT, EU, TE	HRAP systems seemed to be more economically feasible when combined with biofertilizer production instead of biogas.
Ferreira et al (2019) - Spain	LCA to evaluate HRAP systems in different scale analysing: biogas upgrading, biomass production and biomass valorisation.	ReCiPE / SimaPro	All off midpoint and endpoint categories	For the simulated real-scale plant, the biomass recovery showed the highest environmental impacts.

Note: LCI – Life Cycle Inventory. Impact Categories: Abiotic Depletion (AD), Global Warming (GW), Ozone Depletion (OZD), Human Toxicity (HT), Freshwater Ecotoxicity (FWE), Marine Ecotoxicity (ME), Terrestrial Ecotoxicity (TE), Photochemical Oxidation (PO), Acidification (AC), Eutrophication (EU), Resource Depletion (RD) and Greenhouse Gases (GHE).

From IWA's effort on encouraging the use of LCA on STPs, several researches were carried out, mainly for the European scenario (FOLEY *et al.*, 2010; GALLEGO *et al.*, 2008; HOSPIDO *et al.*, 2008; LOPSIK, 2013; TILLMAN *et al.*, 1998). In these studies, different technologies and combinations were evaluated, including: sewage collection (collection network), pumping and treatment (TILLMAN *et al.*, 1998); construction, operation and demolition (DIXON *et al.*, 2003; FOLEY *et al.*, 2010; LUNDIN *et al.*, 2002; RENOU *et al.*, 2008) and only the STP operational phases (CARTES *et al.*, 2018; GALLEGO *et al.*, 2008).

Few studies were published concerning Brazilian and Latin America scenarios. A previous study applied LCA for Brazilian conditions comparing the technologies: facultative ponds, anaerobic ponds, maturation ponds, upflow anaerobic sludge blanket (UASB) and constructed wetlands. The authors concluded that the major differences among the technologies evaluated were related to sludge production and atmospheric emissions. For instance, the emission of methane was 22 times higher for UASB reactors followed by constructed wetland, compared to UASB reactors followed by facultative ponds (GUTIERREZ, 2014). Another important study was performed on the environmental, social and economic LCA in full scale plants, using UASB reactor, to evaluate different options for final disposal of biological sludge and biogas. In this study, the authors evaluated 3 scenarios: i) the use of biogas from UASB, as an energy source for drying and sanitizing the sludge to be used in agriculture; ii) the combustion of sludge to use heat to dry the dewatered sludge, with the destination of the ashes for agriculture and, iii) the same scenario ii, but, the final destination of the ashes being the sanitary landfill. According to the results, the scenario in which biogas was used for drying sludge for agriculture purposes, was the best scenario, due to the indicators for terrestrial acidification and terrestrial ecosystem ozone formation (AMARAL et al., 2019).

Regarding LCA for HRAPs, few studies were published so far. A previous study compared 3 different sewage treatment technologies: i) activated sludge; ii) constructed wetlands and iii) HRAPs. The authors concluded that nature-based solutions were the most environmentally friendly alternatives (constructed wetlands and HRAPs), while the conventional plant showed the worst results, mainly due to the high electricity consumption from mechanical aeration in activated sludge. Moreover, regarding constructed wetland and HRAPs, the systems showed similar results in terms of environmental impact, but HRAPs were less expensive alternatives (GARFÍ *et al*., 2017).

Other researches also performed LCA to evaluate microalgal biomass potential uses. Among the most sustainable valorization pathways the production of biofertilizers and biofuels were preferred (AMARAL, *et al,* 2019; ARASHIRO *et al.*, 2018). In terms of biogas production, anaerobic co-digestion was shown as a viable downstream process, whether or not followed by biomass pre-treatment (CAMPBELL *et al.*, 2011; COLZI LOPES *et al.*, 2018; FERREIRA *et al.*, 2019).

3 HYPOTHESIS / OBJECTIVES

3.1 Hypothesis

Based on a critical analysis of the literature, the following hypotheses were proposed and tested:

1) High Rate Algal Ponds are efficient units for UASB reactors post-treatment, removing organic matter and nutrients. This system applied to tropical climates shows efficient removal rates when operated at 4 hours of HRT.

   Premise: Although in Brazil UASB reactors are widely applied for sewage treatment, the technology requires post-treatment for attaining discharge values of Brazilian legislation. HRAPs have been investigated with successful results for sewage treatment in temperate climate conditions. In these scenarios, during summer, the ponds may operate at 4 days HRT, while during winter, a higher HRT of 8 days is needed. In Brazil, the tropical climate does not show abrupt variations along the year and, therefore, climatic conditions are ideal for a constant growth of microalgae. (Objective 1)

2) Hormones and pharmaceuticals are micropollutants detected in raw sewage. UASB reactors are inefficient in removing these compounds, with less than 50% in terms of concentration. After HRAP pos-treatment (UASB + HRAP system) higher removal efficiencies may be achieved, with values of 80% for hormones and 60% for the pharmaceuticals (Objective 2).

   Premise: The presence of micropollutants in raw sewage has been shown previously. Literature has demonstrated that UASB reactors are inefficient in removing these compounds, obtaining low and even negative removal efficiencies. Organic micropollutants are only considerably eliminated under aerobic conditions. The main routes for removing these pollutants are: sorption (adsorption and absorption), biodegradation, photodegradation, oxidation and volatilization. Biodegradation and sorption are the main routes for the removal of hormones and pharmaceuticals.

3) The microalgal biomass produced and harvested from HRAP used for UASB reactor effluent pos-treatment, when co-digested with raw sewage in UASB reactor, will improve the biogas production and have synergistic effect. (Objective 3)

   Premise: Co-digestion of different organic wastes has been proved efficient for increasing anaerobic digestion rate and biogas production from synergistic factors, as carbon/nitrogen ratio balance, moisture content balance, micronutrients addition, alkalinity, among many others. In UASB+HRAP systems, the mixture of sewage and microalgal biomass is able to promote a better balance in the content of total solids, C/N ratio and micronutrients.

4) The application of thermal pre-treatment using a energy neutral technique, as from solar heating will increase microalgal biomass solubilisation, anaerobic digestion rate and methane yield in UASB reactors. This will improve the energy balance of the STP proposed (Objective 4).

   Premise: Due to microalgae complex and resistant cell wall structure, microalgae requires a pre-treatment step prior to its anaerobic digestion or co-digestion processes. It has been proven that thermal pre-treatment is efficient in disrupting its cell wall and increasing anaerobic biodegradability. However, conventional thermal pre-treatments use external energy, which decreases the energy balance in UASB+HRAP STPs.

5) The life cycle assessment comparing different post-treatment technologies for UASB-based STPs will demonstrate that HRAPs is the best option considering a circular economy approach and the principles of sustainability, particularly for Brazilian scenario (Objective 5).

   Premise: The Brazilian scenario for the sewage treatment shows extensive use of UASB reactors. Usually, to achieve the Brazilian legislation for effluent discharge in water bodies, UASB-based STPs require an aerobic post-treatment step. There are several options already evaluated and consolidated to post-treat UASB reactor effluents. LCA shows advantages and disadvantages of technologies and may be used to compare them. Moreover, HRAP has been proved efficient to post-treat UASB reactor effluents in experimental terms, while there are no studies comparing HRAP with other aerobic post-treatment technologies.

3.2 General Objective

To evaluate the use of HRAPs to post-treat anaerobic effluent from a UASB reactor applied for sewage treatment and to assess anaerobic co-digestion of harvested microalgal biomass and raw sewage in the UASB reactor, with and without thermal solar pretreatment of microalgae.

3.3 Specific Objectives

1- To assess the sewage treatment performance of the UASB + HRAP system in terms of nutrients, organic matter and solids removal, as well as microalgae production, and to validate the BIO_ALGAE 2 model using experimental data and to improve its design parameters.

2- To evaluate the removal of micropollutants in the UASB + HRAP system.

3- To compare the biogas production and composition in the UASB reactor fed with raw sewage and after microalgal biomass recirculation and its anaerobic co-digestion.

4- To compare the biogas production and composition in the UASB reactor fed with raw sewage and after microalgal biomass thermal pre-treatment with solar heating and its anaerobic co-digestion.

5- To investigate the use of life cycle assessment to evaluate HRAP technology in UASB-based STPs in comparison to other post-treatment solutions in terms of its environmental sustainability.

4 DECIPHERING MICROALGAE-BACTERIA INTERACTIONS IN RACEWAYS PONDS TREATING ANAEROBIC EFFLUENT IN TOPICAL CONDITION THROUGH BIOKINETIC MODEELING

Based on: Vassalle, L., Passos, F., Diez-Montero, R., Rueda, E., Rosa-Machado, A.T., Filho, C.R.M., Solimeno, A., Ferrer, I., García, J., 2021. Deciphering microalgae-bacteria interactions in raceways ponds treating anaerobic effluent in tropical conditions through biokinetic modelling. **Final preparation phase**

High rate algal ponds (HRAP) are an effective technology to complement wastewater treatment using UASB reactors. The UASB + HRAP configuration has already been proved to be efficient for pollutant and micropollutant removals, as well as for energy recovery. However, HRAP often operate at non-optimised conditions, with an imbalance between microalgae and heterotrophic bacteria. In this study, mathematical modelling was applied to understand and optimise the symbiotic relation between microalgae and bacteria in anaerobic-aerobic system under tropical conditions. To do this, the BIO_ALGAE model was calibrated considering HRAP treating the effluent of UASB reactors treating Brazilian domestic wastewater. BIO_ALGAE proved to be capable of simulating the system with excellent accuracy normalized root means square error (NRMSQE) (4-20%). Different scenarios were simulated by varying the HRAP hydraulic retention time (HRT) (4, 6 and 8 days). Results obtained showed an efficient removal of COD (70%), TSS (42%), N-NH_4 (57%) and P-PO_4 (30%) in the UASB+HRAP system. For the evaluated scenarios, the operation of HRAP at 4 days of HRT showed to be the optimal in terms of wastewater treatment and energy production with lower area requirement.

4.1 Introduction

In developing countries, sanitation is still a health and environment concern. According to statistics, 75% of Brazilian population is attended by a sewerage, however only 45% of them receive domestic wastewater treatment (i.e. 97 million inhabitants) (BRASIL, 2017, 2018b). Therefore, simplified wastewater treatment systems are important alternatives for enabling universal sanitation. Particularly in tropical countries, anaerobic reactors, as UASB, play an important role as technologies with simple and low cost operation (ANA, 2020; NOYOLA *et al.*, 2012)

Although UASB reactors are considered an attractive technology, since they remove organic matter and solids while generate biogas, the treated effluent usually requires a post-treatment step in order to meet the discharge standards (Chernicharo et al., 2018; Daud et al., 2018). In this context, microalgae-based systems, such as high rate algal ponds (HRAP), have been investigated as potential alternatives with positive results for removing organic matter, nutrients, micropollutants and pathogens. In fact, research on wastewater treatment using HRAPs have exponentially grown in the last 10 years. However, only few studies have been carried out evaluating microalgae-based systems as a post-treatment step following UASB reactors (ESPINOSA *et al.*, 2021; GONÇALVES *et al.*, 2020; SANTIAGO *et al.*, 2017; VASSALLE; DÍEZ-MONTERO; *et al.*, 2020; VASSALLE; GARCÍA-GALAN; *et al.*, 2020). Anaerobic effluents have a high content of ammonium and still significant concentration of dissolved organic matter, which may be degraded through microalgae and bacteria symbiosis. Moreover, microalgal biomass grown in HRAPs may be recovered and processed to generate valuable products, as bioenergy, biofertilizers, natural pigments and bioplastics (MARÍN *et al.*, 2019b; VASSALLE; DÍEZ-MONTERO; *et al.*, 2020).

The symbiotic relation between microalgae and heterotrophic bacteria in HRAPs relies on the exchange of oxygen produced from photosynthetic microorganisms (microalgae) , which is used by heterotrophic microorganisms (bacteria) to degrade organic matter (Solimeno and García, 2019). Recent studies have shown removal efficiencies up to 90% for chemical oxygen demand (COD), 95% for ammonium, and 20% for phosphorus (GONÇALVES *et al.*, 2020) in tropical conditions, treating UASB effluent.. Microalgae production have ranged from 10 g TSS m^{-2} d^{-1} to 40 g TSS m^{-2} d^{-1} (PARK; CRAGGS; SHILTON, 2011; PASSOS *et al.*, 2018; VASSALLE; GARCÍA-GALAN; *et al.*, 2020), depending on the climatic conditions. However, it has been observed an imbalance between microalgae and heterotrophic bacteria populations and activities, which may hamper wastewater treatment (SOLIMENO; GARCÍA, 2019). To minimise this issue, it seems crucial to be able to predict microalgae-bacteria interactions, in order to optimise operational conditions and to maximise the microalgae production and wastewater treatment (MOHSENPOUR *et al.*, 2021).

Mathematical models have been successfully developed and widely used to describe bacterial growth and wastewater treatment in conventional systems, such as activated sludge (HENZE *et al.*, 2006). On the other hand, models used to describe the complex interactions between microalgae and bacteria in wastewater treatment systems are still at its development and calibration stage (SOLIMENO; GARCÍA, 2019). Overall, all models were based on Monod, Lambert and Beer's Law and Arrhenius equations and are able to elucidate the interaction between microalgae-bacteria regarding nutrient availability, light and temperature. However, the models differ by the number of processes considered, the treatment system main goal and the quality of experimental data.

Among the models showed in Table 1, BIO_ALGAE, BIO_ALGAE 2 and ALBA models were the only ones that used data from HRAPs treating domestic wastewater. However, any of them was calibrated using HRAPs as a post-treatment of the effluent of an anaerobic reactor. In addition, the models took into account temperate temperatures, which may differ when considering tropical climate countries.

In this context, this work aimed at applying mathematical modelling to elucidate microalgae-bacteria interactions in HRAPs treating the effluent of an anaerobic reactor. To do this, the BIO_ALGAE 2 model was calibrated using experimental data collected from two pilot HRAPs used as UASB reactor post-treatment, which were operated in tropical climatic conditions for one year. The study evaluated the wastewater treatment efficiency and the ability of the model to predict experimental outputs. Afterwards, the calibrated model was used to simulate different scenarios varying the HRAP hydraulic retention time in terms of wastewater treatment performance and microalgae production. Finally, with the results of the simulations, a technical evaluation of a hypothetical UASB+HRAP system for a population of 10,000 inhabitants was performed, including the land requirement, and biogas and bioenergy production operating the system with anaerobic co-digestion of the harvested microalgal biomass.

4.2 Material and Methods

4.2.1 Experimental data collection in demonstration scale set-up

Experimental data used was obtained in a demonstration-scale system fed with domestic wastewater from a full-scale STP located in Brazil (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020; VASSALLE; GARCÍA-GALAN; *et al.*, 2020), as shown in Figure 4.1.

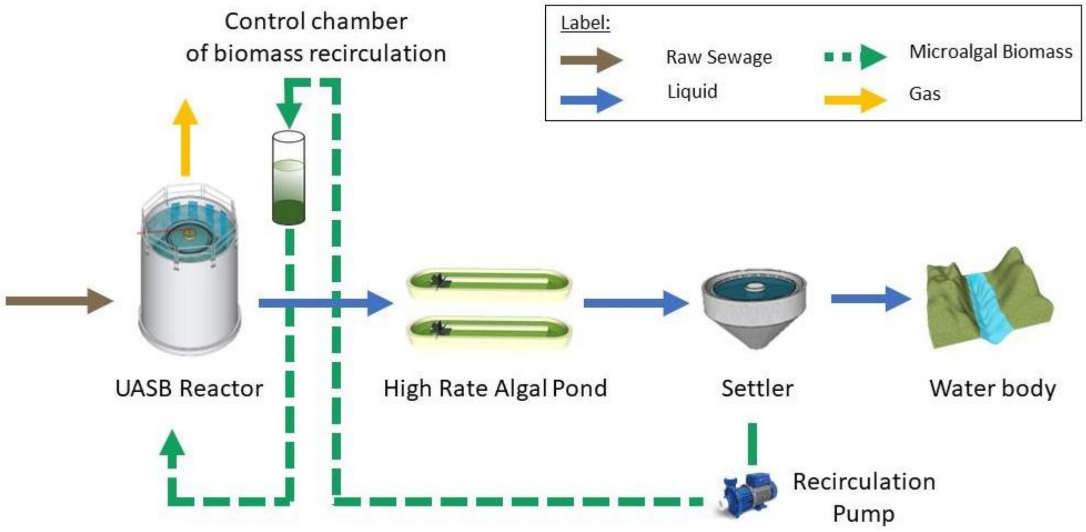

Figure 4.1. Scheme of the demonstration-scale experimental set-up.

The treatment system operated as follows. Wastewater was pumped (Netzsch® Germany) to the UASB reactor after preliminary treatment composed of coarse solids and grit removal. Anaerobic effluent was post-treated in two high rate algal ponds (HRAPs). Microalgal biomass produced and harvested from the ponds in a settler was recirculated to the UASB reactor for anaerobic co-digestion using a positive displacement pump (BCR 2000 – Schneider®).

The UASB reactor was made of fiberglass, with a working volume of 343 L (height 4.0 m; diameter 0.3 m) and was operated at a flow rate of 49 L h^{-1}, hydraulic retention time (HRT) of 7 hours. HRAPs were made of fiberglass with a working volume of 205 L and a surface area of 0.41 m^2 each (height 0.5 m; length 1.7 m; width 0.24 m). Microalgae-based ponds were operated at a flow rate of 25.5 L day^{-1} each, and a hydraulic retention time (HRT) of 8 days. Biomass settler was made of polyvinyl chloride, had a working volume of 30 L (height 0.4 m; diameter 0.1 m) and was operated at a HRT of 14 hours. For anaerobic co-digestion, 12 L of harvested microalgal biomass was pumped to a plexiglass column located 4 m above the UASB reactor and recirculated therefrom to the reactor at flow rate of 0.5 L h^{-1}. The system was operated continuously for one year (from July/2018 to July/2019).

4.2.2 Analytical methods

To evaluate wastewater treatment efficiency, liquid phase samples were collected twice a week (10:00 AM ± 2 h) from raw sewage, UASB and HRAP effluent. HRAP effluent was gathered from the liquid mixture inside the ponds and filtered using glass fiber filters (0.45µm Macherey-Nagel®, GF-2). Physical-chemical parameters analysed were pH, temperature and dissolved oxygen (DO), COD, total and volatile suspended solids (TSS and VSS), Total Nitrogen (TN), ammonium nitrogen ($N\text{-}NH_4^+$), nitrite ($N\text{-}NO_2$), nitrate ($N\text{-}NO_3$) and phosphate ($P\text{-}PO_4$). Temperature, pH and DO were determined in-situ using a portable Hach® sensor - (HQ30D). COD was measured through a Hach® kit COD at high range. TSS and VSS were determined according to Standard Methods (APHA-AWWA-WEF, 2012). TN, $N\text{-}NH_4^+$, $N\text{-}NO_2$, $N\text{-}NO_3$ and $P\text{-}PO_4$ were analysed by ionic chromatography (Metrohm® - 940 professional IC Vario).

For microalgal biomass characterisation, biomass samples were taken twice a week from the settler. Total and volatile solids (TS and VS) and Total Kjeldahl Nitrogen (TKN) were analysed according to standard procedures (APHA-AWWA-WEF, 2012). Total COD was analysed using Hach® kit COD at high range. Carbohydrates were measured through phenol–sulphuric acid method after acid hydrolysis (DUBOIS *et al.*, 1956). For protein content, a conversion factor of 5.95 was used based on the results of TKN (LÓPEZ *et al.*, 2010).

Microalgae production was calculated using the surface area of the ponds as in Equation 4.1, where C_{TSS} corresponds to TSS concentration (g L^{-1}), Q_{HRAP} to HRAPs flow rate (L day^{-1}) and SA_{HRAP} to HRAPs surface area (m^2).

$$Microalgae\ production\left(\frac{g}{m^2 day}\right) = \frac{C_{TSS}\ x\ Q_{HRAP}}{SA_{HRAP}} \qquad \text{(Eq. 4.1)}$$

Climatic data (temperature, solar radiation and precipitation) were obtained from the meteorological station near the WWTP (Brazil National Meteorology Institute, INMET, http://www.inmet.gov.br). Samples for biogas analysis were collected twice a week from the UASB reactor. Biogas production was measured using an automatic meter (Ritter®-Germany). In addition, biogas composition in terms of CH_4, CO_2, O_2, CO and H_2S was analysed using a portable meter (Geotech®-United Kingdom). The results were expressed as methane yield, i.e. volume of methane produced per mass of COD fed to the UASB reactor.

4.2.3 BIO_ALGAE 2 Model calibration and validation

BIO_ALGAE 2 model was calibrated and validated for comparing experimental data with model output. The model was implemented in COMSOL Multiphysics™ software version 5.4, in a computer DELL Inspiron 14 serie 7000 (i14-7460-A20G) laptop, with an Intel® Core™ i7- 7500U CPU @ 2.70GHz 2.90GHz processor and 8GB of RAM. To simplify the hydraulic simulation and reduce the computational costs, a constant stirred tank with perfect mixed was considered. Moreover, a one-dimension domain was used to represent the HRAP. The interface chosen to implement the model was Transport of Diluted Species (TDS). To simulate the transport of dissolved and particulate species and the transfer of gases to the atmosphere the dimension used was 0.41 m of domain, which represented the nominal volume of HRAPs along one dimension.

The complete description of BIO_ALGAE 2 model may be found elsewhere (SOLIMENO; GÓMEZ-SERRANO; ACIÉN, 2019). Notwithstanding, biokinetic processes and values, stoichiometric parameters matrix and physical and chemical parameters are shown in Tables SA.1 to SA.4 (Supplementary Material Section A). The structural parameters selected for model calibration were those related with microalgae growth and decay, since they had a higher impact on model output, as the maximum microalgal and heterotrophic bacteria growth rate ($\mu_{ALG,}$ μ_{H}), the inactivation constant of microalgae and heterotrophic bacteria ($k_{death,ALG,}$ $k_{death,H}$), the saturation constant for nitrogen species ($K_{N,ALG}$) and the saturation constant for phosphorus species ($K_{P,ALG}$). Additionally, structural parameters related with gas transfer to the atmosphere, such as the mass transfer coefficient for oxygen ($k_{a,O2}$), the mass transfer coefficient for CO_2 ($k_{a,CO2}$) and the mass transfer coefficient for NH_3 ($k_{a,NH3}$) were also calibrated.

Calibration was carried out by adjusting the structural parameters values and comparing the simulation results with the experimental data. Afterwards, calibration was validated based on the analysis of the following variables: i) total suspended solids (TSS, calculated as the sum of all the particulate species); ii) dissolved oxygen (DO); iii) chemical oxygen demand (COD) (calculated using the factor 1.05 from TSS (VON SPERLING, M., 2007)); iv) ammonium ($N\text{-}NH_4^+$); v) NOx (calculated as the sum of nitrite ($N\text{-}NO_2^-$) and nitrate ($N\text{-}NO_3^-$) species) and vi) phosphorus ($P\text{-}PO_4^{3-}$). For each variable and for each parameter combination evaluated, the normalized root mean square error (NRMSQE) (Eq.4.2) were calculated to assess the calibration accuracy, where $\theta_{i,j}$ (mg L^{-1}) represent model values for a particular variable of interest (j); $y_{i,j}$ (mg·L^{-1}), represent experimental values of a particular variable of interest (j) at a particular experimental time (i); $\bar{y}_j$ (mg·L^{-1}) represent mean values for experimental data and; N represent the number of experimental values. Finally, the values of the parameters that minimise the NRSQE were selected.

$$\mathrm{NRMSQE} = \frac{\sqrt{\sum_{i=N}^{N} \frac{(y_{i,j}-\theta_{i,j})^2}{N}}}{\bar{y}_j} \qquad \text{(Eq. 4.2)}$$

Calibration was automated through a macro implemented in Excel, which calculates the NRMSE for each experimental variable. The global NRMSE was also calculated. The developed macro enabled to simulate several combinations for structural parameters. The best combination of the calibrated parameters was selected by checking the global NRMSE. This process was repeated for different combination of structural parameters, until the model was able to reproduce the experimental data with a high accuracy (NRMSE< 20%).

4.2.4 Simulation scenarios

After model calibration and validation, simulations were performed considering different scenarios for HRT of the high rate algal ponds, varying the flow and consequently the hydraulic retention time in the unit. The same parameters used for validation were evaluated to verify the impact of HRT on the final effluent quality and microalgae production. Besides the experimental data on an HRT of 8 days, it was considered a flow of 100 L d^{-1} and a HRT of 4 days and a flow of 66.6 L d^{-1} and a HRT of 6 days.

In addition, a simulation for extrapolating the treatment system to provide service to 10,000 inhabitants (i.e. flow of 13.9 L s^{-1}) was performed to verify the impact of reducing the HRT, on area requirement, on microalgae production and finally, on bioenergy production from anaerobic co-digestion. The area requirement was calculated according to Equation 4.3, where, S_A represent the surface area (m^2); PE represent the population equivalent (inhabitants); Wc represent the average water consumption, considered as 150 L inhabitant. d^{-1}; R represent the return factor, considered as 0.8; HRT represent the hydraulic retention time (d) and; W_d represent the water depth in HRAP, considered as 0.30 m for all scenarios.

$$S_A(m^2) = \frac{(E_p \ x \ W_c \ x \ R) \ x \ HRT}{W_d} \qquad \text{(Eq.4.3)}$$

The energy produced from the waste water treatment plant was calculated in terms of the methane yield. In that way, the energy output was calculated following Equation 4.4, where E_o represents the energy output from biogas (kWh d^{-1}); O_L represents the organic loading rate (kg COD d^{-1}); Y represents the average methane yield, considered as 105 L CH_4 kg^{-1}COD (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020); ξ is the lower calorific value of methane, considered as 10 kWh m^{-3} CH_4 and; η_1 is the efficiency for energy generation, considered as 90%.

$$E_o = (O_L Y \ \xi \ \eta_1) \qquad \text{(Eq. 4.4)}$$

4.2.5 Statistical analysis

For comparing the parameters used to calibrate and validate BIO_ALGAE 2 model, Wilcoxon statistical test was used to verify statistical difference. *Statistica 10.0*® software was used to perform all tests and a significance level of 95% was applied.

4.3 Results and discussion

4.3.1 Wastewater treatment efficiency

Physical-chemical characterisation of raw wastewater, UASB reactor effluent and HRAP effluent are summarised in Table 4.1. The main data will be discussed following.

Table 4.1. Physical-chemical characterization (n = 92)

Parameters	**Samples**		
	Raw wastewater **Mean ± SD**	**UASB reactor effluent** **Mean ± SD**	**HRAP effluent** **Mean ± SD**
pH	7.8 ± 0.2	7.2 ± 0.2	9.7 ± 0.4
DO (mg L^{-1})	0.6 ± 0.5	0.2 ± 0.1	8.5 ± 5.1
Temperature (ºC)	25.1 ± 1.6	22.3 ± 1.5	23.1 ± 3.3
COD (mg O_2 L^{-1})	437.3 ±120.3	197.3 ±48.2	144.3 ± 27.7
TSS (mg L^{-1})	242.9 ±56.8	55.1 ± 45.1	142.3 ± 42.6
VSS (mg L^{-1})	182.2 ±48.7	30.9 ± 8.1	96.3 ± 29.1
N-NH_4^+ (mg L^{-1})	32.3 ± 8.8	39.5 ± 7.5	17.0 ± 3.2
N-NO_2 (mg L^{-1})	0.2 ± 0.0	0.9 ± 0.1	0.3 ± 0.0
N-NO_3 (mg L^{-1})	0.2 ± 0.0	0.3 ± 0.1	6.7 ± 0.5
TN (mg L^{-1})	45.6 ± 11.2	48.1 ± 8.2	29.6 ± 6.0
P-PO_4 (mg L^{-1})	4.9 ± 1.8	3.9 ± 0.9	2.7 ± 0.5

Note: DO – Dissolved Oxygem; COD – Chemical Oxygen Demand; TSS – Total Suspended Solids; VSS – Volatile Suspended Solids; N-NH_4^+ - Ammonium; N-NO_2 - Nitrite; N-NO_3 - Nitrate; TN – Total Nitrogen; and P-PO_4 -Phosphate.

The increase on pH and DO observed, may be associated with anaerobic effluent treatment in aerated microalgae-based systems (DE ASSUNÇÃO; VON SPERLING, 2013; VERBYLA; VON SPERLING; MAIGA, 2017). For organic matter, total COD removal was in average 55% for the UASB reactor and 70% for the whole system (UASB+HRAP). Even considering that the UASB reactor received a higher organic load due to microalgal biomass recirculation, the COD removal was consistent to the literature (55-70%) (CHERNICHARO, C. A., 2007). Additionally, the 70% overall removal of COD in the UASB + HRAP system was also in accordance to previous studies

(ESPINOSA *et al.*, 2021; GONÇALVES *et al.*, 2020; SANTIAGO *et al.*, 2017; VILLAR-NAVARRO *et al.*, 2018).

Regarding solids, the results showed TSS and VSS removals of 77 and 84% respectively, in the UASB reactor. Those values are in accordance with previous literature investigating similar systems (CHERNICHARO, C. A., 2007; ESPINOSA *et al.*, 2021; GONÇALVES *et al.*, 2020). For the overall system, solid removals were 42 and 47% for TSS and VSS respectively. It is important to notice that removal efficiencies were calculated using filtered HRAP samples and for final effluent quality microalgal biomass separation is crucial (ORTIZ *et al.*, 2021; VON SPERLING, MARCOS; CHERNICHARO, 2005a). In fact, data not shown here demonstrated that non-filtered analyses from settler showed an increase in TSS and VSS removals by 50% and 46% respectively. In this scenario, final effluent concentrations were 71 mg TSS L^{-1} and 52 mg VSS L^{-1}, which are still lower than optimal values to be achieved in efficient settlers.

Considering nitrogen, the UASB reactor mineralised organic nitrogen, showing an increase in ammonium concentration in the anaerobic effluent (from 32 to 39 mg $N\text{-}NH_4$ L^{-1}). This is a result of the hydrolysis of proteins and urea, leading to an increase of $NH_4^+\text{-}N$ (METCALF & EDDY *et al.*, 2003). In HRAP, a $N\text{-}NH_4^+$ removal average of 57% was observed, with a final effluent concentration of 17 mg $N\text{-}NH_4$ L^{-1}. Previous studies have shown similar removals (64%) for HRAP operated at a HRT of 6 days treating anaerobic effluent (NASCIMENTO, 2001). $N\text{-}NH_4^+$ removal pathway was most probably associated to nitrification and/or volatilization. This was confirmed by the high nitrate concentration in the treated effluent (6.7 mg L^{-1}). The high pH (above 8) evidences the possible formation and volatilization of NH_3. (DOMA *et al.*, 2016). A third mechanism for removing ammonia would be through a biological pathway by microalgae assimilation under conditions of high photosynthetic rate (ARCEIVALA, 1981). The TN removal in the HRAP was in average 39%, with a concentration decrease from 48 mg L^{-1} in the UASB reactor effluent to 29 mg L^{-1} in the HRAP effluent. Same removal efficiency was observed in other UASB+HRAP system treating domestic wastewater and operated at a HRT of 6 days (NASCIMENTO, 2001).

Finally, in terms of phosphate, an average removal of 30% was observed from HRAPs. Efficiencies from 0 to 65% were obtained in microalgae-based ponds (GONÇALVES *et al.*, 2020; YANG *et al.*, 2020). Among the removal pathways, the active mechanism according to literature is through precipitation when the pH is above 8.2 (CROMAR *et al.*, 1992). Thus, phosphorus precipitation and microalgae assimilation might be the main pathways in this work. Additionally, polyphosphate accumulating bacteria (PAO) may also have played a role due to the systems configuration, i.e.

anaerobic followed by aerobic process. The exposition to input carbon alternation can induce the metabolic characteristics of PAOs. In anaerobic environment, carbon substrate is volatile fatty acids produced by fermentative bacteria from sugars and low molecular organic compounds, which are converted and stored as polyhydroxyalkoanates (PHA) (PETERSEN *et al.*, 1998; SANTOS *et al.*, 1999; UBUKATA; TAKII, 1994).

On a whole, the concentrations of pollutants, even without a proper microalgal biomass recovery step, met Brazilian legislation requirements, which are 180 mg COD L^{-1}, 150 mg TSS L^{-1} and 20 mg N-NH_4^+ L^{-1} (MORAIS; SANTOS, 2019; BRASIL, 2011). Considering most restrictive European urban wastewater directive, discharge limits are 125 COD mg L^{-1}, 35 mg TSS L^{-1}, 15 mg TN L^{-1} and 2 mg P L^{-1} (THE COUNCIL OF EUROPEAN COMMUNITIES, 1991). To comply with European legislation, efficient microalgae separation from the mixed liquor should be improved by optimising the settler design and operation.

4.3.2 Microalgal biomass and biogas production

Microalgae production was calculated from average TSS concentration in the HRAP, which was 142 mg L^{-1}. Therefore, microalgae production was 8.8 g TSS m^{-2} day^{-1} in average throughout the year. Nonetheless, values varied among different seasons, even in tropical environment. The averages for autumn/winter was around 10g TSS m^{-2} day^{-1}, while for summer/spring it was 6.35g TSS m^{-2} day^{-1}. This difference was probably associated to seasonality changes and pluviosity. In fact, in tropical regions it is common that warmer months are also those with higher rainfall. On the contrary, a previous study evaluating HRAPs throughout a year in the Mediterranean Region reported higher microalgae production in warmer seasons, when light irradiance was higher (PASSOS *et al.*, 2017).

In terms of biomass characterisation, results reported an average of 3.32 mg TS L^{-1}, 2.40 mg VS L^{-1}, 3,763 mg COD L^{-1}, 186 mg TKN L^{-1}, 412 mg L^{-1} of carbohydrates and 1,107 mg L^{-1} of proteins. Carbohydrates and proteins corresponded to 17% and 46% of the biomass dry weight, respectively. The main microalgae specie found in the harvested biomass was *Kirchneriella* sp. *Scenedesmus* sp., *Westella* sp. and diatoms were also observed, but with a lower frequency. According to previous work, *Scenedesmus* sp. is one of the most common species found in HRAPs treating wastewater (MOHSENPOUR *et al.*, 2021), while *Kirchneriella* sp. and *Westella* sp. were also reported in microalgae-based systems (FULKE *et al.*, 2013; HANAA, 2012). Regarding biomass composition, literature reported a composition of 7% of carbohydrates and 57% of proteins for pure culture of

Kirchneriella sp. (SANTHAKUMARAN; KOOKAL; RAY, 2018). However, our values are much similar to those attained in mixed and complex HRAP (Passos et al., 2017).

Biogas produced in co-digested UASB reactor was in average 165 NL kg^{-1} COD, while methane yield was 106 CH_4 kg^{-1} COD. The biogas composition was 64% CH_4, 7% CO_2, 1.5% O_2, 7 ppmv (parts per million by volume) CO, 1844 ppmv H_2S and 26% of H_2 and N_2 (considering a 100% balance). These values are similar with those reported from UASB reactor treating only domestic wastewater (NOYOLA; MORGAN-SAGASTUME; LÓPEZ-HERNÁNDEZ, 2006; SILVEIRA, 2015).

4.3.3 BIO_ALGAE 2 model calibration and validation

The calibration values for the structural parameters μ_{ALG}, μ_{H}, $k_{death,ALG}$, $k_{death,H}$, $k_{a,O2}$, $k_{a,CO2}$, $k_{a,NH3}$, $K_{N,ALG}$ and $K_{P,ALG}$. are summarised in Table 4.2. The calibrated model was validated using experimental data of pH, DO, TSS, COD, nitrogen and phosphorus species concentrations from experimental set-up.

Table 4.2. Values of calibrated structural parameters for BIO_ALGAE 2 model

Parameter	Description	Value
μ_{ALG}	Maximum specific growth rate of microalgae	1.6 d^{-1}
μ_{H}	Maximum specific growth rate of heterotrophic bacteria	1.0 d^{-1}
$k_{death,ALG}$	Inactivation constant of microalgae	0.15 d^{-1}
$k_{death,H}$	Inactivation constant of heterotrophic bacteria	0.8 d^{-1}
$k_{a,O2}$	Mass transfer coefficient for oxygen	1.45 h^{-1}
$k_{a,CO2}$	Mass transfer coefficient for dioxide carbon	0.8 h^{-1}
$k_{a,NH3}$	Mass transfer coefficient for ammonia	0.3 h^{-1}
$K_{N,ALG}$	Saturation of nitrogen species	0.01 mol m^{-3}
$K_{P,ALG}$	Saturation of phosphorus species	0.02 mol m^{-3}

The climate conditions during the sampling period are shown in Figure 4.2 for temperature and irradiance. Climatic factors directly influence the growth of microalgae, also affecting wastewater treatment efficiency. As may be seen, irradiance values decreased between days 0 and 175. This difference was probably associated with seasonality changes and rainy season (spring-summer). Regarding temperature, it may be observed how the warmest days were also the lowest irradiation values. In fact, in tropical regions it is common that warmer months are included in the rainy season. In this study, spring and summer had an accumulated rainfall of 178 mm and 277 mm, while for autumn and winter (day 175 to 350) it was 37 mm and 18 mm, respectively.

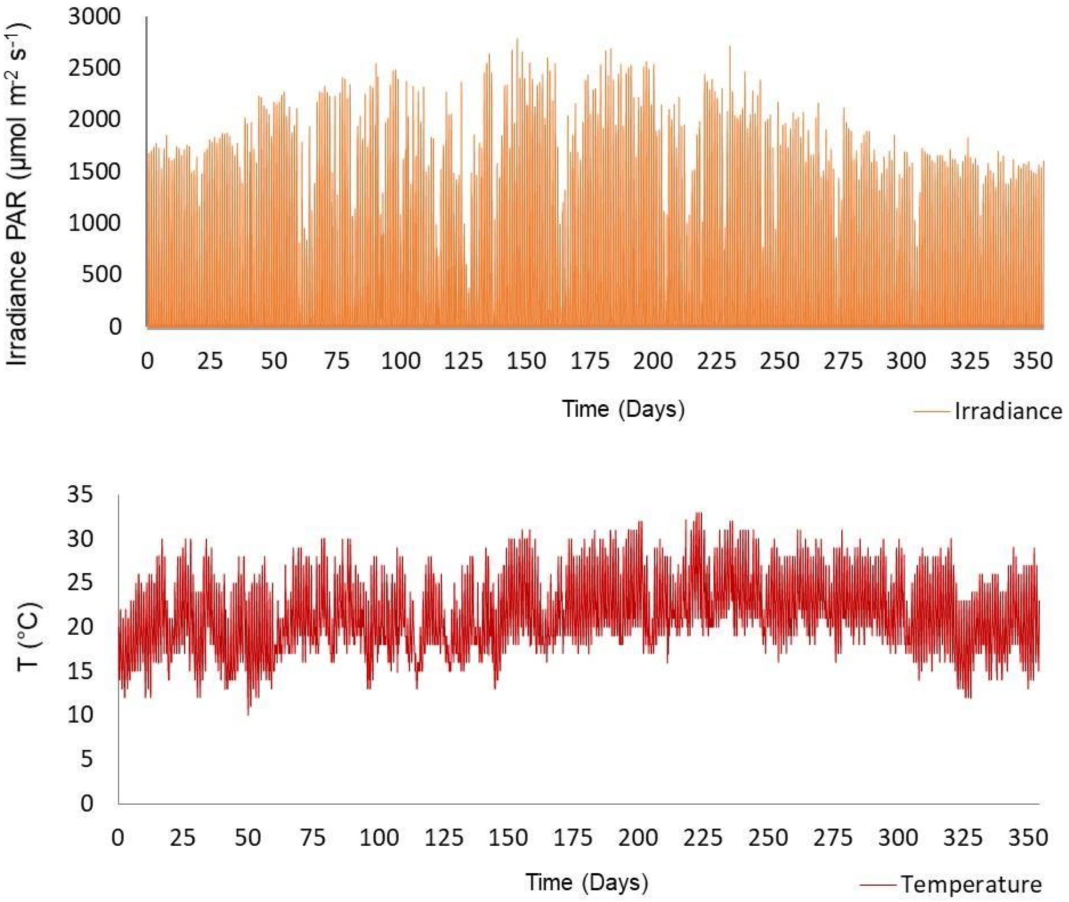

Figure 4.2. Climate conditions during sample period

The experimental data and model output for structural parameters after calibration are shown in Figure 4.3. For each parameter, the NRMSQE were indicated (Figure 4.3). As can be seen, for all parameters analysed, NRMSQE showed good accuracy for the conditions applied to this work. When the NRMSQE is close to 0% respectively, it means that the simulation results are close to what was measured in the experiment. For BIO_ALGAE 2 model calibration, it was observed that the worst simulated parameters were NO_2^- and TSS, each with a NRMSQE of 21%. The pH was the best parameter among those evaluated, a NRMSQE of 4.5%. To illustrate, the simulated pH average was 9.5, while the experimental data showed an average of 9.7.

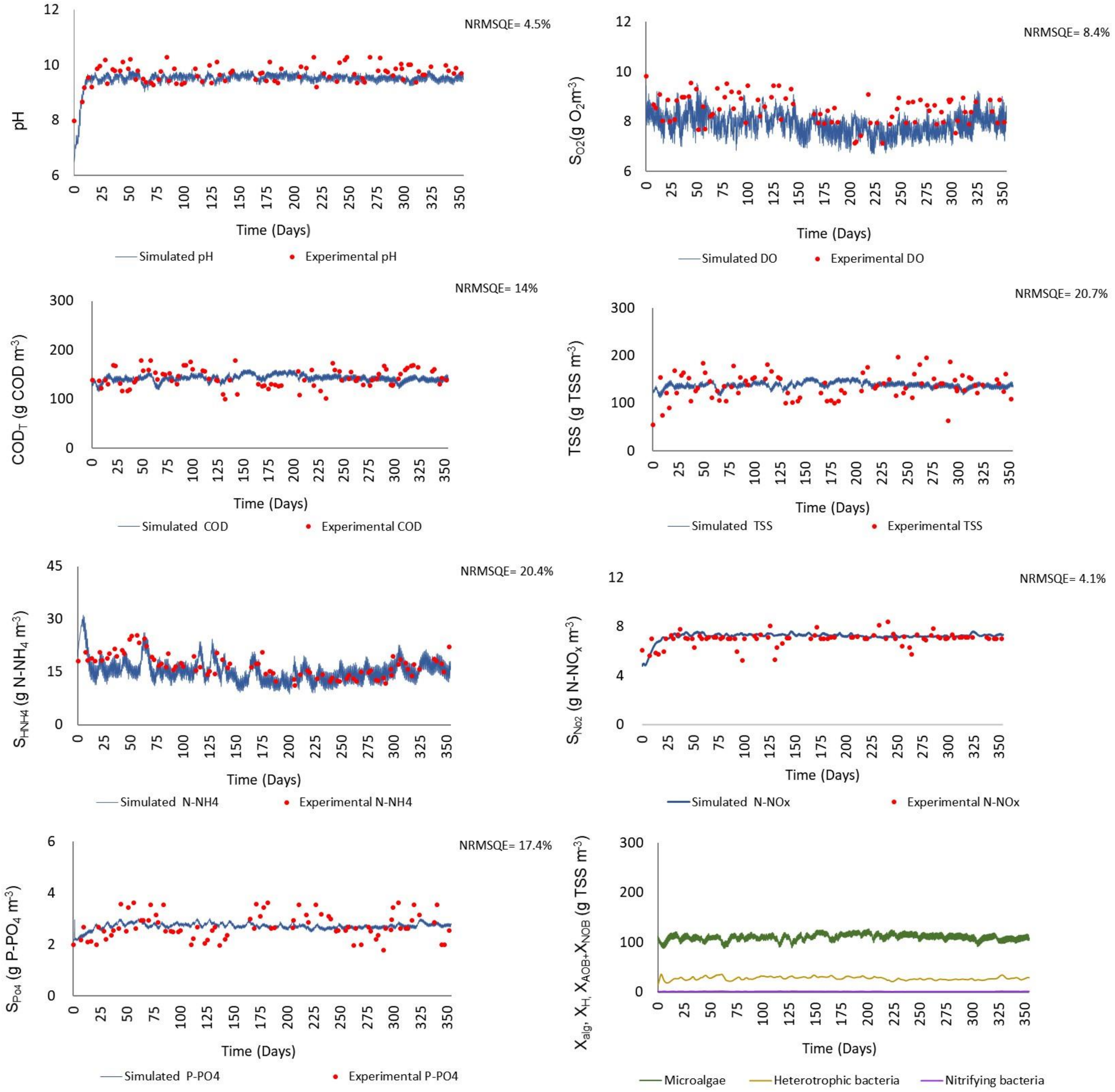

Figure 4.3. Experimental data and model output of structural parameters after calibration

Generally, the trend of simulated and experimental data was quite similar, with few variations throughout the analysed period. The pH increase could be associated with the photosynthetic activity of microalgae, which was related to removal of carbon compounds responsible for basification. Microalgae production increase also led to an increase in the DO concentration. This relationship explained the difference between the experimental and simulated COD, TSS and DO concentration data shown in Figure 4.3. Simulation results slightly underestimated the experimental TSS, which led to a lower production of OD and thus a underestimation of this parameter. However, the differences between the model and the experimental values were minimal.

Simulated ammonium and NOx (nitrate + nitrite) curves matched quite well the experimental data. As for pH, OD, COD and TSS, these parameters were also related to microalgae photosynthesis, showing lower values during daytime. The increase in pH during photosynthetic activity favoured the conversion of ammonium to ammonia. Part of the produced ammonia was probably lost through volatilization. During the dry season (autumn/winter), the average ammonia concentration in the effluent was lower compared to the rainy season (spring/summer). Concerning the results, our data showed a higher influence of rainfall, with lower influence of temperature and irradiance. On the other hand, the trend observed by previous simulation results using BIO_ALGAE 2 showed a higher N removal efficiency in periods with higher solar irradiance (SOLIMENO *et al.*, 2017).

For phosphorus, the average simulated and experimental concentrations were the same (2.7 mg L^{-1}), however throughout the year model overestimated this parameter (Figure 4.3). The greater photosynthetic activity observed in the experimental system in relation to the estimated one, may be the main reason of the value differences. Based on the increase in pH previously mentioned, phosphorus precipitation would increase with pH exceeding 8.2. As the estimated pH was lower than that measured in the experiment, the model simulated lower phosphorus precipitation, showing a slightly higher concentration throughout the historical series. Another explanation may be associated with the metabolism of polyphosphate accumulating bacteria (PAOs). In periods where there was higher concentrations of dissolved oxygen (as shown by the experimental data in relation to the model), which means a higher change of DO between anaerobic and aerobic environments, there was also lower amounts of phosphorus. This higher change on DO may have stimulated the metabolism of polyphosphate accumulating bacteria, leading to a decrease in phosphate concentration, as shown in Figure 4.3.

Regarding microalgae and heterotrophic bacteria, results showed higher microalgae concentration (64%) in respect to heterotrophic bacteria (18%). Nitrifying bacteria biomass was comparatively much lower (2.5%). The remaining TSS may be attributed to slowly biodegraded and inert material (15.5%). Similar proportions were reported for BIO_ALGAE model calibration in a Mediterranean climate and without an anaerobic treatment unit (i.e. 58% of microalgae, 22% of heterotrophic bacteria, 2.5% of nitrifying bacteria and 17.5% of slowly biodegraded and inert material) (SOLIMENO; GARCÍA, 2019).

4.3.4 HRT effect through BIO_ALGAE 2 model response

The results for simulatedscenarios of HRAP operated at 4, 6 and 8 days of HRT are shown in Table 4.3. For HRAP required area, microalgae production and bioenergy production the model was extrapolated to a PE of 10,000 inhabitants. Statistical results showed differences between all scenarios evaluated ($p < 0.05$).

Table 4.3. Treated effluent quality characteristics for each simulated HRT scenario (n = 8500)

Parameters	**Scenarios considering different HRTs**		
	8 days	**6 days**	**4 days**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**
pH	9.5 ± 0.3	9.4 ± 0.3	9.2 ± 0.3
DO (mg L^{-1})	7.9 ± 0.4	7.9 ± 0.4	7.9 ± 0.4
COD (mg L^{-1})	145.6 ± 7.3	145.7 ± 7.7	125.5 ± 13.4
TSS (mg L^{-1})	138.6 ± 7.0	138.8 ± 7.3	119.5 ± 12.8
Microalgae (%)	64%	63%	54%
N-NH$_4^+$ (mg L^{-1})	15.4 ± 3.3	16.7 ± 3.4	24.9 ± 5.3
N-NO$_2$ (mg L^{-1})	0.3 ±0.0	0.3 ±0.0	0.3 ±0.0
N-NO$_3$ (mg L^{-1})	6.9 ± 0.3	7.0 ± 0.2	7.1 ± 0.2
NO$_x$ (mg L^{-1})	7.2 ±0.4	7.3 ±0.3	7.4 ± 0.2
P-PO$_4$ (mg L^{-1})	2.7 ± 0.1	2.8 ± 0.1	3.0 ± 0.1
***HRAP total surface area (m^2)**	32,000	24,000	16,000
***Number of HRAP**	4	3	2
*** Surface area for each HRAP (m^2)**	8,000	8,000	8,000
***Biomass production (g TSS m^{-2} day^{-1})**	5.2	7.0	8.9
***Energy production (kWh d^{-1})**	1608	1609	1458

Note: DO – Dissolved Oxygen; COD – Chemical Oxygen Demand; TSS – Total Suspended Solids; N-NH$_4^+$ - Ammonium; N-NO$_2$ - Nitrite; N-NO$_3$ - Nitrate; TN – Total Nitrogen; and P-PO$_4$ - Phosphorus. *For PE of 10,000-inhabtants

In all the evaluated scenarios, COD and TSS effluent concentration complied Brazilian environmental legislation (180 mg COD L^{-1} and 150 mg TSS L^{-1} respectively), however, for European legislation (125 mg COD L^{-1} and 35 mg TSS L^{-1}), a need for HRAP effluent quality improvement would be required. This would be attained with an efficient biomass separation and concentration, which would further enhance biomass processing and valorisation. Model results showed no difference in the scenarios for HRT of 6 and 8 days. On the other hand, a lower concentration of TSS and COD was observed for the HRT of 4-day. This result was associated with the lower microalgae production in this scenario (SOLIMENO; GARCÍA, 2019).

All nitrogen series showed best final effluent quality for HRAP operated with the highest HRT of 8 days. However, little difference was noted between the scenarios considering HRT of 8 and 6 days. For these cases, Brazilian legislation was reached (20 mg $N\text{-}NH_4$ L^{-1}). For HRT of 4 days, deterioration in the effluent quality was observed. This was possibly due to nitrifying bacteria, which was hindered in such a short retention time. In fact, for this scenario, the legislation was not complied. For phosphate, any of the scenarios showed results complying with European legislation (2 mg L^{-1}). Actually, final phosphate concentration varied only slightly between the three scenarios.

For each scenario, the impact on the required surface area was evaluated, considering a PE 10,000-inhabitants. According to the results, the HRAP operated with an HRT of 6 d had a final effluent quality similar to that operated at 8 d HRT, even with a 25% smaller surface area. For the 4 d HRT, final effluent quality showed deterioration, mainly in relation to evaluated nutrients. However, the area required for this scenario was 100% smaller compared to HRAP operated at a HRT of 8 d and 34% smaller compared to that operated at 6 d. The improvement in final effluent quality may once more be associated with an efficiency biomass separation in optimised settlers. Thus, for locations with tropical climate and smaller surface area available, HRAPs would be capable of operating the HRAP with a 4 days HRT, together with microalgal biomass efficient recovery.

In terms of biogas, the scenario with 4 days HRT would have lower energy production compared to the other two cases. Different uses for biogas produced in UASB reactors in waste water treatment plant have been proposed (MOREIRA *et al.*, 2017). For instance, biogas may be converted into heat using boilers, used for digested sludge dehydration and sanitation, or it can be converted into electricity and heat in a combined heat and power (CHP) plant, or vehicular fuel or purification into biomethane for injection into the grid.

For small systems, due to greater simplification of operation and financial viability, the conversion of energy into heat is more suitable (VALENTE, 2015). A simple conversion alternative may be to sanitize digested sludge, making it suitable for agricultural use (CARTES *et al.*, 2018). A recent study investigating potential UASB WWTPs in rural areas in Brazil showed that the most straight-forward biogas uses were conversion into thermal energy for sludge sanitization, heat for cooking and/or for heating water in the nearby community (PASSOS *et al.*, 2020).

Another alternative would be to use heat produced from biogas for thermal pre-treatment of microalgal biomass. Thermal pre-treatment at low temperatures of 90 ºC have been investigated with success to increase organic matter solubilisation and methane yield (PASSOS; UGGETTI; *et al.*, 2014). In addition, heat produced from biogas could also be used to maintain mesophilic temperatures in the UASB reactor, which could be desirable in cold climate countries or when disinfection is necessary.

As mentioned, energy production was higher when HRAP were submitted to 8 and 6 days of HRT. This means it would be possible to consolidate a 25% smaller HRAP generating the same energy potential. When applying 4 days of HRT, produced energy would decrease by approximately 10%. However, the lower energy production may mean a gain in area demand (between 34% and 100%). Based on the results obtained, empirical combinations were proposed and the most appropriate HRT for each situation are shown in Table 4.4. The results indicate that the best configuration will depend on each context. For example, if the local priority is minimising the Operational Expenditure and Capital Expenditure capacity, maximising the effluent quality, increasing bioenergy production or reducing the area requirement. In any case, HRAP modelling through BIO_ALGAE 2 showed to be an effective tool for process decision and system optimisation.

Table 4.4. Different HRT recommendation for system priorities

	High CAPEX* and OPEX* capacity			**Low CAPEX* and OPEX* capacity**		
Area **Priority**	**Low availability**	**Medium availability**	**High availability**	**Low availability**	**Medium availability**	**High availability**
Standardized Effluent	▲	▲/●	▲/●/♦	▲	▲/●	▲/●
Nutrients Removal	-	●	●/♦	-	●	●
Microalgae to agriculture	▲	▲/●	▲/●/♦	-	▲/●	▲/●
Bioenergy production	▲	▲/●	▲/●/♦	-	-	-

▲ – HRT of 4 days; ● – HRT of 6 days; ♦ – HRT of 8 days; ***OPEX** – Operational Expenditure; **CAPEX** – Capital Expenditure

4.4 Conclusion

The UASB + HRAP system investigated in this study, proved to be suitable for wastewater treatment, with an efficient removal of COD (70%), TSS (42%), N-NH_4 (57%) and P-PO_4 (30%). Microalgae separation was verified as a crucial system unit for improving final effluent quality, by increasing TSS removal by 50%. BIO_ALGAE 2 model was capable of simulating experimental HRAPs used for anaerobic effluent post-treatment and exposed to in tropical climate conditions. The model had accuracy assessed based on the normalized root mean square error (NRMSQE), which varied from 4% to 21% respectively. Additionally, the simulations performed for different HRTs (4, 6 and 8 days) showed that wastewater treatment efficiency was achieved even with a low value of 4 days HRT, which would require much lower area, 100% less if compared with 8-day HRT operation. However, in terms of energy potential, 4 day HRT showed the worst scenario, with an average yield 10% lower. Finally, the results showed how optimal operation will depend on outcome priority, as energy production, final effluent quality or area occupation. In addition, the study demonstrated how BIO_ALGAE 2 model was an important tool on outcome prediction and operation optimisation.

5 CAN HIGH RATE ALGAL PONDS BE USED AS POST-TREATMENT OF UASB REACTORS TO REMOVE MICROPOLLUTANTS?

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The present study evaluated the removal capacity of a UASB-HRAP treatment, combining anaerobic treatment with microalgae-based, aerobic treatment, for eleven micropollutants present in raw sewage, including pharmaceuticals, estrogens and xenoestrogens. The UASB reactor and the HRAP were operated at a hydraulic retention time (HRT) of 7 hours and 8 days, respectively. Samples were collected periodically from the influent and UASB reactor and HRAP effluents. All the target compounds were found in raw sewage, with an occurrence ranging from 70 to 100%. Removal in the UASB reactor was generally incomplete, ranging from no removal (-25.12% for the hormone EE2-ethinylestradiol) to 84.91% (E2 - estradiol), but the overall performance of the UASB+HRAP system was highly efficient for most of the compounds, with removal rates ranging from 64.8% (ibuprofen) to 95% (estrone). Gemfibrozil and bisphenol A were the only exceptions, with overall removal rates of 39% and 43%, respectively. Hormones were the compounds with the highest removal index in the system.

5.1 Introduction

The ubiquity of organic micropollutants in all kind of environmental matrices has become a well-known problem for the scientific community during the last decades, and has derived in a increasing scientific and social concern worldwide (SCHWARZENBACH *et al.*, 2006; SHAO *et al.*, 2019). This is directly associated with all the uncertainties regarding human chronic exposure to these compounds, as their effects at both ecological and human level are not yet fully understood. (EGGEN *et al.*, 2014; NOMAN *et al.*, 2019). It is notorious, though, that these compounds are generating a series of imbalances in non-target organisms causing undesired effects, such as the decrease of entire populations (e.g the case of vultures in Pakistan after diclofenac intake, (Virani et al. 2004)), or endocrine disruption (HOGA; ALMEIDA; REYES, 2018).

Pharmaceuticals (PhACs) and endocrine disruptors (ED) are the organic micropollutants most frequently addressed in studies relating environmental occurrence and the derived ecotoxicity (Kuster et al. 2010; Luo, et al. 2014). Both ED and PhACs reach the environment via excretion, partly in their active form and partly as metabolites, and are also often disposed inappropriately in sanitary appliances or in garbage, especially in developing countries (QUADRA *et al.*, 2019). It has been widely demonstrated in several research studies that conventional sewage treatment, based on activated sludge (CAS), is usually inefficient removing the vast majority of organic micropollutants, including EDs and PhACs (LUO et al., 2014; QUEIROZ et al., 2012). For instance, many of the most commonly used PhACs, such as the anti-inflammatory diclofenac, the antiepileptic carbamazepine or the antibiotic sulfamethoxazole are not completely removed in conventional sewage treatment plants (STP) (GARCÍA-GALÁN et al., 2011; KOSTICH et al., 2014).

The use of upflow anaerobic sludge blanket (UASB) reactors is an alternative to consider, which is already consolidated in developing countries such as Brazil, Colombia, India and Africa (CHERNICHARO, *et al.*, 2018). Indeed, it has permitted that sewage treatment reached populations in places with a low surface availability to install conventional treatment plants, or to cover the needs of small populations. UASB technology essentially allows the removal of suspended or dissolved carbonaceous organic matter from sewage water. However, a post-treatment of the UASB reactor effluent is usually required to improve the effluent quality. Furthermore, regarding organic micropollutants removal, UASB reactors have proved to be inadequate, obtaining low and even negative removals (with higher concentrations in the effluent than in the influent) (BRANDT *et al.*, 2013; STASINAKIS *et al.*, 2013). These same authors have concluded that most PhACs and EDs are only considerably eliminated under aerobic conditions. Even for those compounds with high adsorption coefficients to sludge (k_d), such as the hormone EE2 or the analgesic paracetamol, their deprotonation under anaerobic conditions (due to the operational pH of the UASB) leads to their repulsion from the sludge blanket (also negatively charged), remaining in the liquid phase (SUÁREZ *et al.*, 2008). In addition, the operating conditions of the reactor (low HRT), the physical-chemical characteristics of the pollutant, environmental conditions, sludge concentration and metabolite formation directly influence the removal of micropollutants in UASB reactors (ALVARINO *et al.*, 2014; GONZALEZ-GIL *et al.*, 2016).

Taking all this into consideration, the need to treat UASB effluent is clear. Microalgae-based treatments, specifically in open systems such as high rate algal ponds (HRAP), have demonstrated to be highly adequate to treat secondary effluents (DE GODOS, I. *et al.*, 2016; MULLA *et al.*, 2019). Microalgae-based treatments are gaining a renewed popularity due to their high efficiency removing nutrients and organic matter in a much more sustainable way than conventional treatments. These systems can operate at low operation and maintenance (O&M) costs, as they do not require external aeration due to photosynthesis, or any chemical input (GARCÍA *et al.*, 2006; MUÑOZ, R; GUIEYSSE, 2006). Microalgae biomass grows fixating CO_2 and assimilating the nutrients (mostly nitrogen (N) and phosphorus (P)) present in the raw sewage. Through photosynthesis, microalgae generate the oxygen needed by heterotrophic bacteria to aerobically degrade the organic contaminants present in the water. HRAPs are efficient removing both organic matter and nutrients and also a wide variety of micropollutants by photodegradation, bioadsorption and biodegradation (MATAMOROS et al., 2015; GARCÍA-GALÁN et al. 2018, 2020). Indeed, biodegradation is an important route of removal in HRAPs since the large presence of microalgae and heterotrophic bacteria can remove these compounds intra and extracellularly, by means of adsorption on the cell wall (XIONG; KURADE; JEON, 2018). The removal efficiency of these ponds for different PhACs has been only recently studied by different authors, obtaining good results for most of the target analytes (DE GODOS ET AL., 2012; HOM-DIAZ ET AL., 2017B; GARCÍA-GALÁN ET AL., 2020), but there is still a lack of knowledge regarding the different mechanisms governing these systems (YOUNG; TAYLOR; FALLOWFIELD, 2017). Likewise, the removal of PhACs during UASB treatment, which operates at much lower HRT, needs to be further investigated. Regarding the combination of both systems, to the authors best knowledge, anaerobic-aerobic treatment using UASB and HRAPs has been only barely studied.

The present work aims to evaluate the removal capacity of a treatment system consisting of a UASB reactor (anaerobic treatment) followed by a HRAP (aerobic treatment) for eleven micropollutants: four estrogens, five PhACs and two xenoestrogens.

5.2 Material and Methods

5.2.1 Experimental set-up and operation

The experimental set-up consisted of a UASB reactor followed by two HRAPs, used as post treatment of the UASB effluent, and a settler to separate the microalgal biomass from the liquid phase (see graphical abstract)). The system received real raw sewage from a nearby sewage treatment plant (STP) located in Belo Horizonte (Brazil) designed for a flow rate of 4.5 m^3 s^{-1}.

The UASB reactor was made of fiberglass and had a working volume of 343 L (operational height of 4 m). It operated at a flow rate of 49 L h^{-1} under a HRT of 7 hours and sludge retention time (SRT) of 20 days. The HRAPs were also made of fiberglass, had an operational volume of 205 L each (1.70 m length x 0.3 m depth; 0.68 m^2 surface). The ponds operated at a flow rate of 25.5 L day^{-1} each, corresponding to a HRT of 8 days. The algal biomass produced was harvested in a 30 L volume settler of PVC that operated at a 14 hour HRT. The system was operated continuously for 4 months to reach a steady-state prior to the monitoring campaign.

5.2.2 Analytical methods

5.2.2.1 Chemicals and reagents

Eleven different target compounds, including PhACs and EDs, were selected considering their frequency of detection in similar matrices (raw and treated sewage) and their potential ecotoxicity effects. Five PhACs, namely ibuprofen, diclofenac, naproxen, paracetamol and gemfibrozil; four estrogens, namely estrone (E1), 17β-estradiol (E2), 17α-ethinynestradiol (EE2) and estriol (E3), and two xenoestrogens, nonylphenol and bisphenol A, were studied.

5.2.2.2 Sample collection and preparation

Grab samples of raw sewage, UASB and HRAP effluents samples (1 L) were collected twice per week during 5 weeks between 10:00 am and 11:00 am (n=10 for each point). The sampling was done during the dry season (in May and June – autumn and winter in Brazil). These samples were used both for analysis of the target micropollutants (200 mL) and for physical-chemical analysis. During the sampling campaign, average air temperature was 21.5 ºC, and no precipitation events were registered (Brasil National Meteorology Institute, INMET, http://www.inmet.gov.br).

5.2.2.3 Sewage quality analyses

Water quality parameters such as pH, temperature, dissolved oxygen (DO), total and volatile suspended solids (TSS and VSS), total nitrogen (TN), ammonium (N-NH4+) and chemical oxygen demand (COD) were evaluated daily. pH, DO and temperature were measured using Hach® probes (HQ30D) (Hach, Colorado, US). $N\text{-}NH_4^+$ was analysed by ionic chromatography (IC), using a 940 professional IC Vario instrument (Metrohm®, Herisau, Switzerland). TN was analyzed by a TOC-L analyzer (Shimadzu®, Kioto, Japan). COD was analysed by a Hach® kit for high range. Microalgal biomass was also characterized in terms of volatile solids (VS), carbohydrates, Total Kjeldahl Nitrogen (TKN) and proteins. All of the analyses were done in the laboratory following the Standard Methods (APHA, 2012). pH, temperature and DO, were measured on-site. For qualitative evaluation of microalgae populations, mixed liquor samples were regularly examined under an optic microscope (Olympus BX-50) using a 40x magnification lens and a digital camera (Olympus DP70).

5.2.2.4 GC-MS analysis of the target PhACs and EDs

Samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS), adapting the methodology by Queiroz et al. (2012). Previously, 200 ml of both influent and effluent samples were filtered through glass fiber filters (0.7µm Macherey-Nagel®, GF-3). Then, before the solid phase extraction (SPE), pH was adjusted to 2.0 ± 0.5 with HCl, and the antiquelating agent Ethylenediaminetetraacetic acid (EDTA) was added (50 mg). SPE procedure was adapted from USEPA Method 1694 (Sanson et al., 2014). Briefly, two different cartridges were used, Strata SAX® for ionic compounds (500 mg) and Strata X® for non-polar and aromatic compounds (500 mg), (Phenomenex, California, EUA). SPE was carried out in parallel for each specific cartridge, using 100 mL of each aliquot sample per cartridge. After extraction, Strata SAX cartridges were eluted with 10 mL of ethyl acetate and the Strata X cartridges were rinsed with 10 mL of ultrapure water and then eluted with 10 mL of methanol and 6 mL of a mixture methanol and acetone (1:1). The extracts were collected and evaporated under N_2 flow until they reached 1 mL volume. The extracts were then transferred to vials, completely dried under N_2 flow and frozen until analysis.

Before GC-MS analysis, derivatization of the samples was performed, adding BSTFA (trifluoroacetamide) + 1% TCMS (trimethylchlorosilane). GC/MS analyses were carried out on a QP2010 plus instrument (Shimadzu®). Methodology validation and data statistical analysis

5.2.2.5 GC-MS Conditions

Injection was done in splitless mode, with an injection time and temperature set to 0.5 min and 280 ºC, respectively. The carrier gas was He, with a linear velocity of 45 cm.s-1 and a pressure of approximately 90.7 kPa. A Rtx-5MS column (30 m × 0.25 mm × 0.25 mm) was used. The initial oven temperature was set to 50 ºC during 1 min, then increased to 100 ºC following a gradient of 25ºC.min^{-1}, then increased again to 300 ºC for 5 min, following a gradient of 15ºC.min^{-1}. The gas flow rate through the column was 1.54 mL min-1. Temperature for the MS ionization source was set to 250 °C (interface at 280 °C) and the mass analyser voltage was set at 1.3 kV with a total time analysis of 21.33 min. The adapted methodology was validated in terms of accuracy, calibration range and linearity, sensitivity and selectivity. The characteristics of GS-MS was presented in Table SB.1 (Supplementary Material Section B).

5.2.2.6 Methodology validation and data

Recovery rates (R%), method limit of detection (LOD) and limit of quantification (LOQ) for all the target compounds are given in Table 5. The recover was calculated using equation 5.1. LODs and LOQs were calculated as the minimum detectable amount of analyte with a signal-to-noise ratio of 3 and 10, respectively, in the different samples analyzed. LOQs in the raw sewage ranged from 0.5 ng L^{-1} for estrone to 5.9 ng L^{-1} for naproxen. For the recovery study, 100 mL of each type of sample were fortified to a final concentration of 20 ng mL^{-1} of all the target analytes and were submitted to SPE, following the methodology aforementioned explained. Analyses were performed in triplicate.

$$R(\%)= \frac{C_D + C_B}{C_T} \cdot 100 \qquad \text{(Eq. 5.1)}$$

where C_T corresponds to the theoretical added concentration, C_D is the actual concentration determined in the fortified sample and C_B is the concentration determined in the non-fortified sample (background concentration of the real sample). The R% values obtained were >50% in most cases. Table 5.1 shows the recovery cartridges from different matrices.

Table 5.1. Limits of detection (LOD), limits of quantification (LOQ) and estimated recovery rates (R%) for the different water matrices studied.

Compound	LOD ($ng.L^{-1}$)	LOQ ($ng.L^{-1}$)	R(%) Raw Sewage (%)	R(%) UASB effluent (%)	R(%) HRAP effluent (%)
Estrone	0.2	0.5	64.73	57.54	58.20
17β-estradiol	0.7	2.4	50.60	72.42	64.99
17α-ethinynestradiol	0.6	2.1	48.90	44.46	64.13
Estriol	1.0	3.2	40.20	64.20	53.35
Nonylphenol	0.7	2.5	20.51	56.64	33.16
Bisphenol A	0.2	0.7	58.79	42.78	61.52
Ibuprofen	1.4	4.6	49.82	38.38	52.82
Naproxen	1.8	5.9	54.12	32.00	77.77
Diclofenac	1.4	4.6	66.13	34.11	67.80
Paracetamol	0.4	1.3	50.76	29.07	72.84
Gemfibrozil	1.0	3.4	40.51	66.99	53.36

5.2.3 Statistical Analyses

The Mann-Whitney U-Statistical test was used for independent samples to confirm the statistical difference between influent and effluent samples. Statistica 10.0® software was used, using a significance level for all tests of 95%.

5.3 Results and discussion

5.3.1 Sewage quality parameters

The physical-chemical properties of the raw sewage, UASB effluent and final effluent from the HRAP are summarised in Table 5.2.

Table 5.2. Physical-chemical characterization of the different water samples evaluated: Raw sewage, UASB effluent ($UASB_{eff}$) and final effluent of the treatment system ($HRAP_{eff}$).

Parameter	**Raw Sewage**		**$UASB_{eff}$**		**$HRAP_{eff}$**	
	Mean ± SD	**Min / Max**	**Mean ± SD**	**Min / Max**	**Mean ± SD**	**Min / Max**
TSS ($mg\ L^{-1}$)	366.58 ±134.62	15.12 / 443.46	64.38 ± 41.36	1.03 / 283.94	149.27 ± 92.82	18.32 / 503.94
VSS ($mg\ L^{-1}$)	254.31 ±213.32	92.31 / 389.32	40.38 ± 41.19	0.31 / 219.92	106.32 ± 77.17	16.92 / 410.14
COD ($mgO_2\ L^{-1}$)	518.64 ±123.21	102.80 / 598.43	232.69 ±109.21	111.14 / 482.24	146.08 ±65.40	98.15 / 321.43
pH	7.4 ± 0.2	7.0 / 8.0	7.1 ± 0.4	6.8 / 8.3	8.4 ± 0.7	7.1 / 10.2
DO ($mg\ L^{-1}$)	1.04 ± 0.55	0.05 / 2.33	0.34 ± 0.12	0.13 / 1.36	9.04 ± 2.95	3.45 / 14.45
Temperature (ºC)	24.5 ± 2.5	20.6 / 29.4	23.5 ± 2.6	19.8 / 29.1	21.8 ± 3.3	16.3 / 30.1
TN ($mg\ L^{-1}$)	34.43 ± 9.31	26.21 / 53.12	54.33 ± 5.32	42.21 / 63.95	24.31 ± 11.02	14.13 / 48.04
$N\text{-}NH_4^+$ ($mg\ L^{-1}$)	25.21 ± 8.13/	7.10 / 32.25	34.21 ± 13.43	14.92 / 54.75	14.31 ± 9.13	10.32 / 55.54
VS* ($g\ L^{-1}$)	-	-	-	-	1.01 ± 2.55	0.20 /3.25
TKN* ($mg\ L^{-1}$)*	-	-	-	-	101.37 ± 65.16	29.71 / 235.77
Carbohydrates* ($mg\ L^{-1}$)	-	-	-	-	102.98 ± 86.43	13.89 / 318.82
Proteins* ($mg\ L^{-1}$)	-	-	-	-	633.56 ± 407.25	185.68 / 1,473.56

Note: TS – Total Solids; VS – Volatile Solids; COD – Chemical oxygen demand ; DO – Dissolved Oxygen; TN – Total Nitrogen; $N\text{-}NH_4^+$ - Ammonium - * measured only in HRAP biomass

Average COD values obtained were 232 mg L^{-1} in the UASB reactor effluent and 146 mg L^{-1} in the HRAP effluent, with average removals of 55% and 72% in the UASB reactor and UASB+HRAP, respectively. In general, COD removals between 55 to 65% have been reported for UASB reactors and between 65 and 80% for UASB followed by polishing ponds systems (VON SPERLING 2007) or HRAPs (VILLAR-NAVARRO et al., 2018).

In the UASB reactor, no nitrogen removal was observed, but organic nitrogen was mineralized. Under anaerobic conditions, the decomposition of the organic matter by anaerobic microorganisms leads to the hydrolysis of proteins and urea and the consequent increase of NH_4^+-N (METCALF & EDDY *et al.*, 2003). Mean concentrations found in the raw sewage were 25 mg $N\text{-}NH_4\ L^{-1}$, increasing to 34 mg $N\text{-}NH_4 \cdot L^{-1}$in the UASB effluent. In the HRAPs, an average removal of 44% for

NH_4^+-N was observed, with final effluent concentrations of 14 mg N-NH_4 L^{-1}. The reduction of NH_4^+ in the ponds was due to nitrification and volatilization. Regarding TN, an overall removal of 30% for was observed.

The observed TSS and VSS concentrations increased from the UASB reactor to the HRAP by 130% and 165% respectively. However, the total removal for TSS and VSS was about 59% and 58%, respectively. These results are consistent with the typical values of domestic effluents (Metcalf & Eddy, 2003), UASB reactor effluents (CHERNICHARO 2007) and HRAPs effluents as UASB post treatment (SANTIAGO *et al*. 2017). UASB reactors operating at HRT of 15 hours followed by an HRAP working at HRT of 4- 6 days showed overall removals of 60% for N-NH_4^+, and an average increase of 130% for TSS and 165% for VSS due to microalgae growth (Santiago et al., 2017; Villar-Navarro et al., 2018).

Considering the current Brazilian and Minas Gereais (local where this study was performed) legislation on urban wastewater (CONAMA DIRECTIVE 430/2011 and COPAM DIRECTIVE 01/2008), maximum effluent discharge concentrations for COD, TSS and N-NH_4^+ are set to 180 mg L^{-1}, 150 mg L^{-1} and 20 mg L^{-1} respectively (MORAIS; SANTOS, 2019). The studied system successfully meets all the required limits, parameters, as final concentrations for COD, TSS and N-NH_4^+ of 146 mg L^{-1}, 106 mg L^{-1} and 24 mg L^{-1} were obtained, respectively. On the other hand, considering the most restrictive European urban wastewater Directive (COUNCIL DIRECTIVE 91/271/EEC), with COD, TSS and TN discharge limits in effluents set to 125 mg L^{-1}, 35 mg L^{-1} and 15 mg L^{-1} respectively, the quality of our final effluent would not be compliant. Nevertheless, it is important to note that for a complete analysis of the domestic effluent treatment capacity in natural systems (such as this one), an assessment of at least one year of data would be required to verify the seasonality effect (VON SPERLING, 2007).

The microalgae biomass harvested had a VS concentration of 1.01 g L^{-1}, and 101.4 mg TKN L^{-1}, 102.9 mg L^{-1} of carbohydrates and 635 mg L^{-1} of proteins. Carbohydrates and proteins corresponded to 10% and 62% of the biomass wet weight, respectively. Regarding the different microalgae species present in the HRAPs, *Chlorella vulgaris* was predominant, together with *Scenedesmus* sp., *Westella botryoides* and different species of diatoms.

5.3.2 Occurrence of micropollutants in raw sewage

The concentrations of the targeted micropollutants in the raw sewage are shown in Figure 5.1. The frequency of detection was indicated on top of the box whiskers. Values ranged from 4 ng L^{-1} (E3) to 445 ng L^{-1} (naproxen).

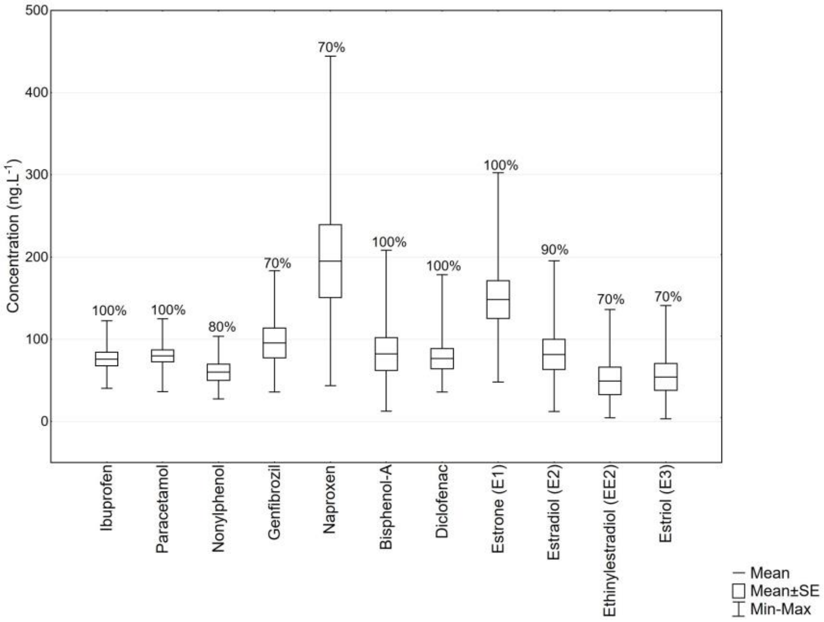


Figure 5.1. Concentrations of the target micropollutants in the raw sewage. Frequencies of detection (%) are shown on top of the box-plots.

In order to simplify the discussion of the results, the target micropollutants were divided into family groups, PhACs (ibuprofen, naproxen, diclofenac, paracetamol and gemfibrozil) estrogens (E1, E2, EE2 and E3), and xenoestrogens (nonylphenol and bisphenol A).

For PhACs, naproxen and gemfibrozil were those detected at the highest concentrations (195.14 and 95.57 ng L^{-1}, respectively). Concentrations for the anti-inflammatories ibuprofen and diclofenac were much lower than those generally found in sewage water (75.82 ng L^{-1} and 76.38 ng L^{-1}, respectively) (AMÉRICO et al., 2012). For gemfibrozil, the values found are within the reported in the literature in different countries (i.e. France, USA and China) (LUO et al., 2014).

For hormones, E1 and E2 were detected at the highest concentrations (148.42 ng L^{-1} and 81.5 ng L^{-1}, respectively). Similar levels have been reported in raw sewage from Brazil (AMÉRICO *et al.*, 2012; FROEHNER *et al.*, 2011). EE2 and E3 were detected at 49.29 ng L^{-1} and 54.05 ng L^{-1}, respectively. When compared to concentrations from different countries, EE2 concentrations are usually lower, ranging from 1 to 3 ng L^{-1} (LUO, et al. 2014; NIE et al. 2012; ZORITA et al. 2009). This difference could be explained by a different consumption pattern the study sites. Regarding the natural estrogens E1, E2 and E3, values found in the present study are within those reported in the literature (LUO, et al. 2014) However, when compared to each other, higher concentrations of E3 than E2 and E1 are usually found in the literature, since the human excretion rate of E3 is far larger than that of E2 or E1 (LIU *et al.*, 2015). But it should be noticed that both E3 and E2 can be dissociated to E1, causing a mass increase of the latter compound (FAN *et al.*, 2011).

EE2 is a hormone formed from E1 by ethinylation, which makes it more stable within the organisms, thus able to exert its contraceptive function. During its metabolism in the organism, it is conjugated with glucuronic and sulfuric acids and so they are excreted and released into sewage in its less active form. However, under anaerobic conditions, these metabolites can revert back to their active form, deconjugating and being transformed back into E1 (BENFENATI *et al.*, 2003; SHORE; GUREVITZ; SHEMESH, 1993). This may also be observed within the pipelines that lead the sewage to the STP (BRANDT *et al.*, 2013).

Regarding the xenoestrogens nonylphenol and bisphenol A, the average concentrations found were 59.86 ng L^{-1} and 82.04 ng L^{-1} respectively, which are similar to those published in previous studies (BRANDT et al. 2013; LUO, GUO, NGO, et al. 2014; NIE et al. 2012; POTHITOU and VOUTSA 2008).

5.3.3 Behavior and fate of micropollutants in the UASB + HRAP system

5.3.3.1 <u>Pharmaceuticals</u>

Concentrations of the five PhACs studied in the effluents from the UASB reactor and HRAP are illustrated in Figure 5.2.

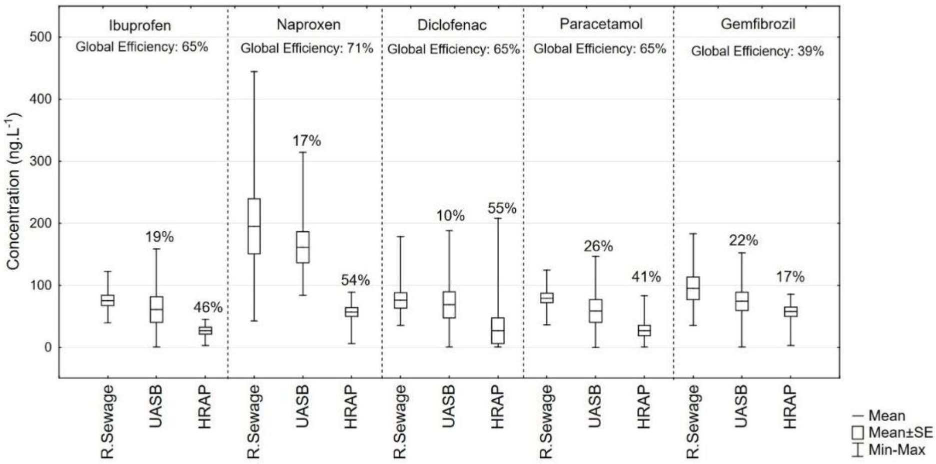


Figure 5.2. Removal efficiency of pharmaceutical in UASB-HRAP system. The percentage placed on top of the plots refers to the average removal observed for the corresponding compound in the corresponding system

Removal rates obtained for the five PhACs in the anaerobic phase of the treatment were lower than 30%, which agrees with previous results (BRANDT *et al.*, 2013; DE GRAAFF *et al.*, 2011; REYES-CONTRERAS *et al.*, 2011). PhACs have pK_a values between 4 and 5 and log K_{ow} between 3 and 4, (with the exception of paracetamol, pK_a = 9.8 and log K_{ow} = 0.46) (CASTRO, 2017; CASTRO *et al.*, 2018). Therefore, sorption could be the main removal mechanism and the ionization of these PhACs may be the factor that causes these compounds to remain in the liquid phase. The low HRT and upflow velocity can cause a detachment of these compounds from the solid phase to the liquid phase. In addition, the repulsion of these compounds (deprotonated) from the sludge blanket (negatively charged) makes them mostly present in the liquid phase in the UASB. Paracetamol follows a different pattern, as it is highly hydrophilic with no tendency to adsorb onto the biosolids. Although it has a high tendency for biodegradation (K_{bio} = 80 $L \cdot gSS^{-1} \cdot d\text{-}^{1}$) (JOSS *et al.*, 2006), the HRT applied to the reactor studied was too low (7 hours) for an efficient biodegradation. Indeed, previous publications obtained removals higher than 90% for paracetamol in a UASB reactor operating at an HRT 30 times higher than that in the present study (DE GRAAFF *et al.*, 2011).

Gemfibrozil was only removed a 20% in the UASB reactor, despite its high tendency to adsorption. This could be explained in terms of its competition with humic substances present in the reactor for the active adsorption sites of the sludge blanket (MAENG *et al.*, 2011).

In the HRAP, removals were greater than 60% for all the anti-inflammatories and analgesics studied (ibuprofen, paracetamol, diclofenac and naproxen). Diclofenac was removed in a 65%. Its low K_{bio} (less than 0,1 $L \cdot gSS^{-1} \cdot d^{-1}$) indicates that biodegradation can be neglected as removal pathway (DE LAURENTIIS *et al.*, 2014). On the other hand the high photosensitivity of this compound has been demonstrated in previous studies, and photodegradation is probably the main elimination route for this anti-inflammatory (GARCÍA-GALÁN et al., 2020; XIONG et al., 2018; IOVINO et al. 2017; ZHANG et al., 2008). Contrary to diclofenac, ibuprofen is highly biodegradable. A recent experiment performed at laboratory scale, using effluent water from a stabilization pond, demonstrated that, regardless the presence or absence of light, the concentration of ibuprofen remained the same when microalgae were not present (LARSEN *et al.*, 2019). On the contrary, when inoculating the system with a pure culture of *Scenedesmus obliquus*, the removal of ibuprofen reached almost a 90%.

Similarly to diclofenac, paracetamol was removed a 65% in the HRAP. It is indeed a readily biodegradable compound (K_{bio} = 80 $L \cdot gSS^{-1} \cdot d\text{-}^{1}$) (JOSS *et al.*, 2006) and removals of up to 90% were obtained in microalgae-based systems *(*ZHOU et al., 2014). Additionally, direct photolysis has also been reported as an important mechanism of paracetamol removal (DE LAURENTIIS et al., 2014). Naproxen was removed a 70%, which is in accordance to previous results (Hom-Diaz et al. 2017; Matamoros et al. 2015). Naproxen removal in conventional STPs is mainly attributed to biodegradation (K_{bio} ~9 $L \cdot gSS^{-1} \cdot d^{-1}$) (SUAREZ; LEMA; OMIL, 2010), whereas sorption processes can be neglected due to its low K_{ow} (KASPRZYK-HORDERN; DINSDALE; GUWY, 2009). Simultaneous removal of NH_4^+-N and naproxen could also take place, as a consequence of the co-metabolic biotransformations induced by autotrophic aerobic bacteria present in the HRAPs (FERNANDEZ-FONTAINA *et al.*, 2012; HELBLING *et al.*, 2012; YI *et al.*, 2006). Some authors suggested that the enzyme ammonium monooxygenase (AMO) is the catalyst responsible for the micropollutants and NH_4^+-N co-metabolism (YI *et al.*, 2006).

For the antilipemic gemfibrozil, an average removal of 40% was obtained. The higher removal of this compound compared to that obtained in the UASB reactor may be related to a lower competition for adsorption sites with the humic substances and a greater availability of active sites in the microalgae biomass. However, the lower removal of this compound compared to the other PhACs studied could be due to the fact that the hydrophobicity of gemfibrozil decreases with an increasing pH, as recently demonstrated by Phan et al., (2018), who observed that the K_d of gemfibrozil was 2.1 when pH was 7, and so gemfibrozil migrated from the solid phase into the aqueous phase.

5.3.3.2 Estrogens

Concentrations of E1, E2, EE2 and E3 in the effluents from the UASB reactor and the HRAPs are shown in Figure 5.3. Removals < 50% were observed for E1 and E3 after the UASB treatment, which is in accordance with previous publications in anaerobic systems such as anaerobic membrane reactors and completed stirred tank reactors (GONZALEZ-GIL *et al.*, 2016; ITO *et al.*, 2016). The low removal of E1 and E3 may be associated with the low HRT (7 hours) and the low sludge retention time (SRT) (20 days) applied in the UASB reactor. Previous studies have demonstrated that SRT between 10-30 days had nearly no effect on the biotransformation of PhACs and estrogens (GONZALEZ-GIL et al., 2016). Nevertheless, E2 was removed a 85% in the UASB reactor, but this elimination was not associated to its biodegradation and full mineralization, but to its transformation to E1 under anaerobic conditions (ADEEL *et al.*, 2017). This could corroborate that the hormone E1, contrary to what is usually reported in the literature, occurs in greater quantities in the raw sewage than E2 due to this dissociation.

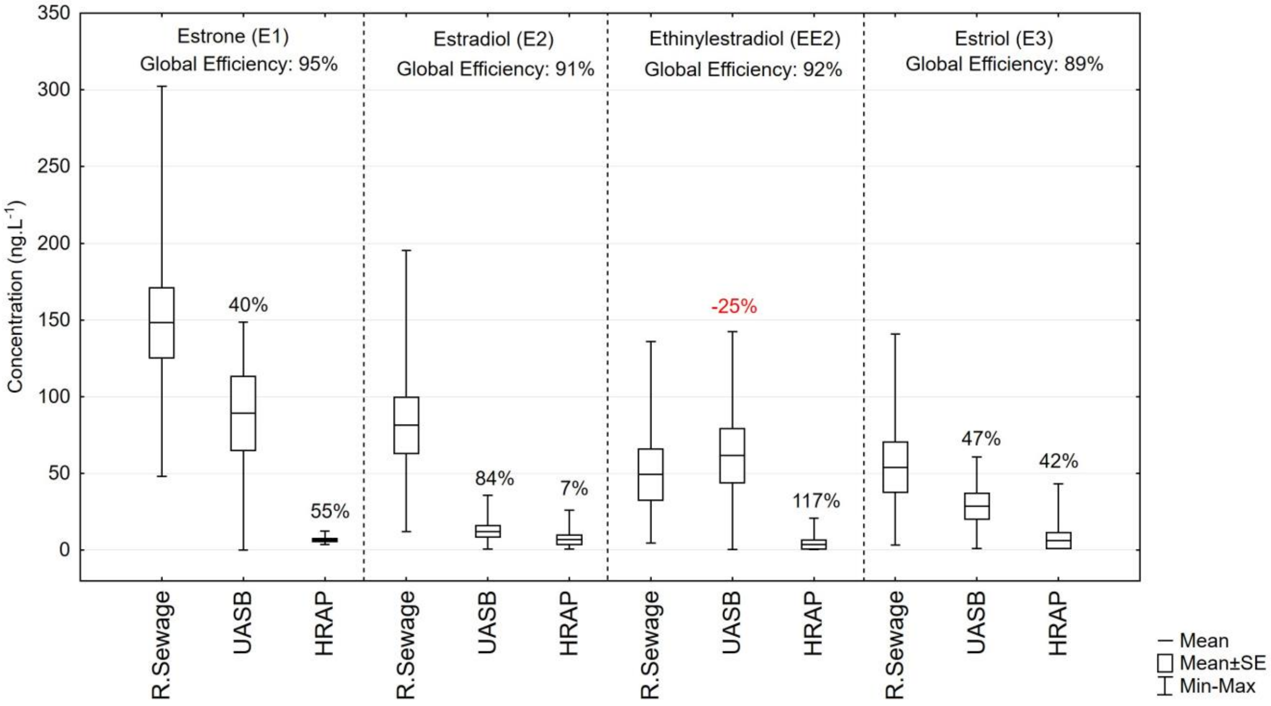


Figure 5.3. Concentrations of estrogens in UASB-HRAP system. The percentage placed on top of the plots refers to the average removal observed for the corresponding compound in the corresponding system.

Despite many studies have addressed the anaerobic degradation of hormones, the reactors evaluated usually operated at HRTs higher than 12 hours, directly affecting the degradation of the compounds. Indeed, two recent studies confirmed that HRT is critical in the removal of micropollutants from UASB reactors, as it is directly related to the contact time between the wastewater and the sludge inside the reactor (QUEIROZ et al. 2012; ALVARINO et al. 2018). The short HRT of the present study may also account for the increased EE2 concentration in the UASB reactor effluent. It is important to point out that in most of the studies carried out only liquid samples, and not solid samples, are analyzed, so a complete mass balance is seldom achieved.

In UASB reactors, the flow rate is applied from the bottom upwards and, therefore, the liquid upflow velocity can cause turbulence in the sludge blanket (ALVARINO *et al.*, 2014). Therefore, lower HRTs mean higher upflow rates applied and, consequently, a higher velocity within the UASB. The turbulence caused in the blanket increases the transfer of lipophilic compounds from the solid to the liquid phase. In addition, under the pH in the UASB (7.1), compounds with high ionization coefficients (pK_a) as EE2 (pK_a = 10,5) tend to ionize (BRANDT *et al.*, 2013). Negatively charged molecules are repelled by the negatively charged biomass and, therefore, remain in the liquid phase (SCHÄFER, ANDREA I. *et al.*, 2011).

No clear trend was observed for anaerobic hormone removal and indeed, most of the previous studies on the removal of these compounds in anaerobic systems reported that estrogens are not significantly removed (BRANDT *et al.*, 2013; CARBALLA *et al.*, 2007; SHI *et al.*, 2010).

In contrast, the results after HRAP treatment showed removals between 88% and 95% for all the hormones evaluated. Similar results were found in the literature for the same compounds (Shi et al., 2010). Removal of micropollutants in HRAPs are related to bioadsorption, biodegradation, photodegradation and volatilization (GARCÍA-GALÁN, MARÍA JESÚS *et al.*, 2020). Hormones have a moderate tendency to adsorb onto solid matrices, (log $K_{ow} > 2.65$), but they are highly biodegradable ($K_{bio(EE2)} = 9 L \cdot gSS^{-1} \cdot d^{-1}$) (JOSS *et al.*, 2006). In microalgae-based treatment systems, the microbiology community acts as a biosorbent (Gadd, 2009). The cell wall of microalgae and bacteria contains polysaccharides and proteins that can provide adsorption sites for the organic contaminants (FOMINA; GADD, 2014; MORITZ; FLEMMING; WINGENDER, 2010). In addition, microalgae may be actively involved in the biodegradation of the organic contaminants, as their enzymes can metabolize these compounds (WANG, Y. *et al.*, 2017). Therefore, for the hormones evaluated in this work, sorption to the solid matrix and biodegradation are probably the two main removal pathways. In particular for EE2, which was the most stable hormone and had the lowest biodegradability coefficient, degradation may also be associated also with the removal of NH_4^+-N (~ 60%, Table 2), as explained for naproxen. Regarding photodegradation, half-lives of 10 days have been estimated for the photolytic degradation of E2 and EE2 (Jurgens et al., 2002). These tests were done on a bench-scale system, using surface water, with daily and direct radiation, suggesting that irradiation could enhance the removal of these hormones. Nevertheless, biosorption to the biomass and biodegradation seem to be the main elimination routes in HRAPs (HOM-DIAZ et al. 2015; YONGLI ZHANG et al. 2014).

5.3.3.3 Xenoestrogens

The concentrations and removals of nonylphenol and bisphenol A in raw sewage and effluents from the UASB reactor and HRAP are shown in Figure 5.4. The removals achieved for both compounds in the UASB were very low, and the statistical test confirmed that there was no significant removal in this treatment unit.

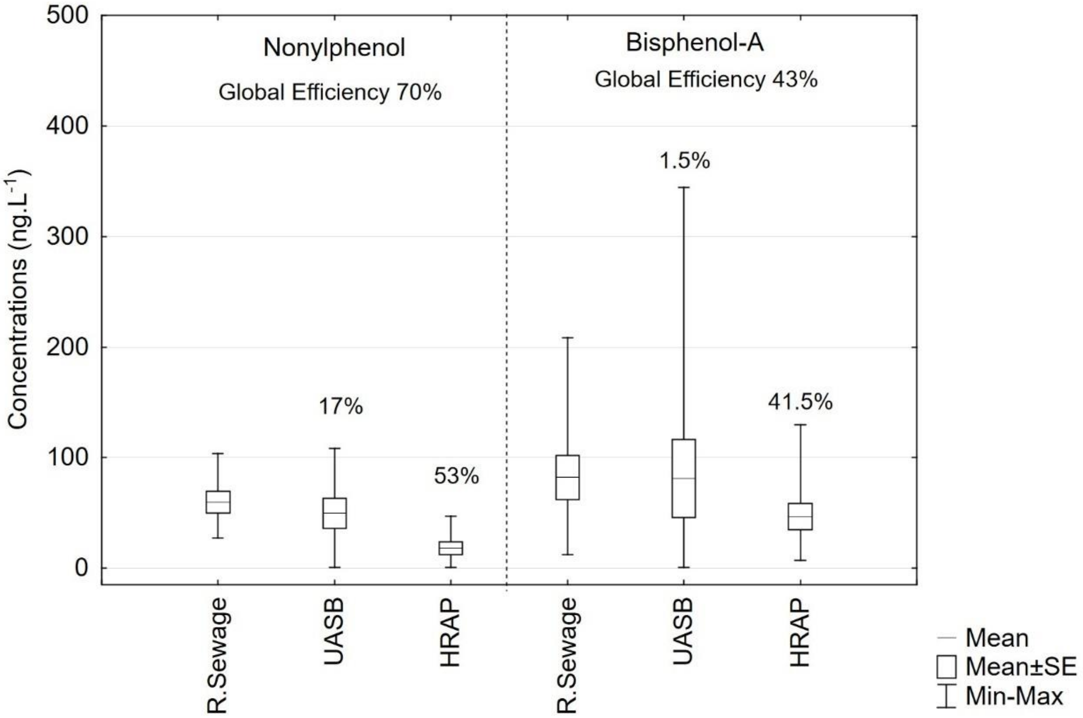


Figure 5.4. Concentrations of xenoestrogens in UASB-HRAP system. The percentage placed on top of the plots refers to the average removal observed for the corresponding compound in the corresponding system.

For nonylphenol, the mean removal was approximately 17%. Nonylphenol is a raw material for the production of non-ethoxylated nonylphenols (NEPO) (VIRKUTYTE; VARMA; JEGATHEESAN, 2010). The recalcitrance of nonylphenol in anaerobic sewage treatment systems is due to the degradation of the NEPOs present in the formulations of cleaning products (AQUINO; BRANDT; CHERNICHARO, 2013). Furthermore, nonylphenol has a slower degradation kinetics in anaerobic environments due to the alkyl chain and the aromatic ring in its molecular structure (SOARES *et al.*, 2008). Despite its pK_a >10, it can be deprotonated and repelled by the solids of the reactor (BRANDT *et al.*, 2013).

Of all the compounds evaluated in this study, bisphenol A was the most recalcitrant to anaerobic treatment. It should be noted, however, that this plasticizer is present in some of the components of the experimental treatment line of this study and thus can be transferred to the liquid phase. causing an increase in concentration. Bisphenol A has a moderate sorption capacity to solid matrices and, besides, the low HRT in UASB reactors led to a shorter contact time of the compound with the sludge blanket, reducing the possibility of sorption. Studies on the biodegradation of this compound in anaerobic environments have shown its recalcitrance under such conditions (RONEN; ABELIOVICH, 2000).

Regarding their removal in the HRAP, an average elimination of 70% for nonylphenol and 44% for bisphenol A was obtained. Removal of Nonylphenol is directly related to biodegradation, and previous studies have shown that it can be absorbed and adsorbed by *Chlorella sp*. (Gao et al., 2011). Photodegradation should not be neglected for this compound. For bisphenol A, Matamoros et al., (2016) obtained higher removals of 60-90% in HRAP. As aforementioned, the different results can be attributed to the concentration and transfer of bisphenol A from the different plastic materials associated to the HRAP. Oxidation of the hydroxyl radicals in the HRAP should also be neglected. with high DO in the HRAP that allows the oxidation

5.4 Conclusions

The efficiency of an anaerobic-aerobic wastewater treatment system, consisting of an UASB reactor followed by an HRAP system, was evaluated, focusing in the removal of 11 selected organic micropollutants. This treatment strategy was efficient treating wastewater in terms of solids, organic matter and nutrients, and also in terms of organic micropollutants. The UASB reactor showed a limited removal for all the targeted compounds, and only the estrogen E2 was removed significantly (85%). The short operation HRTs of the UASB reactor seems to be the most feasible explanation for the low removals obtained. On the contrary, the HRAP system proved to be more efficient in removing estrogens (90%-95%), PhACs (64%-70%) with the only exception of gemfibrozil (39%), and to a lesser extent xenoestrogens, removals reached between 40% and 70%. Irradiance is key in these microalgae-based systems, as it can lead to both direct photodegradation and to an increased growth of microalgae and, in consequence, a higher bio adsorption and biodegradation.

Further research is required to optimize the different operational parameters of this dual system to eventually improve the removal of the studied compounds and a broader range of contaminants. Different types of water and/or microalgae species should also be tested.

6 UPFLOW ANAEROBIC SLUDGE BLANKET IN MICROALGAE-BASED SEWAGE TREATMENT: CO-DIGESTION FOR IMPROVING BIOGAS PRODUCTION

Based on: Vassalle, L., Díez-Montero, R., Machado, A.T.R., Moreira, C., Ferrer, I., Mota, C.R., Passos, F., 2020a. Upflow anaerobic sludge blanket in microalgae-based sewage treatment: Co-digestion for improving biogas production. Bioresour. Technol. 300, 9. https://doi.org/10.1016/j.biortech.2019.122677

Upflow anaerobic sludge blanket (UASB) reactors are widely used to treat domestic sewage and frequently require post-treatment. Little is known about the use of high rate algal ponds (HRAP) for post-treating UASB reactors' effluent. This study aimed to evaluate a UASB reactor followed by a HRAP in terms of sewage treatment efficiency and biogas production, during one year at demonstration-scale. The UASB reactor co-treated raw sewage and the harvested microalgal biomass from the HRAP, which was recirculated to the reactor. An identical UASB reactor, treating only raw sewage, was used as control. The results showed an overall removal of 70% COD and 57% $N\text{-}NH_4^+$ in the system. Furthermore, methane yield was increased by 35% after anaerobic co-digestion with microalgae, from 156 to 211 NL CH_4 kg^{-1} VS. An energy assessment was performed and showed a positive energy balance, with a net ratio of 2.11 to the annual average.

6.1 Introduction

Over the years, the technology of upflow anaerobic sludge blanket (UASB) reactors has been expanding for treating domestic and industrial wastewater. UASB reactors have allowed the expansion of the sewage treatment infrastructure to a vast population, especially in locations with low availability of financial resources, land and/or skilled workers (BRESSANI-RIBEIRO, THIAGO *et al.*, 2019). In this context, this technology have been successfully applied to treat domestic sewage in developing countries, especially with tropical climate like Brazil, Colombia, India and Africa (CHERNICHARO, RIBEIRO; PEGORINI; *et al.*, 2018). Essentially, these reactors remove suspended and dissolved organic matter and, as a result, generate two co-products: a stabilised sludge and biogas. Biogas is mainly composed of methane, which allows its conversion into energy, transforming sewage treatment into a more sustainable platform and contributing to the circular economy.

If compared with complete stirred tank reactors (CSTR) normally used for sludge digestion, the greatest advantage of the UASB technology for treating domestic sewage is its higher rate, allowing a low hydraulic retention time (6-10 hours HRT), but a high solid retention time (~ 30 days SRT) and relatively low cell growth, which implies low sludge generation (DAUD *et al.*, 2018). The applicability of UASB reactors treating sewage in tropical environmental conditions is undeniable in terms of economy, operation and area demand, particularly when compared to activated sludge or stabilisation ponds, for instance. However, the effluent from these reactors often need to be subjected to a post-treatment step in order to remove, above all, nutrients and pathogenic organisms in order to meet worldwide discharge standards (DAUD *et al.*, 2018). Numerous technologies have already been studied and proven efficient for post-treatment of UASB domestic effluent, among them are constructed wetlands, percolating trickling filters and polishing ponds (MUNGRAY; MURTHY; TIRPUDE, 2012). However, there are very few studies evaluating the post-treatment of UASB effluent using high rate algal ponds (HRAP) (DE GODOS. *et al.*, 2016; SANTIAGO *et al.*, 2017; VILLAR-NAVARRO *et al.*, 2018).

The use of UASB reactors followed by HRAPs, may be conceived from the perspective of sustainability and co-products recovery, while promoting sanitation in terms of domestic sewage treatment in tropical developing countries. This suggests that generated co-products may minimise environmental and economic costs and impacts, or even be used for activating the local or regional economy. In the context of this work, microalgal biomass produced in HRAPs, may be converted into biogas and biosolids through anaerobic digestion in the first step of the sewage treatment plant (STP), i.e. the UASB reactors. In this scenario, the recirculation of microalgal biomass to be digested together with the raw sewage, is characterised as anaerobic co-digestion. In spite of the fact that co-digestion of microalgal biomass has been extensively investigated with many substrates, to the authors knowledge there is no studies using domestic sewage as co-substrate (SOLÉ-BUNDÓ; GARFÍ; *et al.*, 2019; UGGETTI *et al.*, 2017). Previous literature shows that co-digestion may increase the biodegradation rate and overall biogas production, due to a more adequate balance of solid to liquid ratio, macro and micronutrients (as carbon to nitrogen balance) and the dilution of toxic and inhibitory compounds, for instance (GONZÁLEZ-FERNÁNDEZ; SIALVE; BERNET; *et al.*, 2012a; GUTIÉRREZ *et al.*, 2016; MATA-ALVAREZ *et al.*, 2014). In addition, it also enables simultaneous treatment infrastructure and final disposal of several residues.

Most studies on microalgae-based STPs have been based on anaerobic co-digestion of microalgal biomass with primary or secondary sludge (SOLÉ-BUNDÓ; PASSOS; *et al.*, 2019). Reported results have shown an increase in methane yield if compared to anaerobic mono-digestion (50-80 %) (SOLÉ-BUNDÓ; GARFÍ; *et al.*, 2019). According to the literature, the methane yield from anaerobic reactors mono-digesting microalgal biomass ranges from 0.10 to 0.25 L CH_4 g VS^{-1} (SOLÉ-BUNDÓ *et al.*, 2018), while those values to sewage sludge substrate are around 0.35 L CH_4 g VS^{-1} (RAMOS-SUÁREZ; CARRERAS, 2014). In terms of microalgal biomass co-digestion with waste activated sludge methane yield was about 0.47 L CH_4 gVS^{-1} (WANG *et al.*, 2013). Moreover, another study also confirmed that the co-digestion of microalgae with sewage sludge (a mixture of primary sludge, biosludge and chemical sludge) increased by 12% the biogas production compared to microalgae anaerobic digestion alone (OLSSON *et al.*, 2014). In addition, the anaerobic co-digestion of microalgae may have specific advantages, as increasing microalgae hydrolysis rate (SOLÉ-BUNDÓ; PASSOS; *et al.*, 2019; WANG. *et al.*, 2013; WANG; PARK, 2015b).

Based on the gaps presented in the literature and in order to propose a sustainable low-cost STP with the recovery of co-products, this study aimed to evaluate a UASB reactor followed by HRAP treating domestic sewage. The first part of this work focused on the sewage treatment efficiency. The second part evaluated the anaerobic digestion of raw sewage and its anaerobic co-digestion with microalgal biomass harvested from the UASB reactor. Finally, an energy assessment was estimated to comprehend the process self-sufficiency. To the authors knowledge, this is the first time that the proposed STP flowsheet is investigated and evaluated in a demonstration-scale facility.

6.2 Material and Methods

6.2.1 Experimental set-up and operation

The demonstration-scale experimental set-up is shown in Figure 6.1. The set-up received raw sewage from a nearby STP located in Belo Horizonte, Brazil (coordinates 19º53'42" S and 43º52'42"W, 800 m of altitude). Sewage reached the system by means of a pump (Netzsch® Germany) after a pre-treatment for the removal of coarse solids and grit. The study was conducted in two UASB reactors, i) $\text{UASB}_{\text{cont}}$: fed only with domestic sewage, and ii) $\text{UASB}_{\text{co-dig}}$: fed with domestic sewage and harvested microalgal biomass and used to evaluate the anaerobic co-digestion. Following, a settler was used to separate and concentrate the microalgal biomass from the HRAP effluent. Co-digestion operation was performed using a centrifuge pump (BCR 2000 – Schneider®) for recirculating the microalgal biomass from the settler to the co-digestion UASB reactor.

The UASB reactors were made of fiberglass, with a working volume of 343 L each. The reactors were operated at a flow rate of 49 L h^{-1}, a HRT of 7 hours, a sludge retention time (SRT) of 35 days and an organic loading rate (OLR) of 0.71 g VS L^{-1} day^{-1}. The HRAPs were also made of fiberglass, with a working volume of 205 L each one and a surface area of 0.41 m^2 each. The ponds received the effluent from one of the UASB reactors, and were operated at a flow rate of 25.5 L day^{-1} each one, and a HRT of 8 days. Treated effluent was conducted by gravity to a 30 L a settler in which microalgal biomass was harvested. The settler was made of PVC and operated at 14 hours HRT. For anaerobic co-digestion, 10 L of microalgal biomass were conveyed to a plexiglass column located 4 m above the UASB and recirculated therefrom at a flow rate of 0.5 L h^{-1} into the bottom of the reactor. The recirculation flow rate was selected in accordance with previous hydraulic tests that showed how higher flow rates (from 0.5 to 10 L h^{-1}) led to the release of the reactor sludge blanket and effluent quality deterioration. The biomass inlet flow was controlled by a needle-type flow-controlled valve. The system was operated continuously for 12 months. The $\text{UASB}_{\text{cont}}$ was fed only with raw sewage, while $\text{UASB}_{\text{co-dig}}$ was fed with raw sewage and harvested microalgal biomass (Figure 6.1).

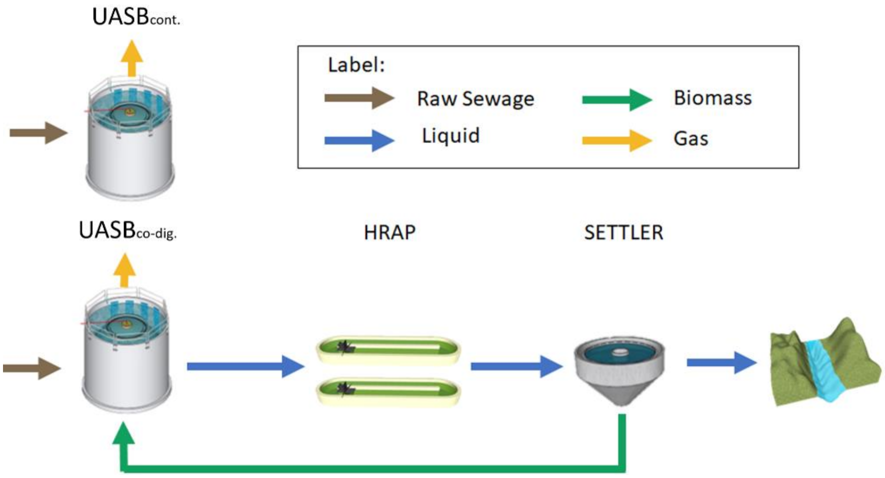


Figure 6.1. Diagram of the demo-scale experimental set-up

6.2.2 Analytical methods

For evaluating the sewage treatment efficiency, four samples were taken from the liquid phase: from the raw sewage, the two UASB reactors effluent and the HRAPs effluent, twice a week. The parameters analysed were pH (at 10 AM), temperature and dissolved oxygen (DO) using a Hach® (HQ30D) probe. COD was analysed by Hach® kit COD at high range. Total and volatile suspended solids (TSS and VSS) were assessed according to Standard Methods (APHA, 2012) and ammonium ($N\text{-}NH_4^+$) by ionic chromatography using Metrohm® - 940 professional IC Vario - ionic chromatography.

For microalgal biomass characterisation, total samples in solid phase were taken once a week from the settler. Total and volatile solids (TS and VS) and Total Kjeldahl Nitrogen (TKN) were analysed according to standard procedures (APHA, 2012). Total COD was analysed by Hach® kit COD at high range. For carbohydrates was measured using a phenol–sulphuric acid method after acid hydrolysis according to literature (DUBOIS *et al.*, 1956). For estimating the protein content, a conversion factor of 5.95 (LÓPEZ *et al.*, 2010) was used based on the results of TKN. The main microalgae species in the HRAPs were identified through optic microscopy (Olympus BX-50) equipped with a camera (Olympus DP70).

Samples for biogas analysis were collected twice a week from both the control and co-digestion UASB reactors. Biogas production was measured twice a week using Ritter® meters. In addition, biogas characterisation in terms of CH_4, CO_2, O_2, CO and H_2S were analysed by means of a Geotech® brand portable meter. The results were expressed as methane yield, i.e. methane volume produced per mass of COD and VS fed to the reactor. Substrate biodegradability was calculated through the ratio between the methane yield in terms of COD_{in} measured and the theoritical methane yield under standard conditions (350 ml CH_4 g $COD_{removed}^{-1}$) (Chernicharo, 2007). Climatic data (temperature, solar radiation and precipitation) were obtained from the meteorological station near the STP (Brasil National Meteorology Institute, INMET, http://www.inmet.gov.br).

6.2.3 Energy Assessment

To assess the STP energy self-sufficiency, an energy balance was estimated from the energy requirement of the proposed system and the energy produced from the average methane yield monitored during the experimental period (12 months). For the calculations, the UASB and HRAP systems were scaled-up and projected for receiving 10,000 population equivalent (PE). For the preliminary treatment of this STP, it was considered manual grid and gravity degritters, that is, without energy costs. The energy input (E_{in}) comprised: i) the energy demand for sewage (and microalgal biomass) pumping ($E_{in,\ UASB}$) and, ii) the energy demand for the HRAP paddle-wheel ($E_{in,\ HRAP}$). For the UASB co-digestion reactor, a total of 300 m^3 d^{-1} (20% from total flow) of microalgal biomass was recirculated during the whole year, since no great differences in microalgal biomass production was noticed due to similar solar irradiation conditions obtained in this study. It is important to note that the operation of the UASB reactor had no external energy requirement, since it was operated at environment temperature during the whole year. The energy produced (E_{out}) was calculated from the methane yield in the control (only domestic sewage) and co-digestion (domestic sewage and microalgal biomass) UASB reactors. This methodology was based and adapted from (PASSOS *et al.*, 2017) and main equations are described following.

6.2.3.1 Energy input

The energy input for the system was calculated from Eq. (6.1).

$$E_{in} = E_{in\ UASB} + E_{in\ HRAP} \tag{6.1}$$

To calculate de energy consumption of the UASB reactor, Eq (6.2) and (6.3) were used. The equations aim to estimate the energy used for pumping wastewater (and biomass, when co-digestion was applied). According to the equations, $E_{pump\ UASB;\ Microalgae}$ is the input energy for the UASB reactor (kWh d^{-1}); Q_p is the pump flow rate (m^3 d^{-1}), Θ is the electricity consumption for pumping (kJ m^{-3}) and 0.000278 is the conversion factor from kJ to kWh.

$$E_{in\ UASB} = E_{pump\ UASB} + E_{pump\ Microalgae} \tag{6.2}$$

$$E_{pump\ UASB}\ or\ E_{pump\ Microalgae} = Q_p \cdot \theta \cdot\ 0.000278 \tag{6.3}$$

In addition, the energy consumed in HRAP was considered to be the energy required for paddle-wheel operation and was calculated according to Equation 6.4, where $E_{paddle\text{-}wheel}$, is the input electricity for the HRAPs paddle-wheel (kWh d^{-1}), Q_{HRAP} is the mixed liquor flow rate in motion ($m^3\ s^{-1}$), γ is the specific weight of water at 20 °C (kN m^{-3}), $h_{f\ channels}$ is the head loss in channels (m), $h_{f\ reversals}$ is the head loss in reversals (m) and ε is the paddle-wheel efficiency.

$$E_{input\ HRAP} = E_{paddle-wheel} = \frac{Q_{HRAP}\cdot\gamma\left(h_{f\ channels} + h_{f\ reversals}\right)\cdot 24}{\varepsilon} \tag{6.4}$$

The HRAP flow (Q_{HRAP}) corresponded to the flow rate through the transversal area of the HRAPs (Eq. (6.5), where υ is the water velocity (m s^{-1}), d is the water depth (m) and W is the channel width (m).

$$Q_{HRAP} = \upsilon\cdot d\cdot W \tag{6.5}$$

The head loss in channels and reversals was calculated according to Eqs. (6.6) and (6.7), respectively, where $h_{f\ channels}$ is head loss in channels (m), L is the channel length (m), n is the Manning factor and, $h_{f\ reversals}$ is the head loss in reversals (m) and g is the gravitational force (m s^{-2}).

$$h_{f\ channels} = \frac{\upsilon^2\cdot L}{\left(\frac{1.486}{n}\right)^2\left(\frac{d\,W}{W+2d}\right)^{1.26}} \tag{6.6}$$

$$h_{f\ \mathrm{reversals}} = 2\cdot\frac{\upsilon^2}{2\cdot g} \tag{6.7}$$

6.2.3.2 Energy output

The energy generated in the STP was calculated in terms of the methane yield produced. To calculate the energy output a lower calorific value of methane of 10 kWh m^{-3} CH_4 (ξ) and an energy conversion efficiency of 90% were considered (η_1) (Eq. 6.8).

$$E_{out} = (O_L Y\ \xi\ \eta_1) \tag{6.8}$$

where E_{output}, is the energy output from biogas (kWh d^{-1}); O_L is organic loading rate of the digester (kg VS d^{-1}); Y is the average methane yield (m^3 CH_4 kg^{-1} VS); ξ is the lower calorific value of methane (kWh·m^{-3} CH_4); and η_1 is the efficiency for energy generation.

6.2.3.3 Energy balance and net energy ratio

Finally, results were expressed in terms of energy balance and net energy ratio (Eq. 6.9 and 6.10). The final energy balance was calculated subtracting the energy output from the energy input, where Δ_E is the final energy balance from methane yield (kWh d^{-1}) (Eq. 6.9).

$$\Delta\text{E} = E_{out} - E_{in} \tag{6.9}$$

While, the net energy ratio (NER) was calculated as the energy output (energy produced by the system) over the energy input (energy consumed by the system) (Eq. (6.10)).

$$NER = \frac{\text{E}_{\text{out}}}{\text{E}_{In}} \tag{6.10}$$

This means that a positive energy balance is when the system has a surplus in terms of energy production (i.e. $\Delta_E > 0$ or NER>1). For the sake of comparison, the assessment was carried out using the same scenarios for control and co-digested UASB reactors.

6.2.4 Statistical analysis

The Mann-Whitney U test for independent samples was used to evaluate COD and ammonia removal, in the complete pilot-scale system by comparing influent and effluent concentration values. For comparing results from anaerobic performance and biogas production in control and co-digestion UASB reactors, the Wilcoxon statistical test was used for paired or dependent samples. To perform the statistical analysis, Statistica 10.0® software was used. The significance level of all tests was 95%.

6.3 Results and discussion

6.3.1 Sewage treatment efficiency

The physical-chemical parameters of raw sewage, $UASB_{cont}$, $UASB_{co\text{-}dig}$, HRAP effluent and microalgal biomass are summarised in Table 6.1. It may be observed that for pH, DO and temperature there was no significant variation for both reactors, indicating that the reactors operated similarly. It should be highlighted that both reactors were continuously fed with the same domestic sewage and that the increase in organic loading rate for the co-digested reactor was due to the addition of microalgal biomass.

Table 6.1. Physical-chemical characterisation of the different sampling points evaluated: Raw sewage, UASB co-digestion ($UASB_{co\text{-}dig}$), control UASB ($UASB_{cont}$) and $HRAP_{eff}$ (n = 92)

Parameters	Raw Sewage Mean ± SD	$UASB_{co\text{-}dig}$ Mean ± SD	$UASB_{cont}$ Mean ± SD	$HRAP_{eff}$ Mean ± SD
pH	7.8 ± 0.2	7.2 ± 0.2	7.3 ± 0.3	9.7 ± 0.4
DO (mg L^{-1})	0.6 ± 0.5	0.2 ± 0.1	0.29 ± 0.13	8.5 ± 5.1
Temperature (ºC)	25.1 ± 1.6	22.3 ± 1.5	23.6 ± 1.9	23.1 ± 3.3
COD (mg O_2 L^{-1})	437.3 ±120.3	197.3 ±48.2	180.6 ±66.6	144.3 ± 27.7
TSS (mg L^{-1})	242.9 ±56.8	55.1 ± 45.1	-	142.3 ± 42.6
VSS (mg L^{-1})	182.2 ±48.7	30.9 ± 8.1	-	96.3 ± 29.1
N-NH_4^+ (mg L^{-1})	32.3 ± 8.8	39.5 ± 7.5	-	17.0 ± 3.2

COD removal in the $UASB_{co-dig}$ was 55%, considering the influent sewage COD and the recycled microalgal biomass COD. The overall removal of COD, after the HRAP, reached 70%. Similar results were found in a previous study carried out with a UASB reactor followed by HRAP, in which a 65% COD removal was obtained (Villar-Navarro et al., 2018a). For the $UASB_{cont}$, the average COD removal was 60%. It may be noticed a lower COD removal in the $UASB_{co-dig}$ when compared with the control. This may have occurred due to microalgal biomass recirculation in the co-digested reactor. Even with a slow recirculation (0.5 L h^{-1}), the upflow biomass movement may have caused a displacement of the UASB sludge blanket, leading to a transport of stabilised organic matter from the solid to the liquid phase. In general, COD removals between 55 to 70% have been reported for UASB reactors and between 65 and 80% for UASB followed by polishing ponds system (VON SPERLING, M., 2007). It may be noticed that in this set-up, the $UASB_{co-dig}$ had slightly lower efficiency in comparison with those reported in the literature for COD removal (54% vs. 55-70%), but when compared the overall removal this data stay within range reported in literature (65% vs. 65-80%).

In the $UASB_{co-dig}$ reactor no total nitrogen removal was observed, but an intense mineralisation of organic nitrogen. Under anaerobic conditions, the decomposition of the organic matter by anaerobic microorganisms, leads to the hydrolysis of proteins and urea and the consequent increase in NH_4^+-N (METCALF & EDDY *et al.*, 2003). Average concentrations observed were 32 mg N-NH_4 L^{-1} for sewage and 39 mg N-NH_4 L^{-1} for UASB effluent. For HRAPs, there was an average removal of 56% of NH_4^+-N, with final effluent concentrations of 17 mg N-NH_4 L^{-1}. According to the literature, the main removal pathway of NH_4^+ in HRAP is due to microalgal biomass assimilation. Nitrification and volatilization could be another removal route. Similar concentrations were found in studies that evaluated the N-NH_4^+ removal in HRAP with similar HRT (DOMA *et al.*, 2016).

TSS and VSS concentrations found in the effluent samples were comparable with the typical values from domestic effluents (120-360 mg TSS L^{-1} and 90-280 mg VSS L^{-1}) (METCALF & EDDY *et al.*, 2003), from UASB reactor effluents (60-160 mg TSS L^{-1} and 30 mg VSS L^{-1}) (CHERNICHARO, 2007) and from HRAPs effluents used as UASB post-treatment units (145 mg TSS L^{-1} and 124 VSS mg L^{-1}) (Santiago et al. 2017) (Table 6.1). The results presented an increase of TSS and VSS values from the UASB reactor to the HRAP by 158% and 220% respectively. However, global removal for TSS and VSS were about 45% and 50%, respectively. Results on HRAP fed with UASB effluent are still incipient in literature. For instance, UASB reactors operating at a higher HRT (15 hours) and HRAP at lower HRT (4 and 6 days) compared to the HRT in both treatment units in this work, showed overall removals of 60% for NH_4^+-N, and an average increase of 130% for TSS and 165% for VSS due to microalgae growth (SANTIAGO *et al.*, 2017; VILLAR-NAVARRO *et al.*, 2018). In respect to studied set-up, both COD and $N\text{-}NH_4^+$ were significantly decreased when influent and effluent values were compared, indicating a removal of these parameters.

6.3.2 Biogas Production

On regard to the characteristics of harvested microalgal biomass recirculated to the $UASB_{co\text{-}dig}$ reactor for co-digestion with a raw sewage, the sample had in average 2,393 mg VS L^{-1}, 172 mg TKN L^{-1}, 395 mg L^{-1} of carbohydrates and 1,023 mg L^{-1} of proteins. Regarding the total COD, these samples presented an average concentration of 3,763 mg L^{-1}. Carbohydrates and proteins corresponded to 17% and 43% of the biomass dry weight, respectively. Regarding the microalgae species present in the HRAPs, *Kirchneriella* sp. was predominant. *Scenedesmus* sp., *Westella* sp. and different species of diatoms were also present in lower frequency. Images of these microalgaes were presented Figures SC.1 and SC.2 (Supplementary Material Section C).

Biogas production and composition of both UASB reactors are shown in Table 6.2. As can be observed, biogas characteristics was typical of UASB reactors. As known, biogas generated from anaerobic treatment in UASB reactors is commonly composed of higher-grade methane and lower concentration by carbon dioxide, due to the high solubility of this gaseous compound in the liquid (VAN HAANDEL; LETTINGA, 1994). The biogas generated in UASB reactors is normally a mixture of gases with volume concentrations of 60-85% methane (CH_4); 5-15% dioxide carbon (CO_2); 2-25% nitrogen (N_2); 0-0.3% carbon monoxide (CO); 0-3% hydrogen (H_2); 0-2% oxygen (O_2); and 1,000-2,000 ppmv (parts per million by volume) of hydrogen sulphide (H_2S) (SILVEIRA, 2015).

Table 6.2. Biogas production and composition (n = 84)

Parameter	$UASB_{co-dig}$		$UASB_{cont}$	
	Mean ± SD	Min/Máx	Mean ± SD	Min/Máx
Biodegradability (%)	30.23 ± 12.97	13.23 / 58.08-	22.16 ±11.59	6.97 / 54.74-
Biogas production ($L\ kg^{-1}$ COD)	165.63 ± 71.44	78.98 / 488.75	149.82 ±64.54	58.29 / 483.57
Methane yield ($LCH_4\ kg^{-1}COD$)	105.81 ± 45.40	45.73 / 283.96	77.56 ± 40.56	24.38 / 292.08
Biogas production ($L\ kg^{-1}$ VS)	331.12 ± 133.97	124.00 / 739.76	304.42 ± 116.11	82.34 / 612.36
Methane yield ($LCH_4\ kg^{-1}VS$)	210.79 ± 78.12	87.37 / 487.06	156.33 ± 45.40	69.69 / 378.54
CH_4 (%)	63.63 ± 7.01	40.80 / 80.40	51.22 ± 9.77	18.40 /70.80
CO_2 (%)	6.74 ±5.67	2.49 / 51.82	6.21 ±3.55	0.20 / 20.62
O_2 (%)	1.39 ±2.07	0.20 /10.80	0.30 ±0.12	0.20 /0.80
CO (ppm)	6.77 ±3.55	0.00 / 35.00	7.82 ±6.03	0.00 / 36.00
H_2S (ppm)	1843.58 ±359.70	362 / 3463	1558.32 ±582.70	104 / 2629
Balance (%) ($N_2 + H_2$)	26.43 ± 9.05	19.36 / 31.52	40.70 ± 16.35	13.44 / 53.33

An increase of H_2S was observed in the biogas of the $UASB_{co-dig}$ reactor. This increase may be related to the increased organic load in UASB reactor, from microalgae. This organic matter is rich in carbohydrates, that digested in anaerobic conditions produce more H_2S.

Produced biogas was in average 304.42 NL kg^{-1} VS (149.81 NL kg^{-1} COD) for $UASB_{cont}$ and 331.12 NL kg^{-1} VS (165.63 NL kg^{-1} COD) for $UASB_{co-dig}$, which represents a 10% increase. Considering the methane yield, the increase after co-digesting sewage with microalgal biomass was 35% (from 156 to 211 NL CH_4 kg^{-1} VS). It is important to note that the increase in the organic content input in terms of VS to the co-digested reactor was in average 9% higher in relation to the $UASB_{cont.}$ Methane yield results in both reactors for the different annual seasons are shown in Figure 6.2. As can be seen, average values were different among the seasons.

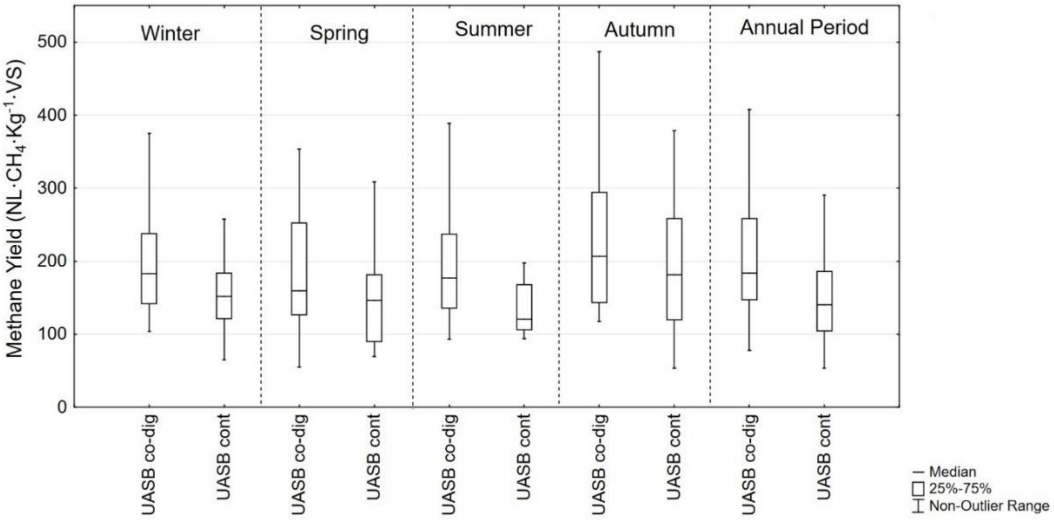


Figure 6.2. Methane yield in UASB reactors with and without co-digestion

Values obtained for anaerobic treatment of raw sewage may be compared with mono-digestion of sewage sludge (110 to 160 NL kg^{-1} VS) (GUNASEELAN, 1997). In terms of co-digestion, previous results have shown that the methane yield increased after treatment with other substrates (12-41% increase) (JANKOWSKA; SAHU; OLESKOWICZ-POPIEL, 2017; SOLÉ-BUNDÓ; GARFÍ; *et al.*, 2019). The results found in this research are within the methane yield range reported in the literature for sewage sludge and microalgae co-digestion (107 – 293 NL CH_4 kg COD^{-1} and 168 – 291 CH_4 kg VS^{-1}) (HLAVÍNEK *et al.*, 2016; JANKOWSKA; SAHU; OLESKOWICZ-POPIEL, 2017; MAHDY *et al.*, 2014a; SOLÉ-BUNDÓ *et al.*, 2018). However, it is important to note that the observed range has a high variation, due to differences in the reactor type, the operation mode, substrate characteristics and to the microalgae species present in the system. Moreover, these data were obtained from CSTR or BMP tests, while this study was conducted using a UASB reactor in demonstrate scale. It is noteworthy that there is an incipient number of publications on microalgal biomass anaerobic digestion using UASB reactors and no publications at all using co-digestion of microalgal biomass and domestic sewage.

Microalgal biomass production in HRAP and solar irradiation during different seasons is shown in Figure 6.3. When compared to Figure 6.2, it may be noticed how the highest methane yield was obtained in winter and autumn, when microalgal biomass production was the highest (Figure 6.3). This difference was probably associated to seasonality changes and pluviosity. Since Summer and Spring were months associated with highest incidence of rainy days. In fact, in tropical regions it is common that warmer months is also the rainy season. This probably affected the microalgal biomass production and concentration. As the recirculation was constant and fixed at a volume of 10 L per day, there was an increase in the recirculated organic load during those phases, leading to an increase in the methane yield during that period. Higher volume of microalgal biomass recirculated to the reactor also may have contributed to the insertion of a portion of oxygen in this reactor. It is known that a micro aeration in anaerobic systems can improve the hydrolysis of organic matter and the acidogenic phase in the reactor, leading to the oxidation of some available substrates by aerobic metabolism (BOTHEJU; BAKKE, 2011). Furthermore, oxygen supplementation in anaerobic digestion is efficient when done in batches and in small quantities, as it was performed in this work. (BOTHEJU; LIE; BAKKE, 2010). Moreover, in terms of biodegradability, the mixed substrate fed to the UASB$_{\text{co-dig}}$ reactor was 26% higher compared to raw sewage (Table 6.2).

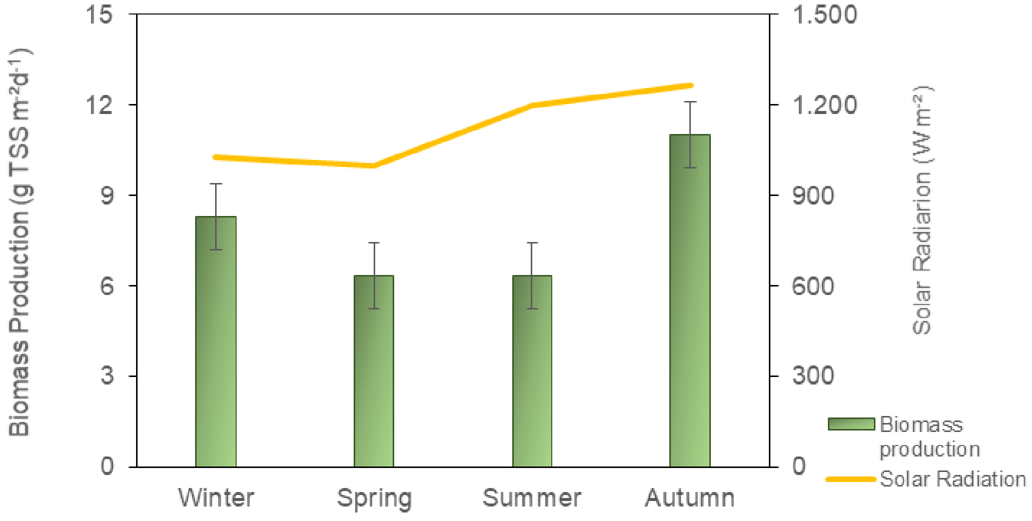


Figure 6.3. Biomass production in the HRAP and solar radiation over the year.

The annual average microalgal biomass production in the HRAP was 8.5 g TSS m^{-2} day. This value is similar to that reported in a study developed in HRAP operated in tropical climate and with the same HRT, where the authors obtained a production of 9.0 g TSS m^{-2} day (PARK; CRAGGS, 2010). However, values between 13 and 35 g TSS m^{-2} day are considered typical to this systems (PARK; CRAGGS, 2011). The lower values may be related to the low availability of carbon dioxide in domestic sewage. The inorganic carbon concentration in the HRAP can be increased by introducing CO_2 into the system. For instance, microalgal biomass production was increased by 15% after addition of CO_2 in HRAP (HEUBECK; CRAGGS; SHILTON, 2007). Another important factor for microalgae growth in HRAPs is the incidence of solar radiation (SOLIMENO *et al.*, 2017). Therefore, the geographic region in which the system is situated may interfere in the biomass production results. In this research, the experiment was performed in the southeast region of Brazil with high solar irradiation throughout the whole year (1000-1200 W m^{-2}). Another important environmental factor is the pluviometry, which causes dilution and biomass production decrease in small-scale systems (~ 200 L). In this study, spring and summer had an accumulated rainfall of 178 mm and 277 mm, while for autumn and winter it was 37 mm and 18 mm, respectively. Probably, due to this difference, biomass production during summer and spring were lower in comparison with the other seasons. In addition, another factor that influences an optimal microalgae growth is the temperature of the ponds, which must be close to 25ºC (SOLIMENO *et al.*, 2017). Temperatures in this study were ranged from 18ºC and 24ºC. Therefore, the temperature was stable and did not seem to be a limiting factor in this case study.

Finally, literature reports that the amount of nutrients, especially ammonium, and pH affects microalgae growth, with a possible inhibition of above 90% when ammonia is higher than 50 mg N-NH_4 L^{-1} and pH is higher than 8 (AZOV; SHELEF; MORAINE, 1982). In this study, HRAPs had average ammonium values of 16 NH_4 L^{-1} and pH of 7.8. Therefore, it can be concluded that low microalgal biomass production was more possibly associated with carbon deficiency and pluviometry.

6.3.3 Energy assessment

The energy assessment for $UASB_{co\text{-}dig}$ and $UASB_{cont}$ was performed to verify the energy self-sustainability of the proposed STP when evaluating biogas production from co-digestion of microalgal biomass with raw sewage. The energy production scenario was developed for a system for 10,000 PE. The results obtained by season and the average annual period are shown in Table 6.3.

Table 6.3. Results of the average seasonal energy assessment UASB+HRAP

Parameters	**Winter**		**Spring**		**Summer**		**Autumn**		**Annual**	
	Co-dig	**Cont**	**Co-dig**	**Cont**	**Co-dig**	**Cont**	**Co-dig**	**Cont**	**Co-dig**	**Cont**
$E_{In\ UASB}$ (kWh d^{-1})	901	751	901	751	901	751	901	751	901	751
$E_{In\ HRAP}$ (kWh d^{-1})	22	0	22	0	22	0	22	0	22	0
E_{In} (kWh d^{-1})	923	751	923	751	923	751	923	751	923	751
E_{Out} (kWh d^{-1})	1875	411	1559	413	1761	359	2589	589	1945	448
Δ E (kWh d^{-1})	952	-340	636	-338	838	-392	1666	-162	1022	-303
NER	2,03	0,55	1,69	0,55	1,91	0,48	2,81	0,78	2,11	0,60

As may be observed from the values, $UASB_{co\text{-}dig.}$ had a positive energy balance for all weather seasons. On the contrary, $UASB_{cont}$ showed all negative values. Thus, it is possible to conclude that the use of microalgal biomass in co-digestion with raw sewage improved the potential in using biogas produced into energy. The energy balance and NER of the UASB$_{co\text{-}dig}$ and UASB$_{cont}$ are shown in Figures 6.4 and 6.5. Positive energy balance was previously reported for an anaerobic system co-digesting microalgal biomass with primary sludge (PASSOS *et al.*, 2017). In the mentioned study, authors obtained energy ratio from 1.01 to 5.31 for different scenarios. It is important to note that the variation in energy production produced in the scenarios was associated with the variation of the OLR input in the UASB reactors as explained in section 6.3.2.

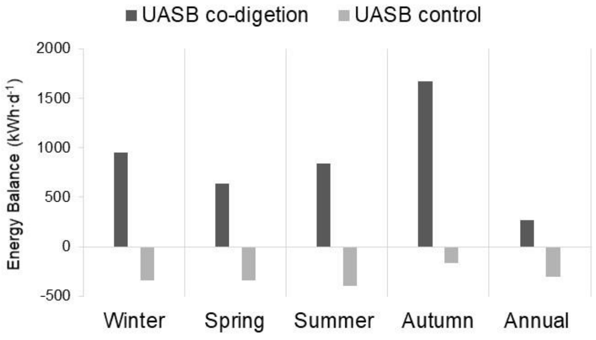


Figure 6.4. Energy balance

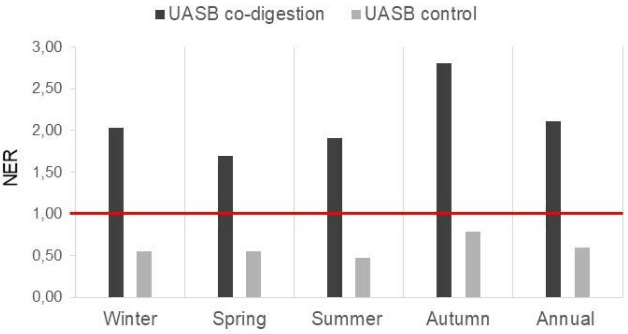


Figure 6.5. NER energy for the system

The energy ratio obtained from UASB$_{co\text{-}dig.}$ varied from 1.7 to 2.8, which means that from 70 to 180% more energy was produced that consumed. The energy produced in this case was much higher compared to the energy produced when only sewage was digested in the reactor (~ 5 times higher). In the latter scenario, more energy was consumed with pumping the sewage and for HRAP paddle-wheels, than the energy produced from the methane yield.

The energy produced by this system has many applications, within the co-digestion STP and also within the nearby community. Three possible energy applications will be discussed below: i) conversion into electricity and heat in a combined heat and power (CHP) plants; ii) conversion into heat using boilers and, iii) conversion into biomethane through biogas upgrading.

Considering conversion of biogas into electricity and heat using a CHP motor, 35% of energy is converted into electricity and 55% into heat. Therefore, in this case, in average 757 kWh d^{-1} of electricity and 1,188 kWh d^{-1} of heat would be produced annually. In this case, the electricity produced could be injected into the grid to reduce the electricity consumption at the plant, covering 82% of the STP demand.

Another option is to produce heat using a boiler. Considering the boiler efficiency 90%, the heat balance would be in average 1.945 kWh d^{-1}. In this case, the electricity required for the STP could be provided through renewable sources, as photovoltaic systems or wind power. Since UASB reactor was operated at ambient conditions, heat should be used for other purposes in or outside STP. This also may be applied for the thermal energy produced using the CHP plant. In the STP, an option is to sanitize the sludge produced, making it suitable for agricultural use. Or by drying and reducing the sludge for its more cost-effective disposal. Previous studies have shown that both applications are viable, with reductions in the sludge volume by 46% and reduction in pathogens by up to 99.9% (CARTES *et al.*, 2018; KACPRZAK *et al.*, 2017; ROSA *et al.*, 2018). Still in STP, the thermal energy could be used for pre-treating microalgal biomass prior to the anaerobic reactor. This application could be used for increasing biomass solubilization and biodegradability, therefore increasing methane yield in the reactor. In fact, previous studies have shown that thermal pretreatment at 70-90 °C increased methane yield of microalgal biomass up to 30% (Passos et al., 2014). Outside the STP, thermal energy produced may also be used for cooking and heating water in the nearby community.

Finally, a third option for converting biogas is through upgrading into biomethane. In this case, purification and removal of CO_2 is necessary to reach acceptable values for injection into the distribution grid (> 95%) (MUÑOZ, RAÚL *et al.*, 2015) and for the use as car engines (96%) (PAPACZ, 2011) . The injection of biomethane into the grid may be used exploited by the nearby population or for the STP car fleet. This scenario would enable a decrease in the use of fossil fuels and, consequently, a decrease in the carbon footprint. In a previously study, biogas purification was achieved in an adsorption column with microalgae and obtained 94-99% of methane content (MARÍN *et al.*, 2019b) . Similar results were reported for biogas upgrading using microalgae grown in HRAPs (MARÍN *et al.*, 2018; POSADAS *et al.*, 2016).

Still in terms of the best energy conversion option, a previous study showed how a STP using UASB reactors, would be more economically viable when converting the energy produced from biogas into electricity and heat, if compared to the same system harnessing only heat for a population equivalent above 200,000 (VALENTE, 2015). In any case, the mentioned study did not consider any co-digestion step. Other approaches consider that biomethane may be more economically viable compared to electricity or heat production (BUDZIANOWSKI AND BUDZIANOWSKA, 2015). However, the mentioned study criticizes the insufficient political and economic incentive given to this technology, leaving it at the margin of CHP or boiler technologies. On the whole, it is safe to say that an energetical assessment should consider each local reality in order to evaluate the complete system viability.

6.4 Conclusion

The propose STP consisting in a UASB + HRAP showed a great potential of organic matter and nutrients removal (65% COD and 61% N-NH4). The evaluated system showed that co-digestion with microalgae improved the methane yield (35% higher than control increase). Moreover, the system showed a positive energy balance, with 70 to 180% more energy produced than consumed throughout the year. Further studies should be performed to verify the real economic impact of the proposed co-digestion on energy production from biogas generated by UASB reactors and consider the implementation of low-cost pre-treatment and energy demand of HRAP microalgal biomass.

7 SOLAR THERMAL PRE-TREATMENT TO IMPROVE THE ANAEROBIC BIODEGRADABILITY OF MICROALGAL BIOMASS IN SEWAGE TREATMENT

Based on: Vassalle, L.[*], Passos, F., Rosa-Machado, A.T, Moreira, C., Reis, M., Freitas, M.P., Ferrer, I. and Mota-Filho, C.R. 2021 - Solar thermal pre-treatment to improve the anaerobic biodegradability of microalgal biomass in sewage treatment: **under review in Algal Research since October2020**

Sustainable sewage treatment plants (STPs) have been intensively investigated in order to identify feasible low-cost and environmental-friendly options. One possibility would be anaerobic-aerobic treatment solutions, as upflow anaerobic sludge blanket (UASB) reactor followed by high rate algal pond (HRAP). This configuration has already proved to be efficient for pollutant and micropollutant removal as well as for energy recovery from the co-digestion of raw sewage and harvested microalgal biomass. Since microalgae cells have complex structures that make them resistant to anaerobic digestion, pre-treatment techniques may be applied to improve microalgal biomass methane yield. Thermal pre-treatment can be an effective method; however, thus far, solar thermal energy has never been investigated for biomass solubilisation. Therefore, this study aimed at evaluating the performance of a solar thermal microalgal biomass pre-treatment system prior to anaerobic co-digestion with raw sewage in UASB reactors. Results showed that chemical oxygen demand (COD) and ammonium (N-NH_4^+) removal efficiencies in the system were 70% and 61%, respectively. The solar pre-treatment system reached a microalgal biomass solubilization of 32% (expressed as COD). Furthermore, methane yield was increased by 45% after anaerobic co-digestion with pre-treated microalgae compared to mono-digestion with raw sewage (from 81 to 117 NL CH_4 kg^{-1} COD). The energy assessment showed a positive energy balance, as the total energy produced was twice the energy consumed in the system.

7.1 Introduction

Studies on microalgae-based sewage treatment plants (STP) have increased in the last 50 years. This is justified, not only because of their high efficiency at removing organic matter and nutrients, but also due to the possibility of recovering microalgal biomass, which may be processed into valuable by-products, such as biofuels, biomethane, biofertilizers, natural pigments and animal proteins (ARASHIRO, LARISSA T. *et al.*, 2020; CRAGGS, R. J. *et al.*, 2011; MARÍN *et al.*, 2019b). In particular, methane yield through anaerobic digestion has been widely investigated and applied as a straight-forward solution for enabling STP with little or no energy input (PASSOS *et al.*, 2017; PASSOS; FERRER, 2014). Moreover, pre-treatment and co-digestion of microalgae with other substrates have been shown to increase the degradation rate and methane yield (PASSOS; UGGETTI; *et al.*, 2014; SOLÉ-BUNDÓ; PASSOS; *et al.*, 2019).

The anaerobic co-digestion of microalgal biomass for biogas production has been widely studied (DEMIRBAS, 2010; MUÑOZ, RAÚL *et al.*, 2015; SOLÉ-BUNDÓ *et al.*, 2018). For instance, the co-digestion of microalgae with sewage sludge increased by 12% the biogas production compared to microalgae mono-digestion in continuous stirred-tank reactor (CSTR) (OLSSON *et al.*, 2014). In STP using UASB reactors followed by high rate algal ponds (HRAP), microalgal biomass can be recirculated to the UASB reactor to be co-digested with raw sewage. In our previous study, the anaerobic co-digestion of sewage with microalgal biomass harvested from HRAP increased the methane yield by 35% (from 156 L CH_4 $kgVS^{-1}$ to 211 L CH_4 $kgVS^{-1}$) as compared to the control UASB reactor without microalgal biomass (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020).

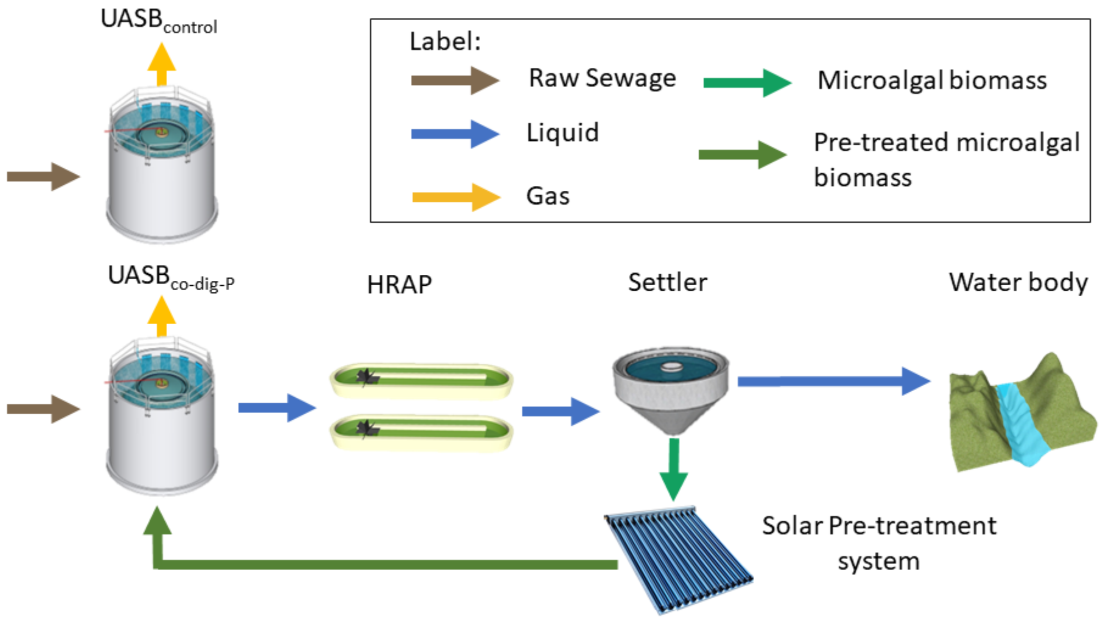
Another strategy to improve microalgae anaerobic biodegradability is through biomass pre-treatment. Microalgae are basically composed of carbohydrates, proteins and lipids, whose proportion in dry mass varies between 4-64%, 6-61% and 2-40%, respectively (DEMIRBAS, 2010). Intracellular carbohydrates have their hydrolysis faster than extracellular, as they are not structural elements of the cell, but they do not become available for anaerobic digestion because microalgae cell walls provide them with physical protection and are resistant by nature, hindering their degradation during anaerobic digestion (CARRILLO-REYES; BARRAGÁN-TRINIDAD; BUITRÓN, 2016; KHAN; SHIN; KIM, 2018). Therefore, pre-treatment of microalgal biomass before anaerobic digestion can be used to break down the cell wall and increase the bioavailability of internal organic matter, as well as to hydrolyse macromolecules (PASSOS; HERNÁNDEZ-MARINÉ; *et al.*, 2014; SOLÉ-BUNDÓ *et al.*, 2018). Several pre-treatment techniques have been applied to microalgal biomass, including thermal, mechanical, biological (enzymatic) and chemical methods (CARRERE *et al.*, 2016; GONZÁLEZ-FERNÁNDEZ; SIALVE; BERNET; *et al.*, 2012a; PASSOS; FERRER, 2014; SARATALE *et al.*, 2018). Among them, thermal pre-treatment has shown its effectiveness (60-220% increase in methane yield) with the need of extra and non-natural energy input to heat the microalgal biomass (GONZÁLEZ-FERNÁNDEZ; SIALVE; STEYER; *et al.*, 2012; PASSOS *et al.*, 2017; PASSOS; FERRER, 2014; SARATALE *et al.*, 2018). Results obtained with low temperature pretreatment of micro-algal biomass, produced in high-rate algal ponds treating municipal sewage, show increasing the methane yield of about 70% for temperatures between 75 and 95°C, and 10 hours of exposure however, using an electrical heater to pre-treat the microalgal biomass (PASSOS *et al.*, 2017). The disadvantage of conventional thermal systems for pretreating microalgal biomass is that the extra energy expenditure required from this technique is deducted from the final yield in the system's energy balance. The solar system to pre-treat microalgal biomass from sewage treatment, is extra energy free and has never been studied for this purpose. This system has a great potential of applicability to pre-treatment microalgal biomass in countries without significant variations in temperature (such as countries with tropical climate).

Based on the drawback of the conventional thermal pretreatment for microalgal biomass, this study aimed at evaluating a system to pre-treat the microalgal biomass using solar energy, without introducing any extra electrical energy to increasing the anaerobic biodegradability of microalgal biomass for co-digestion with raw sewage in UASB reactor. The entire system was built at demonstration scale and was fed with real sewage. The first part of this work focused on the sewage treatment efficiency. The second part evaluated the pretreatment efficiency and the biogas production from anaerobic co-digestion of raw sewage with microalgal biomass pretreated in UASB reactor. Finally, an energy assessment was estimated to comprehend the process’ self-sufficiency. To the author’s knowledge it is the first time that a solar thermal treatment system (extra electrical energy free) is put forward to improve the microalgal biomass solubilization and also the first time that the solar pre-treated biomass is evaluated in co-digestion with raw sewage in UASB reactors.

7.2 Material and Methods

7.2.1 Experimental set-up and operation

The demonstration scale experimental set-up is shown in Figure 7.1. The system received real raw sewage from a full-scale STP located in Belo Horizonte, Brazil.



Note: UASB - Upflow Anaerobic Sludge Blanket;
UASB$_{co\text{-}dig\text{-}P}$ - Upflow Anaerobic Sludge Blanket with co-digestion of raw sewage and pre-treated microalgal biomass
HRAP – High Rate Algal Pond

Figure 7.1. Demonstration scale experimental set-up flowchart

Sewage was pumped (Netzsch® Germany) to the system after a pre-treatment for the removal of coarse solids and grit. The study was conducted in two UASB reactors, namely UASB$_{control}$, fed only with raw sewage, and UASB$_{co\text{-}dig\text{-}P}$, fed with raw sewage and pre-treated microalgal biomass, which was used to evaluate anaerobic co-digestion of both streams. The effluent from the UASB$_{co\text{-}dig\text{-}P}$ was post-treated in two HRAP. The produced microalgal biomass was harvested in a settler, and then conducted by gravity to the solar pre-treatment unit. Pre-treated biomass was recirculated to the UASB reactor using a positive displacement pump (BCR 2000 – Schneider®) (Figure 7.1).

The UASB reactors were made of fiberglass, with a working volume of 343 L each (height 4.0 m ; diameter 0.3 m). The reactors were operated at a flow rate of 49 L h^{-1}, hydraulic retention time (HRT) of 7 hours, and sludge retention time (SRT) of 35 days. Both HRAP were made of fiberglass with a working volume of 205 L and a surface area of 0.41 m² each (height 0.5 m; length 1.7 m; width 0.24 m). HRAP were operated at a flow rate of 25.5 L day^{-1} each, and a HRT of 8 days. The settler was made of polyvinyl chloride, it had a working volume of 30 L (height 0.4 m; diameter 0.1 m) and operated at 14 hours of HRT. For anaerobic co-digestion, 12 L of pre-treated microalgal biomass were pumped to a plexiglass column located 4 m above the UASB$_{co\text{-}dig\text{-}P}$ and recirculated therefrom at a flow rate of 0.5 L h^{-1} into the bottom of the reactor. The biomass inlet flow was controlled by a needle-type flow control valve. The system was operated continuously for 9 months (July/2019 to March/2020).

7.2.2 Analytical methods

To evaluate the sewage treatment efficiency, liquid phase samples were collected twice a week (around 10am) from: i) raw sewage; ii) effluent from both UASB reactors; iii) effluent from the HRAP; and iv) supernatant effluent from the settler. The physico-chemical parameters analysed were pH, temperature and dissolved oxygen (DO) using a Hach® (HQ30D) probe, COD (using Hach® kit COD at high range), total and volatile suspended solids (TSS and VSS) (according to Standard Methods - APHA, 2012), Total Nitrogen (TN) and ammonium ($N\text{-}NH_4^+$) (using ionic chromatography, Metrohm® - 940 professional IC Vario).

For microalgal biomass characterisation (with and without pre-treatment), biomass samples were taken twice a week from the settler (before the thermal pre-treatment) and from the solar pre-treatment boiler unit. Total and volatile solids (TS and VS) and Total Kjeldahl Nitrogen (TKN) were analysed according to standard procedures (APHA-AWWA-WEF, 2012). Total COD was analysed by Hach® kit COD at high range. Carbohydrates were measured using a phenol–sulphuric acid method after acid hydrolysis, (DUBOIS *et al.*, 1956). For estimating the protein content, a conversion factor of 5.95 was used based on the results of TKN (LÓPEZ *et al.*, 2010). To evaluate the effect of the solar pre-treatment on biomass solubilisation, soluble VS and soluble COD were analysed before and after the pre-treatment unit. Samples were filtered through glass fiber filters with nominal retention capacity of 0.7 µm (Macherey-Nagel®, GF-3). The dominant microalgae species in the HRAP were identified through optic microscopy (Olympus BX-50) equipped with a camera (Olympus DP70).

Samples for biogas analysis were collected twice a week from both the $UASB_{control}$ and $UASB_{co\text{-}dig\text{-}P}$ reactors. Biogas production was measured twice a week using an automatic meter (Ritter®-Germany). In addition, biogas composition in terms of CH_4, CO_2, O_2, CO and H_2S was analysed using a portable meter (Geotech®-United Kingdom). The results were expressed as methane yield, i.e. volume of methane produced per mass of COD and VS fed to the reactor. The substrate anaerobic biodegradability was calculated as the ratio between the measured methane yield (expressed in terms of COD_{in}) and the theoretical methane yield under standard conditions (350 ml CH_4 g $COD_{removed}^{-1}$) (METCALF & EDDY *et al.*, 2003) .

The microalgal biomass production (MBP) related to the surface area of the ponds is demonstrated in Equation 7.1. Where C_{TSS} corresponds to TSS (g L^{-1}) concentration, Q_{HRAP} (L day^{-1}) and SA_{HRAP} (m^2) correspond, respectively, to flow and surface area from HRAP.

$$MBP\left(\frac{g}{m^2 day}\right) = \frac{C_{TSS}\ x\ Q_{HRAP}}{SA_{HRAP}} \qquad \text{(Eq. 7.1)}$$

Climatic data (temperature, solar radiation and precipitation) were obtained from the meteorological station near the STP (Brasil National Meteorology Institute, INMET, http://www.inmet.gov.br).

7.2.3 Solar thermal microalgal biomass pre-treatment

The solar pre-treatment unit was composed of 4 vacuum tempered glass tubes with a working volume of 3 L each and an insulated fiberglass boiler with useful capacity of 16 L. The total capacity of the system was 28 L. The pre-treatment unit was operated at a HRT of 13 hours. The system was placed in a shadow-free location at a 20° angle in relation to the ground towards the north to maximize direct sunlight exposure. The position and inclination were based on the evaluation of monthly average solar irradiation, performed using the *SunData*® software. This software uses mathematical models to calculate the local levels of solar radiation based on the Brazilian Atlas of Solar Energy. An automatic temperature probe with data logger (Elitech® - Model RC-4 - Brasil) was inserted in the boiler and data were registered every 15 minutes.

Pre-treatment effectiveness was determined by means of the organic matter solubilisation after the solar pre-treatment. It was calculated by determining the increase in soluble COD after pre-treatment in relation to the particulate COD before pre-treatment, as expressed in Equation 7.2, where COD_s corresponds to soluble COD and the sub-indexes refer to pre-treated (p) and non-pre-treated (n).

$$S(\%) = \frac{(COD_S)_p - (COD_S)_n}{COD - (COD_S)_n}\ x\ 100 \qquad \text{(Eq. 7.2)}$$

7.2.4 Energy Assessment

An energy assessment was carried out to estimate what the energy balance would be in a full-scale STP using the same system. This was done by calculating the energy requirement of the proposed system and the energy produced, based on the average methane yield determined during the experimental period. A population equivalent (PE) of 10,000 was used for the design of the scaled-up UASB and HRAP systems. Gravity grit chambers were adopted for pre-treatment, as they do not demand any energy.

The energy input (E_{in}) was estimated as the energy demand for sewage and microalgal biomass pumping ($E_{in,\ UASB}$), and the energy demand for the HRAP paddle-wheel ($E_{in,\ HRAP}$). For the $UASB_{co\text{-}dig\text{-}P}$, recirculated microalgal biomass was assumed to be 300 $m^3\ day^{-1}$ (20% from the total flow). It is important to note that the anaerobic reactors were operated at ambient temperature during the whole experimental period, thus, without external energy input for heating.

The energy output (E_{out}) was estimated as the methane yield in the $UASB_{control}$ (treating domestic sewage only) and $UASB_{co\text{-}dig\text{-}P}$ (treating domestic sewage and pre-treated microalgal biomass) reactors. The results were expressed as net energy ratio (NER) and energy balance (ΔE), according to Equations 7.3 and 7.4, respectively. If NER is higher than 1 and if ΔE is positive, more energy for sewage treatment is generated than consumed. This methodology was adapted from (PASSOS *et al.*, 2017; VASSALLE; DÍEZ-MONTERO; *et al.*, 2020).

$$NER = \frac{E_{out}}{(E_{in,UASB} + E_{in,HRAP})} \quad \text{(Eq. 7.3)}$$

$$\Delta E\ (kWh\ d^{-1}) = (E_{in,UASB} + E_{in,HRAP}) - E_{out} \quad \text{(Eq. 7.4)}$$

Energy assessment results were discussed and compared with our previous study, which evaluated the co-digestion in a UASB fed with raw sewage and microalgal biomass without pre-treatment (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020).

7.2.5 Statistical analysis

Mann-Whitney U test for independent samples was used to evaluate COD and ammonia removals in all units of the demonstration-scale system by comparing influent and effluent concentrations. Wilcoxon statistical test for paired or dependent samples was used to evaluate anaerobic performance and biogas production in the $UASB_{control}$ and $UASB_{co\text{-}dig\text{-}P}$ reactors. The same test was used to compare the physico-chemical parameters in both anaerobic reactors and soluble organic matter of pre-treated and non-pre-treated microalgal biomass. Pearson's statistical correlation test was used to verify the correspondence between the temperature of the solar pre-treatment and the organic matter solubilisation. *Statistica 10.0*® software was used to perform all statistical tests, at a significance level of 95%.

7.3 Results and discussion

7.3.1 Sewage treatment efficiency

Results from physico-chemical analyses for raw sewage, $UASB_{control}$, $UASB_{co\text{-}dig\text{-}P}$, HRAP and final effluent (settler supernatant) are summarized in Table 7.1.

Table 7.1. Physico-chemical characterisation from the different sampling points of the experimental set-up (n = 55 for all columns, except for parameters marked with *, for which n = 32)

Parameter	**Raw Sewage** **Mean ± SD**	**UASB$_{control}$** **Mean ± SD**	**UASB$_{co-dig-P}$** **Mean ± SD**	**HRAP$_{eff}$** **Mean ± SD**	**Final Effluent** **Mean ± SD**
pH	7.7 ± 0.2	7.3 ± 0.3	7.6 ± 0.4	8.2 ± 0.7	7.7 ± 0.6
DO (mg L^{-1})	0.8 ± 0.9	0.3 ± 0.1	0.4 ± 0.2	9.5 ± 3.1	8.4 ± 5.2
Temperature (ºC)	24.9 ± 1.9	23.3 ± 1.7	23.4 ± 2.2	23.1 ± 3.4	24.8 ± 4.1
COD (mg O_2 L^{-1})	409.2 ±119.3	182.7 ±58.8	226.7 ±72.9	-	124.7 ±40.2
TSS (mg L^{-1})	237.0 ±76.8	53.1 ± 13.4	72.6 ± 46.0	141.4 ± 44.7	71.4 ± 21.3
VSS (mg L^{-1})	187.8 ±64.7	31.3 ± 8.7	45.2 ± 27.9	95.6 ± 31.1	51.8 ± 27.6
TN (mg L^{-1})*	39.9 ± 10.8	-	44.5 ± 5.9	-	26.5 ± 8.0
N-NH_4^+ (mg L^{-1})*	30.9 ± 9.5	-	35.5 ± 7.1	-	14.0 ± 5.7

Note: DO – Dissolved Oxygem; COD – Chemical Oxygen Demand ; TSS – Total Suspended Solids; VSS – Volatile Suspended Solids; TN – Total Nitrogen; N-NH_4^+ - Ammonium.

For both UASB reactors, no significant differences (Wilcoxon statistical test) were shown for pH, DO and temperature, indicating that both units operated under the same physio-chemical conditions, despite microalgae recirculation to the UASB$_{co-dig-P}$ reactor. An average of COD (total) removal were 55% for the UASB$_{control}$ and 48% for the UASB$_{co-dig-P}$. It is important to note that the UASB$_{co-dig-P}$ reactor received a higher organic load, considering both the influent sewage and microalgal biomass, which represented 105 mg COD day^{-1} (25% higher compared to the UASB$_{control}$). The overall removal of COD in the system (UASB + HRAP) was 70%. These results are similar to those presented in our previous work with the same experimental set-up but without microalgae pre-treatment (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020), suggesting that the recirculation of pre-treated microalgal biomass did not overload the UASB or HRAP units under the evaluated conditions. In general, COD removals between 55 and 70% have been reported for UASB reactors and between 65 and 80% for UASB reactors followed by polishing ponds (VON SPERLING, M., 2007). Moreover, the overall COD removal achieved in this study (70%) is similar to previous studies carried out with UASB followed by HRAP, which obtained the same 70% COD removal (VASSALLE; GARCÍA-GALAN; *et al.*, 2020; VILLAR-NAVARRO *et al.*, 2018).

As expected, the UASB reactor evaluated (UASB$_{co-dig-P}$) mineralized organic nitrogen, showing an increase in ammonium concentration in the reactor effluent (from 30 to 35 mg N-NH_4 L^{-1}). This is a result of the hydrolysis of proteins and urea, leading to an increase of NH_4^+-N (METCALF & EDDY *et al.*, 2003). An average N-NH_4^+ removal of 61% was observed for the HRAP, with an effluent concentration of 14 mg $N\text{-}NH_4$ L^{-1}. The observed $N\text{-}NH_4^+$ removal in the open ponds was most probably associated to nitrification and/or volatilization (DOMA *et al.*, 2016). The average TN removal in the HRAP was 40% (the TN concentration was reduced from 45 mg L^{-1} in the $UASB_{co\text{-}dig\text{-}P}$ effluent to 27 mg L^{-1} in the HRAP effluent). Similar results were observed in UASB+HRAP laboratory-scale systems treating sewage, in which the effluent from the UASB reactor showed 40 mg $N\text{-}NH_4 \cdot L^{-1}$and the HRAP effluent showed 12 mg $N\text{-}NH_4 \cdot L^{-1}$ (SANTIAGO *et al.*, 2017).

Regarding the quantification of solids in the system, the results showed that for both TSS and VSS the concentrations varied in the range of 120-360 mg TSS L^{-1} and 90-280 mg VSS L^{-1} for raw sewage, 60-160 mg TSS L^{-1} and 30 mg VSS L^{-1} in the UASB reactor effluents, and 145 mg TSS L^{-1} and 124 VSS mg L^{-1} in the HRAP effluents (Table 1). All these values are within commonly reported ranges for similar systems (CHERNICHARO, C. A., 2007; SANTIAGO *et al.*, 2017). The mean TSS concentration in the supernatant from the settler was 70 mg L^{-1} (Table 7.1).

On the whole, the concentrations of pollutants met current local legislation requirements, which are set at 180 mg L^{-1}, 150 mg L^{-1} and 20 mg L^{-1} for COD, TSS and $N\text{-}NH_4^+$, respectively (MORAIS; SANTOS, 2019) (Conama Directive 430/2011 and Copam directive 01/2008). Considering the most restrictive European urban wastewater Directive (Council Directive 91/271/EEC), COD, TSS and TN discharge limits are 125 mg L^{-1}, 35 mg L^{-1} and 15 mg L^{-1} respectively; hence COD and $N\text{-}NH_4^+$ would also comply with European legislation, whereas TSS separation from the mixed liquor should be improved by optimising the settler design and operation.

7.3.2 Microalgal biomass: Efficiency of solar pre-treatment for anaerobic co-digestion

7.3.2.1 Microalgal biomass production

Average TSS and VSS concentrations in the HRAP were 142 mg L^{-1} and 96 mg L^{-1}, respectively. The mean microalgae production was 8.7 mg TSS m^{-2} day^{-1}, which was stable over the year (Figure 7.2). This value is in agreement with the production obtained in HRAP operated under similar conditions (9.0 g TSS m^{-2} day^{-1}) (PARK; CRAGGS, 2010), yet it is still lower than optimal values indicated in the literature for HRAP (between 13 and 35 g TSS m^{-2} day^{-1}) (PARK; CRAGGS, 2011). Inorganic carbon limitation in domestic sewage may be one of the reasons for these lower values. A mathematical simulation for microalgae growth in open ponds showed that inorganic carbon supplementation to these systems can result in an increase of 15% in biomass production (SOLIMENO; GARCÍA, 2019; SOLIMENO; GÓMEZ-SERRANO; ACIÉN, 2019). Another relevant factor may be rainfall, since it is a demonstrate scale system (205 L) and therefore, influenced by dilution.

Microalgal biomass production in the HRAP and solar irradiation during the experimental period are shown in Figure 7.2. The two months with the lowest microalgal biomass productivity showed the highest total rainfall in the operating period (December / 2019 with 8.08 g TSS m^{-2} day^{-1} and 349 mm, and January / 2020 with 7.66 g TSS m^{-2} day^{-1} and 740 mm). The accumulated rainfall values were presented in the Supplementary Material Section D (Figure SD.1).

Microspora sp. was the dominant microalgae species present in the HRAP throughout the experiment. *Scenedesmus* sp., *Westella* sp. and other species of diatoms were also present. Figure of biomass was presented in Supplementary Material Section D (Figure SD.2). Carbohydrates and proteins represented 42% and 20% of total microalgal biomass macromolecular composition, respectively.

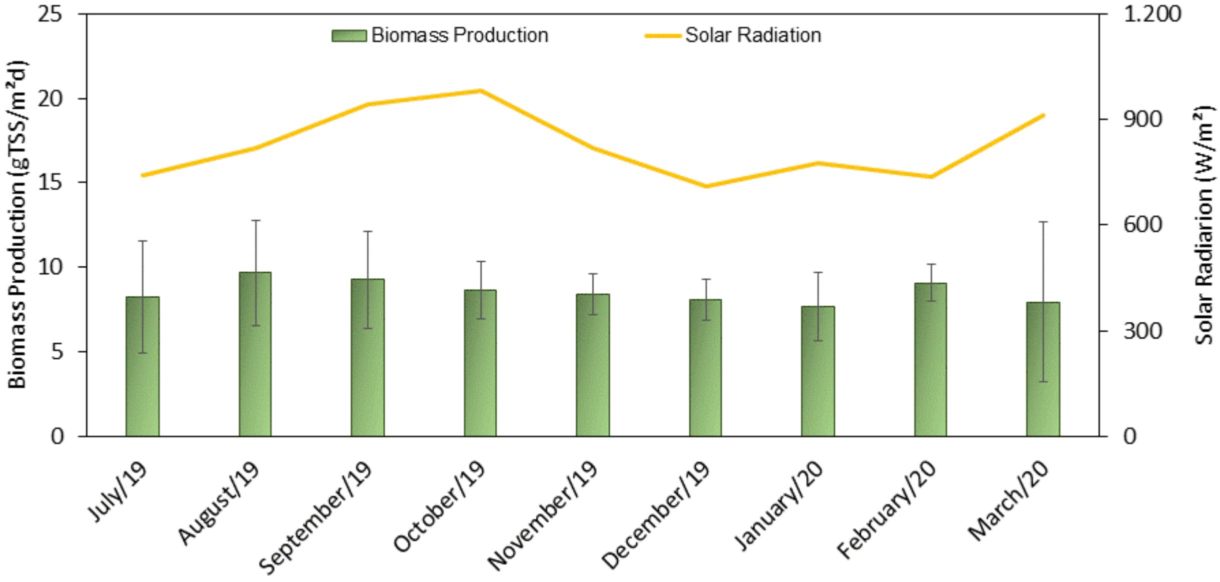


Figure 7.2. Biomass production and solar radiation.

7.3.2.2 Solar thermal pre-treatment effectiveness

Soluble COD increased by 32% after the solar thermal pre-treatment (eq. 7.2). Previous studies on conventional thermal pre-treatment of microalgae at 55 °C for 12 and 24 hours reported a COD solubilisation increase of 29% (ALZATE *et al.*, 2012). The microalgal biomass was analysed before and after the solar thermal pre-treatment. The results are summarised in Table 7.2.

Table 7.2. Microalgal biomass composition before and after solar thermal pre-treatment (n = 55)

Parameter	Microalgal Biomass before the Solar Thermal Pre-treatment		Microalgal Biomass after the Solar Thermal Pre-treatment	
	Mean ± SD	**Min/Max**	**Mean ± SD**	**Min/Max**
COD_{total} ($g\ L^{-1}$)	3.5 ± 2.3	0.3 / 8.9	3.3 ±2.1	0.3 / 8.3
COD_S ($g\ L^{-1}$)	0.5 ± 0.4	0.1 / 1.4	1.3 ± 0.8	0.1 / 3.1
TS ($g\ L^{-1}$)	3.2 ± 2.6	0.4 / 9.8	3.0 ± 2.6	0.1 / 9.5
VS ($g\ L^{-1}$)	2.4 ± 2.1	0.3 / 7.3	2.1 ± 1.8	0.1 / 7.3
VS/TS (%)	73.6 ± 11.8	27.4 / 87.1	72.5 ± 13.1	38.7 / 95.2
TKN ($mg\ L^{-1}$)	172.1 ±176.7	9.1 / 739.3	182.2 ± 236.3	10.7 /1043.5

Note: COD_{total} – Total Chemical Oxygen Demand; CODs – Soluble Chemical Oxygen Demand TS – Total Solids; VS – Volatile Solids; VS/TN – Ratio of Total Solids and Volatile Solids TKN - Total Kjeldahl Nitrogen

As thermal pre-treatment was carried out using solar energy, the applied temperature changed slightly depending on climate conditions. Figure 7.3 shows the temperature reached and the solubilisation increase. As can be observed and was confirmed through statistical test (Person's statistical test) a strong correlation (P value < 0.05 and R^2 of 0.9) between temperature and biomass solubilisation was reported. Furthermore, it was not observed great variation in temperature during the experimental period, even though the pre-treatment system depended on sunlight. It is important to note that this experiment was carried out in a tropical country. In fact, the small temperature variability justified and made the system more reliable in guaranteeing the thermal pre-treatment of microalgal biomass. The applied temperature of 55 ºC represents and average value, as there was solar variation during the pre-treatment time (01 AM to 12 PM). The system reported minimum values of 36 °C in the evening and maximum values of 89 °C when the sun was at its peak. The daily solar temperature variation can be seen in the supplementary material section D (Figure SD.3).

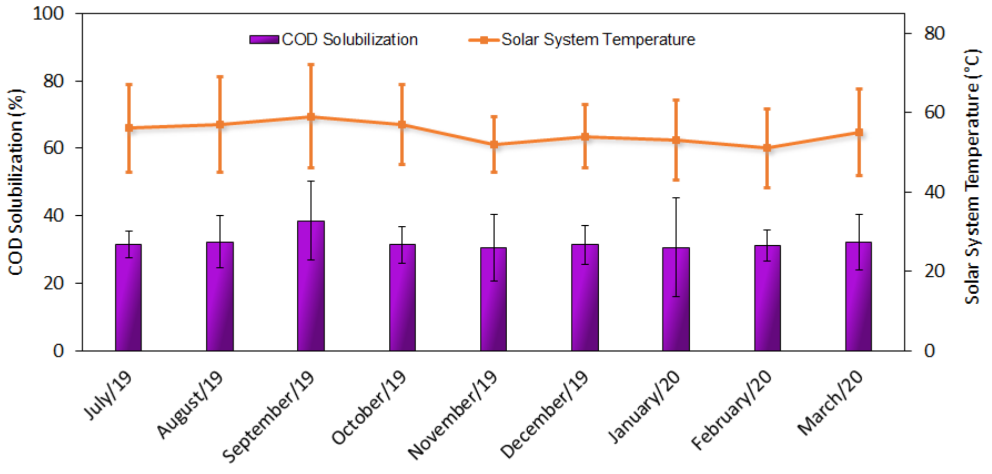


Figure 7.3. Chemical oxygen demand (COD) solubilization and average solar system temperature

7.3.2.3 Anaerobic digestion performance

Biogas production and composition for both UASB reactors are shown in Table 7.3. Biogas characteristics were within the typical range of biogas from UASB reactors treating sewage (SILVEIRA, 2015), with 60-85% methane (CH_4); 5-15% carbon dioxide (CO_2); 2-25% nitrogen gas (N_2); 0-0.3% carbon monoxide (CO); 0-3% hydrogen (H_2); 0-2% oxygen (O_2); and 1,000-2,000 ppmv (parts per million by volume) of hydrogen sulphide (H_2S). Wilcoxon test indicated that biogas composition for both reactors was significantly different only for methane content, which was higher in the $UASB_{co\text{-}dig\text{-}P}$ (68% vs. 57% in the $UASB_{control}$). This difference had already been observed in our previous study and was associated with improved organics degradation due to the presence of small amounts of oxygen introduced by the recirculation of microalgal biomass to the $UASB_{co\text{-}dig\text{-}P}$ reactor (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). Micro aeration in batches and in small quantities can improve the hydrolysis of organic matter and the acidogenic phase in anaerobic systems, leading to the oxidation of some available substrates by aerobic metabolism (BOTHEJU; BAKKE, 2011; BOTHEJU; LIE; BAKKE, 2010). The methane yield in the $UASB_{co\text{-}dig\text{-}P}$ was 41% higher than in the $UASB_{control}$ (252 vs. 179 NL CH_4 kg^{-1} VS), and 20% higher if compared with the same reactor co-digesting raw sewage and microalgal biomass without pre-treatment (252 vs. 211 NL CH_4 kg^{-1} VS) (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020).

No previous studies using solar pre-treatment of microalgal biomass have been published to date. Nevertheless, previous studies using conventional system to pretreat microalgal biomasss at 55 °C for 12 hours showed that the results obtained depended on the type of microalgae present in the system, with soluble COD increases of 4% for *Microspora* sp. and 3% for a mixed biomass composed of *Acutodesmus obliquus, Oocystis* sp., *Phormidium* sp. and *Nitzschia* sp. (ALZATE *et al.*, 2012). Upon co-digestion with primary sludge, the conventional thermal pre-treatment of microalgal biomass (at 75 °C for 10 hours) increased the methane yield by 5% (from 237 to 252 NL CH_4 kg^{-1} VS) in biochemical methane potential (BMP) tests (ARASHIRO, LARISSA T. *et al.*, 2019), and by 16% (from 291 to 339 NL CH_4 kg^{-1} VS) in semi-continuos lab-scale reactors (SOLÉ-BUNDÓ *et al.*, 2018) if compared the co-digestion with pre-trated and non-pre-treated microalgal biomass. In the present study, the solar pre-treatment at an avergae temperature of 55 ºC reached a higher methane yield increase (20%) using a demonstration scale facility operated continuously.

The anaerobic biodegradability in the $UASB_{co\text{-}dig\text{-}P}$ was on average 24% higher than in the $UASB_{control}$ (69% versus 45%) (Table 7.3). If compared to the UASB co-digesting raw sewage and non-pre-treated microalgal biomass ($UASB_{co\text{-}dig}$), the anaerobic biodegradability in the $UASB_{co\text{-}dig\text{-}}$

$_P$ increased by 15% as soluble COD (69% versus 54%) (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). A previous study evaluating pure microalgae cultures, reported an increase in the anaerobic biodegradability of 24% after pre-treatment at 70 ºC and 48% after pre-treatment at 90 °C for 3 hours (GONZÁLEZ-FERNÁNDEZ; SIALVE; BERNET; *et al.*, 2012b).

Results from mathematical modelling for biogas production in UASB reactors treating sewage (LOBATO; CHERNICHARO; SOUZA, 2012) showed similar results to experimental results. For the UASB$_{control}$ the mean methane yield was 152.85 NL CH_4 kg^{-1} COD$_{removed}$, while the modelled yield was 158.3 NL CH_4 kg^{-1} COD$_{removed}$. The methane yield in the UASB$_{co-dig-P}$ was 265.3 NL CH_4 kg^{-1} COD$_{removed}$, 35% higher than the modelled yield for the most optimistic scenario in the model (196 NL CH_4 kg^{-1} COD$_{removed}$) (LOBATO; CHERNICHARO; SOUZA, 2012). This indicated that the co-digestion of raw sewage with pre-treated microalgal biomass in solar pre-treatment system interfered positively on the methane yield in the UASB reactor.

Table 7.3. Biogas production and composition (n = 55)

Parameter	**UASB$_{control}$** **Mean ± SD**	***UASB$_{co-dig}$** **Mean ± SD**	**UASB$_{co-dig;P}$** **Mean ± SD**
Anaerobic biodegradability (%)	44.9 ±25.9	53.6 ± 20.9	69.2 ± 35.1
Biogas yield (L kg^{-1} COD)	141.4 ±73.8	165.6 ± 71.4	174.9 ± 68.1
Methane yiel (LCH_4 kg^{-1}COD)	81.3 ± 45.6	105.8 ± 45.4	117.9 ± 46.3
Biogas yield (NL kg^{-1} VS)	309.4 ± 140.3	331.1 ± 134.0	375.1 ± 135.5
Methane yield (NLCH_4 kg^{-1}VS)	178.5 ± 90.0	210.8 ± 78.1	252.4 ± 90.5
CH_4 (%)	56.8 ± 7.2	63.6 ± 7.0	67.5 ± 4.7
CO_2 (%)	5.8 ±1.2	6.7 ±5.7	6.7 ±2.4
O_2 (%)	0.6 ±1.3	1.4 ±2.1	0.9 ±4.4
CO (ppm)	23.3 ±9.7	6.8 ±3.5	26.2 ±16.1
H_2S (ppm)	1603.5 ±465.6	1843.6 ±359.7	1649.5 ±317.4
Balance (%) (N_2 + H_2)	36.7 ± 6.8	26.4 ± 9.1	24.9 ± 7.0

Note: CH_4 – Methane; CO_2 - Carbon Dioxide; O_2 – Oxygen; CO - Carbon Monoxide; H_2S - Hydrogen sulphide; N_2 – Nitrogen; H_2 – Hydrogen

* Data used for comparison purposes, already published by the same authors of this study (Vassalle et al 2020a).

7.3.3 Energy assessment

The energy assessment was performed to verify the energy self-sustainability of the proposed system with regards to the biogas production from the co-digestion of pre-treated microalgal biomass with raw sewage. The data used for calculations were collected from this study ($UASB_{control}$ and $UASB_{co\text{-}dig\text{-}P}$) and from our previous experiment that investigated raw sewage and non-pre-treated microalgal biomass co-digestion using the same UASB reactor ($UASB_{co\text{-}dig}$) (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). The results obtained are shown in Table 7.4.

Table 7.4. Average annual energy assessment for the UASB+HRAP system

Parameters	**Annual**		
	$UASB_{control}$	**$UASB_{co\text{-}dig}$***	**$UASB_{co\text{-}dig\text{-}P}$**
$E_{In\ UASB}$ ($kWh\cdot day^{-1}$)	751	901	901
$E_{In\ HRAP}$ ($kWh\cdot day^{-1}$)	0	22	22
E_{In} ($kWh\cdot day^{-1}$)	751	923	923
E_{Out} ($kWh\cdot day^{-1}$)	456	1945	2316
ΔE ($kWh\cdot day^{-1}$)	-294	1022	1393
NER	0.61	2.11	2.51

Note: Ein UASB – Energy In on Upflow Anaerobic Sludge Blanket; Ein HRAP – Energy In on High Rate Algal Ponds; Ein – Energy in on system; E-Out – Energy Out from System; ΔE –Difference between Energy In and Energy Out; NER - Net Energy Ratio.

* Data used for comparison purposes, already published by the same authors of this study (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020)

For the $UASB_{control}$, the system consumed more energy than it produced from biogas (i.e. NER < 1 and $\Delta E < 0$). It is important to highlight that pumping requirements were considered for feeding sewage, and that it was the only energy demand for the control scenario. If the conception of the project allowed flow entirely by gravity, the energy demand for this scenario would be zero, thus having a positive energy balance.

For the UASB reactors fed with microalgal biomass and raw sewage, the energy balance was positive, due to the increase in organic loading and methane yield. When comparing the $UASB_{co\text{-}dig}$ with the $UASB_{co\text{-}dig\text{-}P}$, an increase about 20% in the NER was observed (from 2.11 to 2.52) after pre-treatment (Figure 7.4), due to a further increase in the obtained methane yield from biomass solubilisation. A previous study assessing the energy balance of anaerobic digestion of microalgal biomass with and without pre-treatment at 75 °C for 10 hours reported an increase in the NER from 1.09 to 1.27 (17%) (PASSOS; FERRER, 2014). In another study evaluating the anaerobic digestion of microalgal biomass pre-treated (75°C for 10 hours) and the co-digestion with primary sludge, the authors showed an increase in the NER from 1.2 to 4.0 (230%) (SOLÉ-BUNDÓ *et al.*, 2018).

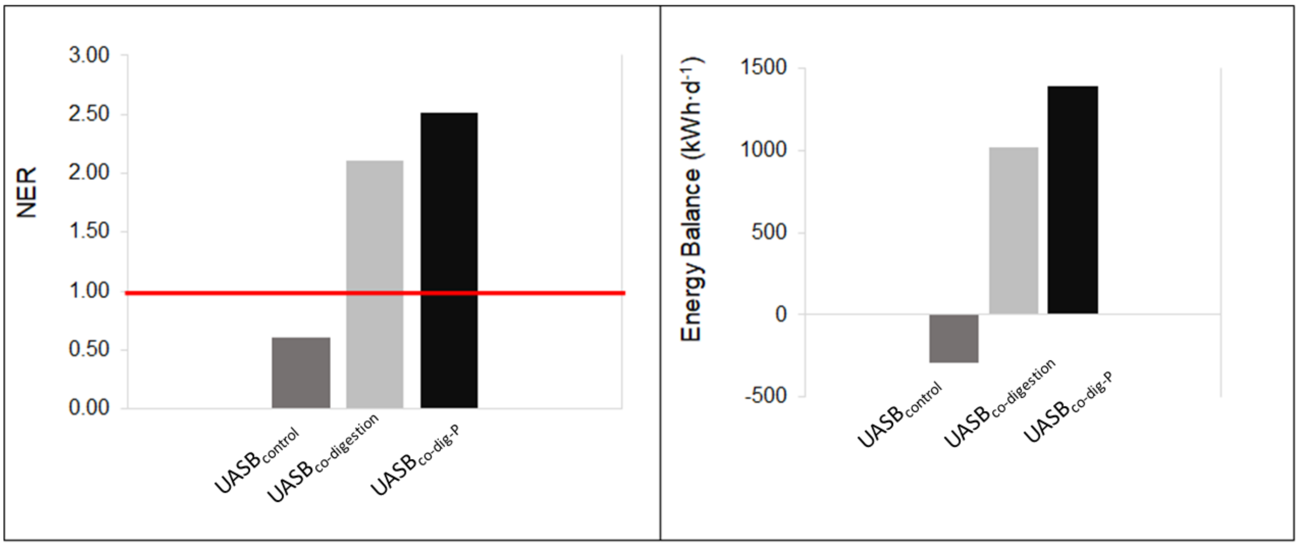


Figure 7.4. Net Energy Ratio (NER) and Energy balance for the system

The positive energy balance achieved may indicate a potential use of the energy produced within the same STP and/or in other applications. Many different uses for biogas produced in UASB reactors in STPs have been proposed (*deutsche gesellschaft für internationale zusammenarbeit* (GIZ) guide) (MOREIRA *et al.*, 2017). For instance, the conversion of biogas into heat using boilers, biogas use for dehydration and sanitation of the anaerobic reactor sludge, the conversion of biogas to electricity and heat in a combined heat and power (CHP) plant, the conversion of biogas into vehicular fuel or the production and injection of biomethane into the grid.

The biogas produced from the system could be used to produce heat (2.316 kWh day^{-1}, considering boiler efficiency of 90%). Alternatively, biogas could be used to generate heat to complement the solar heating system and achieve higher pre-treatment temperatures, which could further increase the biomass solubilisation and methane yield. In addition, biogas could be used to produce part of the heat necessary to maintain mesophilic temperatures in the UASB reactor, which could be desirable in cold climate countries or when disinfection is necessary. One of the main perceived advantages of the proposed UASB+HRAP+Solar Heater (for microalgal biomass pre-treatment) system is that it produces treated effluent of high quality (secondary standard) and generates only UASB sludge as by-product. This is possible because the other by-product, i.e. microalgal biomass (non-stabilised sludge), is pre-treated and stabilised in the UASB reactor, where its organic matter is converted into biogas and anaerobic sludge. UASB sludge may be used for agricultural purposes following sanitation, for example by using heat with a rotary dryer (AMARAL, K. G. C. DO;

AISSE; POSSETTI, 2019). However, the application of sewage sludge in agriculture can be repulsed by society and have legal barriers due to very restrictive legislation. Alternatively, UASB sludge can be dehydrated with heat to reduce its volume and minimise transportation costs for landfilling. Previous studies have shown that reductions in sludge volume of 46% can be achieved (CARTES *et al.*, 2018; KACPRZAK *et al.*, 2017).

Approximately 35% of biogas energy can be converted into electricity and 55% into heat using a CHP engine. Therefore, for the proposed STP with co-digestion of raw sewage with pre-treated microalgal biomass in solar system the average heat production was estimated as 1,415 kWh day^{-1} and electricity production estimated as 901 kWh day^{-1}. Considering that the electricity demand for the evaluated scenario was 923 kWh day^{-1}, almost all energy input (98%) would be supplied by electricity generated by CHP unit.

This electrical energy produced by CHP can be injected into the grid with direct exchange with a local trader. This practice, called distributed generation, is regulated by Brazilian law (ANEEL 482-2012, modified in 2017). However, distributed generation is not economically feasible for STP smaller than 100,000 population-equivalent (MOREIRA *et al.*, 2017) or 200,000 people equivalent (VALENTE, 2015). No strategy for improving the methane yield was applied in those studies and, therefore, the methane yield in small STP may be underestimated. It is strongly recommended to carry out an economic assessment to understand the viability of each scenario taking into account local conditions.

Finally, another use for biogas could be the direct injection into the grid or its use as vehicle fuel. However, biogas should be previously purified, which would let to an increase on the technological unit for biogas pre-treatment and the costs for energy conversion. Purification of biogas for that purpose is focused on the removal of hydrogen sulphide and carbon dioxide. Some techniques for biogas purification such as micro-aeration, filter membranes, adsorption and even columns with microalgae have been widely evaluated (MARÍN *et al.*, 2018, 2019b; MOREIRA *et al.*, 2017; POSADAS *et al.*, 2016). Following H_2S removal, biogas can be converted into biomethane, with a CH_4 concentration over 95% (MUÑOZ, RAÚL *et al.*, 2015; PAPACZ, 2011). This scenario would enable a decrease in the use of fossil fuels and, consequently, a decrease in the carbon footprint.

7.4 Conclusion

The proposed system showed adequate efficiency in removing COD (70%) and $N\text{-}NH_4$ (61%). The microalgal biomass was harvested and pre-treated in solar system. The solar pretreatment system of microalgal biomass proved to be efficient. Biomass solubilisation and methane yield increase by 32% (COD) and 45% respectively. Finally, the energy assessment showed a positive balance, with 150% more energy produced than consumed throughout the year when compared with current systems, without using microalgal biomass (control system of this study). The proposed system produces treated effluent of high quality and generates only UASB sludge as solid by-product. This is possible because the microalgal biomass is pre-treated and stabilised in the UASB reactor, where its organic matter is converted into biogas and anaerobic sludge.

8 COMPARATIVE LIFE CYCLE ASSESSMENT OF UASB REACTORS COUPLED WITH LOW-COST TECHNOLOGIES FOR SEWAGE TREATMENT

Based on: Vassalle, L.[*], Ferrer, I., Passos, F., Mota-Filho, C.R., Rosa-Machado, A.T., Freitas, M.P., and Garfi, M.. 2021 - 8 Comparative life cycle assessment of UASV reactors coupled with low cost technologies for sewage treatment: **Final preparation phase**

The Latin America scenario for sewage treatment shows that decentralized domestic wastewater treatment systems seem to be preferred in respect to conventional centralized plants. For this scenario, Upflow anaerobic sludge blanket (UASB) reactors are widely used to treat domestic sewage and frequently require post-treatment. Little is known about the post treatment of UASB reactors effluent by high rate algal ponds. This study aimed to assess the environmental impact of using HRAP post-treating UASB reactor effluent. A life cycle analysis was made comparing this technology with three others used for the same purpose, namely trickling filters, polishing ponds and constructed wetlands. The results showed that among the 8 categories evaluated, the high rate algal ponds showed better environmental performance in 4 and great economical potential, when used to recover energy. It be concluded that this technology can be considered as a potential for post treatment of effluents from UASB reactors and may also have better environmental performance when used appropriate materials and construction techniques.

8.1 Introduction

The rates of sewage collection and treatment in Brazil show that 75% of the population is attended by a sewerage, and of this amount collected, only 45% receive some type of treatment, that's mean about 97 million of people does not have treated sewage (BRASIL, 2017, 2018b). The difficulty of accessing certain places, but mainly the lack of investment, aggravates the situation. The absence of sewage treatment is a serious environmental and public health problem and can be considered a waste of resources, given the great energy potential of this by-product. The use of this resource could help to balance the Brasil energy matrix, which is currently based on non-renewable sources (57%) (BRASIL, 2018a).

Simplified conceptions of the sewage collection and treatment system are growing as a solution for universal sanitation. In this context, decentralized sewage treatment plants were reported to be the most common solution with respect to conventional (e.g. activated sludge systems). In a study performed for Latin America, 2,734 sewage treatment plants were evaluated, 67% of the STPs had treatment capacity below 25 L s^{-1}, being classified as small size. The most present technologies reported for sewage treatment were stabilization ponds , activated sludge and upflow anaerobic sludge blanket reactors (UASB) (NOYOLA *et al.*, 2012). In Brazil, study showed that in a universe of 2,187 STP, 41% are polishing ponds, 37% UASB reactors with post treatment and 11% activated sludge or other systems (Von Sperling, 2016) . It is worth noticing that , UASB reactors although only implemented from the mid-1980s, is already among the most available technologies in the region (Chernicharo, *et al.*, 2018). The investment in technologies that involved UASB reactors with post treatment in many developing countries may be associated with the lower capital and operational expenditure (CAPEX and OPEX) of this technology, when compared to conventional treatment systems such as activated sludge systems) (CHERNICHARO, C. A. DE L.; RIBEIRO; GARCIA; *et al.*, 2018). Moreover, UASB reactors are considered an interesting technological option since it removes efficiently organic matter and solids from the raw sewage at low cost and low area, while generating biogas (CHERNICHARO, C. A. DE L.; RIBEIRO; PEGORINI; *et al.*, 2018). The recovered biogas from the UASB reactors is usually converted into heat using boilers, electricity and heat using combined heat and power plants or even purified into biomethane and injected into the gas grid. In small communities of the Brazilian and Latin American rural areas, the recovered biogas may be used as sustainable fuel for cooking or heating. Moreover, biosolids can be sanitized and used as biofertilizer, but often this use can be restricted by environmental legislation based on the precautionary principle (SCHÄFER, A. I.; BEDER, 2006). However, the UASB effluent usually requires a post-treatment step to remove organic matter, nutrients and pathogens in order to meet the discharge requirements (DAUD *et al.*, 2018). A recent study evaluating 1667 sewage treatment plants in Brazil, showed that 667 of them applied UASB reactors followed by post-treatment. Trickling filters (TKF) and polishing ponds (PP) the most common technologies (around 65%) (CHERNICHARO, C. A. DE L.; RIBEIRO; GARCIA; *et al.*, 2018). Recently, constructed wetlands (CW) were shown to be another suitable option for improving UASB effluent quality for small scale plants (SEZERINO *et al.*, 2015). High rate algal ponds are shallow, paddlewheel mixed, raceway ponds where treatment is carried out by a consortium of microalgae and bacteria which assimilate nutrients and degrade organic matter (PARK; CRAGGS, 2010). In the recent years, these systems have been gaining popularity since resources might be recovered from the produced biomass. In fact, microalgae grown in the shallow ponds may be harvested and valorized to produce biofuels (e.g. biogas), biofertilizers or animal feed (GONZÁLEZ-FERNÁNDEZ *et al.*, 2011). When HRAPs follow a UASB reactor, microalgal (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020) biomass can be recirculated to the latter and co-digested anaerobically with raw sewage increasing biogas production and methane yield up to 10% and 35%, respectively (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). Even though technical feasibility of UASB reactor coupled with HRAP have been already proved, environmental benefits due to their implementation have to be demonstrated yet.

To select an appropriate flowchart for a wastewater treatment scenario is a complex process, which takes into account several variables that affect the required levels of treatment and overall costs (BRESSANI-RIBEIRO, THIAGO *et al.*, 2019). There is no single solution applicable to all cases, the definition of a sewage treatment flowchart should be based on a balance between economic, environmental and technical criteria (VON SPERLING, MARCOS; CHERNICHARO, 2005b). The selection of inadequate sewage treatment technologies may result in low treatment performance and operational and maintenance difficulties (VAN LIER; LETTINGA, 1999).

To facilitate this challenging decision, accounting the numerous variables that can interfere with the cost of implementation and, especially, the environmental sustainability of wastewater treatment, the life cycle assessment (LCA) tool may assist the decision-making process in the sanitation field. This tool enable to compare different technologies (renowned and innovative) in order to improve the decision-making and, consequently, the success in sewage treatment processes. (FERREIRA, S. *et al.*, 2014; GARFÍ; FLORES; FERRER, 2017).

Based on the challenge and gaps presented in the literature and in order to propose a sustainable flowchart STP using UASB reactor and a post-treatment unit, the aim of this study was to assess, for the first time, the environmental impacts of UASB reactor coupled with high rate algal ponds (UASB + HRAP) using the life cycle assessment methodology. Moreover, they were compared to other technologies which have already been well established or studied in rural areas of Brasil (i.e. UASB + Trickling Filter and UASB + Polish Pond, UASB + Constructed Wetlands).

8.2 Material and Methods

8.2.1 Sewage treatment plants description

The systems were hypothetical sewage treatment plants based on extrapolation performance from pilot-scale studies (up to 100 m^2) located in Belo Horizonte, Brasil (coordinates 19º53'42" S and 43º52'42"W, 800 m of altitude - Sanitation Research and Training Center) (DE ASSUNÇÃO; VON SPERLING, 2013; DORNELAS; MACHADO; VON SPERLING, 2009; PONTES *et al.*, 2003; VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). They were designed to serve a population equivalent of 10,000 p.e. and to treat a flow rate of 18 L s$^{-1,}$ taking into account the sewage characteristics and project parameters reported in literature (CHERNICHARO, C. A., 2007; CRAGGS, R. J. *et al.*, 2003; METCALF & EDDY *et al.*, 2003; VON SPERLING, M., 2007). All project parameters considered for each system, was presented in the supplementary material section E (Tables E.1 to E.8),the original worksheets used to calculate each unit considered in this study were presented, without changes or translations.

For all scenarios, a pre-treatment was considered (manual grid, grit chamber and Parshall flume). Subsequently, the sewage was fed to UASB reactor. The four scenarios differed in the technology used to post-treat the UASB reactor effluent, which are (Figure 8.1): a) trickling filter (UASB + TKF), b) high rate algal pond (UASB + HRAP), c) polishing pond (UASB + PP) and d) horizontal sub-surface flow constructed wetlands (UASB + CW).

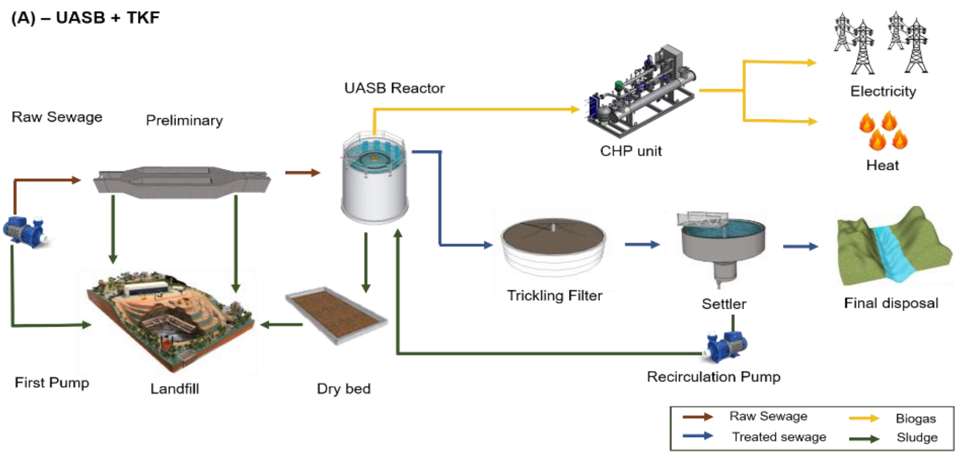
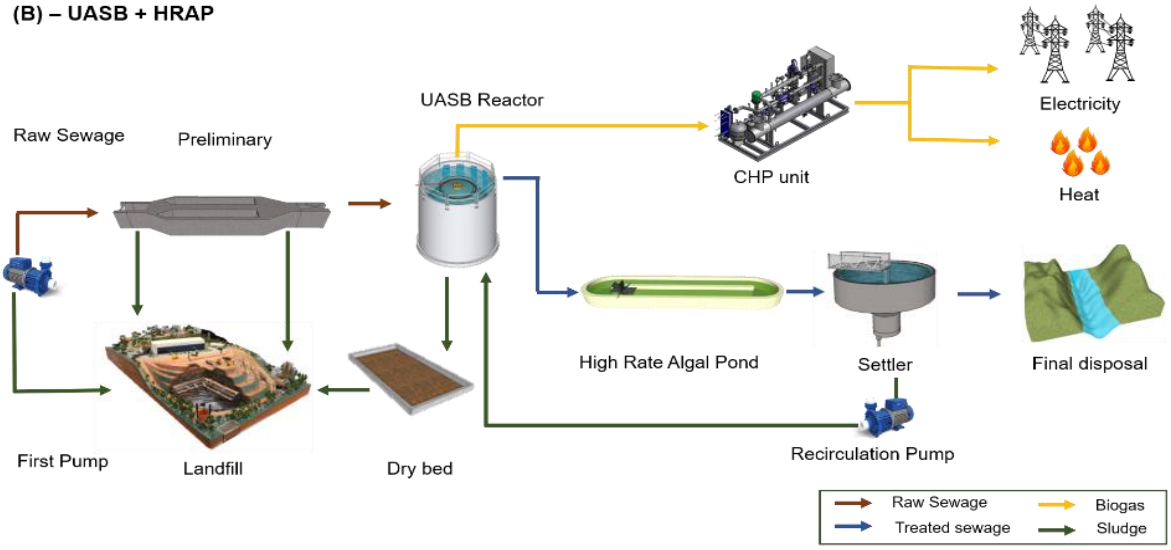
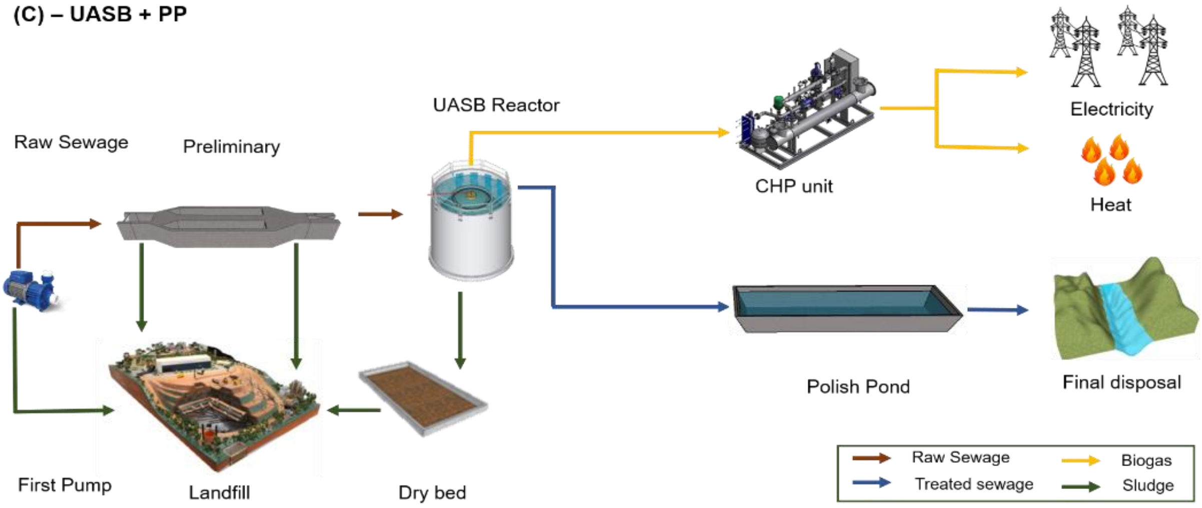


Figure 8.1 (A). Diagram of the STP scenarios: (A) UASB+TKF

(B) – UASB + HRAP



(C) – UASB + PP



(D) – UASB + CW

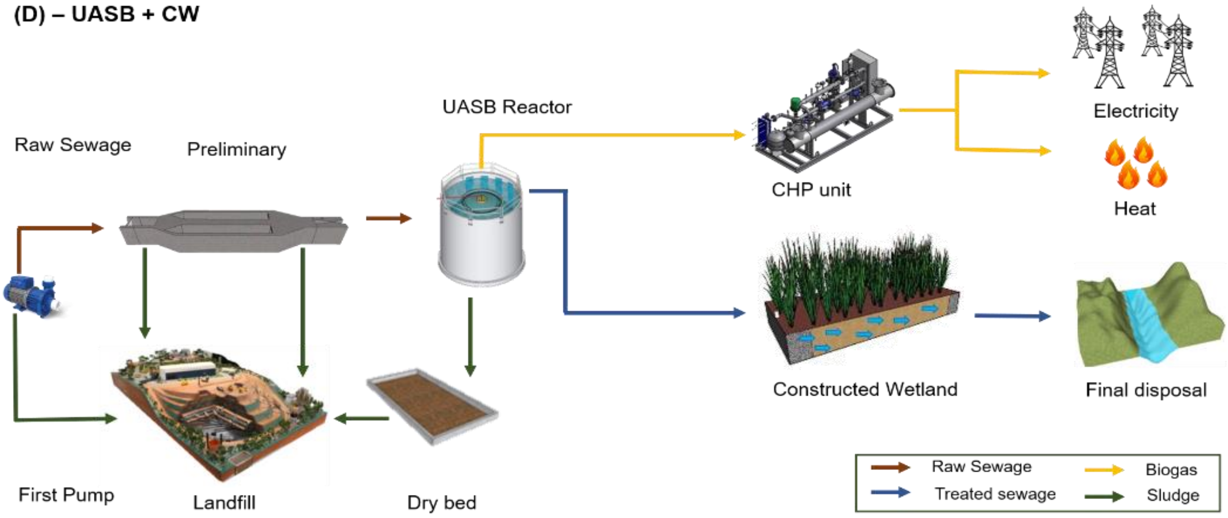


Figure 8.2 (B to D). Diagram of the STP scenarios: (B) UASB+HRAP; (C) UASB+PP; (D) UASB+CW

To characterize the raw sewage, used to design the scenarios, analyzes were performed during one year from a full STP located in Belo Horizonte, Brazil. The methodology was describe in previous study (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). In the trickling filter (UASB + TKF) and high rate algal ponds (UASB + HRAP) scenarios, the secondary effluent goes through a gravity settler for biomass concentration (sludge and microalgae, respectively). The harvested biomass is then recirculated (10%) to the UASB reactor in order to increase the biogas production (35%) (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020), while treated sewage is discharged into the environment. In the polish pond (UASB+PP) and constructed wetlands (UASB+CW) scenarios, the final effluent is directly discharged into a water body. In all scenarios, biogas produced by the UASB reactor is converted in a combined heat and power (CHP) unit. The sludge from the same reactor is treated in drying beds and then landfilled together with the material separated from the pre-treatment (sand and grid material) (BRESSANI-RIBEIRO, THIAGO *et al.*, 2019). Table 8.1 summarises the design and operational parameters of each unit of the sewage treatment plants.

Table 8.1. Design and operational conditions of the units

Design and operational conditions – Flow: 17.36 L s^{-1}						
Parameter	**Units**					
	Preliminary*[*]	**UASB reactor**	**Trickling Filter**	**High Rate Algal Pond**	**Polish Pond**	**Constructed Wetlands**
Water deep (m)	1.5	4.1	2.5	0.3	0.8	0.3
Width (m)	1	10	-	12	40	40
Length (m)	12	12	-	625	160	80
Diameter (m)	-	-	10	-	-	-
Surface Area each unit (m²)	12	120	78.5	7952	6400	3200
Total HRT[**]	-	7 hours	-	8 days	12 days	10 days
Units (n)	1	1	1	4	4	7
Drying bed (n)	-	-	2	3	2	2

* Preliminary was composed with 67 grids of 5 mm thick and 10 mm spaced. The bars were designed with inclination of 60º and length of 1.62 meters. In addition, were considered a grit chamber with 1 meter of width and 2 of length and a Parshall flume with 2 inches. ** HRT – Hydraulic retention time

In relation to the generated by-products, it was considered for all cases that the treated effluent was disposed in water bodies and the solid material, landfilled, as these are traditional alternatives adopted in most STP of this size in Latin America. In all scenarios, the biogas produced from the UASB reactor was considered to produce heat and electricity by CHP motor. To increase the biogas production two, among the four scenarios studied, had a recirculation of organic matter to the UASB reactor, increasing methane yield, i.e. UASB + TKF and UASB + HRAP. The return of excess sludge produced in the filter to the reactor was assumed according to previous literature (PONTES *et al.*, 2003). Similarly, harvested microalgal biomass was recirculated and co-digested with the raw sewage in the UASB reactor (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). In these scenarios, a recirculation pump was designed. Another pumping station was considered in all scenarios, to conduct the sewage from the interceptor pipe until the pre-treatment.

Effluent quality and treatment efficiencies of each alternative where taken from previous pilot-scale studies. For the UASB + TKF scenario, removal efficiencies of 81% for COD, 87% for TSS and 20% for TN and TP were considered (Pontes et al., 2003, Oliveira and von Sperling, 2002; Von Sperling, 2007). For the UASB + HRAP scenario, removal efficiencies of 67% for COD, 44% for TSS, 32% for TN and an 11% increase (assimilation) for TP were considered (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). For the UASB + PP scenario, the removal efficiencies of 72% for COD and TSS and 44% for TN and an 12% increase (assimilation) for TP were considered (DE ASSUNÇÃO; VON SPERLING, 2013). Finally, for the UASB + CW scenario removal efficiencies of 94% for COD, 98% for TSS, 31% for TN and 55% for TP were taken into account (DORNELAS; MACHADO; VON SPERLING, 2009).

The production of sand and grid material separated from the pre-treatment were estimated for pre-treatment and UASB reactor. For the pre-treatment, a retention rate of 0.064 L m^{-3} for the manual grid, 0.030 L m^{-3} for the grit unit and 0.012 L m^{-3} for the pump were adopted according to the literature (METCALF & EDDY *et al.*, 2003). On the other hand, the amount of solid retained was obtained by multiplying the flow rate applied to the unit by the respective retention rates. Secondly, for the sludge produced in the UASB reactor were calculated considering a solids coefficient of 0.15 kg SST kg COD^{-1} (CHERNICHARO, C. A., 2007).

Methane yield was calculated considering previous pilot-scale studies. A specific methane yield of 0.16 L CH_4 g^{-1} VS was considered for the UASB+TKF, UASB+PP and UASB+CW scenarios. On the other hand, for the UASB+HRAP scenarios, methane yield was 30% higher due to the recirculation of sludge and microalgae, respectively (0.21 L CH_4 g^{-1} VS) (Vassalle et al., 2020).

Influent and effluent characteristics as well as sludge and biogas production of each alternative are summarized in Table 8.2.

Table 8.2. Influent and effluent characteristics as well as sludge and biogas production of each alternative

System characteristics	Unit	UASB+TKF	UASB+HRAP	UASB+PP	UASB+CW
Flow	L s^{-1}	17.36	17.36	17.36	17.36
COD $_{\text{Influent}}$	mg O_2 L^{-1}	437.0	437.0	437.0	437.0
COD $_{\text{effluent}}$	mg O_2 L^{-1}	83.0	144.3	127.2	36.1
TSS $_{\text{Influent}}$	mg L^{-1}	243.0	243.0	243.0	243.0
TSS $_{\text{effluent}}$	mg L^{-1}	32.2	142.3	71.2	5.2
VS $_{\text{Influent}}$	mg L^{-1}	207.0	207.0	207.0	207.0
VS $_{\text{effluent}}$	mg L^{-1}	19.0	96.0	41.05	4.12
TN $_{\text{Influent}}$	mg L^{-1}	45.6	45.6	45.6	45.6
TN $_{\text{effluent}}$	mg L^{-1}	25.2	29.6	25.2	31.6
TP $_{\text{Influent}}$	mg L^{-1}	4.9	4.9	4.9	4.9
TP $_{\text{effluent}}$	mg L^{-1}	2.90	3.29	3.25	1.34
Solid production	m^3 $year^{-1}$	183.03	483.03	172.03	172.03
Methane yield	m^3 CH_4 day^{-1}	83.07	292.32	50.01	50.01
Total surface area	m^2 PE^{-1}	0.05	3.00	2.60	2.60

8.2.2 Life Cycle Assessment

The LCA was conducted following the ISO standards (ISO, 2000; ISO, 2006) in order to evaluate and quantify the potential environmental impacts of the investigated scenarios. It consisted of four main stages: i) goal and scope definition, ii) inventory analysis, iii) impacts assessment and iv) interpretation of the results (ISO, 2006). The following sections describe the specific content of each phase.

8.2.2.1 Goal and scope definition

The goal of this study was to determine the potential environmental impacts of the UASB reactor coupled with HRAPs for wastewater treatment and resources recovery in small-communities in Brazil. This configuration was compared to other low-cost technologies that have already been established in the country. Overall, four alternatives were compared:

i) UASB reactor coupled with a trickling filter (scenario UASB+TKF);

ii) UASB reactor coupled with high rate algal ponds (scenario UASB+ HRAP);

iii) UASB reactor coupled with polish ponds (scenario UASB+PP);

iv) UASB reactor coupled with sub-surface flow constructed wetlands (scenario UASB+CW).

The functional unit (FU) for this study was set as 1 m^3 of treated water, since the main function of the technologies proposed is to treat sewage. The cradle-to-grave boundaries included systems construction, operation and maintenance over a 20-years period (ARASHIRO, LARISSA TERUMI *et al.*, 2018; GARFÍ; FLORES; FERRER, 2017; PÉREZ-LÓPEZ *et al.*, 2017). Input and output flows of materials (i.e. construction materials) and energy resources (heat and electricity) were systematically studied for all scenarios. Direct greenhouse gases (GHG) emissions and NH_4^+ volatilization associated with wastewater treatment were also included in the boundaries (GUTIERREZ, 2014; SØVIK *et al.*, 2006). Direct emissions to water were also considered, since treated wastewater is discharged into a water body. Regarding the sludge produced in all scenarios, transportation of dried sludge to the landfill was also taken into account (20 km) (HOSPIDO, ALMUDENA *et al.*, 2004) were accounted for. The end-of-life of infrastructures and equipment were neglected, since the impact would be marginal compared to the overall impact. Since the studied scenarios would generate by-products (i.e. biogas), the system expansion method has been used following the ISO guidelines (GUINÉE, 2006). In this method, by-products are supposed to avoid the production of conventional products. Thus, the impacts associated with the production of conventional products were withdrawn from the overall impact of the systems (Arashiro et al., 2018; Sfez et al., 2015; ISO 2006). Specifically, the avoided impacts of using heat and electricity produced by the biogas cogeneration instead of heat from natural gas and electricity from the grid were considered.

8.2.2.2 Inventory analysis

Inventory data for the studied scenarios are summarized in Table 8.3. In all scenarios, inventory data regarding construction materials and operation were based on the detailed engineering designs performed in the frame of this study. As mentioned above, effluents characteristics were estimated considering the removal efficiencies and experimental results of previous studies. In the case of the trickling filters (UASB + TKF) and constructed wetlands (UASB+CW) scenarios, direct CH_4 and

N_2O emissions were estimated using emission factors from the literature (Gutierrez, 2014; Søvik et al., 2006). NH_4 volatilization in the high rate algal pond (UASB+HRAP) scenario was estimated through nitrogen mass balance (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). In the case of the polish pond (UASB+PP) scenario, NH_3 emissions were calculated taken into account the factor of 3% for ammonia removed in polish pond (DE ASSUNÇÃO; VON SPERLING, 2013). Heavy metals were not detected in the raw sewage, they were not considered in any scenarios. As mentioned above, to estimate electricity and heat production from biogas cogeneration, biogas production obtained in pilot-scale experiments was taken into account. Background data (i.e. data of construction materials, chemicals, energy production, transportation, sludge landfilling) were obtained from the Ecoinvent 3.1 database (MORENO RUIZ *et al.*, 2013; WEIDEMA *et al.*, 2013).

Table 8.3. Summary of the inventory for all scenarios to post-treat UASB Effluent. Values are referred to the functional unit (1 m^3 of water).

Parameter	**Unit**	**Scenario**			
		UASB+TKF	**UASB+HRAP**	**UASB+PP**	**UASB+CW**
Input					
Construction materials*					
Concrete	$m^3\ m^{-3}$	1.92E-05	6.06E-04	8.79E-06	4.33E-04
Steel	$kg\ m^{-3}$	1.49E-03	4.88E-02	6.60E-04	6.94E-04
Fiber Glass	$kg\ m^{-3}$	1.84E-04	1.84E-04	1.84E-04	1.84E-04
Gravel	$kg\ m^{-3}$	1.89E-04	-	-	9.21E-01
PEAD	$kg\ m^{-3}$	-	-	4.11E-03	-
Operation					
Energy consumption	$kwh\ m^{-3}$	4.87E-02	8.73E-02	4.47E-02	4.47E-02
Output					
Emissions to water**					
Total COD	$g\ m^{-3}$	8.30E+01	1.48E+02	1.27E+02	3.60E+01
TSS	$g\ m^{-3}$	3.20E+01	1.42E+02	7.10E+01	5.00E+00
TN	$g\ m^{-3}$	2.52E+01	3.18E+01	2.52E+01	3.16E+01
TP	$g\ m^{-3}$	2.90E+00	3.29E+00	3.25E+00	1.34E+00
Emissions to air**					
CH_4	$g\ m^{-3}$	2.68 E+00	-	-	2.64E+00
N_2O	$g\ m^{-3}$	2.30E-01	-	-	1,05E-01
NH_3	$g\ m^{-3}$	-	5.10E-01	5.80E-01	-
Emission to soil**					
Soil to landfill	$kg\ m^{-3}$	3.18E-01	8.77E-01	2.97E-01	2.97E-01
Avoid products (from biogas cogeneration)					
Electricity	$kwh\ m^{-3}$	1.93E-01	7.92E-01	1.16E-01	1.16E-01
Heat	$kwh\ m^{-3}$	3.03E-01	1.25E+00	1.82E-01	1.82E-01

* The materials of preliminary treatment composed by grids, grit chamber and Parshall flume were considered for all scenarios. ** Annual averages.

8.2.2.3 Impact assessment

This LCA study has been performed using the SimaPro® 8 software (Pré Sustainability, 2014). The potential environmental impacts were calculated using the ReCiPe midpoint method (hierarchist approach) (GOEDKOOP *et al.*, 2009). In this study, characterization phase was performed considering the following impact categories: Climate Change, Ozone Depletion, Terrestrial acidification, Freshwater Eutrophication, Marine Eutrophication, Metal Depletion, Terrestrial ecotoxicity and Fossil Depletion. These impact categories were selected according to the most relevant environmental issues related to sewage treatment (ARASHIRO, LARISSA TERUMI *et al.*, 2018; CARTES *et al.*, 2018; FLORES *et al.*, 2019; GARFÍ; FLORES; FERRER, 2017; GUTIERREZ, 2014). Normalisation was carried out in order to compare all the environmental impacts at the same scale. This provides information on the relative significance of the indicator results, allowing a fair comparison between the impacts estimated for each scenario (ISO, 2006). In this study, the European normalization factors have been used (Europe ReCiPe H) (GOEDKOOP *et al.*, 2009).

8.2.3 Economic Assessment

The economic assessment was performed comparing the capital cost and the operation and maintenance cost of all scenarios. The capital and operation and maintenance costs were based on costs reported for systems composed of a UASB reactor followed by post-treatment for the Brazilian reality (Von Sperling, 2014). The published values (in reais – R$) for the year 2010 were adjusted to present values (2020) based on the Brazilian index, of construction cost (INCC) (correction index used for cost of capital) and consumer prices (INPC) (correction index used for operation and maintenance). Equation 8.1 was used to calculate the correction index for present value. Where super index V means costs and sub-indices A and 0 mean current and initial respectively.

$$V_A = V_0 \cdot \left(1 + \frac{I_A - I_o}{I_o}\right) \qquad \text{(Eq. 8.1)}$$

The correction rates (2010 to 2020) for INCC and IPCC were 81.46% and 71.25% respectively. The costs in € were converted from the costs in Brazilian reais (R$) using the exchange rate of € 1.00 = R$ 6.26 (19/May/2020, Central Bank of Brazil). For the calculation of avoided eletrical energy expenditure in each scenario, due to the production of biogas, a tariff of 0.11€ Kwh^{-1} was considered.

8.3 Results and discussion

8.3.1 Characterization

The potential environmental impacts associated with each alternative are shown in Figure 8.2. Comparing all the scenarios (UASB +TKF, UASB + HRAP, UASB +PP and UASB + CW), the results showed that the high rate algal ponds (UASB + HRAP) scenario was the most environmental friendly alternative in 4 out of 8 impact categories (i.e. Climate Change, Ozone Depletion, Terrestrial ecotoxicity and Fossil Depletion potentials). The trickling filters (UASB + TKF) scenario was the most environmental friendly in marine eutrophication and terrestrial acidification impact categories. On the other hand, the constructed wetlands (UASB+CW) and polishing ponds (UASB+PP) were the best alternative in freshwater eutrophication and metal depletion impact categories, respectively.

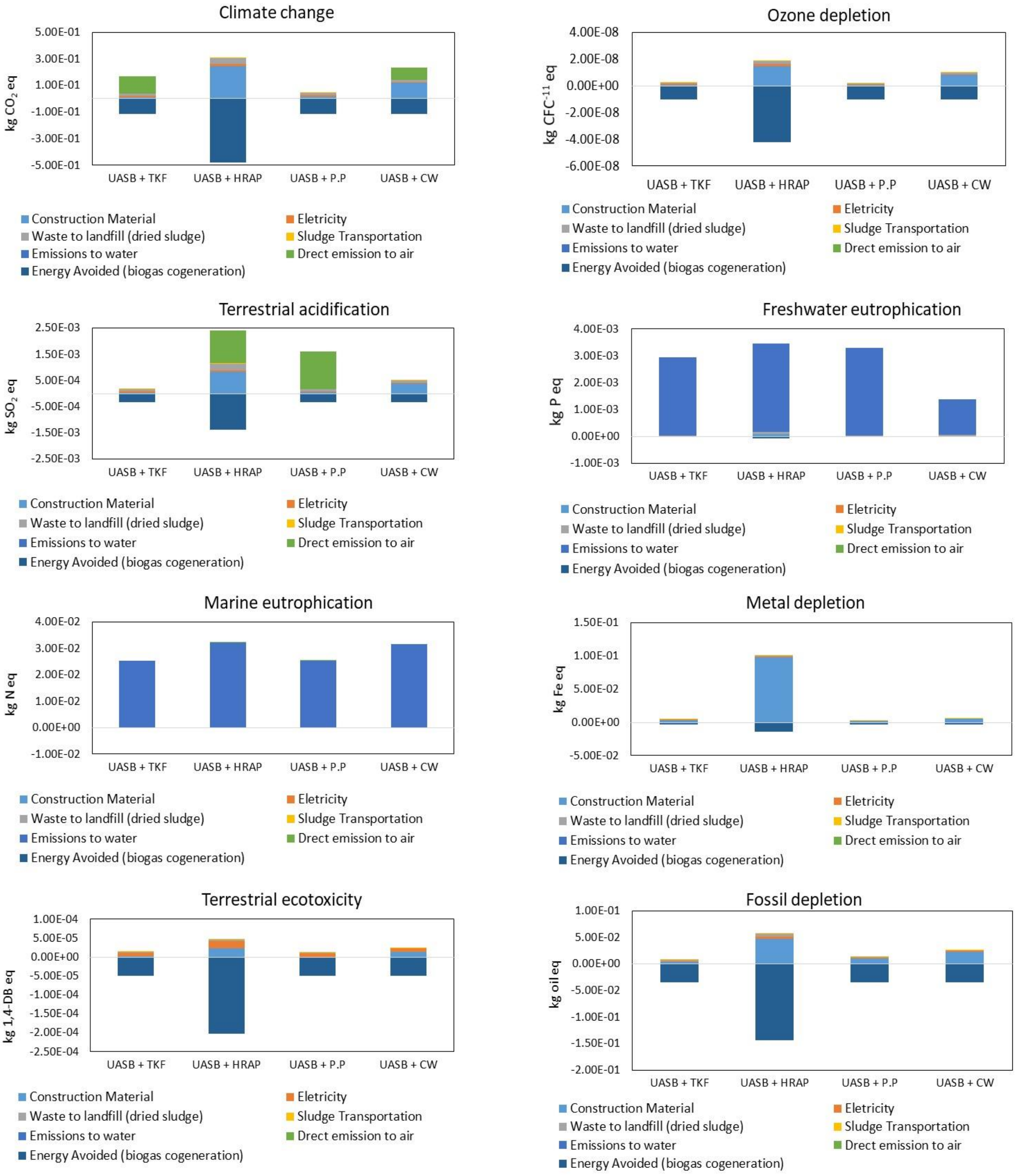


Figure 8.3. Potential environmental impacts for the four scenarios using different technologies to post treat UASB reactor: Trickling filter (UASB+ TKF), high rate algal ponds (UASB+HRAP), Values are referred to the functional unit (1 m^3 of water).

Regarding the climate change impact category, the higher impacts of trickling filters (UASB+TKF) and constructed wetlands (UASB+CW) were mainly due to the methane and nitrous oxide gases emissions which are associated with these technologies. These emissions were responsible for 77% and 38% of the overall impact in those scenarios, respectively. Moreover, in the high rate algal ponds (UASB+HRAP) scenario the high impact caused by the large amount of construction materials (79% of the overall impact) is completely offset by the avoided energy due to biogas cogeneration. It is mean that the recirculation of microalgal biomass in the UASB reactor (co-digestion with raw sewage) to produce biogas and avoid energy expense have a great potential to be used and make this scenario eco-friendlier.

Anaerobic co-digestion of microalgal biomass with primary or secondary sludge in the treatment of sewage or others wastewater is widely studied and those have showed that this practice allows an increase methane production in biogas from digestors (MONTINGELLI; TEDESCO; OLABI, 2015; PASSOS *et al.*, 2013; UGGETTI *et al.*, 2017). Methane production values in biogas from anaerobic microalgae co-digestion reported in the literature ranges from 0.10 to 0.46 L CH_4 gVS^{-1} (MAHDY *et al.*, 2014b; UGGETTI *et al.*, 2017) against 0.24 L CH_4 gVS^{-1} without co-digestion (WANG, M.; PARK, 2015b).

In fact, previous studies observed a positive energy balance (from -303 kWh d^{-1} in reactor without microalgal biomass to 1,022 kWh d^{-1} in reactor with co-digestion) when microalgal biomass was co-digested with raw sewage and the biogas was used to cogenerate electricity and heat (VASSALLE; DÍEZ-MONTERO; *et al.*, 2020). In particular to the high rate algal ponds (USAB + HRAP) scenario, the avoided energy was around 4 times higher compared to the other scenarios (Table 2). The results obtained for this category are in accordance with previous studies which analysed the environmental impacts of high rate algal ponds coupled with anaerobic digestion (ARASHIRO, LARISSA TERUMI *et al.*, 2018).

Concerning to the ozone depletion and terrestrial ecotoxicity categories for all scenarios the impact caused was completely offset by the avoided energy. As happened in the climate change category, high rate algal ponds (USAB + HRAP) scenario had the lowest impact among the evaluated scenarios, due to its greater potential for energy production with the use of microalgal biomass being co-digested with raw sewage.

As far as terrestrial acidification potential is concerned, the alternatives eco-friendlier, after the trickling filters (TKF), were constructed wetlands (CW), high rate algal ponds (HRAP) and polish ponds (PP) respectively. For this category, the impact for environment was 7 times less in the scenario with trickling filters (TKF) when compared to high rate algal ponds (HRAP) and polish ponds (PP). For these scenarios (HRAP and PP), ammonia emissions were responsible for 52% and 89% of the environmental impact in this category. The terrestrial acidification penalizes ammonia emissions to the air and this type of emission is associated with pond systems in the treatment of sewage, due to the high pH (> 9) of operation of these units, since the balance between ammonium ion and free ammonia in these conditions tends to shift towards the formation of NH_3, which is very unstable and volatilizes. It is estimated that 3% of the removed ammonia is volatile in ponds system (DE ASSUNÇÃO; VON SPERLING, 2013). One way to control the pH is the injection of CO_2 in the ponds, this practice can increase up to 15% the production of microalgae in these systems (SOLIMENO; GÓMEZ-SERRANO; ACIÉN, 2019).

About the freshwater and marine eutrophication potentials, the high rate algal ponds alternative (UASB+HRAP scenario) showed a slightly higher environmental impact with respect to the other scenarios. Eutrophication is associated with the excessive supply of nutrients (nitrogen and phosphorus) in a water body (VON SPERLING, M., 2007). This corroborates with the results obtained with this study, where the configuration with higher nutrients concentration effluent (HRAP) showed higher impacts in those categories. High nutrients discharge (phosphorous and nitrogen, respectively) concentrations in the HRAPs effluent (Table 3) impacting , freshwater and marine eutrophication categories in life cycle assessment studies as also shown in previous studies (ARASHIRO, LARISSA T. *et al.*, 2019; GARFÍ; FLORES; FERRER, 2017; GUTIERREZ, 2014). The high concentration of nutrients in the HRAP effluent may be associated with the concentration of microalgae present in the final effluent, that were not separated by the settler. The higher concentration of total solids in the final effluent (Table 3) is a strong indication that there was a difficulty in separating the microalgae for disposal of the effluent. Microalgae assimilate nitrogen and phosphorus during sewage treatment, thus, possibly these nutrients were discharged in the final effluent due to loss of microalgal biomass. The difficulty in sedimentation of microalgal biomass has already been reported in the literature and associated with the size of the cell, which is very small and tends to remain in suspension (GUPTA *et al.*, 2017). To increase the sediment ability of microalgae, coagulants can be used (GUPTA *et al.*, 2017). Due to the negative charges that the microalgae assume at neutral pH, cationic chemical coagulants are normally used (CHATSUNGNOEN; CHISTI, 2016). However, the introduction of chemical products into a natural system is a concern due to the assimilation of these products by algae and the possibility of producing a hazardous sludge (GUPTA *et al.*, 2017). This can be avoided with the use of natural coagulants, which increase the recovery of microalgal biomass by up to 90% and do not limit the application of this recovered by-product. (GUPTA *et al.*, 2017; GUTIÉRREZ *et al.*, 2015).

Regarding the metal depletion potential, the higher impact of high rate algal ponds (USAB + HRAP) was due to the large amount of construction materials used (98% of the overall impact). Indeed, HRAPs had a high surface area requirement compared to the other alternatives (3.0 m^2 PE^{-1}). Moreover, for high rate algal ponds (USAB + HRAP) scenario, was considered the use of concrete and steel, which are responsible of a high environmental burden. To compare, in this category, the most environmentally friendly scenario was polish pond (UASB + PP). This could be related to the construction technique considered, which was basically for excavation and waterproofing the terrain. Thus, possibility to minimize the impacts caused by HRAP for this category would be the use of same construction technique considered to polish ponds (excavation and waterproofing the terrain). In addition, the use of metals in paddle-well can also increase the impact in this category, even if to a slighter extent. One way to reduce this impact would be to change the paddle-well for another material, such as high-density polyethylene. Regarding the fossil depletion impact category, the impacts were mainly associated with the electricity required and construction material of each system. As for the climate change category, avoided energy is an important factor in mitigating the impact of fossil depletion, so in this scenario the lower impact of HRAPs was due to the higher biogas production compared to the other alternative. This was in accordance with previous studies that demonstrated the environmental and economic benefits of biogas recovery from sewage (AMARAL, K. G. C. DO; AISSE; POSSETTI, 2019; AMARAL, K. C. *et al.*, 2018).

It is important to note that the objective of this study was to compare technologies for the post treatment of UASB reactors, based on the Brazilian reality, so it was decided not to include conventional activated sludge systems as a comparison scenario. However, in order to place this technology within the evaluated scenarios, it was observed in a study that evaluated the same impact categories of this present work, that the activated sludge technology was the least ecofriendly among HRAP and constructed wetlands. In addition, in this same study, an economic assessment showed that the costs of implantation (capital), operation and maintenance of the conventional systems can be twice times more spent than HRAP and constructed wetlands (GARFÍ; FLORES; FERRER, 2017). Another study, for communities of 10,000 inhabitants, also showed that the conventional configuration is more expensive, impact and makes circular economy unfeasible, which does not minimize the impacts generated (ARASHIRO, LARISSA TERUMI *et al.*, 2018).

In conclusion, UASB reactor coupled with high rate algal ponds showed to be an environmentally friendly solution for sewage treatment in small communities in Brazil, especially if the propose of the plant is treat raw sewage and produce energy. Despite being more impactful in terms of eutrophication due to the deterioration of the final effluent because to the loss of microalgal biomass in settler, the system delivers treated sewage under Brazilian legislation. Based on this conclusion, it is strongly recommended that further studies will carried out on the separation of microalgal biomass from the final effluent, to minimize the impact on eutrophication and permit that most of the microalgal biomass can be harvest to produce more energy to be reverted to the nearby community. Furthermore, further studies should be carried out to evaluate the socio-economic benefits associated with this technology.

8.3.2 Normalization

The normalized results are summarized in Figure 8.3. Results show that Freshwater Eutrophication and Marine Eutrophication are the most significant impact categories for all the scenarios considered. For the Freshwater Eutrophication category, the constructed wetlands technology showed to be the most environmentally friendly alternative and the others performed similarly, which also occurs for the Marine Eutrophication category. It is important to note that in all scenarios the final effluent is in accordance with Brazilian legislation. As previously mentioned, algae-based systems to treat sewage require a better separation of the microalgal biomass by settler, which would lead to a better recovery of the biomass to be reused and an improvement in the quality of the final effluent. The results found in our study are in accordance with previous LCAs on sewage treatment (ARASHIRO, LARISSA TERUMI *et al.*, 2018; HOSPIDO, ALMUDENA *et al.*, 2004).

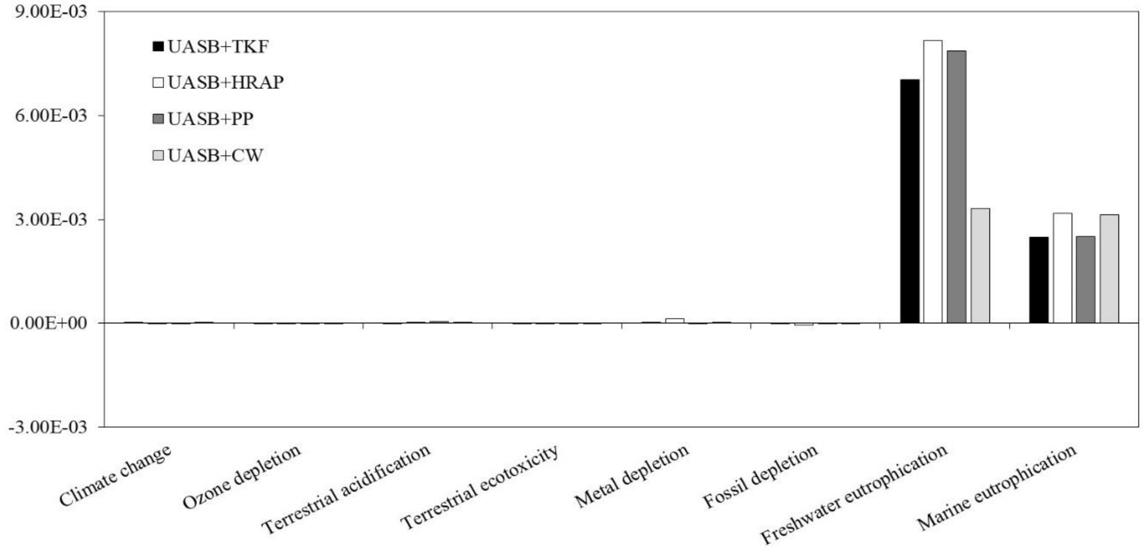


Figure 8.4. Normalized potential environmental impacts for the four scenarios: i) trickling filter (UASB + TKF), ii) high rate algal pond (UASB + HRAP), iii) polishing pond (UASB + PP) and iv) horizontal sub-surface flow constructed wetlands (UASB + CW)

8.3.3 Economic Assessment

The results of economic assessment are shown in Table 8.4. Regarding capital costs, the trickling filters scenario (UASB + TKF) was the least expensive among the 4 evaluated. The capital cost is associated with the area occupied by each technology and, consequently, with the need for construction material of the units. The ponds, due to the large area needed (3 m² p.e.$^{-1}$) and the greater use of construction material had a somewhat unfavourable scenario in relation to polish ponds (PP) and constructed wetlands (CW). Regarding the cost of operation and maintenance, the constructed wetland (CW) scenario was the best with the lowest cost. The simplicity of operation and less need for maintenance make this scenario the most attractive in operational terms. Due to greater automation and complexity of operation, the HRAP scenario proved to be the most expensive among the evaluated scenarios, however it is observed that the difference between the evaluated technologies was very slight. However, due to the greater potential for biogas production and consequently energy, the costs avoided with operation and maintenance (119%) were considerably higher in the HRAP scenario, which makes this technology more attractive when the objective of the plant, is treat sewage and produce energy from biogas. It is observed that this is a hypothetical scenario, since under Brazilian legislation, the exchange of electricity with the concessionaire is limited to what has been consumed, and the excess energy produced is not monetized, but generated as a credit with a validity of 5 years. In addition, it is known that the energy costs in a sewage treatment plant are around 12% of the total maintenance operation costs. For this scenario, energy form of heat was not considered. This energy could be used in the plant to clean the sludge or used by neighbours for cooking, which would have a favourable social impact on the HRAP scenario. Finally, it is important to note that this economic evaluation is based on values that can be more detailed to confirm the indicated results, therefore, a more in-depth economic study is recommended.

Table 8.4. Results of the economic assessment for UASB post-treatment options

Parameters	**Unit**	**UASB+TKF**	**UASB+HRAP**	**UASB+PP**	**UASB+CW**
Capital costs	€ p.e.$^{-1}$	72.47	130.44	115.95	86.96
Operation and maintenance	€ m$^{-3}_{water}$	0.09	0.10	0.07	0.06
Price of electricity sold back to the grid	€ m$^{-3}_{water}$	0.03	0.12	0.02	0.02
Expenditure avoided due energy production	%	32	119	25	29

8.4 Conclusion

This study aimed at comparing different systems for post-treating the effluent from UASB reactors treating sewage by means of the life cycle assessment tool. The chosen scenarios were based on technologies already used in Latin America, and were: i) UASB + Trickling Filter (TKF), ii) UASB + High Rate Algal Ponds (HRAP), iii) UASB + Polish Ponds (PP) and, iv) UASB +Constructed Wetlands (CW). The LCA evaluated eight different categories: Climate Change, Ozone Depletion, Terrestrial acidification, Freshwater Eutrophication, Marine Eutrophication, Metal Depletion, Terrestrial ecotoxicity and Fossil Depletion. The results showed that among the categories evaluated, UASB + HRAP system is the most environmental friendly, with the lowest environmental impacts of 4 from the 8 categories evaluated. However, it was observed that due to the difficulty in separating microalgal biomass, the system was penalized in the categories of eutrophication, even delivering effluent within the legislation. In addition, this study also indicated that pH control with CO_2 injection to reduce NH_3 emissions can minimize the impact and improve microalgae growth. Finally, it was observed that HRAP could be integrated in the sewage treatment system in a circular economy perspective, by recirculating harvested microalgal biomass into the UASB reactor to increase biogas production in the reactors, converting the system into a more sustainable process with a positive energy balance and economically attractive.

9 CONCLUSIONS

This PhD thesis evaluated five main aspects of UASB reactors followed by HRAPs system to treat sewage, namely: i) its performance regarding organic matter and nutrients removal for attaining discharge standard limits; ii) its ability to remove micropollutants; iii) its achievement for operating with anaerobic co-digestion of raw sewage with microalgal biomass on biogas production in UASB reactors; iv) its achievement for operating with anaerobic co-digestion of raw sewage with microlgal biomass after solar thermal pre-treatment on biogas production in UASB reactors and; v) its potential in terms of environmental sustainability compared to other UASB reactor post-treatment units using LCA. After this study period it can be concluded that:

i. The use of UASB followed by HRAP treatment system was efficient at treating sewage in terms of solids, organic matter and nutrients. The evaluated parameters were in accordance to Brazilian environmental standards for effluent discharge. Mathematical modelling using BIO_ALGAE 2 showed that HRAP in tropical conditions and post-treating UASB reactor effluent, can operate successfully at 4 days HRT. In comparison with operation at 8 days HRT, results showed a decrease of 100% in the area required.

ii. The combined system evaluated was efficient in removing micropollutants, as estrogens and pharmaceuticals. The UASB reactor showed a limited removal for all the targeted compounds, while only estrogen E2 was removed significantly (85%). This was due to the short operational HRTs. On the contrary, the HRAP was more efficient in removing estrogens (90%-95%) and PhACs (64%-70%), with the only exception for gemfibrozil (39%) and xenoestrogens (40-70%). Solar irradiance was detected as the probable key factor for removal in open ponds, enhancing direct photodegradation and bio adsorption and biodegradation from higher microalgae growth.

iii. Anaerobic co-digestion of raw sewage with microalgal biomass improved the methane yield by 35%compared to UASB reactor fed only with raw sewage. The system showed a positive energy balance throughout the year.

iv. Thermal solar pre-treatment of microalgal biomass increased organic matter solubilization in 32%. Consequently, methane yield was further increased, attaining 45% higher values compared to UASB reactor fed only with raw sewage. The system showed a positive energy balance, with 2-fold of energy produced compared to the energy consumed.

v. HRAP as UASB reactor pos-treatment technology was the most environmentally friendly, achieving the lowest environmental impacts in 4 of the 8 categories evaluated, compared to the other pos-treatment solutions evaluated (trickling filter, polish pond and constructed wetland). HRAP was mostly penalized due to the construction material (i.e. steel). Other material could be an alternative, as an underground unit with polypropylene waterproofing and paddles of high density polyethylene. If HRAP is conceived under a circular economy approach, it also appears as the most sustainable option in evaluated UASB-based STPs.

10 RECOMMENDATIONS FOR FUTURE RESEARCH

Important scientific information was achieved in this research, which could contribute to the implementation of HRAP as an efficient and sustainable pos-treatment for UASB-based STPs in Brazil and, generally, in developing countries with tropical climate conditions. However, this PhD also opened more gaps to be fulfilled in further investigation. Thus, as recommendations for future research, the following topics were enumerated:

i. Evaluation of other HRAP operational conditions, as microalgal biomass recirculation and paddle-wheel velocity, for optimization of microalgae sedimentability and production, understanding the outcome data by means of mathematical modelling tool.

ii. Optimization of the settler design to improve microalgae separation and concentration and to increase biomass recovery in the system. It is recommended to evaluate operational and hydraulic parameters, as well as to assess materials in order to reduce the adhesion of microalgae to the settler wall.

iii. Identification of micropollutants removal pathways in HRAPs and consolidation of decay routes and mass balance of these compounds in the system. Associate this investigation with molecular techniques quantification.

iv. Improvement in the solar tubes design to increase performance for biomass thermal treatment. For instance, avoiding biomass loss due to adhesion on the wall of the tubes. Evaluation of the economic and technical feasibility for scaling-up the solar thermal pre-treatment, in order to consolidate this technology as an option also for other organic waste anaerobically digested.

v. Development of environmental, social and economic LCA for UASB+HRAP system, also for other scales (medium and large) and considering by-product recovery in STP.

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SUPPLEMENTARY-MATERIAL

Section A – *Model Bio_Algae_2*

Table SA.1. Matrix of stoichiometric parameters that relates processes and components through stoichiometric coefficients

	S_{NH4}	S_{NH3}	S_{NO3}	S_{NO2}	S_{CO2}	S_{HCO3}	S_{CO3}	S_{PO4}	S_{O2}	S_H	S_{OH}	S_S	S_I	X_{ALG}	X_S	X_I	X_H	X_{AOB}	X_{NOB}
ρ_{1a}	$v_{1,1a}$				$v_{5,1a}$			$v_{8,1a}$	$v_{9,1a}$	$v_{10,1a}$				$v_{14,1a}$					
ρ_{1b}			$v_{3,1b}$		$v_{5,1b}$			$v_{8,1b}$	$v_{9,1b}$	$v_{10,1b}$				$v_{14,1b}$					
ρ_{2}	$v_{1,2}$				$v_{5,2}$			$v_{8,2}$	$v_{9,2}$	$v_{10,2}$				$v_{14,2}$					
ρ_{3}	$v_{1,3}$				$v_{5,3}$			$v_{8,3}$	$v_{9,3}$	$v_{10,3}$				$v_{14,3}$	$v_{15,3}$	$v_{16,3}$			
ρ_{4a}	$v_{1,4a}$				$v_{5,4a}$			$v_{8,4a}$	$v_{9,4a}$	$v_{10,4a}$		$v_{12,4a}$					$v_{17,4a}$		
ρ_{4b}			$v_{3,4b}$		$v_{5,4b}$			$v_{8,4b}$	$v_{9,4b}$	$v_{10,4b}$		$v_{12,4b}$					$v_{17,4b}$		
ρ_{5}				$v_{4,5}$	$v_{5,5}$			$v_{8,5}$		$v_{10,5}$		$v_{12,5}$					$v_{17,5}$		
ρ_{6}			$v_{3,6}$		$v_{5,6}$			$v_{8,6}$		$v_{10,6}$		$v_{12,6}$					$v_{17,6}$		
ρ_{7}	$v_{1,7}$				$v_{5,7}$			$v_{8,7}$	$v_{9,7}$	$v_{10,7}$							$v_{17,7}$		
ρ_{8}	$v_{1,8}$		$v_{3,8}$	$v_{4,8}$	$v_{5,8}$			$v_{8,8}$		$v_{10,8}$							$v_{17,8}$		
ρ_{9}															$v_{15,9}$	$v_{16,9}$	$v_{17,9}$		
ρ_{10}	$v_{1,10}$			$v_{4,10}$	$v_{5,10}$			$v_{8,10}$	$v_{9,10}$	$v_{10,10}$								$v_{18,10}$	
ρ_{11}			$v_{3,11}$	$v_{4,11}$	$v_{5,11}$			$v_{8,11}$	$v_{9,11}$	$v_{10,11}$									$v_{19,11}$
ρ_{12}	$v_{1,12}$				$v_{5,12}$			$v_{8,12}$	$v_{9,12}$	$v_{10,12}$						$v_{16,12}$		$v_{18,12}$	
ρ_{13}	$v_{1,13}$				$v_{5,13}$			$v_{8,13}$	$v_{9,13}$	$v_{10,13}$						$v_{16,13}$			$v_{19,13}$
ρ_{14a}															$v_{15,14a}$	$v_{16,14a}$		$v_{18,14a}$	
ρ_{14b}															$v_{15,14b}$	$v_{16,14b}$			$v_{19,14b}$
ρ_{15}	$v_{1,15}$				$v_{5,15}$			$v_{8,15}$		$v_{10,15}$		$v_{12,15}$	$v_{13,15}$		$v_{15,15}$				
ρ_{16}					$v_{5,16}$	$v_{6,16}$				$v_{10,16}$									
ρ_{17}						$v_{6,17}$	$v_{7,17}$			$v_{10,17}$									
ρ_{18}	$v_{1,18}$	$v_{2,18}$								$v_{10,18}$									
ρ_{19}										$v_{10,19}$	$v_{11,19}$								
ρ_{20}									$v_{9,20}$										
ρ_{21}					$v_{5,21}$														
ρ_{22}		$v_{2,22}$																	

Table SA.2. Mathematical description of the processes of the model (processes rates).

Processes	**Process rate [M L^{-3} T^{-1}]**
Microalgae (X_{ALG}) processes	
1a. Growth of X_{ALG} on S_{NH4}	$\rho_{1a} = \frac{\mu_{ALG} \cdot {I_{av}}^{n}}{{I_{k}}^{n} + {I_{av}}^{n}} \cdot \varphi(T_{ALG}) \cdot \eta_{PS}(S_{O2}) \cdot \varphi(pH_{ALG}) \cdot \frac{S_{CO2} + S_{HCO3}}{K_{C,ALG} + S_{CO2} + S_{HCO3} + \frac{{S_{CO2}}^{2}}{I_{CO2,ALG}}} \cdot \frac{S_{NH3} + S_{NH4}}{K_{N,ALG} + S_{NH3} + S_{NH4}} \cdot \frac{S_{PO4}}{K_{P,ALG} + S_{PO4}} \cdot X_{ALG}$
1b. Growth of X_{ALG} on S_{NO3}	$\rho_{1b} = \frac{\mu_{ALG} \cdot {I_{av}}^{n}}{{I_{k}}^{n} + {I_{av}}^{n}} \cdot \varphi(T_{ALG}) \cdot \eta_{PS}(S_{O2}) \cdot \varphi(pH_{ALG}) \cdot \frac{S_{CO2} + S_{HCO3}}{K_{C,ALG} + S_{CO2} + S_{HCO3} + \frac{{S_{CO2}}^{2}}{I_{CO2,ALG}}} \cdot \frac{S_{NO3}}{K_{N,ALG} + S_{NO3}} \cdot \frac{K_{N,ALG}}{K_{N,ALG} + S_{NH3} + S_{NH4}} \cdot \frac{S_{PO4}}{K_{P,ALG} + S_{PO4}} \cdot X_{ALG}$
2. Endogenous respiration of X_{ALG}	$\rho_{2} = k_{resp,ALG} \cdot \varphi(T_{ALG}) \cdot \varphi(pH_{ALG}) \cdot \frac{S_{O2}}{K_{O2,ALG} + S_{O2}} \cdot X_{ALG}$
3. Decay of X_{ALG}	$\rho_{3} = k_{death,ALG} \cdot \varphi(T_{ALG}) \cdot \varphi(pH_{ALG}) \cdot X_{ALG}$
Heterotrophic bacteria (X_{H}) (aerobic and denitrifying activity)	
4a. Aerobic growth of X_{H} on S_{NH4}	$\rho_{4a} = \mu_{H} \cdot f_{T,MB}(T) \cdot \varphi(pH_{H}) \cdot \frac{S_{S}}{K_{S,H} + S_{s}} \cdot \frac{S_{O2}}{K_{O2,H} + S_{O2}} \cdot \frac{S_{NH4} + S_{NH3}}{K_{N,H} + S_{NH4} + S_{NH3}} \cdot \frac{S_{PO4}}{K_{P,H} + S_{PO4}} \cdot X_{H}$
4b. Aerobic growth of X_{H} on S_{NO3}	$\rho_{4b} = \mu_{H} \cdot f_{T,MB}(T) \cdot \varphi(pH_{H}) \cdot \frac{S_{S}}{K_{S,H} + S_{s}} \cdot \frac{S_{O2}}{K_{O2,H} + S_{O2}} \cdot \frac{S_{NO3}}{K_{N,H} + S_{NO3}} \cdot \frac{S_{PO4}}{K_{P,H} + S_{PO4}} \cdot X_{H}$
5. Anoxic growth of X_{H} on S_{NO2} (denitrification on S_{NO2})	$\rho_{5} = \mu_{H} \cdot \eta_{H} \cdot f_{T,MB}(T) \cdot \varphi(pH_{H}) \cdot \frac{S_{S}}{K_{S,H} + S_{s}} \cdot \frac{K_{O2,H}}{K_{O2,H} + S_{O2}} \cdot \frac{S_{NO2}}{K_{NO2,H,anox} + S_{NO2}} \cdot \frac{S_{PO4}}{K_{P,H} + S_{PO4}} \cdot X_{H}$
6. Anoxic growth of X_{H} on S_{NO3} (denitrification on S_{NO3})	$\rho_{6} = \mu_{H} \cdot \eta_{H} \cdot f_{T,MB}(T) \cdot \varphi(pH_{H}) \cdot \frac{S_{S}}{K_{S,H} + S_{s}} \cdot \frac{K_{O2,H}}{K_{O2,H} + S_{O2}} \cdot \frac{S_{NO3}}{K_{NO3,H,anox} + S_{NO3}} \cdot \frac{S_{PO4}}{K_{P,H} + S_{PO4}} \cdot X_{H}$
7. Aerobic endogenous respiration of X_{H}	$\rho_{7} = k_{resp,H} \cdot f_{T,MB}(T) \cdot \varphi(pH_{H}) \cdot \frac{S_{O2}}{K_{O2,H} + S_{O2}} \cdot X_{H}$
8. Anoxic endogenous respiration of X_{H}	$\rho_{8} = k_{resp,H} \cdot \eta_{H} \cdot f_{T,MB}(T) \cdot \varphi(pH_{H}) \cdot \frac{K_{O2,H}}{K_{O2,H} + S_{O2}} \cdot \frac{S_{NO3} + S_{NO2}}{K_{NO3,H,anox} + S_{NO2} + S_{NO3}} \cdot X_{H}$
9. Decay of X_{H}	$\rho_{9} = k_{death,H} \cdot f_{T,MB}(T) \cdot \varphi(pH_{H}) \cdot X_{H}$
Autotrophic bacteria (nitrifying activity)	
10. Growth of X_{AOB}	$\rho_{10} = \mu_{AOB} \cdot \varphi(T_{N}) \cdot \varphi(pH_{N}) \cdot \frac{S_{O2}}{K_{O2,AOB} + S_{O2}} \cdot \frac{S_{NH3} + S_{NH4}}{K_{NH4,AOB} + S_{NH4} + S_{NH3}} \cdot \frac{S_{CO2} + S_{HCO3}}{K_{C,AOB} + S_{CO2} + S_{HCO3}} \cdot \frac{S_{PO4}}{K_{P,AOB} + S_{PO4}} \cdot X_{AOB}$
11. Growth of X_{NOB}	$\rho_{11} = \mu_{NOB} \cdot \varphi(T_{N}) \cdot \varphi(pH_{N}) \cdot \frac{S_{O2}}{K_{O2,NOB} + S_{O2}} \cdot \frac{K_{I,NH4}}{K_{I,NH4} + S_{NH4} + S_{NH3}} \cdot \frac{S_{NO2}}{K_{NO2,NOB} + S_{NO2}} \cdot \frac{S_{CO2} + S_{HCO3}}{K_{C,NOB} + S_{CO2} + S_{HCO3}} \cdot \frac{S_{PO4}}{K_{P,NOB} + S_{PO4}} \cdot X_{NOB}$
12. Endogenous respiration of X_{AOB}	$\rho_{10} = k_{resp,AOB} \cdot \varphi(T_{N}) \cdot \varphi(pH_{N}) \cdot \frac{S_{O2}}{K_{O2,AOB} + S_{O2}} \cdot X_{AOB}$
13. Endogenous respiration of X_{NOB}	$\rho_{13} = k_{resp,NOB} \cdot \varphi(T_{N}) \cdot \varphi(pH_{N}) \cdot \frac{S_{O2}}{K_{O2,NOB} + S_{O2}} \cdot X_{NOB}$
14a. Decay of X_{AOB}	$\rho_{14a} = k_{death,AOB} \cdot \varphi(T_{N}) \cdot \varphi(pH_{N}) \cdot X_{AOB}$

14b. Decay of X_{NOB}	$\rho_{14b} = k_{death,NOB} \cdot f_{T,MB}(T) \cdot \varphi(pH_N) \cdot X_{NOB}$
Hydrolysis, Chemical equilibrium and Transfer of gases	
15. Hydrolysis	$\rho_{15} = k_{HYD} \cdot \frac{X_S/X_H}{K_{HYD} + (X_S/X_H)} \cdot X_H$
16. Chemical equilibrium $CO_2 \leftrightarrow HCO_3^-$	$\rho_{16} = k_{eq,1} \cdot (S_{CO2} - S_H S_{HCO3}/K_{eq,1})$
17. Chemical equilibrium $HCO_3^- \leftrightarrow CO_3^{2-}$	$\rho_{17} = k_{eq,2} \cdot (S_{HCO3} - S_H S_{CO3}/K_{eq,2})$
18. Chemical equilibrium $NH_4^+ \leftrightarrow NH_3$	$\rho_{18} = k_{eq,3} \cdot (S_{NH4} - S_H S_{NH3}/K_{eq,3})$
19. Chemical equilibrium $H^+ \leftrightarrow OH^-$	$\rho_{19} = k_{eq,w} \cdot (1 - S_H S_{OH}/K_{eq,w})$
20. S_{O2} transfer to the atmosphere	$\rho_{20} = K_{lal,O2} \cdot \left(S_{O2}^{WAT} - S_{O2}\right)$
21. S_{CO2} transfer to the atmosphere	$\rho_{21} = K_{lal,CO2} \cdot \left(S_{CO2}^{WAT} - S_{CO2}\right)$
22. S_{NH3} transfer to the atmosphere	$\rho_{22} = K_{lal,NH3} \cdot (-S_{NH3})$

Table SA.3. Values of biokinetic, chemical and physic parameters.

Parameters	**Description**	**Value**	**Unit**	**Reference**
Microalgae (X_{ALG})				
μ_{ALG}	Maximum growth rate of X_{ALG}	1.6	d^{-1}	CALIBRATED
$k_{resp,ALG}$	Endogenous respiration constant	0.05	d^{-1}	BIO_ALGAE
$k_{death,ALG}$	Decay constant	0.15	d^{-1}	CALIBRATED
$K_{C,ALG}$	Saturation constant of X_{ALG} on S_{CO2}	4E-3	$gC\ m^{-3}$	Novak and Brune, 1985
$I_{CO2,ALG}$	Inhibition constant of X_{ALG} on S_{CO2}	120	$gC\ m^{-3}$	Silva and Pirt, 1984
$K_{N,ALG}$	Saturation constant of X_{ALG} on nitrogen	0.01	$gN\ m^{-3}$	CALIBRATED
$K_{O2,ALG}$	Saturation constant of X_{ALG} on S_{O2}	0.2	$gO_2\ m^{-3}$	Reichert et al., 2001
$K_{P,ALG}$	Saturation constant of X_{ALG} for S_{HPO4}	0.02	$gP\ m^{-3}$	CALIBRATED
Heterotrophic bacteria (X_H)				
μ_H	Maximum growth rate of X_H	1.0	d^{-1}	CALIBRATED
η_H	Anoxic reduction factor for X_H	0.6	–	Gujer et al., 1999
$k_{resp,H}$	Endogenous respiration rate of X_H	0.3	d^{-1}	Reichert et al., 2001
$K_{O2,H}$	Saturation constant of X_H for S_{O2}	0.2	$gO_2\ m^{-3}$	Reichert et al., 2001
$K_{N,H}$	Saturation constant of X_H for S_N	0.2	$gN\ m^{-3}$	Reichert et al., 2001
$K_{S,H}$	Saturation constant of X_H for S_S	20	$gCOD\ m^{-3}$	Henze et al., 2000
$K_{NO3,H,anox}$	Saturation constant of X_H for S_{NO3}	0.5	$gN\ m^{-3}$	Reichert et al., 2001
$K_{NO2,H,anox}$	Saturation constant of X_H for S_{NO2}	0.2	$gN\ m^{-3}$	Reichert et al., 2001
$k_{death,H}$	Decay constant of X_H	0.8	d^{-1}	CALIBRATED
Autotrophic bacteria: ammonia oxidizing bacteria (X_{AOB}) and nitrite oxidizing bacteria (X_{NOB})				
μ_{AOB}	Maximum growth rate of X_{AOB}	0.63	d^{-1}	Gujer et al., 1999
μ_{NOB}	Maximum growth rate of X_{NOB}	1.1	d^{-1}	Gujer et al., 1999
$K_{O2,AOB}/K_{O2,NOB}$	Saturation constant of X_{AOB} / X_{NOB} for S_{O2}	0.5	$gO_2\ m^{-3}$	Reichert et al., 2001
$K_{NH4,AOB}$	Saturation constant of X_{AOB} on S_{NH4}	0.5	$gN\ m^{-3}$	Reichert et al., 2001
$K_{I,NH4}$	Ammonia inhibition constant of X_{NOB}	5.0	$gN\ m^{-3}$	Henze et al., 2000
$K_{NO2,NOB}$	Saturation constant of X_{NOB} for S_{NO2}	0.5	$gN\ m^{-3}$	Henze et al., 2000
$K_{C,AOB}/K_{C,NOB}$	Saturation constant of X_{AOB} / X_{NOB} for S_{HCO3}	0.5	$gC\ m^{-3}$	Henze et al., 2000
$k_{resp,AOB}/k_{resp,NOB}$	Endogenous respiration rate of X_{AOB} /X_{NOB}	0.05	d^{-1}	Reichert et al., 2001
$k_{death,AOB}/k_{death,NOB}$	Decay constant of X_{AOB} and X_{NOB}	0.2	d^{-1}	Henze et al., 2000
Hydrolysis				
k_{HYD}	Hydrolysis rate constant	3.0	d^{-1}	Reichert et al., 2001
Photorespiration factor of microalgae				
K_{PR}	Inhibition constant of photorespiration	0.03	–	Solimeno et al., 2018
τ	Excess of S_{O2} coefficient	3.5	–	Fernández et al., 2014
S_{O2}^{SAT}	S_{O2} air saturation	9.07	$gO_2\ m^{-3}$	Fernández et al., 2014
Light factor of microalgae				
α	Activation rate	1.9E-3	$(\mu E\ m^{-2})^{-1}$	Wu and Merchuk, 2001
β	Inhibition rate	5.7E-7	$(\mu E\ m^{-2})^{-1}$	Wu and Merchuk, 2001
γ	Production rate	0.14	s^{-1}	Wu and Merchuk, 2001
δ	Recovery rate	4.7E-4	s^{-1}	Wu and Merchuk, 2001
K_I	Biomass extinction coefficient	0.07	$m^2\ g^{-1}$	Molina et al., 1994

pH cardinal factor				
$pH_{ALG,max}$	Maximum pH value for X_{ALG}	12.3	–	Experimental
$pH_{ALG,min}$	Minimum pH value for X_{ALG}	4	–	Experimental
$pH_{ALG,opt}$	Optimum pH value for X_{ALG}	8.8	–	Experimental
$pH_{H,max}$	Maximum pH value for X_H	11.2	–	Experimental
$pH_{H,min}$	Minimum pH value for X_H	2	–	Experimental
$pH_{H,opt}$	Optimum pH value for X_H	8.2	–	Experimental
$pH_{N,max}$	Maximum pH value for X_{AOB} and X_{NOB}	11	–	Experimental
$pH_{N,min}$	Minimum pH value for X_{AOB} and X_{NOB}	2	–	Experimental
$pH_{N,opt}$	Optimum pH value for X_{AOB} and X_{NOB}	8.5	–	Experimental
Temperature cardinal factor				
$T_{ALG,max}$	Maximum temperature value for X_{ALG}	46	–	Experimental
$T_{ALG,min}$	Minimum temperature value for X_{ALG}	7	–	Experimental
$T_{ALG,opt}$	Optimum temperature value for X_{ALG}	26	–	Experimental
$T_{N,max}$	Maximum temperature value for X_{AOB} and X_{NOB}	40	–	Experimental
$T_{N,min}$	Minimum temperature value for X_{AOB} and X_{NOB}	13	–	Experimental
$T_{N,opt}$	Optimum temperature value for X_{AOB} and X_{NOB}	31	–	Experimental
Heterotrophic bacteria thermal factor				
$T_{H,opt}$	Optimum temperature value for X_H	20	°C	Reichert et al., 2001
θ	Temperature coefficient for XH	1.07		Von Sperling, 2005

Parameters	**Equations**
Chemical equilibrium $CO_2 \leftrightarrow HCO_3^-.$	$K_{eq,1} = 10^{17.843-\frac{3404.71}{273.15+T}-0.032786(273.15+T)}$
Chemical equilibrium $HCO_3^- \leftrightarrow CO_3^{2-}$	$K_{eq,2} = 10^{9.494-\frac{2902.39}{273.15+T}-0.02379(273.15+T)}$
Chemical equilibrium $NH_4^+ \leftrightarrow NH_3$	$K_{eq,3} = 10^{2.891-\frac{2727}{(273.15+T)}}$
Chemical equilibrium $H^+ \leftrightarrow OH^-$	$K_{eq,w} = 10^{-\frac{4470.99}{273.15+T}+12.0875-0.01706(273.15+T)}$

Kinetics parameters				
$k_{eq,1}$	Dissociation constant of $CO_2 \leftrightarrow HCO_3^-.$	10000	d^{-1}	Reichert et al., 2001
$k_{eq,2}$	Dissociation constant of $HCO_3^- \leftrightarrow CO_3^{2-}$	1000	d^{-1}	Reichert et al., 2001
$k_{eq,3}$	Dissociation constant of $NH_4^+ \leftrightarrow NH_3$	1000	d^{-1}	Reichert et al., 2001
$k_{eq,w}$	Dissociation constant of $H^+ \leftrightarrow OH^-$	1000	$g\ m^{-1}\ d^{-1}$	Reichert et al., 2001
Transfer of gases to the atmosphere				
$K_{lal,O2}$	Mass transfer coefficient for S_{O2}	1.45	h^{-1}	Calibrated
$K_{lal,CO2}$	Mass transfer coefficient for S_{CO2}	0.8	h^{-1}	Calibrated
$K_{lal,NH3}$	Mass transfer coefficient for S_{NH3}	0.3	h^{-1}	Calibrated

Table SA.4 Values of fractions of carbon, hydrogen, oxygen and nitrogen in microalgae and bacteria biomass.

Parameters	Description	Value	Unit	Source
Fractions of microalgal biomass (X_{ALG})				
$i_{C,ALG}$	Fraction of carbon in microalgae	0.387	$gC\ gCOD^{-1}$	Reichert et al., 2001
$i_{H,ALG}$	Fraction of hydrogen in microalgae	0.075	$gH\ gCOD^{-1}$	Reichert et al., 2001
$i_{O,ALG}$	Fraction of oxygen in microalgae	0.269	$gO_2\ gCOD^{-1}$	Reichert et al., 2001
$i_{N,ALG}$	Fraction of nitrogen in microalgae	0.065	$gN\ gCOD^{-1}$	Reichert et al., 2001
$i_{P,ALG}$	Fraction of phosphorus in microalgae	0.01	$gP\ gCOD^{-1}$	Reichert et al., 2001
Fractions of bacteria biomass (X_H, X_{AOB}, X_{NOB})				
$i_{C,BM}$	Fraction of carbon in bacteria	0.323	$gC\ gCOD^{-1}$	Reichert et al., 2001
$i_{H,BM}$	Fraction of hydrogen in bacteria	0.060	$gH\ gCOD^{-1}$	Reichert et al., 2001
$i_{O,BM}$	Fraction of oxygen in bacteria	0.077	$gO_2\ gCOD^{-1}$	Reichert et al., 2001
$i_{N,BM}$	Fraction of nitrogen in bacteria	0.075	$gN\ gCOD^{-1}$	Reichert et al., 2001
$i_{P,BM}$	Fraction of phosphorus in bacteria	0.018	$gP\ gCOD^{-1}$	Reichert et al., 2001
Fractions of slowly biodegradable substrates (X_S)				
$i_{C,XS}$	Fraction of carbon in X_S	0.318	$gC\ gCOD^{-1}$	Reichert et al., 2001
$i_{H,XS}$	Fraction of hydrogen in X_S	0.045	$gH\ gCOD^{-1}$	Reichert et al., 2001
$i_{O,XS}$	Fraction of oxygen in X_S	0.077	$gO_2\ gCOD^{-1}$	Reichert et al., 2001
$i_{N,XS}$	Fraction of nitrogen in X_S	0.034	$gN\ gCOD^{-1}$	Reichert et al., 2001
$i_{P,XS}$	Fraction of phosphorus in X_S	0.005	$gP\ gCOD^{-1}$	Reichert et al., 2001
Fractions of inert particulate organics (X_I)				
$i_{C,XI}$	Fraction of carbon in X_I	0.327	$gC\ gCOD^{-1}$	Reichert et al., 2001
$i_{H,XI}$	Fraction of hydrogen in X_I	0.037	$gH\ gCOD^{-1}$	Reichert et al., 2001
$i_{O,XI}$	Fraction of oxygen in X_I	0.075	$gO_2\ gCOD^{-1}$	Reichert et al., 2001
$i_{N,XI}$	Fraction of nitrogen in X_I	0.016	$gN\ gCOD^{-1}$	Reichert et al., 2001
$i_{P,XI}$	Fraction of phosphorus in X_I	0.005	$gP\ gCOD^{-1}$	Reichert et al., 2001
Fractions of readily biodegradable substrates (S_S)				
$i_{C,SS}$	Fraction of carbon in S_S	0.318	$gC\ gCOD^{-1}$	Reichert et al., 2001
$i_{H,SS}$	Fraction of hydrogen in S_S	0.045	$gH\ gCOD^{-1}$	Reichert et al., 2001
$i_{O,SS}$	Fraction of oxygen in S_S	0.078	$gO_2\ gCOD^{-1}$	Reichert et al., 2001
$i_{N,SS}$	Fraction of nitrogen in S_S	0.034	$gN\ gCOD^{-1}$	Reichert et al., 2001
$i_{P,SS}$	Fraction of phosphorus in S_S	0.005	$gP\ gCOD^{-1}$	Reichert et al., 2001
Fractions of soluble inert organics (S_I)				
$i_{C,SI}$	Fraction of carbon in S_I	0.327	$gC\ gCOD^{-1}$	Reichert et al., 2001
$i_{H,SI}$	Fraction of hydrogen in S_I	0.037	$gH\ gCOD^{-1}$	Reichert et al., 2001
$i_{O,SI}$	Fraction of oxygen in S_I	0.075	$gO_2\ gCOD^{-1}$	Reichert et al., 2001
$i_{N,SI}$	Fraction of nitrogen in S_I	0.016	$gN\ gCOD^{-1}$	Reichert et al., 2001
$i_{P,SI}$	Fraction of phosphorus in S_I	0.005	$gP\ gCOD^{-1}$	Reichert et al., 2001
Fractions of inert produced by biomass degradation				
f_{ALG}	Production of X_I in endogenous resp. of X_{ALG}	0.1	$gCOD\ gCOD^{-1}$	Sah et al., 2011
f_{XI}	Production of X_I in endogenous resp. of X_H	0.1	$gCOD\ gCOD^{-1}$	Sah et al., 2011
Yield of biomass				
Y_{ALG}	Yield of X_{ALG}	0.62	$gCOD\ gCOD^{-1}$	Reichert et al., 2001
Y_H	Yield of X_H on S_{O2}	0.6	$gCOD\ gCOD^{-1}$	Reichert et al., 2001
$Y_{H,NO3}$	Yield of X_H on S_{NO3}	0.5	$gCOD\ gCOD^{-1}$	Reichert et al., 2001

$Y_{H,NO2}$	Yield of X_H on S_{NO2}	0.3	gCOD gCOD^{-1}	Reichert et al., 2001
Y_{AOB}	Yield of X_{AOB}	0.13	gCOD gCOD^{-1}	Reichert et al., 2001
Y_{NOB}	Yield of X_{NOB}	0.03	gCOD gCOD^{-1}	Reichert et al., 2001
K_{HYD}	Hydrolysis saturation constant	1	gCOD gCOD^{-1}	Reichert et al., 2001

Table SA.5. Mathematical expressions of the stoichiometric coefficients of each process.

Stoichiometric coefficients	**Unit**
Growth of X_{ALG} on S_{NH4}	
$v_{1,1a} = -i_{N,ALG}$	gN gCOD^{-1}
$v_{5,1a} = -i_{C,ALG}$	gC gCOD^{-1}
$v_{8,1a} = -i_{P,ALG}$	gP gCOD^{-1}
$v_{9,1a} = (8i_{C,ALG}/3 + 8i_{H,ALG} - i_{O,ALG} - 12i_{N,ALG}/7 + 40i_{P,ALG}/31)/2$	gO_2 gCOD^{-1}
$v_{10,1a} = i_{N,ALG}/14 - 2i_{P,ALG}/31$	gH gCOD^{-1}
$v_{14,1a} = 1$	gCOD gCOD^{-1}
Growth of X_{ALG} on S_{NO3}	
$v_{3,1b} = -i_{N,ALG}$	gN gCOD^{-1}
$v_{5,1b} = -i_{C,ALG}$	gC gCOD^{-1}
$v_{8,1b} = -i_{P,ALG}$	gP gCOD^{-1}
$v_{9,1b} = (8i_{C,ALG}/3 + 8i_{H,ALG} - i_{O,ALG} + 20i_{N,ALG}/7 + 40i_{P,ALG}/31)/2$	gO_2 gCOD^{-1}
$v_{10,1b} = -\ i_{N,ALG}/14 - 2i_{P,ALG}/31$	gH gCOD^{-1}
$v_{14,1b} = 1$	gCOD gCOD^{-1}
Endogenous respiration of X_{ALG}	
$v_{1,2} = i_{N,ALG} - f_{ALG}\ i_{N,XI}$	gN gCOD^{-1}
$v_{5,2} = i_{C,ALG} - f_{ALG}\ i_{C,XI}$	gC gCOD^{-1}
$v_{8,2} = i_{P,ALG} - f_{ALG}\ i_{P,XI}$	gP gCOD^{-1}
$v_{9,2} = ((i_{O,ALG} - f_{ALG}\ i_{O,XI}) - 8(i_{H,ALG} - f_{ALG}\ i_{H,XI}) - 8/3\,(i_{C,ALG} - f_{ALG}\ i_{C,XI})$ $+ 12/7\,(i_{N,ALG} - f_{ALG}\ i_{N,XI}) - 40/31\,(i_{P,ALG} - f_{ALG}\ i_{P,XI}))/2$	gO_2 gCOD^{-1}
$v_{10,2} = -\ 1/14\,(i_{N,ALG} - f_{ALG}\ i_{N,XI}) + 2/31\,(i_{P,ALG} - f_{ALG}\ i_{P,XI})$	gH gCOD^{-1}
$v_{14,2} = -1$	gCOD gCOD^{-1}
$v_{16,2} = f_{ALG}$	gCOD gCOD^{-1}
Decay of X_{ALG}	
$v_{1,3} = i_{N,ALG} - (1 - f_{ALG})Y_{ALG}\ i_{N,XS} - f_{ALG}Y_{ALG}\ i_{N,ALG}$	gN gCOD^{-1}
$v_{5,3} = i_{C,ALG} - (1 - f_{ALG})Y_{ALG}\ i_{C,XS} - f_{ALG}Y_{ALG}\ i_{C,ALG}$	gC gCOD^{-1}
$v_{8,3} = i_{P,ALG} - (1 - f_{ALG})Y_{ALG}\ i_{P,XS} - f_{ALG}Y_{ALG}\ i_{P,ALG}$	gP gCOD^{-1}
$v_{9,3} = -\ ((i_{O,ALG} - f_{ALG}\ i_{O,XI}) - 8(i_{H,ALG} - f_{ALG}\ i_{H,XI}) - 8/3\,(i_{C,ALG} - f_{ALG}\ i_{C,XI})$ $+ 12/7\,(i_{N,ALG} - f_{ALG}\ i_{N,XI}) - 40/31\,(i_{P,ALG} - f_{ALG}\ i_{P,XI}))/2$	gO_2 gCOD^{-1}
$v_{10,3} = -\ 1/14\,(i_{N,ALG}\,(1 - f_{ALG})Y_{ALG}\ i_{N,XS} - f_{ALG}Y_{ALG}\ i_{N,XI})$ $+ 2/31\,(i_{P,ALG}\,(1 - f_{ALG})Y_{ALG}\ i_{P,XS} - f_{ALG}Y_{ALG}\ i_{P,XI})$	gH gCOD^{-1}
$v_{14,3} = -1$	gCOD gCOD^{-1}
$v_{15,3} = (1 - f_{ALG})Y_{ALG}$	gCOD gCOD^{-1}
$v_{16,3} = f_{ALG}Y_{ALG}$	gCOD gCOD^{-1}
Aerobic growth of X_H on S_{NH4}	
$v_{1,4a} = i_{N,SS}/Y_H - i_{N,BM}$	gN gCOD^{-1}
$v_{5,4a} = i_{C,SS}/Y_H - i_{C,BM}$	gC gCOD^{-1}
$v_{8,4a} = i_{P,SS}/Y_H - i_{P,BM}$	gP gCOD^{-1}
$v_{9,4a} = -((1 - Y_H)/\ Y_H)/2$	gO_2 gCOD^{-1}
$v_{10,4a} = -\ 1/14\,(i_{N,SS}/Y_H - i_{N,BM}) + 2/31\,(i_{P,SS}/Y_H - i_{P,BM})$	gH gCOD^{-1}
$v_{12,4a} = -1/Y_H$	gCOD gCOD^{-1}
$v_{17,4a} = 1$	gCOD gCOD^{-1}

Aerobic growth of X_H on S_{NO3}	
$v_{3,4b} = i_{N,SS}/Y_H - i_{N,BM}$	gN gCOD^{-1}
$v_{5,4b} = i_{C,SS}/Y_H - i_{C,BM}$	gC gCOD^{-1}
$v_{8,4b} = \left(i_{P,SS}/Y_H - i_{P,BM}\right)$	gP gCOD^{-1}
$v_{9,4b} = -((1 - Y_H)/\ Y_H)/2$	gO$_2$ gCOD^{-1}
$v_{10,4b} = -\,1/14\left(i_{N,SS}/Y_H - i_{N,BM}\right) + 2/31\left(i_{P,SS}/Y_H - i_{P,BM}\right)$	gH gCOD^{-1}
$v_{12,4b} = -\,1/Y_H$	gCOD gCOD^{-1}
$v_{17,4b} = 1$	gCOD gCOD^{-1}
Anoxic growth of X_H on S_{NO2}	
$v_{4,5} = -(1 - Y_{H,NO2})/(1.71Y_{H,NO2})$	gN gCOD^{-1}
$v_{5,5} = \left(i_{C,SS}/Y_{H,NO2} - i_{C,BM}\right)$	gC gCOD^{-1}
$v_{8,5} = \left(i_{P,SS}/Y_{H,NO2} - i_{P,BM}\right)$	gP gCOD^{-1}
$v_{10,5} = 1/24\left(i_{O,SS}/Y_{H,NO2} - i_{O,BM}\right) - 1/3\left(i_{H,SS}/Y_{H,NO2} - i_{H,BM}\right)$ $\quad - 1/9\left(i_{C,SS}/Y_{H,NO2} - i_{C,BM}\right) + 1/93\left(i_{P,SS}/Y_{H,NO2} - i_{P,BM}\right)$	gH gCOD^{-1}
$v_{12,5} = -\,1/Y_{H,NO2}$	gCOD gCOD^{-1}
$v_{17,5} = 1$	gCOD gCOD^{-1}
Anoxic growth of X_H on S_{NO3}	
$v_{3,6} = -(1 - Y_{H,NO3})/(1.14Y_{H,NO3})$	gN gCOD^{-1}
$v_{4,6} = (1 - Y_{H,NO3})/(1.14Y_{H,NO3})$	gN gCOD^{-1}
$v_{5,6} = \left(i_{C,SS}/Y_{H,,NO3} - i_{C,BM}\right)$	gC gCOD^{-1}
$v_{8,6} = \left(i_{P,SS}/Y_{H,NO3} - i_{P,BM}\right)$	gP gCOD^{-1}
$v_{10,6} = 1/14\left(i_{N,SS}/Y_{H,NO3} - i_{N,BM}\right) + 2/31\left(i_{P,SS}/Y_{H,NO3} - i_{P,BM}\right)$	gH gCOD^{-1}
$v_{12,6} = -\,1/Y_{H,NO3}$	gCOD gCOD^{-1}
$v_{17,6} = 1$	gCOD gCOD^{-1}
Aerobic endogenous respiration of X_H	
$v_{1,7} = i_{N,BM} - f_{XI}\ i_{N,XI}$	gN gCOD^{-1}
$v_{5,7} = i_{C,BM} - f_{X1}\ i_{C,XI}$	gC gCOD^{-1}
$v_{8,7} = i_{P,BM} - f_{X1}\ i_{P,XI}$	gP gCOD^{-1}
$v_{9,7} = -(1 - f_{X1})/2$	gO$_2$ gCOD^{-1}
$v_{10,7} = -\,1/14\left(i_{N,BM} - f_{XI}\ i_{N,XI}\right) + 2/31\left(i_{P,BM} - f_{XI}\ i_{P,XI}\right)$	gH gCOD^{-1}
$v_{16,7} = f_{XI}$	gCOD gCOD^{-1}
$v_{17,7} = -1$	gCOD gCOD^{-1}
Anoxic endogenous respiration of X_H	
$v_{1,8} = i_{N,BM} - f_{XI}\ i_{N,XI}$	gN gCOD^{-1}
$v_{3,8} = (f_{XI} - 1)/1.14$	gN gCOD^{-1}
$v_{4,8} = (1 - f_{XI})/1.14$	gN gCOD^{-1}
$v_{5,8} = i_{C,BM} - f_{XI}i_{C,XI}$	gC gCOD^{-1}
$v_{8,8} = i_{P,BM} - f_{XI}i_{P,XI}$	gP gCOD^{-1}
$v_{10,8} = 1/40\left(i_{O,BM} - f_{XI}i_{O,XI}\right) - 1/5\left(i_{H,BM} - f_{XI}i_{H,XI}\right) - 1/15\left(i_{C,BM} - f_{XI}i_{C,XI}\right)$ $\quad + 1/35\left(i_{N,BM} - f_{XI}i_{N,XI}\right)\ - 1/31\left(i_{P,BM} - f_{XI}i_{P,XI}\right)$	gH gCOD^{-1}
$v_{16,8} = f_{XI}$	gCOD gCOD^{-1}
$v_{17,8} = -1$	gCOD gCOD^{-1}
Decay of X_H	
$v_{15,9} = (1 - f_{XI})c$	gCOD gCOD^{-1}
$v_{16,9} = f_{XI}Y_H$	gCOD gCOD^{-1}
$v_{17,9} = -1$	gCOD gCOD^{-1}

Growth of ammonia oxidizing bacteria (X_{AOB})	
$v_{1,10} = -1/Y_{AOB} - i_{N,BM}$	gN gCOD^{-1}
$v_{4,10} = 1/Y_{AOB} - i_{N,BM}$	gN gCOD^{-1}
$v_{5,10} = -i_{C,BM}$	gC gCOD^{-1}
$v_{8,10} = -i_{P,BM}$	gP gCOD^{-1}
$v_{9,10} = (1 - 3.43/Y_{AOB})/2$	gO$_2$ gCOD^{-1}
$v_{10,10} = 2/14Y_{AOB} - 1/14\left(i_{N,BM}\right) - 2/31\left(i_{P,BM}\right)$	gH gCOD^{-1}
$v_{18,10} = 1$	gCOD gCOD^{-1}
Growth of nitrite oxidizing bacteria (X_{NOB})	
$v_{3,11} = 1/Y_{NOB} - i_{N,BM}$	gN gCOD^{-1}
$v_{4,11} = -1/Y_{NOB}$	gN gCOD-1
$v_{5,11} = -i_{C,BM}$	gC gCOD^{-1}
$v_{8,10} = -i_{P,BM}$	gP gCOD^{-1}
$v_{9,11} = (1 - 1.14/Y_{NOB})/2$	gO$_2$ gCOD^{-1}
$v_{10,11} = -1/14\left(i_{N,BM}\right) - 2/31\left(i_{P,BM}\right)$	gH gCOD^{-1}
$v_{19,11} = 1$	gCOD gCOD^{-1}
Endogenous respiration of X_{AOB}	
$v_{1,12} = i_{N,BM} - f_{XI}\, i_{N,XI}$	gN gCOD^{-1}
$v_{5,12} = i_{C,BM} - f_{XI} i_{C,XI}$	gC gCOD^{-1}
$v_{8,12} = i_{P,BM} - f_{XI} i_{P,XI}$	gP gCOD^{-1}
$v_{9,12} = -(1 - f_{XI})/2$	gO$_2$ gCOD^{-1}
$v_{10,12} = -1/14\left(i_{N,BM} - f_{XI}\, i_{N,XI}\right) + 2/31\left(i_{P,BM} - f_{XI}\, i_{P,XI}\right)$	gH gCOD^{-1}
$v_{16,12} = f_{XI}$	gCOD gCOD^{-1}
$v_{18,12} = -1$	gCOD gCOD^{-1}
Endogenous respiration of X_{NOB}	
$v_{1,13} = i_{N,BM} - f_{XI}\, i_{N,XI}$	gN gCOD^{-1}
$v_{5,13} = i_{C,BM} - f_{XI} i_{C,XI}$	gC gCOD^{-1}
$v_{8,13} = i_{P,BM} - f_{XI} i_{P,XI}$	gP gCOD^{-1}
$v_{9,13} = -(1 - f_{XI})/2$	gO$_2$ gCOD^{-1}
$v_{10,13} = -1/14\left(i_{N,BM} - f_{XI}\, i_{N,XI}\right) + 2/31\left(i_{P,BM} - f_{XI}\, i_{P,XI}\right)$	gH gCOD^{-1}
$v_{16,13} = f_{XI}$	gCOD gCOD^{-1}
$v_{19,13} = -1$	gCOD gCOD^{-1}
Decay of X_{AOB} and X_{NOB}	
$v_{15,14a} = (1 - f_{XI})Y_{AOB}$	gCOD gCOD^{-1}
$v_{16,14a} = f_{XI} Y_{AOB}$	gCOD gCOD^{-1}
$v_{18,14a} = -1$	gCOD gCOD^{-1}
$v_{15,14b} = (1 - f_{XI})Y_{NOB}$	gCOD gCOD^{-1}
$v_{16,14b} = f_{XI} Y_{NOB}$	gCOD gCOD^{-1}
$v_{19,14b} = -1$	gCOD gCOD^{-1}
Hydrolysis	
$v_{1,15} = -(1 - f_{SI})i_{N,SS} - f_{SI} i_{N,SI} + i_{N,XS}$	gN gCOD^{-1}
$v_{5,15} = i_{C,XS} - (1 - f_{SI})Y_{HYD} i_{C,SS} - f_{SI} Y_{HYD} i_{C,SI}$	gC gCOD^{-1}
$v_{8,15} = i_{P,XS} - (1 - f_{SI})Y_{HYD} i_{P,SS} - f_{I,XS} Y_{HYD} i_{P,SI}$	gP gCOD^{-1}
$v_{10,15} = -1/14\left(i_{N,XS} - (1 - f_{SI})Y_{HYD} i_{N,SS} - f_{SI} Y_{HYD} i_{N,SI}\right) + 2/31\left(i_{P,XS} - (1 - f_{SI})Y_{HYD} i_{P,SS} - f_{SI} Y_{HYD} i_{P,SI}\right)$	gH gCOD^{-1}
$v_{12,15} = (1 - f_{SI})Y_{HYD}$	gCOD gCOD^{-1}
$v_{13,15} = (f_{SI})Y_{HYD}$	gCOD gCOD^{-1}
$v_{15,15} = -1$	gCOD gCOD^{-1}

Chemical equilibria $\mathbf{CO_2 \leftrightarrow HCO_3^-}$	
$v_{5,16} = -1$	gC gC^{-1}
$v_{6,16} = 1$	gC gC^{-1}
$v_{10,16} = 1/12$	gH gC^{-1}
Chemical equilibria $\mathbf{HCO_3^- \leftrightarrow CO_3^{2-}}$	
$v_{6,17} = -1$	gC gC^{-1}
$v_{7,17} = 1$	gC gC^{-1}
$v_{10,17} = 1/12$	gH gC^{-1}
Chemical equilibria $\mathbf{NH_4^+ \leftrightarrow NH_3}$	
$v_{1,18} = -1$	gN gN^{-1}
$v_{2,18} = 1$	gN gN^{-1}
$v_{10,18} = 1/14$	gH gN^{-1}
Chemical equilibria $\mathbf{H^+ \leftrightarrow OH^-}$	
$v_{10,19} = 1$	gH gH^{-1}
$v_{11,19} = 1$	gH gH^{-1}
Oxygen transfer to the atmosphere	
$v_{9,20} = 1$	–
Carbon dioxide transfer to the atmosphere	
$v_{5,21} = 1$	–
Ammonia transfer to the atmosphere	
$v_{2,22} = 1$	–

Section B – *Micropollutants*

Table SB.1. GC-MS Operational conditions

GC-MS	
Parameter	**Value**
Injector	
Temperature	280 °C
Injection mode	*splitless*
Time of *splitless*	0.5 min
Column heater	
Column	Rtx-5MS (30 m × 0,25 mm × 0,25 mm)
Temperature ramp	50°C (1 min), improve to 100 °C a
Movel Fase	
Carrier gas	Helium
Flow control	Linear Velocity
Pression	~ 90,7kPa
Total Flow	37,3mL min^{-1}
Column Flow	1,54mL min^{-1}
Linear Velocity	45,0 cm s^{-1}
Split ratio	20
Mass Spectrometer	
Ionization source	250 °C
Interface	280 °C
Solvent cut-off time	5min
Detector voltage	1.3kV
Total analysis time	21.33 min

Section C – *Microalgae Co-digestion*

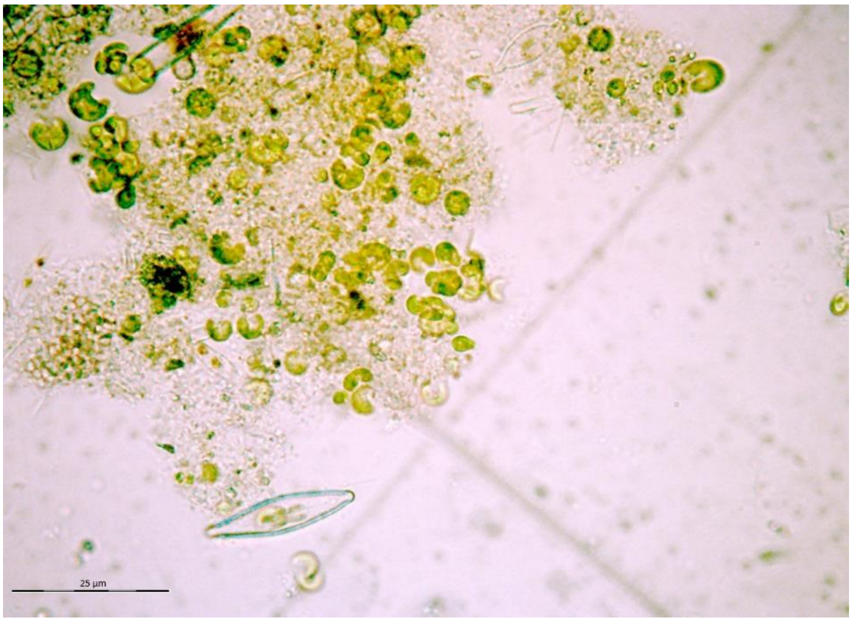


Figure SC.1. Microscopic image of microalgal biomass mainly composed by Kirchneriella sp. and diatoms.

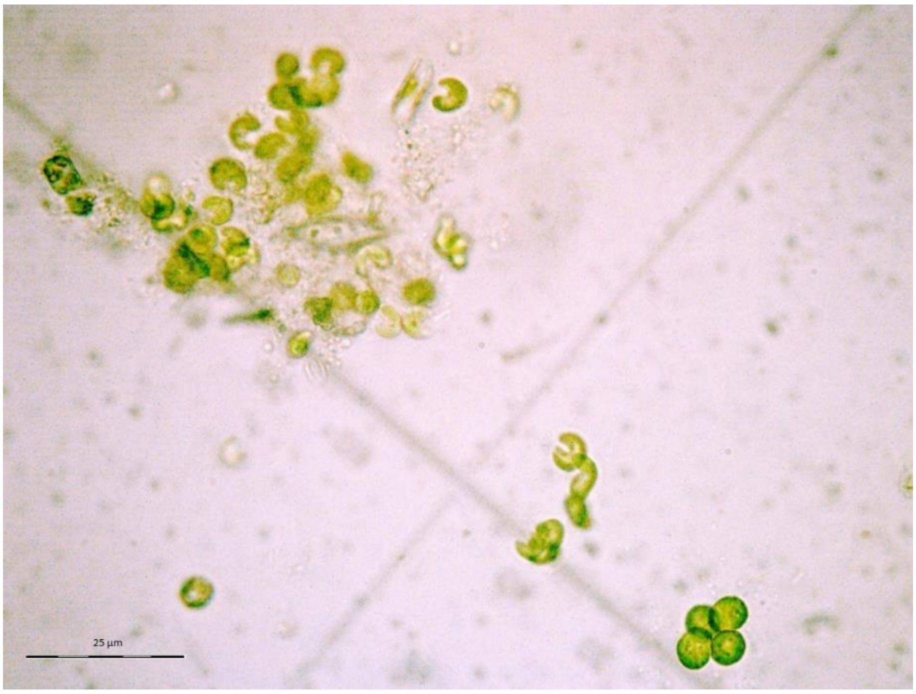


Fig. SC.2. Microscopic image of microalgal biomass mainly composed by Kirchneriella sp. and Westella sp

Section D – *Microalgae Pre-treatment*

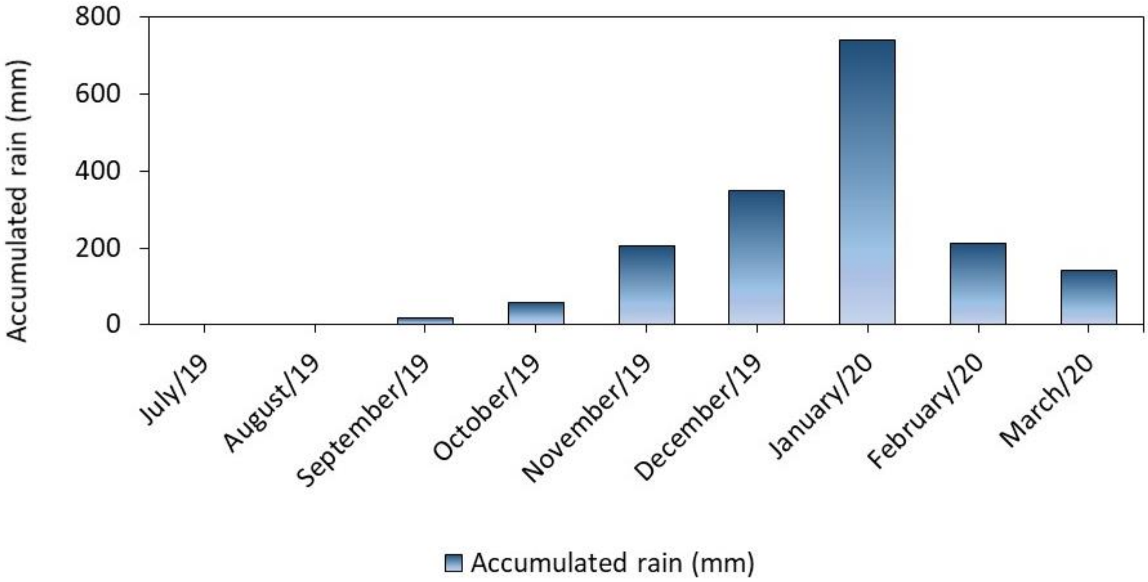


Figure SD1. Accumulated rain (mm) in the sampling months

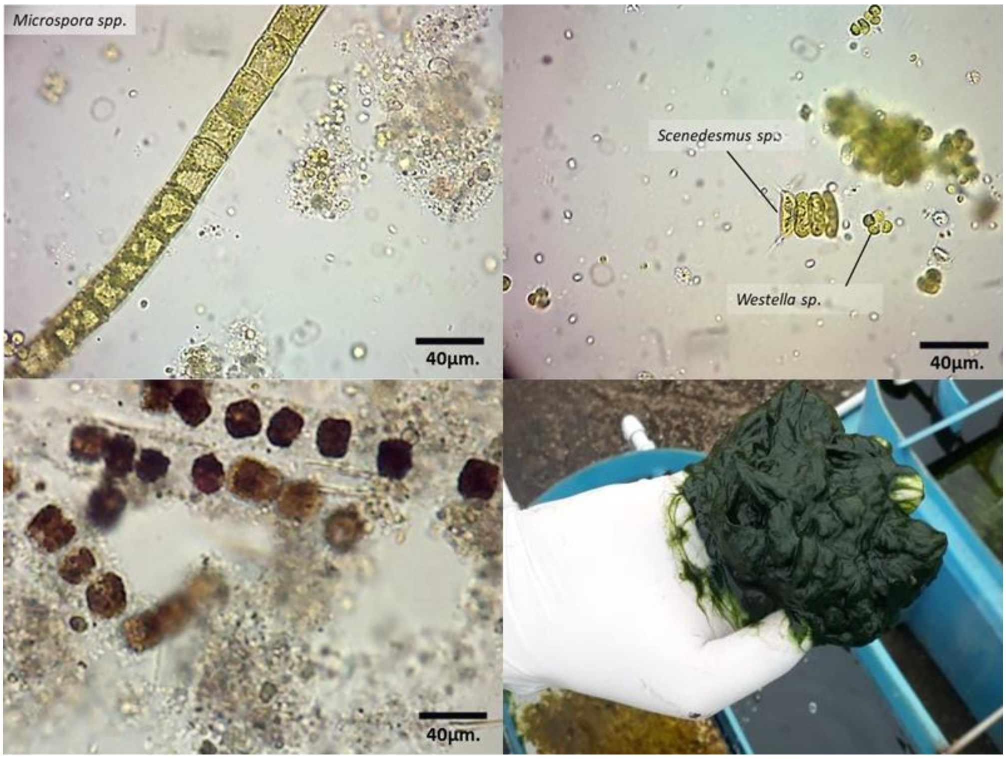


Figure SD2. Biomass characterization

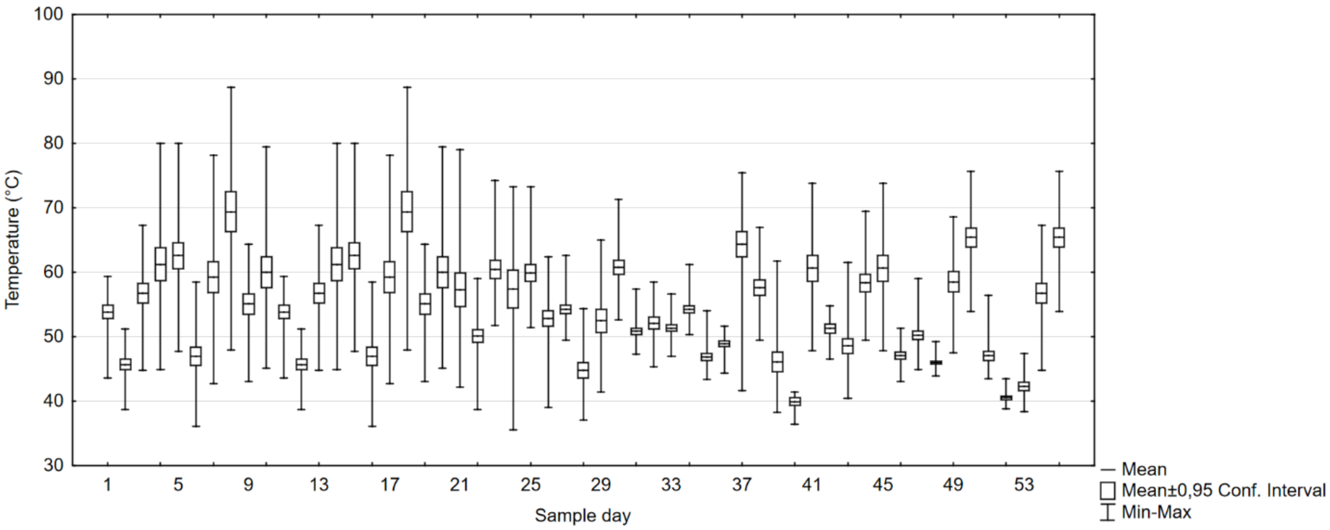


Figure SD3. Temperature variation on sample days

Table SE.1. Calculation memorial: Preliminary treatment

Vazão máxima horária final de projeto (l/s)	**17,36**	
Vazão máxima final de projeto (l/s)	**17,36**	
CAIXA DE AMORTIZAÇÃO		
Largura (m)	1,00	
Comprimento (m)	2,00	
Altura (m) $Q=1{,}4H^{5/2} \rightarrow H=\left(\frac{Q}{1{,}4}\right)^{2/5}$	1,50	
Volume (m³)	3	
Tempo de retenção hidráulica	TRH ≤ 3,0 min.	OK
TRH (s)	172,80	
TRH (min)	2,88	
CALHA PARSHALL		
Dimensão da calha (pé)	2	
W=	0,610	
λ=	1,426	Dados correlacionados à dimensão da calha
n=	1,550	
Qdimens (CAPACIDADE)=	11,89 a 936,7	
Largura do canal a montante (m)	0,500	0,8
Altura máxima da lâmina (m)	0,058	$H=(Q/\lambda)^{1/n}$
Velocidade máxima a montante (m/s)	0,60	
Intervalo recomendado para velocidade no canal	0,60<v<1,00 m/s	OK
Perda de carga (m)	0,010	**Azevedo pg 462**
Altura lâmina a jusante (m)	0,048	
Velocidade a jusante (m/s)	**0,45**	
VERTEDOR CAIXA DE AREIA		
VAZÃO (l/s)	17,36	
LARGURA (cm)	100,00	
ALTURA (cm)	4,47	
ALTURA (m)	0,045	m
GRADE FINA LIMPEZA MANUAL		
Largura do canal (m)	1,00	
Profundidade do canal (m)	1,40	
Altura do canal (m)	1,50	
Espaçamento entre barras (mm)	10,00	
Espessura da barra (mm)	5,00	
Largura da barra (mm)	20,00	
Taxa de material retido (l/m³)	0,042	
Inclinação	60º	
Tamanho da barra	1,620	
Lâmina a jusante da grade (m)	0,045	
Área da seção na grade (m²)	0,045	
Eficiência da grade	66,67%	
Área útil canal (m²)	**0,030**	
Velocidade na grade (m/s) (0,40 a máx 1,20 m/s)	**0,58**	
Velocidade à montante da grade (m/s)(entre 0,30 a 1,40m/s)	**0,39**	
Perda carga grade limpa (m)	0,014	
Perda carga grade 50% obstruída (m)	0,055	
Altura lâmina a montante da grade limpa (m)	**0,058**	
Altura lâmina a montante da grade (50% obstruída) (m)	**0,100**	
Volume do material gradeado início do plano (l/dia)	82,50	
Volume do material gradeado final do plano (l/dia)	82,50	

Table SE.2. Calculation memorial: Solids from preliminary treatment

MATERIAL RETIDO NO CESTO - ELEVATÓRIA	
Vazão inicial 2.020 (l/s)	17,36
Vazão final 2.040 (l/s)	17,36
Volume retido no gradeamento (l/m^3)	0,012
Volume de material retido acumulado (m^3/ano)	**6,57**
MATERIAL RETIDO NO GRADEAMENTO GROSSO DO TRATAMENTO PRELIMINAR	
Vazão inicial 2.020 (l/s)	17,36
Vazão final 2.040 (l/s)	17,36
Espaçamento entre as barras (cm)	4,00
Taxa de material retido (l/m^3)	0,009
Volume de material retido acumulado - 2.040 (m^3)	**98,54**
MATERIAL RETIDO NO GRADEAMENTO FINO DO TRATAMENTO PRELIMINAR	
Vazão inicial 2.020 (l/s)	17,36
Vazão final 2.040 (l/s)	17,36
Espaçamento entre as barras (cm)	1,20
Taxa de acúmulo de areia (l/m^3)	0,055
Volume de material retido acumulado - 2.040 (m^3)	**632,32**
MATERIAL RETIDO NA CAIXA DE AREIA DO TRATAMENTO PRELIMINAR	
Vazão inicial 2.020 (l/s)	17,36
Vazão final 2.040 (l/s)	17,36
Taxa de acúmulo de areia (l/m^3)	0,030
Volume de material retido acumulado - 2.040 (m^3)	**328,48**
PRODUÇÃO DE RESÍDUOS SÓLIDOS NO LEITO DE SECAGEM	
Volume do lodo por descarte 2.020(m^3)	124,56
Volume do lodo por descarte 2.040 (m^3)	124,56
Número de descarte por ano	20
Umidade do lodo afluente (%)	96
Umidade do lodo seco (%)	70
Volume de material retido acumulado - 2.040 (m^3)	**2.615,72**
VOLUME TOTAL DE RESÍDUOS SÓLIDOS NO ATERRO (m^3)	**3.353,15**

Table SE.3. Calculation memorial: UASB Reactors

ETE - CÁLCULO DA MODULAÇÃO DOS REATORES

ACV - 10.000

DISCRIMINAÇÃO	SIGLA	UNID	1ª etapa		2ª etapa	
			2.020	2.029	2.030	2.039
Vazão afluente média (com vazão de infiltração e industrial)	$Q_{\text{méd}}$	l/s	17,36	17,36	17,36	17,36
Volume útil necessário	Vutil	m^3	500	500	500	500
Modulação adotada	N	unid	**1**	**1**	**1**	**1**
Volume útil necessário/módulo	Vnec	m^3	500,00	500,00	500,00	500,00

DIMENSIONAMENTO DO MÓDULO

DISCRIMINAÇÃO	SIGLA	UNID	ADOTADO
Volume útil (adotar no máximo 1.500 m^3 por módulo)	Vutil adot	m^3	492,00
Altura útil (Adotar entre 4 e 6 metros NBR 12209/2011)	Hútil	m	**4,10**
Comprimento	Comp	m	**12,00**
Largura	Larg	m	**10,00**
Área	Área	m^2	120,00

DADOS POR MÓDULO

ITEM	PARÂMETROS	FÓRMULA	$Q_{\text{MÉD}}$			
			2.020	2.029	2.030	2.039
1	Vazão média (com vazão de infiltração e industrial) por módulo (m^3/h)	$Q_{\text{méd c/ inf e ind}}$	62,50	62,50	62,50	62,50
2	Vazão máxima (com vazão de infiltração e industrial) por módulo (m^3/h)	$Q_{\text{máx c/ inf e ind}}$	62,50	62,50	62,50	62,50
3	Carga por módulo (kgDBO/dia)	Pop. Atend x 0,054 / (N)	402,00	402,00	402,00	402,00
4	Concentração DBO (mg/l)	(3)x1000 / 24x(1)	268	268	268	268
5	Concentração DQO (kgDQO/m^3) ou (g/l)	(5) x 1,7/1000	0,456	0,456	0,456	0,456
6	Concentração ST (mg/l)	Medido	252	252	252	252
7	Concentração SV (mg/l)	Medido	208	208	208	208
8	Carga SV Kg/dia	(7)*Q(l/s) /1000 /1000	312	312	312	312
9	Carga ST Kg/dia	(6)*Q(l/s) /1000 /1001	378	378	378	378

VERIFICAÇÃO DAS CONDIÇÕES OPERACIONAIS DO REATOR - (POR MÓDULO)

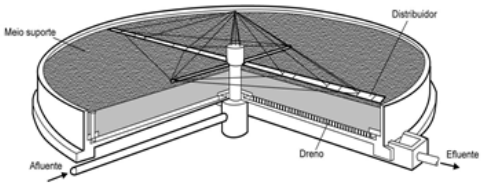
ITEM	PARÂMETROS	FÓRMULA	$Q_{\text{MÉD}}$				OBSERVAÇÕES
			2.020	2.029	2.030	2.039	
10	Velocidade Ascencional - Qméd (m/h)	(1) / (A)	0,5	0,5	0,5	0,5	Velocidades preferencialmente na faixa de 0,5 a 0,7m/h e sempre inferior a 2,0 m/h
11	Velocidade Ascencional - Qmáx (m/h)	(2) / (A)	0,5	0,5	0,5	0,5	Velocidades maiores que Q méd e inferiores de 1,2 m/h
12	Tempo de detenção hidráulica (h)	(Vutil) / (1)	7,9	7,9	7,9	7,9	O TDH mínimo de 8 h para temp média do esgoto de 18 a 21 ºC
13	Carga hidráulica volumétrica (m^3/(m^3xdia))	(1) x24 / (Vútil)	3,0	3,0	3,0	3,0	Deve ser inferior a 5 m^3/(m^3xdia)
14	Carga orgânica volumétrica (kgDQO/(m^3xdia))	(1) x (5) x24 / (Vútil)	1,4	1,4	1,4	1,4	Deve ser inferior a 5 kgDQO/(m^3xdia) em esgotos estritamente domésticos
15	Estimativa eficiência de remoção de DBO (%)	100 x(1-0,708x$(8)^{-0,5}$)	61,0	61,0	61,0	61,0	As eficiências esperadas para os reatores UASB geralmente estão na faixa de 60 a 75%
16	Estimativa eficiência de remoção de DQO (%)	Medição	52,0	52,0	52,0	52,0	As eficiências esperadas para os reatores UASB geralmente estão na faixa de 55 a 70%
17	Produção de gás metano	(8)*Taxa de produção	50	50	50	50	Taxa de produção de 0,16 m^3CH4/kgSV
18	Produção de biogás	(13) / efi(%)	92	92	92	92	Concentração de CH_4 no biogás 70-80%
19	Taxa biogás (m^3/biogás/(m^2 x dia)	(14) / (A)	0,8	0,8	0,8	0,8	A taxa de produção de biogás deve ser no mínimo de 1,0 e no máximo entre 3,0 e 5,0.
20	Concentração DBO efluente (mg/l)	(4)-((11)x(4))	105	105	105	105	
21	Concentração DQO efluente (mg/l)	Medido	180	180	180	180	

Table SE.4. Calculation memorial: Trickling Filters

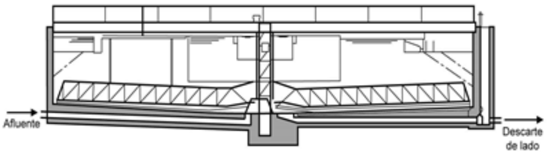
1) DADOS GERAIS DE ENTRADA				
População atendida - hab (P)	10.000	10.000	10.000	10.000
Qméd total - Qméd Total (l/s)	17,36	17,36	17,36	17,36
Qméd diária total - com k1 - Qméd diária Total(l/s)	17,36	17,36	17,36	17,36
Qmáx horária total - com k1 e k2 - Qmáx Total (l/s)	17,36	17,36	17,36	17,36
Qtotal 1 - sem k1 - incluindo Qlodo - l/s (Qt1)	17,39	17,39	17,39	17,39
Qtotal 2 - com k1 - incluindo Qlodo - l/s (Qt2)	17,39	17,39	17,39	17,39
Qtotal 3 - com k1 e k2 - incluindo Qlodo - l/s (Qt3)	17,39	17,39	17,39	17,39
Carga orgânica efluente do reator UASB (kgDBO/d)	210,60	210,60	210,60	210,60
Concentração média de DBO do efluente do reator UASB - incl. Lodo retorno - So (mgDBO/L)	140,17	140,17	140,17	140,17
Coeficiente de produção de lodo no FBP - Y (kgSST/kgDBOremov)	0,75	0,75	0,75	0,75
Concentração esperada para o lodo de descarte do decantador secundário - C (%)	1,00	1,00	1,00	1,00
Densidade do lodo - (kgSST/m^3)	1.020	1.020	1.020	1.020
2) DIMENSIONAMENTO DOS FILTROS BIOLÓGICOS PERCOLADORES	**Filtro de Alta Taxa (pós reator UASB)**			
Ano/etapa	**1ª ETAPA**		**2ª ETAPA**	
	2.020	**2.029**	**2.030**	**2.039**
a) Critérios e parâmetros adotados				
Profundidade do meio suporte - H (m) - até 3,0 m (NBR12209/2011)	2,50	2,50	2,50	2,50
Concentração DBO_5 afluente - So (mg/L)	140,17	140,17	140,17	140,17
Taxa de recirculação do efluente (percentagem da vazão afluente)	50	50	50	50
Carga orgânica volumétrica - Cv (kgDBO/m3.d) - 0,5 a 1,2 kgDBO/m^3.d (NBR12209/2011)	0,8	0,8	0,8	0,8
b) Determinação do volume de meio suporte				
V = (Qméd x So) / Cv	263,25	263,25	263,25	263,25
c) Determinação das dimensões do filtro				
Determinação da área superficial do filtro (m^2) - A = V / H	105,30	105,30	105,30	105,30
Número de filtros (un)	1	1	1	1
Área superficial de cada filtro (m^2)	105,30	105,30	105,30	105,30
Diâmetro do FBP (m)	11,58	11,58	11,58	11,58
Diâmetro corrigido do FBP (m)	10,00	10,00	10,00	10,00
Área superficial útil corrigida de cada filtro (m^2)	78,50	78,50	78,50	78,50
d) Resumo das dimensões do FBP				
Profundidade do meio suporte do filtro (m)	2,50	2,50	2,50	2,50
Diâmetro de cada FBP (m)	10,00	10,00	10,00	10,00
Área corrigida de cada filtro (m^2)	78,50	78,50	78,50	78,50
Volume de cada filtro (m^3)	196,25	196,25	196,25	196,25
e) Verificação das cargas aplicadas				
Carga hidráulica p/ Qméd sem k1e com lodo de retorno (m^3/m^2.d) (verificar: 15 a 20 m^3/m^2.d)	19,140	19,140	19,140	19,140
Carga hidráulica p/ Qméd com k1 e lodo de retorno (m^3/m^2.d) (verificar: 18 a 23 m^3/m^2.d)	19,140	19,140	19,140	19,140
Carga hidráulica p/ Qmáx com k1e k2 e lodo de retorno (m^3/m^2.d) (verificar: 25 a 30 m^3/m^2.d)	19,141	19,141	19,141	19,141
Carga hidráulica p/ Qméd com k1 + lodo de retorno + recirculação do efluente (m^3/m^2.d)	19,140	19,140	19,140	19,140
Cargas orgânicas recomendadas (kgDBO/m^3.d)	**0,50 a 1,00**			
Carga orgânica (kgDBO/m^3.d)	1,07	1,07	1,07	1,07
f) Estimativa da concentração de DBO no efluente final				
Eficiência de remoção de DBO esperada - E = 100 / [1+0,443 x (Cv/F)^0,5]	65,6	65,6	65,1	60,0
Concentração esperada de DBO5 no efluente - Se (mg/L)	48,22	48,22	48,92	56,07
Eficiência do Sistema (%)	82,0	82,0	81,7	79,1

Table SE.5. Calculation memorial: Settler

3) DIMENSIONAMENTO DOS DECANTADORES SECUNDÁRIOS				
a) Critérios e parâmetros adotados				
Profundidade útil junto à parede - H (m) - mín 3,50m (NBR12209/2011)	4,00	4,00	4,00	4,00
Taxa de aplicação superficial (para Qmédia) - 16 a 32 $m^3/m^2.d$	24	24	24	24
b) Determinação das dimensões do decantador				
Cálculo da área de decantação necessária - A = Qméd / TAS (m^2)	62,60	62,60	62,60	62,60
Número de decantadores (un)	1	1	1	1
Área de cada decantador (m^2)	62,60	62,60	62,60	62,60
Diâmetro de cada decantador (m)	8,93	8,93	8,93	8,93
Diâmetro corrigido para o decantador (m)	8,00	8,00	8,00	8,00
Área corrigida de cada decantador (m^2)	50,24	50,24	50,24	50,24
Volume de cada decantador - desconsiderando-se a inclinação de fundo (m^3)	200,96	200,96	200,96	200,96
Verificação da taxa de aplicação (16 a 32 $m^3/m^2.d$)	29,91	29,91	29,91	29,91

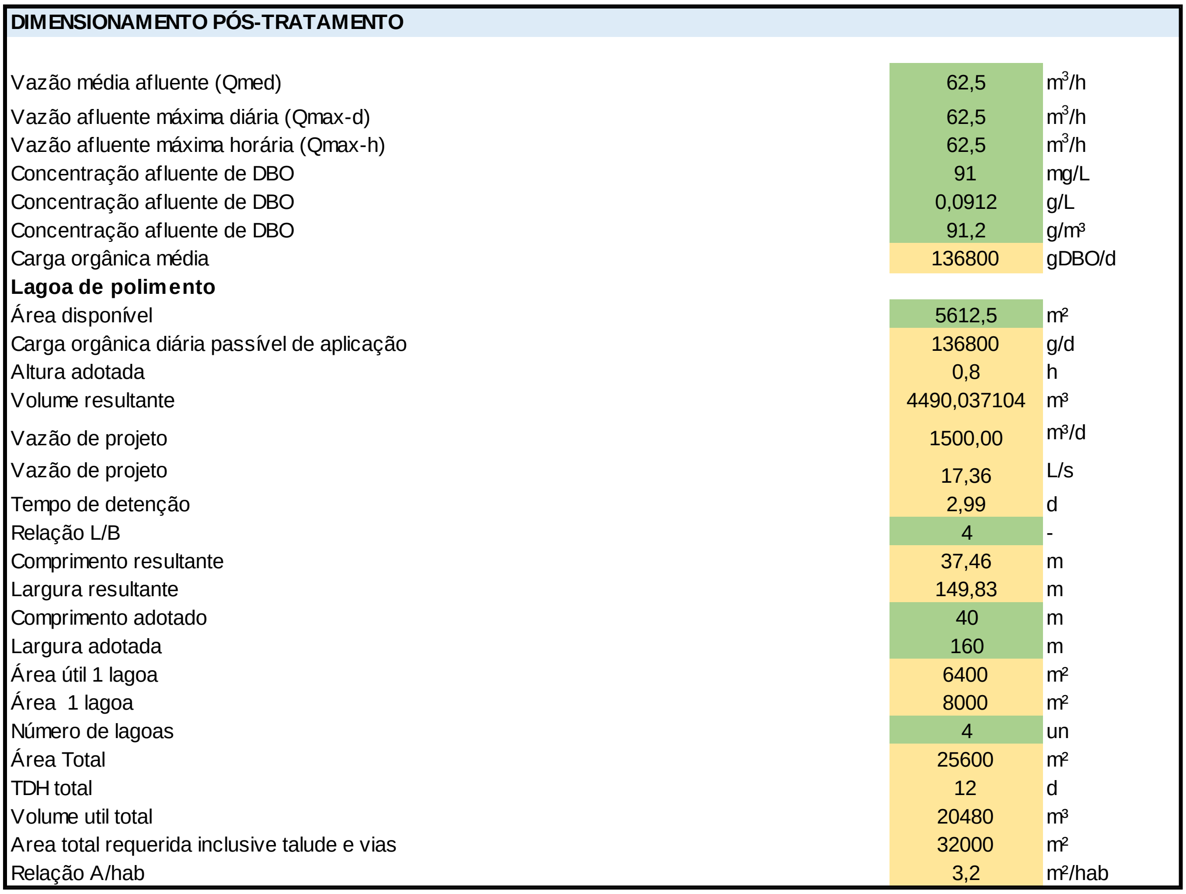


Esquema Filtro Biológico Percolador



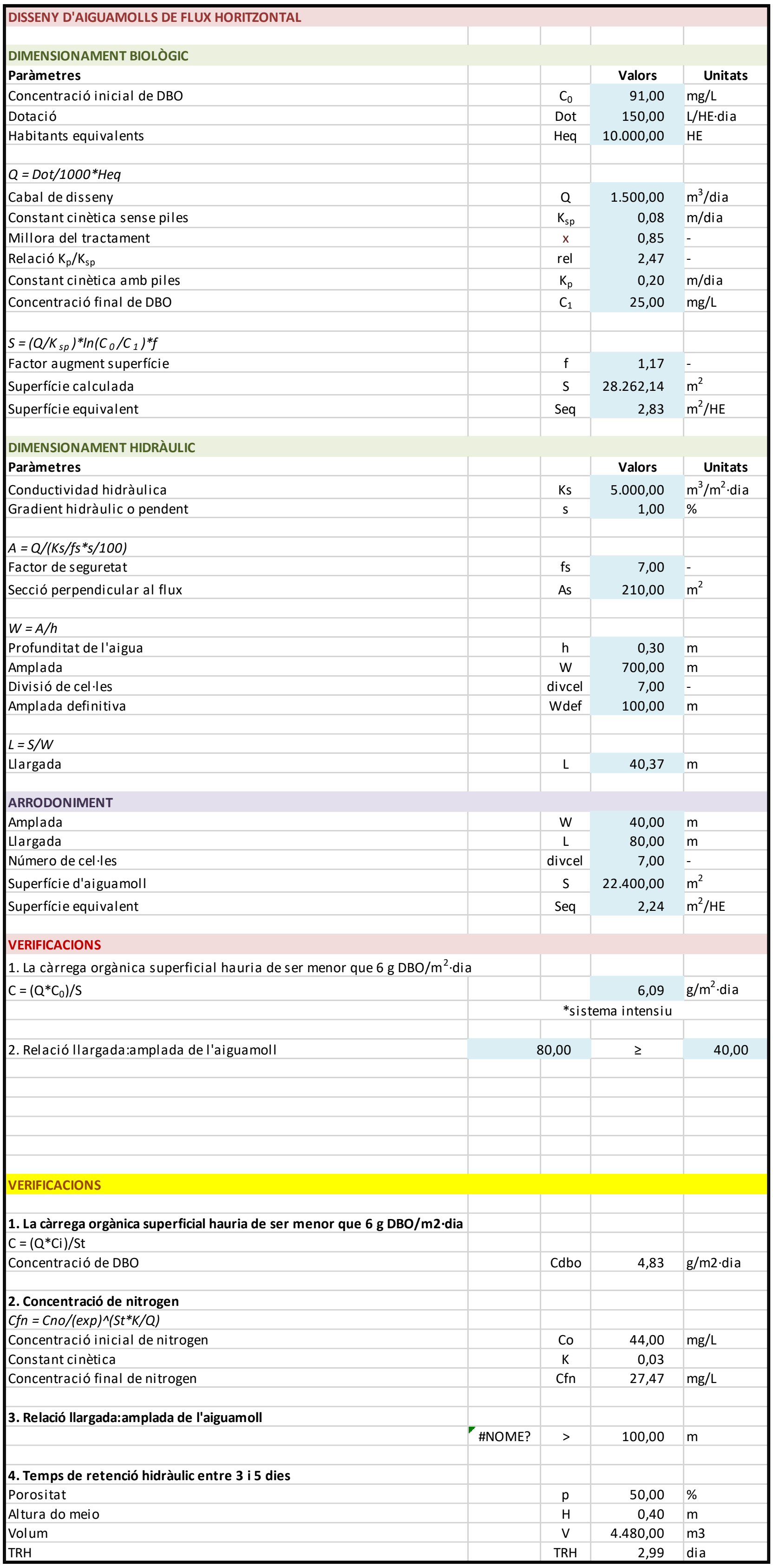
Esquema Decantador Secundário

Table SE.6. Calculation memorial: Polish Ponds



DIMENSIONAMENTO PÓS-TRATAMENTO		
Vazão média afluente (Qmed)	62,5	m^3/h
Vazão afluente máxima diária (Qmax-d)	62,5	m^3/h
Vazão afluente máxima horária (Qmax-h)	62,5	m^3/h
Concentração afluente de DBO	91	mg/L
Concentração afluente de DBO	0,0912	g/L
Concentração afluente de DBO	91,2	g/m^3
Carga orgânica média	136800	gDBO/d
Lagoa de polimento		
Área disponível	5612,5	m^2
Carga orgânica diária passível de aplicação	136800	g/d
Altura adotada	0,8	h
Volume resultante	4490,037104	m^3
Vazão de projeto	1500,00	m^3/d
Vazão de projeto	17,36	L/s
Tempo de detenção	2,99	d
Relação L/B	4	-
Comprimento resultante	37,46	m
Largura resultante	149,83	m
Comprimento adotado	40	m
Largura adotada	160	m
Área útil 1 lagoa	6400	m^2
Área 1 lagoa	8000	m^2
Número de lagoas	4	un
Área Total	25600	m^2
TDH total	12	d
Volume util total	20480	m^3
Area total requerida inclusive talude e vias	32000	m^2
Relação A/hab	3,2	m^2/hab

Table SE.7. Calculation memorial: Constructed Wetlands



DISSENY D'AIGUAMOLLS DE FLUX HORITZONTAL				
DIMENSIONAMENT BIOLÒGIC				
Paràmetres			**Valors**	**Unitats**
Concentració inicial de DBO		C_0	91,00	mg/L
Dotació		Dot	150,00	L/HE·dia
Habitants equivalents		Heq	10.000,00	HE
*Q = Dot/1000*Heq*				
Cabal de disseny		Q	1.500,00	m^3/dia
Constant cinètica sense piles		K_{sp}	0,08	m/dia
Millora del tractament		x	0,85	-
Relació K_p/K_{sp}		rel	2,47	-
Constant cinètica amb piles		K_p	0,20	m/dia
Concentració final de DBO		C_1	25,00	mg/L
$S = (Q/K_{sp})*ln(C_0/C_1)*f$				
Factor augment superfície		f	1,17	-
Superfície calculada		S	28.262,14	m^2
Superfície equivalent		Seq	2,83	m^2/HE
DIMENSIONAMENT HIDRÀULIC				
Paràmetres			**Valors**	**Unitats**
Conductividad hidràulica		Ks	5.000,00	m^3/m^2·dia
Gradient hidràulic o pendent		s	1,00	%
*A = Q/(Ks/fs*s/100)*				
Factor de seguretat		fs	7,00	-
Secció perpendicular al flux		As	210,00	m^2
W = A/h				
Profunditat de l'aigua		h	0,30	m
Amplada		W	700,00	m
Divisió de cel·les		divcel	7,00	-
Amplada definitiva		Wdef	100,00	m
L = S/W				
Llargada		L	40,37	m
ARRODONIMENT				
Amplada		W	40,00	m
Llargada		L	80,00	m
Número de cel·les		divcel	7,00	-
Superfície d'aiguamoll		S	22.400,00	m^2
Superfície equivalent		Seq	2,24	m^2/HE
VERIFICACIONS				
1. La càrrega orgànica superficial hauria de ser menor que 6 g DBO/m^2·dia				
C = (Q*C_0)/S			6,09	g/m^2·dia
		*sistema intensiu		
2. Relació llargada:amplada de l'aiguamoll	80,00		≥	40,00
VERIFICACIONS				
1. La càrrega orgànica superficial hauria de ser menor que 6 g DBO/m2·dia				
C = (Q*Ci)/St				
Concentració de DBO		Cdbo	4,83	g/m2·dia
2. Concentració de nitrogen				
*Cfn = Cno/(exp)^(St*K/Q)*				
Concentració inicial de nitrogen		Co	44,00	mg/L
Constant cinètica		K	0,03	
Concentració final de nitrogen		Cfn	27,47	mg/L
3. Relació llargada:amplada de l'aiguamoll				
	#NOME?	>	100,00	m
4. Temps de retenció hidràulic entre 3 i 5 dies				
Porositat		p	50,00	%
Altura do meio		H	0,40	m
Volum		V	4.480,00	m3
TRH		TRH	2,99	dia

Table SE.8. Calculation memorial: High Rate Algal Ponds

HRAP design

Items		Quantity	Unit	Comments
		HRAP 8 days		*Different HRT*
Person emission rate		54	g BOD/HE·day	*Design parameter*
Wastewater load		540	kg BOD/day	
Design organic loading rate		140,00	kg BOD/ha·day	*Design parameter*
Efficiency of primary treatment (BOD removal)		75%	-	
Influent load - HRAP		135	kg DBO/day	
Total surface area	St	0,96	ha	
		9.643	m2	
Hydraulic retention time	TRH	8	day	
Treatment volume	V	12.000	m3	
Number of HRAP	-	4	-	
Volume 1 HRAP	VHRAP	3.000	m3	
Water depth	h	0,40	m	
Surface 1 HRAP	S	7.500	m2	
Width 1 HRAP	a	12	m	*7 - 12*
Length 1 HRAP	l	625,00	m	
Perimeter 1 HRAP	P	1.274	m	*Rectangular HRAP*
Depth of the walls	h	0,50	m	*10 cm more than treatment capacity*
Concrete/walls thickness	e	0,20	m	
Concrete volume (walls)	Vm	509,60	m3	
Concrete volume (basement)	Vb	6.000	m3	
Total concrete volume	Vf	**6.509,60**	m3	For the inventory the 4 HRAPs must be considered
Steel needed	Ma	**520.768**	kg	
Total surface area needed	St	**30.000**	m2	(all of the are implemented)
Paddlewheel 1 HRAP - steel	Ma	381,36	kg	
Paddlewheel 1 HRAP - Fiberglass	Mfv	976,52	kg	
Number of paddlewheels	-	8	-	
Total steel mass for Paddlewheels	Mat	**3.050,85**	kg	For the inventory the 4 HRAPs must be considered
Total fiberglass mass for Paddlewheel	Mfvt	**7.812,17**	kg	(all of the are implemented)

Head loss in channels and in reversals

Items		Quantity	Unit	Comments
Surface 1 HRAP	S	7.500	m2	
Width	a	12	m	
Length	l	625,00	m	
Manning coefficient	n	0,025	-	
Water depth	h	0,40	m	
Water velocity	v	0,15	m/s	
Head loss in channels 1 HRAP	Δd1	0,014	m	$\Delta d_1 = \frac{v^2 \cdot l}{\left(\frac{1{,}486}{n}\right)^2 \cdot \left(\frac{h \cdot a}{a+2h}\right)^{1{,}26}}$ $\Delta d = \frac{v^2 L}{\left(\frac{1.486}{n}\right)^2 \left(\frac{d W}{W+2d}\right)}$
Number of reversals	-	2,00	-	
Head loss in reversals 1 HRAP	Δd2	0,0023	m	$\Delta d_2 = \frac{v^2}{2g}$ $\Delta d = \frac{v^2}{2g}$
Total head loss 1 HRAP	Δd	0,0160	m	$\Delta d = \Delta d_1 + \Delta d_2$

Energy consumption in HRAP

Items		Quantity	Unit	Comments
Water flow	Qm	0,72	m3/s	
Specific weight of water at 20ºC	γ	9,78	kN/m3	
Total head loss 1 HRAP	Δd	0,0160	m	
Paddlewheel efficiency	E	0,5	-	*0,2- 0,6*
Number of HRAP	-	4	-	
Power requirement	P	0,9009	kW	
		0,0751	W/m3	
Energy consumption	Ce	22,4230	kWh/day·ha	
		2,2423	Wh/day·m2	
		1,8019	Wh/day·m3	