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TRACE METALS ON ANAEROBIC REACTORS TREATING DOMESTIC SEWAGE

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TRACE METALS ON ANAEROBIC REACTORS TREATING DOMESTIC SEWAGE

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ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE JULLIANA ALVES DA SILVA, DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM ENGENHARIA CIVIL E AMBIENTAL, DA FACULDADE DE ENGENHARIA - CÂMPUS DE BAURU.

Aos 02 dias do mês de setembro do ano de 2021, às 12:00 horas, por meio de Videoconferência, realizou-se a defesa de TESE DE DOUTORADO de JULLIANA ALVES DA SILVA, intitulada **TRACE METALS ON ANAEROBIC REACTORS TREATING DOMESTIC SEWAGE**. A Comissão Examinadora foi constituida pelos seguintes membros: Prof. Dr. GUSTAVO HENRIQUE RIBEIRO DA SILVA (Orientador(a) - Participação Virtual) do(a) Departamento de Engenharia Civil e Ambiental / Faculdade de Engenharia de Bauru - UNESP, Profª Drª PAULA LOUREIRO PAULO (Participação Virtual) do(a) Faculdade de Engenharias, Arquitetura e urbanismo e Geografia - FAENG / Universidade Federal de Mato Grosso do Sul, Prof. Dr. GERMÁN BUITRÓN MÉNDEZ (Participação Virtual) do(a) Instituto de Engenharia / Unidade Acadêmica de Juriquilla / Universidad Nacional Autónoma de México - UNAM Mexico, Profa. Dra. RACHEL BIANCALANA COSTA (Participação Virtual) do(a) Instituto de Química - Depto de Bioquímica e Química Orgânica / Universidade Estadual Paulista , Prof. Dr. ANTONIO SERRANO MORAL (Participação Virtual) do(a) Instituto de la Grasa, Bioprocesses for the Circular Economy Research Group / Consejo Superior de Investigaciones Cientificas - C.S.I.C.. Após a exposição pela doutoranda e arguição pelos membros da Comissão Examinadora que participaram do ato, de forma presencial e/ou virtual, a discente recebeu o conceito final: Aprovada. Nada mais havendo, foi lavrada a presente ata, que após lida e aprovada, foi assinada pelo(a) Presidente(a) da Comissão Examinadora.

Prof. Dr. GUSTAVO HENRIQUE RIBEIRO DA SILVA

Preface

Silva, J. A. (2021) **Trace metals on anaerobic reactors treating domestic sewage.** Doctoral Thesis, São Paulo State University, Brazil.

The use of anaerobic bioreactors for wastewater treatment depends on the adequate set-up and control of the physical, chemical, and biological parameters. In order to keep stable and suitable performance, it is essential to know the characteristics of the wastewater to be treated to choose the best treatment and reactor configuration, find the optimal hydraulic retention time (HRT), operational conditions and nutrients requirement.

Regarding nutrients, deficiencies or excess of macro or micronutrients in anaerobic wastewater treatment systems are not a rare phenomenon. Knowing the wastewater composition and the biomass trace metals requirement in the system are necessary to achieve a maximal substrate conversion. Hence, providing a rational trace metal supplementation to the reactor.

Therefore, in this thesis, the trace metal profile of domestic sewage (DS) treated in an innovative configuration of anaerobic reactor, the anaerobic structured-fixed bed reactor (ASTBR) is studied, together with the boundary conditions for stable operation, according to the organic matter load and trace metals contribution. In addition, a UASB reactor is operated in parallel as a model reactor to compare the ATBSR performance and as a source of sludge for the optimization of trace metals in DS anaerobic digestion, improving methane production.

The results are presented in three individual papers, two published in international journals.

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Chapter 1

1.1 GENERAL INTRODUCTION

1.1.1 The need sustained by the opportunity- Methane (CH_4)

Greenhouse gases (GHG) are considered gases released to the atmosphere, which components absorb longer wavelength radiation, contributing to the global atmospheric temperature increase. GHG includes water vapor (H_2O), carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF_6) (Smoot and Baxter, 2002). The accumulation of GHG in the atmosphere from human and industrial activities is recognized as one of humanity's main problems due to global climate changes (Ruiz-Ruiz et al., 2020).

According to the United States Environmental Protection Agency (EPA, 2018), in 2018, 28% of the GHG emission came from transportation, 27% from electricity production, 22% from industry, 12% from human commercial and residential activities, and 10% from agriculture, totalizing 6.677 million metric tons of CO_2 equivalent, all related to non-renewable sources of energy.

Consequently, the need to transition the world energy matrix to clean and sustainable alternatives is eminent. It is necessary to find technologies eco-friendly and economically viable to compensate for the environmental crisis through a greener approach (Sivasankar et al., 2019).

Clean water resources are becoming limited as the population grows, raising wastewater generation and environmental contamination (James et al., 2020). In 2018, the International Water Association (IWA) in the Wastewater Report (IWA and Association, 2018), claimed that 80% of all world wastewater is discharged into the water bodies without any treatment.

The negative impacts of untreated wastewater discharge on the environment and humans are well known. It includes the death of aquatic life, eutrophication, toxicity from chemical contaminants, the significant contribution to GHG emissions, as emissions from untreated sewage represents three times the emissions of DS treated by conventional systems (IWA and Association, 2018), and toxicity from micropollutants and emerging contaminants (Kümmerer, 2011).

Technologies are making resource recovery from wastewater commercially feasible to face these problems. From anaerobic wastewater treatment, it is possible to reduce water bodies contamination, obtain clean water, organic compounds, phosphates, nitrogen, organic acids, rare earth elements, and other resources to produce fertilizer, plastics and, perhaps a source of electricity through biogas (Zeeman et al., 2008; IWA and Association, 2018; Sivasankar et al., 2019).

Therefore, the biogas obtained from AD, using wastewater as substrate, can be considered a gadget to produce electricity due to the energetic feasibility of CH_4, its main gas constituent (Salomon and Lora, 2009).

The main advantages of using CH_4 for energy production are the proximity of the generating source; the possibility of additional profit due to the production and sale of energy; the reduction of the consumption from external electricity supply; the reduction of GHG emissions to the atmosphere (Salomon and Lora, 2009); lower sale price compared with diesel and petrol (Mao et al., 2015).

1.1.2 Domestic sewage treatment

Among the types of pollution found worldwide, water pollution is considered a severe threat to the environment. It involves several types of wastewaters, including residential, industrial, agricultural, and hospital, discharged in water bodies without treatment (Metcalf and Eddy, 2003).

DS is produced by urban activities, including residences, institutions, and commerce discharges (Von Sperling, 2007). Those encompass toilets, personal wash (shower, bath, dishes, laundry), cook, swimming pools discharged, chemical toilets. In some cases, infiltration and industrial effluents may collaborate on the volume of DS produced (Von Sperling, 2007).

Due to the different sources, DS usually has characteristics that oscillate from one source to another. In general, it contains water; solids; bio and non-biodegradable organic matter; nutrients (nitrogen and phosphorus), microorganisms; inorganic dissolved solids, and metals (e.g.,arsenic (As), aluminium (Al), cadmium(Cd), chrome (Cr), copper (Cu), iron (Fe), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn) (Von Sperling, 2007; Metcalf and Eddy, 2003); and pharmaceutical residues and hormones, which fluctuate in concentration.

Organic matter can be found in concentrations from 250 to 1000 mg L^{-1} as Chemical Oxygen demand (COD), Ammonia from 12 to 50 mg L^{-1}, Sulfate from 20 to 50 mg L^{-1} (Metcalf and Eddy, 2003). Metals such as Cr in concentrations of 0.315 mg L^{-1}, Ni of 0.275 mg L^{-1}, Cu of 0.421 mg L^{-1}, Zn of 1.282 mg L^{-1} and Pb of 0.063 mg L^{-1} (Souza et al., 2014).

Contaminants such as antibiotic compounds are considered emerging, which can be found in various environmental matrices at concentrations of μg L^{-1} or even lower (ng L^{-1}) (Carneiro et al., 2019).These pollutants are hazardous to the environment, humans, and living beings. They also change the water's physicochemical conditions, letting the pH out of neutrality, adding color, smell, among other characteristics. Therefore, the DS's depth characterization and treatment are mandatory before discharging the DS into the waterbodies.

Usually, DS sewage is treated by biological treatment, through aerobic, anaerobic or anaerobic/aerobic (union of the processes) treatment. For a long period, aerobic systems were considered the most appropriate techniques to treat DS, followed by the misconception that the lower efficiency of the anaerobic systems was attributable to a lower metabolic capacity of anaerobic microorganisms. Therefore, a long retention time was necessary (Van Haandel et al., 2006). In 1969, Young and McCarty showed the high potential of anaerobic systems, using an up-flow anaerobic filter to treat rum distillery wastewater (Young and McCarty, 1969).

Anaerobic digestion (AD) consists of a metabolic process associated with microorganisms that require non-oxygen conditions to transform organic substrates into CH_4 and CO_2. This process is divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Speece, 1996); each one is performed by different groups of syntrophic microorganisms, which require specific conditions for optimal activity.

In AD, the complex organic compounds present in the substrate (lipids, polysaccharides, and proteins) are digested into simpler and soluble substances. By the hydrolytic or fermentative bacteria, extracellular enzymes are released, hydrolyzing the compounds (Surendra et al., 2014). The simpler hydrolyzed compounds are then used by different anaerobic and facultative bacteria, which release short-chain organic acids and molecules, which are oxidized to H_2, CO_2 (Sikora et al., 2017). In the last stage, in strictly anaerobic conditions, the H_2 and acetate are converted to CO_2 and CH_4 through the action of methanogenic archaea. The two last steps of the AD works with syntrophic associations between hydrogen-producing acetogenic bacteria and hydrogenotrophic methanogens (Sikora et al., 2017).

1.1.3 Trace elements influence on anaerobic digestion

The greater the efficiency of biological treatment is, the greater the production of CH_4 by the system and, consequently, the capacity to obtain energy (Jiang et al., 2013; Deng et al., 2014).

In principle, all anaerobic reactors are capable of digest organic compounds, resulting in CH_4 (Chernicharo, 2007). However, it is well established that the AD needs adequate operational conditions and also, essential macro and micronutrients (trace metals) concentrations for microorganisms' growth, resulting in CH_4 production. During the AD of any organic substrate to biogas-CH_4, the availability or lack of trace metals (TMs) are significant in keeping a stable and an efficient conversion process (Demirel and Scherer, 2011). Some substrates contain sufficient concentrations of trace metals, e.g. swine wastewater (Amaral et al., 2014), and others, such as methanol, demand metals addition.

Beyond macronutrients (C, N, P, S, K, Mg), anaerobic microorganisms require small concentrations of trace metals (TMs). The importance of TMs, such Fe, Ni, cobalt (Co), Zn, Cu, Se, Pb, is related to the role they play in the metabolism of microorganisms. Some TMs are essential components of cofactors and enzymes of anaerobic metabolism (Speece et al., 1983; Fermoso et al., 2019).

The operational conditions of anaerobic reactors govern the availability of TMs for AD (e.g., pH, temperature, HRT and redox potential) (Mudhoo and Kumar, 2013). The assessment of the behavior of TMs presented in the substrate such as DS could be an essential tool to avoid losses in the performance and efficiency and improve the performance. Improved digester performance due to TMS supplementation has been demonstrated at laboratory, pilot and full scale. Reduced VFA accumulation and higher biogas output are the main benefits reported (Roussel et al., 2018).

1.1.4 The Anaerobic Structured-fixed bed reactor (ASTBR) in DS treatment

Several anaerobic reactor configurations have already been used or tested for the treatment of DS. As examples: septic tanks, Imhoff tanks, anaerobic ponds, anaerobic filters, up-flow anaerobic sludge bed (UASB) reactor, expanded granular sludge bed (EGSB) reactor, anaerobic baffled reactor (ABR), fixed-film fluidized bed reactor, expanded bed reactor, among others.

Despite the development of the anaerobic reactors, each one has its own advantages and disadvantages, and further investigations are still necessary to supplant limitations of the existing reactor configurations and process designs to increase both organic matter digestion and energy recovery through CH_4 (Fuess et al., 2017).

Anaerobic reactors like UASB (e.g.,EGSB, ABR) typically contain self-immobilized biomass with microbial consortia granules (Mockaitis et al., 2014). It also includes the three-phase system, which separates the gas produced from the liquid and solid parts, which guarantees solids sedimentation conditions. Inclined devices, called deflectors, and a three-phase separator is present in these reactors. The three-phase separator allows the gas to escape at the top, the solid phase returns to the sludge blanket, and the liquid part is collected at a level above the deflectors already treated (Lettinga et al., 1980; Chernicharo, 2007).

Reactors that contains biomass immobilized on inert materials (such as low-density polyethylene, polyurethane foam, and ceramic), like ASTBR, form a biofilm

(Carneiro et al., 2019) and are designed as fixed, expanded, or fluidized beds (Mockaitis et al., 2014).

The fixed bed reactors were introduced to the DS treatment under anoxic conditions by Coulter et al.(1957). The traditional packed-bed biofilm reactor, in which the support is randomly distributed in the bed, culminated in the structured fixed bed reactor due to its operational problems. Packed-bed biofilm reactor operation usually results in the reduction of HRT due to the clogging of the interstices, channeling, hydraulic short circuit zones, and higher resistance to gas transfer from the reaction zone to the headspace is observed (Carneiro et al., 2019).Therefore, to avoid these possible problems, the ASTBR emerges as an attractive alternative to order the flow using a parallel organized configuration of the reactor bed (Camiloti et al., 2014; Mockaitis et al., 2014).

According to Mockaitis et al.(2014), the ASTBR is an alternative to the typical anaerobic treatment systems to optimize biomass retention in the reactor with less washout of microorganisms, maintaining high values of sludge retention time, even under low HRT. In relation to DS treatment, few studies focused on the ASTBR performance and stability. It is necessary to explore several applications of the ASTBR, in diverse operation conditions to elucidate robustness and efficiency (Cunha et al., 2020).

1.2 OUTLINE OF THE THESIS

The main objective of this thesis is to analyze different operational conditions applied to two anaerobic reactors treating DS, in terms of operational stability and trace metals profile. This thesis also aims to answer the following questions: “What is the statically significant effect of TMs on maximum methane production from DS?

Chapter 2 focuses on the evaluation of a UASB reactor treating DS, operated at four different HRTs. Also, the investigation of TMs in the influent and effluent.

Chapter 3 focuses on the identification of statistically significant TMs on the maximum methane production from DS. The statistically-based Plackett-Burman experimental design was adopted to screen 7 TMs: Barium (Ba), Co, Cu, Fe, Manganese (Mn), Ni and Se. The anaerobic granular sludge obtained in the operated UASB, presented in the previous chapter, are used in batch reactors to design an optimal dosage. The influence of Fe in a biochemical methane potential is evaluated in different concentrations.

The focuses of chapter 4 are the evaluation of an innovative anaerobic reactor configuration, the ASTBR, applied in DS treatment, operated at four different HRTs. Also, the investigation of TMs in the influent, effluent, initial and final TM content in the biomass and support material. Moreover, the results acquired in chapter 3 are used in chapter 4 in an optimal dosing strategy, i.e.step addition of the statistically significant concentrations of iron added in the last HRT operated.

Finally, chapter 5 summarizes the main results and discussions of this thesis in a general conclusion and list recommendations for future research.

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Chapter 2

ANALYSES OF TRACE METALS IN UASB REACTOR TREATING DOMESTIC SEWAGE

ABSTRACT

The present study aimed at evaluating the efficiency of a UASB reactor in removing organic matter in terms of Chemical oxygen demand (COD) in different Hydraulic retention times (HRT) treating domestic sewage (DS), verify if the reactor remains stable during the long term operation (718 days) and also, investigate the concentrations of trace metals (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn) in the influent and effluent of the UASB reactor. Four decreasing HRT were applied (12; 8; 6 and 5 hours), based in the steady states. The DS treated by the system showed a COD average concentration of 587 ± 116 mg L^{-1}, with COD removal efficiencies of: 72 ± 8 % (HRT of 12 hours), 76 ± 5 % (HRT of 8 hours), 73 ± 6 % (HRT of 6 hours), 70 ± % (HRT of 5 hours). During the operation, the UASB reactor was capable of maintain a steady conditions, with neutral PH values, and steady concentration of volatile fatty acids in the effluent. The trace metals effluent concentrations found in this study are below with the Brazilian legislation for water bodies.

2.1 INTRODUCTION

Intensives city growth, with deficient planning, and industrialization may be considered as the main causes of water bodies pollution. Among the types of pollution, water contamination is considered a serious threat to the environment because it involves several forms of waste, including the depletion and degradation of natural resources

(Moreira and Fazza, 2008). Currently, the wastewater treatment cannot be considered as an option anymore, but an obligation to industries and city halls.

The water sources have been severe polluted by several pollutants such as inorganic ions, organic pollutants, organometallic compounds and nanoparticles (Walker, 2012). Among these pollutants, metals emerged in anthropogenic activities as mining, smelting, foundries, and other industries that are metal-based, also, leaching of metals from landfills, waste dumps, excretion, livestock and chicken manure, automobiles and roadworks (Briffa, 2020).

Metals are considered as toxic because due to its chemical properties, they tend to form covalent bonds, generating toxic effects when they bind to nonmetallic elements of cellular macromolecules. They are nonbiodegradable, so they create bonds within a protein or deposit in intracellular granules (Briffa, 2020).

In anaerobic digestion, metals will be removed from the wastewaster by complex interactions among the liquid and solid phase in the reactor. The uptake of metals by microorganisms is assumed to proceed mainly via the transport of free metal ions across the cell membrane (Zandvoort, 2206).

Anaerobic treatment technology is applicable and widespread around the world, being the main secondary treatment unit in tropical countries - e.g., UASB (Upflow Anaerobic Sludge Blanket) (Chernicharo et al., 2015). Thus, anaerobic system performance for removal of metals needs to be evaluated. In this context, this study aimed at evaluating the efficiency of a UASB reactor in removing organic matter in terms of Chemical oxygen demand (COD) in different Hydraulic retention times (HRT) treating domestic sewage (DS), verify if the reactor remains stable during the long term operation (718 days) and also, investigate the concentrations of trace metals (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn) in the influent and effluent.

2.2 MATERIALS AND METHODS

2.2.1 Experimental set up

An 11.3 L acrylic UASB reactor was used in this study (Fig 1) operated for 648 days. The operation conditions were divided in four phases, with decreasing HRTs, based on the steady states (Table 1). The reactor was fed with DS collected from the São Carlos municipal sewage network, through a sewer line at the experimental facilities of São Paulo University, São Carlos, SP, Brazil, deviated to a private system compound by a storage tank (220 L.), sieve screen (0.5 mm) and the UASB reactor.

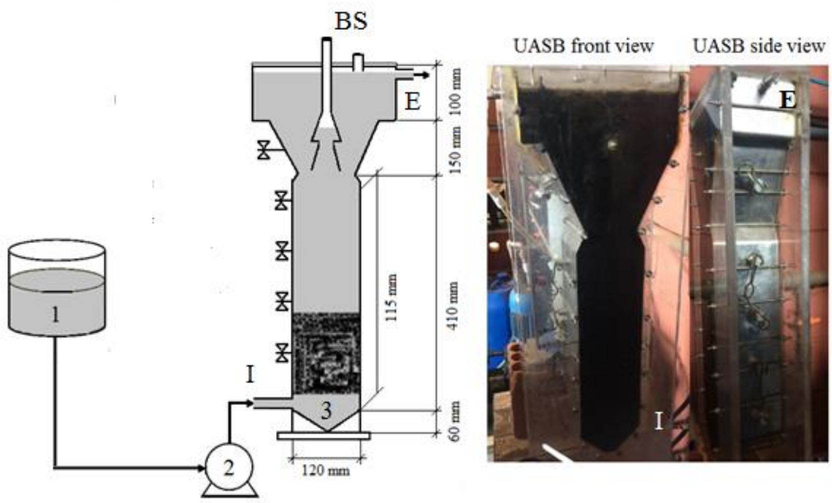

Figure 1. Layout of the experimental facilities: Legend: 1 – raw domestic sewage (storage tank - 220 L.), 2 – pump, 3 – UASB reactor (11.3 L.); BS - Biogas exits; I - Influent; E - Effluent.

Table 1. Operational conditions of the UASB reactor

Phase	Period (days)	Flow (L h^{-1})	HRT (hours)	Liquid temperature (°C)	
				Influent	Effluent
1	123	0.94	12	26 ± 2	28 ± 2
2	181	1.41	8	28 ± 3	29 ± 2
3	193	1.88	6	27 ± 6	29 ± 5
4	201	2.26	5	29 ± 2	28 ± 2

2.2.2 Inoculation and start-up procedures

The inoculum (32.8 gTVS L^{-1}) used in this study was acquired from real scale UASB reactor, located in poultry slaughterhouse (Dacar S.A., Tietê, SP, Brazil), used in the treatment of poultry processing wastewater.

For the inoculation, 3.76 L of inoculum (1/3 of the reactor's volume) was used, left to rest inside the reactor for 24 hours. After this, the reactor was filled with the DS, remaining at rest for 12 h, then the continuous feeding started with an HRT of 12 h. Procedures were based in Chernicharo, (2007).

2.2.3 Analytical methods

The system was evaluated in terms of operational stability of the physicochemical parameters, organic matter removal and metals concentration, in a long-term operation (718 days). The samples were analyzed by Chemical Oxygen Demand (COD), Total suspended solids (TSS), pH (APHA et al., 2005); Alkalinity (Partial and Intermediate - PA and IA) (Ripley et al., 1986) and Volatile fatty acids (VFA) (Dilallo and Albertson 1961).

The trace metals concentration in DS (influent and effluent) were divided in wet and dry seasons. It was considered dry season from March to July 2017 (HRT of 12 hours) and January to July of 2018 (HRT of 6 hours). The wet season was from August to December of 2018 (HRT of 8 hours) and September to March of 2019 (HRT of 5 hours). These classifications were based on São Carlos's forecast and average precipitation. (World Weather & Climate Information, 2018).

The samples were initially digested in a microwave (Four Speed, Berghof GmbH, Germany) to remove any residual solids, filtered (Whatman® 589/1, Sigma-Aldrich, MO, USA), and diluted to 25 mL in volumetric flasks. After, the samples were analyzed by

Inductively coupled plasma optical emission spectrometry - ICP/OES (Optima 8000, Perkin Elmer, USA) (Braga et al., 2017). An internal standard (yttrium at 1 mg L^{-1}) was added as an internal standard (Y 324.227 axial) and the trace metals analyzed were: Al (308.215 nm radial), Ba (233.527 nm axial), Cd (226.502 nm axial), Co (228.616 nm axial), Cr (267.716 nm axial), Cu (324.752 nm axial), Fe (238.204 nm radial), Mn (257.610 nm axial), Ni (231.604 nm axial), Pb (217.000 nm axial), Se (196.026 nm axial) and Zn (206.205 nm axial). The digestion and ICP analysis were based in Braga et al. (2017).

2.2.4 Statistical Analyses

Statistical analyses were used to compared the results of COD removal efficiency (%) through the different HRT and trace metals through seasons.

COD removal efficiency, among the different HRT operated, were tested for normality using the Kolmogorov-Smirnov test (K-S). Outliers and extremes were not found among the data. The parameters were statically analyzed using the One way-ANOVA, followed by the Tuckey HSD (honestly significant difference), verify whether the HRT had a significant statistical effect on COD removal efficiency at a 90% confidence level, using the software Statistica 10 (Stat Soft, Inc., Tulsa, USA).

2.3 RESULTS AND DISCUSSION

2.3.1 Performance and stability assessment of the UASB reactor

The temporal profiles of COD (influent and effluent), COD removal efficiency; TSS (influent and effluent) are presented in Figure 2.

The COD average values of the influent, per HRT, were: 630 ± 126 mg L^{-1} (HRT of 12 hours), 621 ± 76 mg L^{-1} (HRT of 8 hours), 525 ± 82 mg L^{-1} (HRT of 6 hours) and 583 ± 86 mg L^{-1} (HRT of 5 hours). The DS used to feed the reactor was considered as medium strength according to Metcalf and Eddy Inc., (2003).

Even with the considerable oscillation in COD influent concentrations, the UASB reactor maintained steady concentrations in the effluent during most of the operation time.

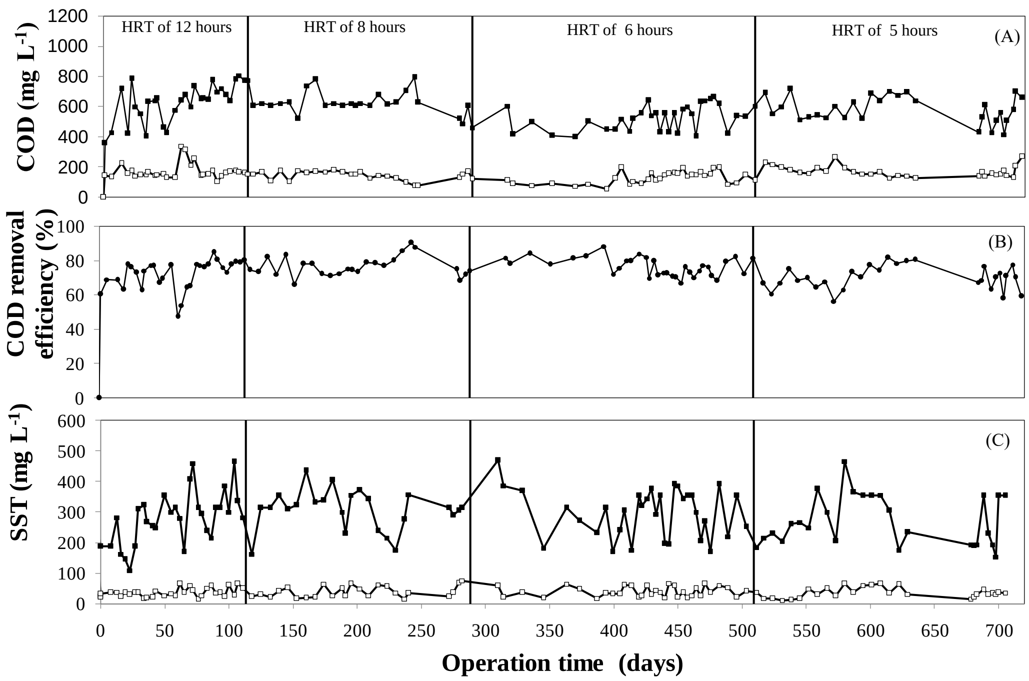

Figure 2. Temporal profiles of COD (mg L^{-1}) (A), COD removal efficiency (%) (B), TSS (mg L^{-1}) (C). Legend: ▪ Influent; ▫ Effluent.

Typical COD removal efficiencies for UASB reactors treating DS are between 60–70% (Chernicharo, 2007). During the start-up period (HRT of 12 h) the COD removal efficiency remained around 65% until de 20th day of operation, increasing to 77% on the 22^{sd} day, and keeping an average value of 75 ± 6% until the end of HRT of 12 hours, indicating a stable operation.

However, among days 65 to 70, the COD removal efficiency decreased to 64%, with efficiency recovery on the 76th day, with COD removal efficiency value of 80%.

These decrease influenced the COD effluent concentration, which increased from 131 mg L^{-1} to 313 mg L^{-1}.

Oscillations in the COD removal efficiency during start-up period is awaited. The start-up may be considered as the most critical step in the operation of anaerobic digestion processes (Ike et al., 2010) as the biomass is undergoing adaptations and formation of new community. The sludge used as inoculum was adapted for an high strength wastewater, with a COD concentration around 4000 mg L^{-1} (poultry slaughterhouse), which is very different from the COD present in the DS (587 ± 116 mg L^{-1}) used in this study, which may contributed for the variations on COD removal efficiencies in the HRT of 12 hours. During the HRT of 8 and 6, no abrupt changes were observed in the COD removal efficiency, showing average values of 76 ± 5 % (HRT of 8 hours) and 74 ± 6 % (HRT of 6 hours).

In relation to the HRT of 5 hours, after the HRT change, during the 509th and 517th days, the COD removal efficiency decreased from 81% to 60%. During the same period, the COD effluent concentration increased from 111 to 230 mg $L^{-1.}$

This decrease of efficiency may be related to the HRT change (6 to 5 hours), which may affect the system. It could be related to the COD influent concentration increase (600 to 690 mg L^{-1}), however this oscillation was considered mild compared to the TDHs in general. Statistically, the normally distributed data showed a significant difference on HRT of 12 hours.

Considering the SST, the average values of the effluent, per HRT, were: 34 ± 13 mg L^{-1} (HRT of 12 hours), 38 ± 17 mg L^{-1} (HRT of 8 hours), 39 ± 17 mg L^{-1} (HRT of 6 hours) and 35 ± 16 mg L^{-1} (HRT of 5 hours). Compared with a general average concentration in the influent of 286 ± 86 mg L^{-1}, it is possible to affirm that the UASB reactor retained solids or part was digested by the system, as a high fraction of the solids are organic particles (Chernicharo, 2015).

One of the main features of UASB reactors is their great capacity for biomass retention when operated under suitable operating conditions (Chernicharo, 2015), resulting in low concentrations in the effluent and a greater degree of sludge stabilization.

Table 2. Average concentrations in the affluent and effluent of the UASB reactor for pH, Volatile Fatty Acids (VFA*)* and PA/IA ratio.

HRT (hours)	**pH**		**VFA (mg L^{-1})**		**PA/IA**
	Influent	**Effluent**	**Influent**	**Effluent**	**Effluent**
12	7.1± 0.1	7.2 ± 0.1	42 ± 9	19 ± 7	0.30 ± 0.13
8	7.2 ± 0.1	7.4 ± 0.1	41 ± 8	18 ± 9	0.34 ± 0.09
6	7.1 ± 0.2	7.1 ± 0.2	50 ± 14	24 ± 13	0.31 ± 0.12
5	7.1± 0.1	7.2 ± 0.1	46 ± 6	21 ± 8	0.28 ± 0.12

The UASB reactor presented low variability of the monitoring parameters (Tab. 2). The average value of the IA/PA ratio at the effluent was around 0.3 in all HRT, considered as stable, with no disturbances in the process (Ripley et al., 1986; Foresti, 1994).

The pH values in each HRT (Table 2) indicated that the influent values were in the neutral range and optimal for the methanogens (6.8 and 7.2) (Mudrak and Kunst, 1986). The UASB reactor maintained the stable values in the effluent, during the operation.

The VFA values lower in the effluent (Tab. 2) demonstrate a consumption of acids, linked to the stability of the pH values.

2.3.2 Trace metals assessment of the UASB reactor

The average concentration of trace metals in both influent and effluent, divided in dry and wet seasons are presented in Table 3.

Table 3. Average concentrations of trace metals in influent and effluent (dry and wet seasons)

Samples			**Wet season (mg L^{-1})**			
	Al	Ba	Fe	Mn	Pb	Zn
Influent	0.95 ± 0.55	0.08 ± 0.03	0.99 ± 0.21	0.09 ± 0.03	0.06 ± 0.01	0.22 ± 0.03
Effluent	0.43 ± 0.26	0.04 ± 0.02	0.46 ± 0.29	0.05 ± 0.06	0.04 ± 0.04	0.09 ± 0.04
			Dry season (mg L^{-1})			
	Al	Ba	Fe	Mn	Pb	Zn
Influent	1.29 ± 0.60	0.11 ± 0.04	1.29 ± 0.65	0.12 ± 0.05	0.09 ± 0.04	0.31 ± 0.10
Effluent	0.66 ± 0.16	0.06 ± 0.02	0.71 ± 0.29	0.06 ± 0.06	0.05 ± 0.04	0.14 ± 0.04

The current results showed that trace metals Cd, Cr, Cu, Ni and Se were not detected in the DS samples. It is observed that the concentrations in wet season are lower than the wet season, what is expected. Also, that the trace metals concentration in the effluent is smaller than those in the influent, indicating that the UASB reactor promoted the reduction of its levels, probably by retention by the sludge.

The metals presented in the reactor have to be retained by the sludge in order to prevent losses with the effluent and to be available for the biomass in the granules . The main mechanisms involved different mechanisms such ion exchange, adsorption, inorganic microprecipitation (Zandvoort et al., 2006). Studies about the concentrations in the sludge are necessary.

Similar results about the concentrations in DS were discuss by Pipi et al. (2018), analyzing samples of raw and treated DS, from two treatment plants, Candeia (Bauru, São Paulo, Brazil) and Tibiriça (Tibiriça, São Paulo, Brazil), with no season divisions.

The effluent concentrations found in this study are in agreement with the Brazilian Resolution CONAMA no. 430, 2011 (National Environmental Council, 2011), which sets standards maximum metals values allowed for effluent discharge.

2.4 CONCLUSIONS

The UASB reactor demonstrate robustness for treatment of domestic sewage, even with variations in the influent COD and TSS concentrations. It was capable of maintain a steady operation, with levelheaded COD removal efficiency, being statistically different only in the HRT of 12 hours, correlated to the start-up period. Among the long-term operation, the UASB reactor reached COD removal efficiencies higher than 70%.

The trace metals effluent concentrations found in this study are below with the Brazilian legislation for water bodies.

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Chapter 3

EVALUATION OF THE INFLUENCE OF TRACE METALS ON METHANE PRODUCTION FROM DOMESTIC SEWAGE, USING THE PLACKETT-BURMAN EXPERIMENTAL DESIGN

A modified version of this chapter has been published as: Silva, J A.; Braga, A. F. M., Fermoso, F. G.; Zaiat, M.; Silva, G. H. R. (2021) Evaluation of the influence of trace metals on methane production from domestic sewage, using The Plackett-Burman experimental desing, Journal of Environmetal Management, 294.

ABSTRACT

Methanogenesis involves several enzymes with trace metal components that catalyze major metabolic pathways and, therefore, requires a sufficient supply of micronutrients. The statistically-based Plackett-Burman experimental design was adopted in this study to identify which trace metal have a statistically significant effect on the maximum methane production from domestic sewage (DS). The addition of Barium (Ba), Cobalt (Co), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni) and Selenium (Se) was tested in batch reactors using DS as the substrate and sewage sludge as the inoculum. The results showed that the addition of Fe was statistically significant, positively affecting the maximum CH_4 production (*p-value* 0.05). The results are expressed in L of CH_4 per g of Chemical Oxygen Demand added, increasing it from 0.10 to 0.13 LCH_4 $gCOD_{add}^{-1}$. In L of CH_4 per g of Volatile Solids, increased it from 0.031 to 0.040 LCH_4 gVS^{-1}. The addition of Se was statistically significant, but with a negative effect on the maximum methane production (*p-value* 0.002), which decreased from 0.13 to 0.10 LCH_4 $gCOD_{add}^{-1}$. Moreover, six Fe concentrations (0, 40, 80, 120, 160 and 200 mg L^{-1}) were tested, showing that the addition of 120 mg L^{-1} had the greatest effect for statistically improving the maximum methane production, with 33% improvement (0.12 ± 0.03 to 0.16 ± 0.01LCH_4 $gCOD_{add}^{-1}$) compared to no addition of Fe and the specific CH_4 production to 0.040 ± 0.001 LCH_4. gVS^{-1}.

3.1 INTRODUCTION

Anaerobic digestion (AD) is a set of processes in which several types of microorganisms decompose biodegradable organic matter into its final products (Chernicharo, 2007) in the absence of oxygen gas. During the AD process, microorganisms break down the organic matter into biogas, a mixture of mainly CH_4 and CO_2 (Bachmann, 2015). CH_4 is released in the last step of the process, called methanogenesis. AD has been proven as a suitable technology for the treatment of several types of wastewaters, including domestic sewage (DS), applied through high-rate anaerobic reactors. Among the different kind of high-rate anaerobic reactors, such as the up-flow anaerobic sludge bed reactor (UASB), expanded granular sludge bed (EGSB) reactor, anaerobic baffled reactor (ABR), fixed-film fluidized bed reactor and the expanded bed reactor, the UASB stands out as a very important system for DS treatment, in tropical regions (Van Haandel et al., 2006; Santos et al., 2016) and as the most widely applied system worldwide (Chong et al., 2012)

Beyond the removal organic matter and pollutants, anaerobic wastewater treatment may provide nutrients for fertilizers, treated wastewater for reuse, acids for industry and biogas (mainly CH_4) for energy generation, that also allow renewable energy generation. The CH_4 obtained through the metabolism of methanogens is responsible for 71% of the total global atmospheric CH_4. When captured, CH_4 can be used as a fuel source (Holmes and Smith, 2016), promoting a sustainable cycle of energy production-consumption in domestic sewage treatment plants, thus reducing CH_4 emissions into the atmosphere.

Brazil has the largest number of installed UASB reactors treating sewage in the world (Passos et al., 2020); however, very few use CH_4 recovery. Thus, they don't use the environmental aid full potential of the anaerobic treatment systems as a biogas energy source. Energy recovery from sewage treatment in Brazil is still an incipient activity

(Lopes et al., 2019). Biogas is usually not recovery due to the low CH_4 concentration in the biogas and the low volume produced, related to inadequate performance and sewage characteristics.

According to Lopes et al. (2019), most UASB reactors in Brazil are concentrated in the States of Minas Gerais, São Paulo, and Paraná. Only Parana State has 182 sewage treatment plants based on UASB reactor, with no biogas energy recovery. The authors related that, in theoretical calculations, these STPs have a biogas energy potential varying from 3 GJ d^{-1} to 380 GJ d^{-1}. Considering a conversion yield of 30% by the power generator, it could produce 677 MWh d^{-1} of electricity using biogas as source, which is enough to supply the energy demand of a city around 111.000 inhabitants, approximately.

It is known that for the process of AD to occur properly, with the consequent efficient production of CH_4, it is necessary to maintain steady physical-chemical conditions, such as neutral pH, adequate temperature and the presence, in optimal concentrations, of organic matter and macro and micronutrients.

The nutritional conditions required by microorganisms that digest organic matter in anaerobic systems play an important role in the process, as they interfere with their growth speed, direct the metabolic routes and, consequently, determine the efficiency of conversion of organic matter and methane production. In addition to macronutrients such as C, N, P, S, K, and Mg anaerobic microorganisms require small concentrations of trace metals (MT), which consist of essential components of cofactors and enzymes of anaerobic metabolism (Speece et al., 1983; Thanh et al., 2016; Fermoso et al., 2019).

Proper TM supplementation for digesters can be an option for increase CH_4 production. TM supplementation in AD has been studied for almost 40 years (Roussel et al., 2018), and it is known that TMs are part of the structure of the enzymes that direct anaerobic metabolism (Oleszkiewicz and Sharma, 1990). Fundamental TMs for methanogenesis and CH_4 oxidation include Fe, Ni, Co, Mo, W and Zn (Glass and Orphan, 2012).

Co can be considered one of the most crucial TMs for AD due to its participation in acetogenesis and methanogenesis (Fermoso et al., 2008). Ni is also essential because it is a constituent of the enzyme F430, exclusively found in methanogens (Pobeheim et al., 2011). Fe is an essential TM in AD, crucial in acetrotrophic pathways, acting on acetate oxidation (Ho et al., 2014). Cu is crucial in acidogenesis, being related to the hydrogenases of facultative anaerobic microorganisms (Oleszkiewicz and Sharma, 1990). Mn acts as a stabilizer of the methyltransferase in methanogenesis (Oleszkiewicz and Sharma, 1990) and as an electron donor for methane formation from CO_2 (Qiao et al., 2015). Like Cu, Se is also related to hydrogenases (Oleszkiewicz and Sharma, 1990) and, according to Yirong et al., (2015), also participates in propionate oxidation. Regarding Ba, even if it is not considered to be an essential TM for AD (Wyman et al., 2019), those authors have reported that it enhances enzymatic hydrolysis (Muñoz et al., 2016). Therefore, a deficient concentration of any of these TMs may significantly limit AD, reducing the CH_4 yield (Ortner et al., 2015). On the other hand, the excess may also inhibit the microorganisms involved in anaerobic digestion processes.

The positive impact of TMs on AD systems had been shown by several studies, and it was reported that productivity, process stability and CH_4 rates were significantly improved (Ortner et al., 2015). Evranos and Demirel (2015) studied the impact of TM supplementation, namely Ni, Co and Mo, on CH_4 yield from a batch of mesophilic AD of maize silage. The authors used 0.5 mg L^{-1}, 0.5 mg L^{-1} and 0.25 mg L^{-1} of Co, Ni and Mo, respectively, and obtained the highest CH_4 yield of 0.429 L CH_4 gVolatile $Solids_{added}^{-1}$. Pinto-Ibieta et al. (2016) used Co to improve CH_4 production of the anaerobic digestion of olive mill solid waste. The authors reached 23% of improvement in CH_4 production and 30% in CH_4 yield, using a concentration range of 0.018 to 0.035 mg L^{-1}.

The Design of Experiments (DOE) is a statistical tool applied in studies where it is necessary to verify the effect of several factors. Unlike the one-factor-at-a-time design, which is traditionally used and investigates one factor in each experimental round while the others remain constant, the multiple-factor DOE (factorial design) allows researchers to delineate the interactions between different factors (Wang and Wan, 2009). Factorial designs enable researchers to work with many factors, notably the fractional factorial designs (FFD), an alternative for studies where the number of runs is not workable (Wang and Wan, 2009). A fractional factorial design considers the effects of individual factors on a specific response, and can be studied under economic and practical conditions (Luftig and Jordan, 1998). The particular saturated FFD described by Plackett and Burman (1946)is the concomitant investigation of multiple factors with a minimum number (n) of experiments, up ton=48. It runs the behavior of several factors on the parameter of interest, which is then considered as the response, to determine the factors that have a statistically significant influence on the performance of a given process. This approach is based on a matrix of two design levels, a high level (+1) and a low level (-1), where several main effects can be assessed at once.

The FFD was used by Jiang et al. (2017) for Co, Ni, Mo, Se, Fe and W. The FFD helped these authors to identify selenium as a key TM in promoting the degradation rates of both acetic and propionic acids, and nickel as inhibitor of the degradation of Volatile Fatty Acids (VFA), possibly due to its toxicity. Ezebuiro et al. (2018) used a factorial experimental design to test Ni, Co, Se, Mo and VFA, focusing on investigating interactions in the AD of maize silage. Using the FFD, these authors determined synergistic interactions between metals for the VFA degradation rate. The present study aimed to screen the effects of the addition of Barium (Ba), Cobalt (Co), Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni), and Selenium (Se) in the process of biochemical CH_4 production (BMP) on the AD of DS, using a fractional factorial experimental design and thus, verify if there is an improvement in the production of CH_4 for the purposes of energy production in sewage treatment systems.

3.2 MATERIALS AND METHODS

3.2.1 Inoculum and substrate source

To assemble the experiment, raw DS samples were collected from a sewer line built on Campus 2 of the University of São Paulo (São Carlos, SP, Brazil), which deviated part of the flow from the São Carlos municipal sewage network. The DS was used as substrate in the experiment, considerate as feedstock. As source of inoculum, sewage sludge sample was collected from a bench-scale UASB reactor, installed in the same campus.

The UASB (11.3 L), a bench-scale reactor, had been operating for 380 days, with a 6-hour HRT. It was operated as a source of sludge (inoculum) and to this, was start-up with a 12-hour HRT for 123 days, reducing to 8 hours for 189 days and then to 6 hours for 165 days, treating DS from the São Carlos municipal sewage network. The sewage sludge sample was stored at 4°C until the experiment was assembled. Since the inoculum remained in contact with the substrate for a long period (380 days), it was fully adapted to the substrate. The physical-chemical (Organic Matter in terms of total Chemical oxygen demand (tCOD); pH; Total and Volatile Solids (TS; TVS); Total and Volatile Suspended

Solis (TSS; VSS)) and TMs characterizations from DS and sewage sludge samples are described in Table 1.

Table 1. Characterization of the DS and sewage sludge (inoculum) samples used in the BMP assays.

Parameter	Raw DS		Sewage sludge
	Sample 1	**Sample 2**	
tCOD (mg $L^{-1)}$	635 ± 3	335 ± 4	-
pH	7.28 ± 0.04	7.25 ± 0.05	-
VFA (mg L^{-1})	61.9 ± 0.1	57.5 ± 0.2	-
TSS (mg L^{-1})	215 ± 32	271 ± 98	-
VSS (mg L^{-1})	155 ± 27	191 ± 13	-
TS (g L^{-1})	-	-	23 ± 3
TVS (g L^{-1})	-	-	14.9 ± 0.10
Al[a]	0.80 ± 0.55	1.07 ± 0.45	8.73 ± 1.80
As[a]	n.d. [b]	n.d. [b]	n.d. [b]
Ba[a]	0.01 ± 0.01	0.01 ± 0.10	0.002 ± 0.420
Cd[a]	n.d. [b]	n.d. [b]	n.d. [b]
Co[a]	0	0.01 ± 0.21	0.005 ± 0.000
Cr[a]	n.d. [b]	n.d. [b]	0.08 ± 0.05
Cu[a]	0.01 ± 0.03	0.02 ± 0.09	0.51 ± 0.32
Fe[a]	0.71 ± 0.05	0.80 ± 0.08	10.4 ± 2.0
K[a]	0.25 ± 0.05	0.27 ± 0.06	2.22 ± 0.90
Mg[a]	0.04 ± 0.01	0.05 ± 0.01	5.61 ± 1.91
Mn[a]	0	0.01 ± 0.01	0.97 ± 0.04
Ni[a]	0	0	0.02 ± 0.03
Pb[a]	0.001 ± 0.002	0.001 ± 0.001	0.11 ± 0.09
Se[a]	0.01 ± 0.05	0.01 ± 0.02	0.02 ± 0.04
Zn[a]	n.d. [b]	n.d. [b]	3.3 ± 2.5

[a]Sewage sludge in m ggTS^{-1} and raw DS in mg L^{-1}; [b] n.d.: not-detected

3.2.2 Biochemical methane potential experiment set up

The Biochemical Methane Potential (BMP) approach was used to evaluate the effect of TMs on CH_4 production from domestic sewage through two different assays. The first BMP assay was used to screen metals with significant effects on CH_4 production. The Placket-Burman fractional factorial experimental design (Plackett. and Burman, 1946) was used to outline the BMP assay (BMP-PB). A second BMP assay was carried out to determine the concentration range of the metal identified in the BMP-PB assay which had the highest CH_4 production. According to the response obtained in the first BMP-PB assay, six different Fe concentrations were evaluated in this second BMP (BMP-Fe), as follows: 0, 40, 80, 120, 160 and 200 mg L^{-1}.

The BMP-PB experiment was set up using 100 mL glass bottles, in duplicate. Of this 100 mL, a working volume of 58 mL was filled with a mix of DS (45 mL), sludge (6.1 mL), a solution of TMs with different concentrations established by the PB matrix (varying volume) and ultrapure water (Milli-Q, Merck-Millipore, USA) (complementary volume), with the remaining volume of 42 mL of filled with head space. The working volume was calculated for each bottle to ensure that all bottles had the same COD. The BMP-Fe experiment was set up using 100 mL glass bottles, in triplicate. Of this 100 mL, a working volume of 58 mL was filled with DS (43 mL), sludge (3.2 mL), Fe solution varying from 0 to 5mL and ultrapure water (complementary volume). The remaining volume of 42 mL of filled with head space. Calculations for adding the inoculum were based on a TVS : tCOD ratio of approximately 3.3:1. These procedures were based on Braga et al.(2018).

After the assembly of the bottles´ contents, but before they were sealed, they were flushed with an Ar/CO_2 gas mixture (80/20) for 3 min to remove any residual oxygen inside them. Both BPM assays were maintained in a chamber under a controlled temperature (31.6±0.9ºC), and manual shaking was applied before measuring the pressure inside the bottles. A set of duplicates without adding any metal solution was used as a control group (CG).

The selection of the chamber's temperature was based on the average temperature of São Carlos city, where the experiment was carried out. During the test run, daily, the temperature of the chamber was checked, obtaining the average value.

3.2.3 Analytical methods

The analyses of TS, TVS, COD and pH were performed according to APHA et al.(2005). VFA analysis was performed according to Ripley et al. (1986). The digestion of samples and TM analysis followed the methodology used by Braga et al.(2017).

The biogas production was monitored through the pressure in the bottles, and the composition was determined using a gas chromatograph (Shimadzu GC 2010, Kyoto, Japan). The procedures and equipment for evaluating the biogas production of each BMP were based on Braga et al.(2018).

3.2.4 Kinetic and statistical analysis

The BMP results were estimated by adjusting the accumulated CH_4 production to the modified non-linear Gompertz equation (Equation 1) (Zwietering et al., 1990)

$$B = P * exp\left\{-exp\left[\frac{Rm * e}{P}(L - t) + 1\right]\right\} \quad \text{(Eq. 1)}$$

where:

B = cumulative methane production over a given digestion time *t* ($LCH_4\ gCOD_{add}^{-1}$);

P = maximum CH_4 production ($LCH_4\ gCOD_{add}^{-1}$);

Rm = production velocity ($LCH_4\ d^{-1}$);

t = cumulative time for methane production (days);

L= lag phase period or minimum time to initiate methane production (days).

Through adjustment to the Gompertz equation, the response P – maximum CH_4 production ($LCH_4\ gCOD_{add}^{-1}$) was used to assess the TM's effect on the AD of DS.

The response obtained with the BMP-PB assays was included in the PB matrix to fit a linear equation as a function of the factors and then the ANOVA test was performed, determining significant factors at a 90% confidence level.

For the BMP-Fe assay response, the adjustment using the Gompertz equation was applied to calculated P, using the cumulative methane production over a given digestion time *t* (LCH_4 $gCOD_{add}^{-1}$), however, it was also used to adjust the specific CH_4 production over a given digestion time *t* (LCH_4 gVS). The maximum CH_4 production (LCH_4 $gCOD_{add}^{-1}$), specific CH_4 (LCH_4 gVS), the CH_4 proportion in the biogas, mean final COD concentrations and removal efficiency and pH were statically processed using the one-way ANOVA followed by Tukey's HSD, considering a 95% confidence level among the concentrations studied. All tests were made using the Statistica 10 software (Stat Soft, Inc., Tulsa, USA).

The seven TMs evaluated in the screening were added to the BMP in different concentrations, according to the matrix following the PB design, built using the Statistica 10 software (Stat Soft, Inc., Tulsa, USA). The PB matrix (Table 2) was built using the highest level of concentration.

Table 2. PB matrix for high level metals addition of the seven factors screened for the BMP-PB assay.

Conditions	**Concentration added to the bottles (mgL^{-1})**						
	Ba	**Co**	**Cu**	**Fe**	**Mn**	**Ni**	**Se**
1	0.00	0.00	0.00	15.00	0.27	0.29	0.00
2	0.00	0.00	1.00	15.00	0.00	0.00	1.58
3	0.00	0.29	0.00	0.00	0.27	0.00	1.58
4	0.00	0.29	1.00	0.00	0.00	0.29	0.00
Center Point	343.30	0.14	0.50	7.50	0.13	0.14	0.79
5	686.60	0.00	0.00	0.00	0.00	0.29	1.58
6	686.60	0.00	1.00	0.00	0.27	0.00	0.00
7	686.60	0.29	0.00	15.00	0.00	0.00	0.00
8	686.60	0.29	1.00	15.00	0.27	0.29	1.58

The metals concentration at the high level was based on the range adopted by Braga et al. (2018), which used as substrate simulated black water, i.e., mixture of DS slaughterhouse effluent. As the use of DS as a substrate for supplementation in the production of methane is scarce, Braga et al. (2018) was chose due to the use of a substrate more similar to DS, however with the following adaptations in the concentrations: the high levels used for Ba and Mn were the center points defined by Braga et al. (2018), as they have a negative effect on the methanogens present in the inoculum. The high levels for Cu and Fe adopted a concentration 20% higher than the sludge and raw DS (S+RS) because the concentrations used in Braga et al. (2018) were much lower than those found in the S+RS. The low level had no external metal addition, and the only source of metals was the concentration measured in the S+RS itself (Table 1). A center point was also added to include a curvature factor to the linear equation used to fit the response in function of the factors, thus permitting verification of whether one or more factors produced a maximum response within the range tested.

3.3 RESULTS AND DISCUSSION

3.3.1 Screening of the effect of metals on P (maximum CH_4 production) – BMP-PB

The BMP-PB assays were carried out for 34 days until the CH_4 accumulate production stabilized. The final mean pH and COD values were 7.21 ± 0.14 mg L^{-1} and 146 ± 2 mg L^{-1}, respectively. The mean accumulated CH_4 production was 0.11 ± 0.04 LCH_4 $gCOD_{add}^{-1}$.

The effect of the TMs supplementation on CH_4 production kinetics was tested to Lag phase duration, production velocity and maximum production, however only the maximum production showed a significant effect, with an adequate adjustment (R^2 = 0.99; R_{adj} = 0.98).

According to the statistical analysis of the PB design, the effect of the metals Co (*p-value* 0.31), N (*p-value* 0.34), Cu (*p-value* 0.51) and Ba (*p-value* 0.39) was not statistically significant on P of the UASB sludge using raw DS as the substrate (Figure 1). Mn (*p-value* 0.07) had a response close to the 0.05 significance level. Fe was statistically significant (*p-value* 0.01), with a positive effect on P of the UASB sludge, and Se was statistically significant (*p-value* 0.002), although with a negative effect on P (Figure 1).

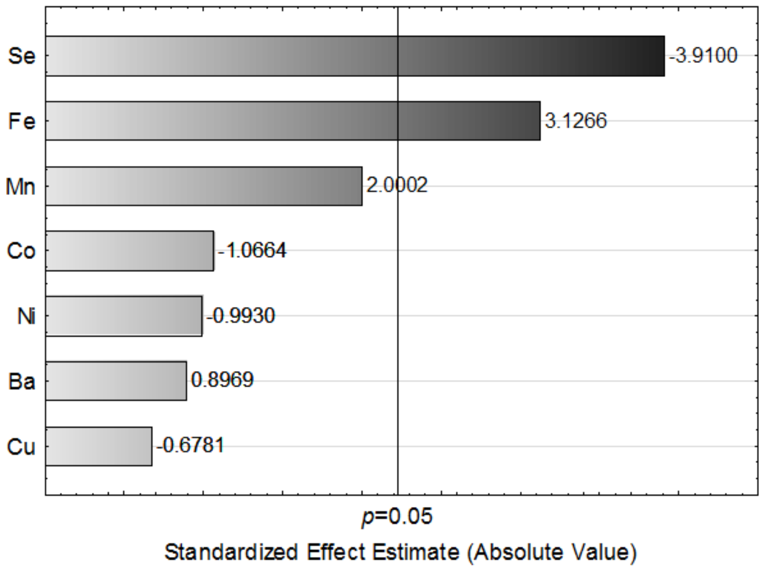

Figure 1. Pareto chart of standardized effects of seven screened metals affecting P in the BMP-PB assay.

Considering the TM concentrations found in the inoculum and DS (Table 1), plus the additional supplementations (Table 2) used in the present study, Co, Ni, Cu and Ba were evaluated at the concentration ranges from 0.012 to 0.302 mg L^{-1}, 0.066 to 0.356 mg L^{-1}, 1.249 to 2.249 mg L^{-1} and 0.008 to 686.6 mg L^{-1}, respectively. The lack of statistical significance for these four metals in our study indicates that no inhibition or improvement of CH_4 production occurred in the concentrations studied. To the best of our knowledge, the literature on the addition of TMs affecting methanogenesis using substrates with the organic load and characteristics similar to DS – as used in this study – is limited.

Braga et al. (2018) reported that Co (0.03-0.62 mg L^{-1}) did not show a significant effect on CH_4 production from simulated blackwater. However, Pinto-Ibieta et al.(2016) observed positive effects in the range from 0.24 to 0.65 mg L^{-1} using olive mill solid waste (OMSW) as the substrate. The maximum Co concentration used in this study (0.296 mg L^{-1}) was similar to the minimum concentration range studied by Pinto-Ibieta et al. (2016), which might explain its lack of significance.

Experiments using sewage sludge from UASB as the inoculum demonstrated that the minimum Ni requirement to support the successful CH_4 fermentation of acetate (30 g L^{-1}) was 0.2 mg L^{-1} (Takashima and Speece, 1989). However, Paulo et al.(2017) found no effect of Ni on acetoclastic or hydrogenotrophic activity when testing at concentrations of 0.117, 0.235 and 0.470 mg L^{-1}. Their results suggest that Ni supplementation may not be required for either methanogenesis or in previous AD stages using DS as a substrate.

Muñoz et al. (2016) reported that Ba supplementation enhanced enzymatic hydrolysis, which may indirectly benefit methanogenesis. However, Wyman et al.(2019) showed that Ba might not be considered an essential micronutrient for AD. Their assays tested the impact of Ba supplementations on pure and complex substrates, ranging between 2 and 2000 mg L^{-1} in each AD stage. The authors inferred that the addition of Ba affected the hydrolysis, but did not affect the following steps of AD.

Regarding Cu supplementation, the tested concentrations (from 1.249 to 2.249 mg L^{-1}) did not statistically affect CH_4 production. However, according to Yenigün et al. (1996), the inhibition of the acidogenic phase during glucose AD already occurs with a concentration of 1 mg L^{-1}, which can indirectly affect CH_4 production. For this study, it was found that the concentration added and the substrate used may influence methanogenesis and the ideal Cu concentrations when analyzing the acidogenic stage.

Mn was not considered significant (*p-value* 0.07) at concentrations between 2.361 and 2.631 mg L^{-1}; however, this result indicates that Mn deserves more attention in CH_4 production from sewage. Cai et al.(2017) obtained improvement in CH_4 yield from rice straw by supplementation of 0.5 mg L^{-1} of Mn, which increased the VFA degradation and may be beneficial to CH_4 production. In this case, further studies of ideal concentrations are needed.

Fe was tested in concentrations ranging from 25.969 to 40.969 mg L^{-1}, and showed a positive effect with statistical significance (*p-value* 0.05). According to Glass and Orphan (2012), methanogens have expressive Fe requirements, more than in the case of other TMs. Choong et al. (2016) cited in their review that Fe is the most common TM studied in AD supplementations. Besides, Fe acts in promoting CH_4 production through the utilization of VFAs and H_2 in the mesophilic environment (Sai Ram et al., 2000). One crucial fact is that the method of Fe supplementation can increase the propionate conversion rate (Meng et al., 2013). This fact is fundamental because propionate and butyrate, to a greater degree than other VFAs, are precursors of acetate used in methanogenesis (Choong et al., 2016). After adjusting the linear model of the response (P) as a function of Fe (R^2 = 0.88; R_{adj}= 0.81), and considering Fe supplementation (Table 2), it was estimated that P increased from approximately 0.10 to 0.13 LCH_4 $gCOD_{add}^{-1}$ under -1 and +1 conditions (Fig. 2).

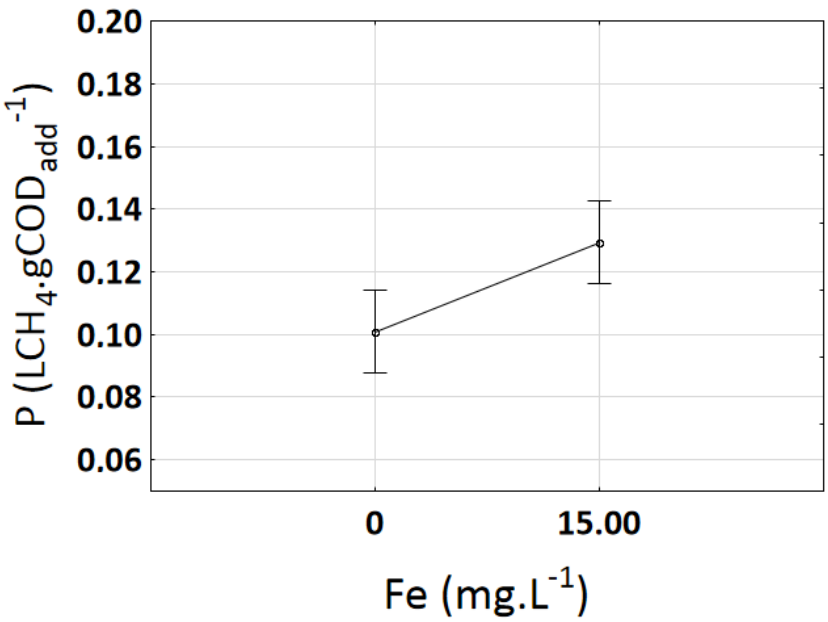

Figure 2. Plot of marginal means of Fe considering the supplementation (level -1 and +1) of the PB design affecting P.

Se was evaluated in this study in the concentration range from 0.059 to 1.639 mgL^{-1}, with a negative effect on P, indicating that the addition reached the level of inhibition. Ariunbaatar et al. (2016) reported an optimal Se concentration for AD of foodwaste in the range of 0.025 to 0.050mgL^{-1}, enhancing BMP by more than 30%. Cai et al. (2018) studied the effects of Mo, Se and Mn on the AD of rice straw. Focusing on Se, these authors added concentrations of 0.001, 0.01, 0.1, 1, 10, and 100 mgL^{-1}, and obtained a significant improvement in CH_4 production with 0.1 mgL^{-1} of Se, followed by the gradual decrease in response with higher Se concentrations, reaching toxic effects at 100 mgL^{-1}. These previous studies corroborate the negative effect observed in the present study for Se by analyzing the metal´s effect through the application of PB to BMP assays.

The response (P) adjusted to the non-linear model, taking into account the Se supplementations (Table 2), showed a decrease from approximately 0.13 to 0.10 $LCH_4gCOD_{add}^{-1}$, under -1 and +1 conditions (Fig. 3).

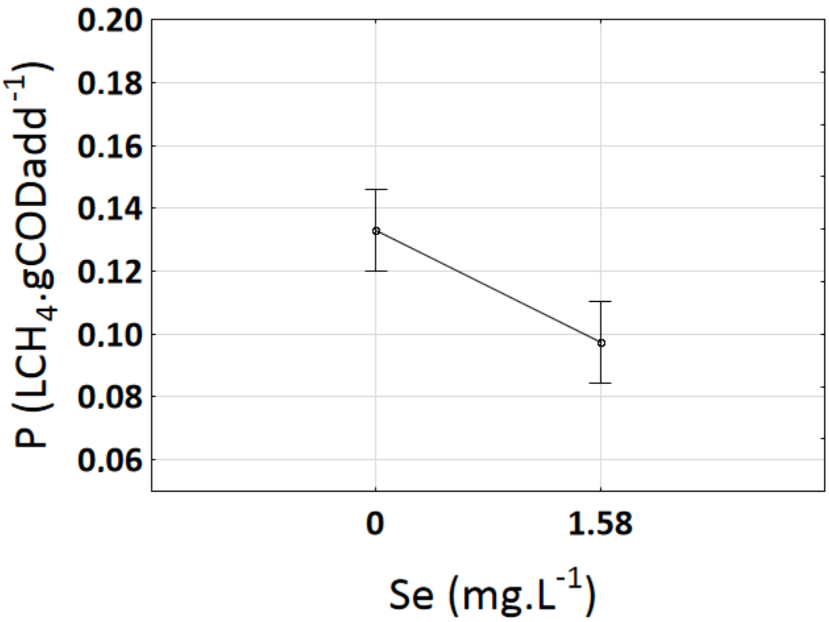

Figure 3. Plot of marginal means of Se considering the supplementations (levels -1 and +1) of the PB design affecting P.

3.3.2 Biochemical methane potential for Fe– BMP-Fe

Considering the response obtained in the BMP-PB assay, Fe was tested in six different concentrations. The pH values at the end of the assays, for the conditions tested, remained in the neutral range, between 7.20 and 7.30 (Tab. 3), with no statistical difference between them. The control group (0 mg L^{-1}), showed a 97.0 ± 0.4 mg L^{-1} final COD concentration, with 74.8 ± 0.1% removal efficiency, 120 mg L^{-1} as the concentration with the highest removal efficiency (78.1 ± 0.2%) and an 84.5 ± 0.9 mgL^{-1} final COD concentration. The values of the final COD concentration and COD removal efficiency were statistically significantly different from all other conditions.

Regarding the CH_4 proportion in the biogas, the control group showed that 44.7 ± 0.8% of the biogas was comprised of CH_4. The concentrations of 180 and 200 mg L^{-1}, with 47.4 ± 3.5% and 45.2 ± 2.7%, respectively, showed similar percentages compared with the control group. Concentration conditions of 80 and 120 mg L^{-1} showed higher CH_4

proportions in the biogas, with 54.5 ± 2.8% and 59.2 ± 0.6%, respectively (Table 3). Comparing the conditions to each other, 120 mg L^{-1} was significantly higher statistically.

Table 3. CH_4 content in biogas, COD and removal efficiency and pH at the end of the BMP-Fe assay using different Fe supplementations.

Fe (mg L^{-1})	pH	tCOD (mg L^{-1})	tCOD Removal efficiency (%)	CH_4 proportion on biogas (%)
0	7.21 ± 0.02	97.0 ± 0.5	75.8 ± 0.1	44.7 ± 0.8
40	7.31 ± 0.02	93.5 ± 0.7	75.7 ± 0.2	47.3 ± 4.3
80	7.32 ± 0.05	93.0 ± 2.7	75.9 ± 0.7	54.5 ± 2.8
120	7.25 ± 0.02	84.5 ± 0.9	78.1 ± 0.2	59.2 ± 0.6
160	7.22 ± 0.06	91.0 ± 1.0	76.4 ± 0.3	47.4 ± 3.5
200	7.28 ± 0.05	91.5 ± 1.7	76.2 ± 0.4	45.2 ± 2.7

For the response P, expressed LCH_4 gVS^{-1}, the control group obtained a mean of 0.031 ± 0.003 LCH_4 $gCOD_{add}^{-1}$. Concentration conditions of 160 and 200 mg L^{-1} showed a lower mean P than the control group and statistically lower *p*-values of 0.003 and 0.008, respectively. Conditions of 40 and 80 mg L^{-1} showed mean P values similar to the control group, with no statistical difference. The highest mean was achieved at 120 mg L^{-1} (0.163 ± 0.007 LCH_4 $gCOD_{add}^{-1}$) which was 33% more than the control group, and had a statistically significant difference compared with the P values obtained for the other conditions. These findings indicate that the most appropriate Fe concentration on the AD of DS was 120 mg L^{-1}, which improved CH_4 production and substrate conversion.

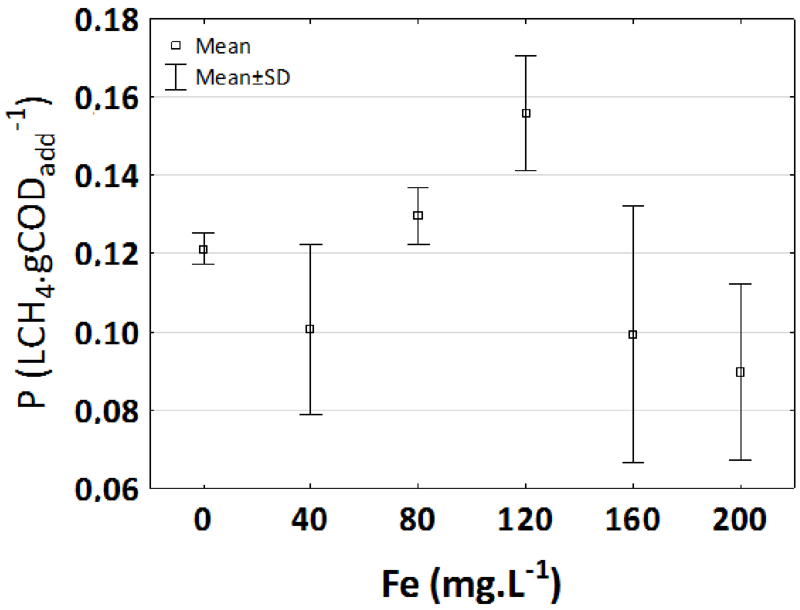

Figure 4. Six different Fe concentrations studied, affecting the maximum CH_4 production (P).

For the response specific CH_4 production, expressed LCH_4 gVS^{-1}, the control group obtained a mean of 0.031 ± 0.001 LCH_4 gVS^{-1}. Concentration conditions of 40, 160 and 200 mg L^{-1} showed a lower mean P than the control group and statistically lower *p*-values of 0.002, 0.003 and 0.007, respectively. Conditions of 80 mgL^{-1} showed mean P values (0.033 ± 0.001 LCH_4 gVS^{-1}) similar to the control group, with no statistical difference. The highest mean was achieved at 120 mg L^{-1} (0.040 ± 0.003 LCH_4 gVS^{-1}), with statistically significant difference compared with the P values obtained for the other conditions. These findings also indicate that the most appropriate Fe concentration was 120 mg L^{-1}, which improved the specific CH_4 production.

According to Meng et al., (2013), Fe supplementation may improve the propionate conversion rate, by having more propionate-utilizing and homoacetogenic bacteria, what may consequentially increase CH_4 production. Fe is mainly present as Fe-S clusters for catalysis and/or electron transport (Hijazi et al., 2020) and has an irreplaceable role in the synthesis of essential coenzymes or cofactors in methanogenic pathways (Zhang et al., 2015), besides being constituent of the enzymes Fe-Fe hydrogenase, related to acidogenesis, and methane monooxygenase, related to CH_4 production (Fermoso et al., 2009). Therefore, the production of CH_4 may have been improved by the addition of Fe due to its action on methanogenic enzymes, stimulating the metabolism of acids during methanogenesis.

Braga et al. (2018) reported that Fe (0.81-25.19 mg L^{-1}) did not show a significant effect on CH_4 production from simulated blackwater. However The improvement in producing CH_4 through Fe supplementation in sewage sludge AD was also reported in Qin et al. (2019). The authors tested four different Fe concentrations (100, 200, 400 and 800 mg L^{-1}) in CH_4 production from the anaerobic digestion of sewage sludge diluted in deionized water (10.5 ± 0.2% TS; 66.0 ± 0.6% VS/TS). The highest cumulative CH_4 production reached was 0.364 LCH_4 $gTVS^{-1}$ with 200 mg L^{-1}, which was 28.9% higher than the control. However, the cumulative CH_4production decreased to 174.7 LCH_4 $gTVS^{-1}$ and 194.3 LCH_4 $gTVS^{-1}$ with the supplementation of 400 and 800 mg L^{-1}, respectively. The best Fe concentration was 200 mg L^{-1}. The authors reported that the results suggested that appropriate Fe dosage was beneficial to CH_4 production, whereas excessive dosages inhibited methane production. A similar concentration was found in the present study, indicating the possibility of improve CH_4 production by adding appropriate Fe concentrations.

In Ketheesan et al. (2016), a carbohydrate-protein substrate was used as the synthetic wastewater, composed of glucose, peptone, meat extract, sodium bicarbonate and essential minerals: K, Co, Fe, Mn, Na, Ni and Mg. The authors tested different Fe doses (0–100 mg L^{-1}) in the degradation of VFA into methane (propionate– 1.5 g COD L^{-1}) in a batch/semi-batch anaerobic reactor. Statistically significant (p-value< 0.05) improvement was found in the kinetics of propionate to CH_4 with a 10–30 mg L^{-1} Fe concentration, although 10 mg L^{-1} was found to be optimum

The relevance of improving CH_4 production in DS treatment plants applies to the concept of promoting a sustainable cycle of energy production through anaerobic digestion of a waste, minimizing the need of fossil fuels and also reducing the emissions of CH_4 in the atmosphere. Therefore, more sustainable options must be investigated for TM supplementation, such as the co-digestion using wastewater with Fe content, to reduce the environmental risks and ensure the economic benefit of an increase in CH_4 production through Fe additions. Co-digestion with high Fe content substrate could make the process even more favorable.

Speece (1996) reports that adding nails scraped with file increased methane production in an anaerobic digester. Casals et al. (2014) describe that iron oxide nanoparticles (NPs) in anaerobic digesters increased the biogas production in 180% and 234% CH_4 production.

3.4 CONCLUSIONS

The Placket-Burman fractional factorial design (PB) was useful and suitable for selecting the statistically significant metals in CH_4 production, through a practicable run of tests, using domestic sewage (DS) as substrate.

It was assumed that Ba (0.008-686.6 mg L^{-1}), Co (0.012 - 0.302 mg L^{-1}), Cu (1.249-2.249 mg L^{-1}) and Ni (0.066-0.356 mg L^{-1}), in the concentrations studied, neither inhibited nor improved the CH_4 production on the AD of DS, due to the lack of statistical significance.

Mn (2.361- 2.631 mg L^{-1}) was statistically significant in CH_4 production (*p*-value=0.07); however, further studies on optimal concentrations are required. Supplementation with Fe (25.969 - 40.969 mg L^{-1}) and Se (0.059 - 1.639 mg L^{-1}) was statistically significant on CH_4 production, with a positive effect for Fe concentrations and a negative effect for those of Se. It was concluded that Se achieved the inhibition level.

An improvement of 33% in CH_4 production was evident (Figure 4) with single Fe supplementations at a concentration of 120 mg L^{-1}, compared to the control test (0 mg L^{-1}). At 120 mg L^{-1}, the COD removal efficiency was raised by 4.3%. This Fe concentration also improved the specific CH_4 production to 0.040 ± 0.001 LCH_4 gVS^{-1}.

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Chapter 4

LONG-TERM ANAEROBIC STRUCTURED-FIXED BED REACTOR OPERATION FOR DOMESTIC SEWAGE TREATMENT: PERFORMANCE AND METALS DYNAMICS

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ABSTRACT

This study evaluated a recent configuration of anaerobic reactor known as anaerobic fixed-structured bed reactor (ASTBR) in the treatment of domestic sewage (DS). The initial studies with ASTBR focused in improve anaerobic packed bed reactors, due the random distribution of the media, that tends to limit their application to solid-rich wastewaters and since then, it has been studied with greater emphasis with high-strength, however, up to knowledge there are no studies in the literature treating DS. Beyond that, the anaerobic digestion (AD) requires well-balanced operation conditions to achieve optimal performance, steady physical-chemical conditions, and adequate nutrients concentration. Large attention is given to N and P however, metals are, usually, not monitored throughout the operation of wastewater treatment systems or even characterized in the influent. Thus, four decreasing hydraulic retention time (HRT) were applied (12; 8; 6 and 5 hours), to evaluate the organic matter removal efficiency and impact on physical-chemical parameters in a long-term operation (614 days). Moreover, the dynamics of 15 metals (Al, As, Ba, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Se, and Zn) was monitored in the influent and effluent, and in the biomass (initial and final content in the support material). In general, the results highlighted the feasibility of applying the ASTBR to DS treatment, indicating an average of chemical oxygen demand (COD) removal efficiency of 70%. The ASTBR showed a stable long-term operation (614 days), even for oscillating volatile fatty acids (VFA) concentrations in the influent, being capable of adaptation,

buffering the system. The average concentration of VFA in the effluent, during the 614 days was 20.4± 3.3 mg L^{-1} and with an average pH value of 7.16 ± 0.16. In relation to the metals content in the influent, divided in wet season and the dry season, Cd, Co, Cr, Cu, Ni, and Se were not detected, with higher concentrations of Zn and Pb in the wet season.

4. 1 INTRODUCTION

Domestic sewage (DS) is a type of wastewater generated in urban activities. In general, it contains water and different pollutants substances such inorganic dissolved solids, organic matter, microorganisms and metals (Metcalf and Eddy, 2003).

Due to the characteristics of the compounds found in the DS, the typical process used to treat it is the biological treatment, through aerobic or anaerobic digestion (AD).

The high potential of anaerobic systems was proved by Young and McCarty (Young and McCarty, 1969), who successfully operated an up-flow anaerobic filter, treating concentrated mainly soluble rum distillery wastewater. Since then, several anaerobic reactor configurations have already been used on the real scale in wastewater treatment stations or tested on a lab-scale to treat DS and different types of wastewaters.

According to the National Water Agency, (2020), there are 3.419 DS treatment plants in operation in Brazil, distributed in 1.893 cities. The majority are anaerobic reactors, with 1.389 plants, which possess different configurations, 1.307 have different types of lagoons, and 407 possess activated sludge as the main treatment from the total number of plants system. However, despite the advance of anaerobic reactors, further investigations are still required to surpass limitations of the available reactor configurations and process designs.

Anaerobic fixed bed reactors show important advantages compared to other configurations, such as the possibility of increasing biomass concentration in a reactor and shock loads (Cuel et al., 2011; Mockaitis et al., 2014). Traditionally, they are randomly packed (packed-bed reactors), similar to the anaerobic filters from which they evolved. The random distribution of the media limits its application due to the continuous growth of biomass leads to clogging of the bed in long-term operations, channeling and hydraulic short circuit zones. Therefore, the development of the anaerobic structured-fixed bed reactor (ASTBR)originated from the need to prevent these hydrodynamic issues common to packed-bed bioreactors like (de Aquino et al., 2017; Carneiro et al., 2019).

The ASTBR consists of a design where the biomass is vertically structured immobilized on inert materials fixed and positioned along the reactor, forming a biofilm. It was proposed by Mockaitis et al. (2014) with the application in the treatment of low-strength complex synthetic wastewater and by Camiloti et al.(2014), which used the ASTBR for the treatment of complex synthetic wastewater aiming to reduce sulfate and oxidate organic matter. The ASTBR main advantages are the easy solids separation and sedimentation, high concentration of biomass (De Araujo et al., 2016), high performance and stability in low HRTs and low content of suspended solids effluent (Cuel et al., 2011; Mockaitis et al., 2014).

The type of support material influences the biomass adhesion and reactor's hydrodynamics, affecting overall system performance. Many types of support materials for ASTBR have been studied, including polyurethane foam, polypropylene, polyethene, polyethene terephthalate (PET) rings, plastic plates, and ceramic matrix (De Araujo et al., 2016). According to De Araujo et al. (2016), polyurethane foam promotes a gradient of substrate concentration and electron donors, contributing to establishing the microorganism’s community essential to complete anaerobic digestion.

Studies have already been carried out with the ASTBR to remove nitrogen from the synthetic substrate (Santos et al., 2016); brewery wastewater treatment with high volumetric organic loading rate (OLR) (De Araujo et al., 2016); production of hydrogen from synthetic wastewater (Blanco et al., 2017); treatment of sugarcane vinasse (Fuess et al., 2017). However, despite the recent development associated with this reactor configuration, several applications are still required to elucidate robustness under diverse operating conditions (Cunha et al., 2020) and different substrates, such as DS.

Up to knowledge, few studies focused on DS as a substrate in ASTBR operation. Carneiro et al. (2019) studied the performance of the ASTBR treating synthetic wastewater and real DS, focusing on removal of Sulfamethoxazole (SMX) and Ciprofloxacin (CIP), two antibiotics frequently detected in DS. According to the authors, the ASTBR, is a promising technology to remove recalcitrant compounds, with high efficiency under HRT, and they can be applied successfully in DS treatment.

Carneiro et al. (2020) investigated the influence of the OLR, hydraulic retention time (HRT), sludge retention time and liquid superficial velocity on CIP and SMX biodegradation, removal kinetics, and spatial profile of the bioreactors using synthetic wastewater. The authors stated that adjusting the operational parameters might enhance the biodegradation of SMX and CIP during anaerobic digestion in DS treatment. According to the authors, the increase in the organic loading rate (0.6 to 2.0 kgCOD m^{-3} d^{-1}) negatively impacted the CIP and SMX biodegradation by the lower antibiotic - biofilm contact time (higher HRT) in the bed and higher washout suspended biomass, which resulted in the decrease of sludge retention time.

It is currently well established that anaerobic digestion requires well-balanced operation conditions to achieve optimal performance, steady physical-chemical conditions, and adequate macro and micronutrients concentration. Considerable attention has traditionally been paid to N, P (macronutrients) and organic matter, related to the effects on the treatment systems and effluent discharge. Even with a lower concentration in DS (in order of 10-30 mg L^{-1}) than N and P, K still needs appropriate management and monitoring (Arienzo et al., 2009). K and Mg are also considered macronutrients, required in larger amounts by the microorganisms than the micronutrients or trace metals (TMs) (Madigan et al., 2012). TMs are usually not monitored throughout the operation of wastewater treatment systems or even characterized in the influent.

TMs, such as Co, Ni, or Se, influence microbial metabolism and can inhibit activity in cases of excessive concentrations (Roussel et al., 2018). In biological processes, each TM requirement in AD is determined by the anaerobic reactor, type of substrate, temperature, and other factors (Thanh et al., 2016). They are responsible for cell growth, and enzymatic reactions and their relevance in anaerobic systems are classified according to the requirements in methanogenesis, i.e., Fe >> Zn > Ni > Cu ≈ Co ≈ Mo > Mn (Choong et al., 2016; Zhang et al., 2015). Anaerobic bioreactors require a range of TMs with different degrees of affinity, and their concentration profile may change under different operating conditions.

In addition to the fact that TMs are essential in AD, they may be toxic in certain concentrations, raising great concern due to the impacts that high concentration discharges can cause to the environment, living beings and human health. According to Lambert and Leven (2000), TMs such As, Cd, Cr, Cu, Pb, Ni, and Zn have been substantially mined, smelted and consumed by industries, and the wastewaters generated are left behind to pollute surface and groundwater. These TMs and other ones, such as Co, S, Al, are hazardous in certain concentrations. Due to the importance of TMs concerning microbial metabolism in the AD process and the environmental relevance they present, their monitoring and quantification in wastewaters are essential.

In this context, the present study aimed to evaluate the ASTBR applied in DS treatment, operated during 614 days, at four decreasing HRTs (12, 8, 6 and 5 h) based on steady states achieved in each HRT. Moreover, the dynamics of 13 TMs (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn), including K and Mg in the reactor was evaluated by monitoring influent and effluent concentration. The initial and final metal content in the biomass and support material was also evaluated.

4.2 MATERIAL AND METHODS

4.2.1 Experimental set-up

An ASTBR made of acrylic, with a total working volume of 5.2 L discounting support material, was operated in a continuous up-flow model. The stems for biomass immobilization were composed of 9 fixed strips of polyurethane foam with internal plastic rods. Each polyurethane foam strip contained a length of 600 mm and a cubic shape with 10x10 (mm), fixed to the upper part of the reactor by a metallic grid. The porosity of the bed was 98.4%.

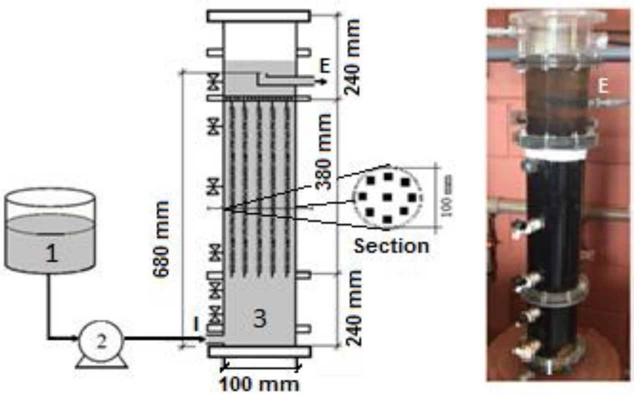

Figure 1. Layout of the ASTBR. Legend: 1 – raw DS (storage tank), 2 – pump, 3 – ASTBR, I - Influent, E - Effluent. Section: Strips distribution.

4.2.2 Reactor startup and operation

The granular sludge seed (32.8 gTVS L^{-1}) was obtained from a UASB reactor treating wastewater from a poultry slaughterhouse (Dacar S.A., Tietê, SP, Brazil). The seed was crushed for 2 min, in a blender. Then the polyurethane strips (ASTBR bed) were immersed in the crushed seed for 2 hours for biomass attachment. After this period, the strips were fixed inside the reactor filled with the DS, remained resting for 12 h (Mockaitis et al., 2014). Then the continuous operation started at an HRT of 12 h and mesophilic ambient temperature (23 ± 3 to 28 ± 2°C).

The ASTBR was operated for 614 days at decreasing HRTs, i.e., 12, 8, 6 and 5 h (Table 1). The influent flow rate was controlled using the diaphragm pump. The HRTs changes were based on steady states periods.

Table 1. Operational conditions of ASTBR

Phase	Period (days)	Flow (L h^{-1})	HRT (hours)	Liquid temperature (°C)	
				Influent	Effluent
1	123	0.43	12	27 ± 2	28 ± 2
2	179	0.65	8	25 ± 3	26 ± 2
3	155	0.86	6	28 ± 6	29 ± 5
4	157	1.04	5	29 ± 2	28 ± 2

The ASTBR was fed with DS collected from a sewer line built in São Paulo University (São Carlos, SP, Brazil), which deviated part of the São Carlos municipal sewage network flow. Every 48 hours, a sufficient volume to feed the ASTBR was transferred to a storage tank and sieve screen (0.5 mm).

4.2.3 Performance assessment: Analytical and Statistical analysis

4.2.3.1 Treatment efficiency measurements

The organic matter concentrations in terms of chemical oxygen demand (COD), total and volatile suspended solids (TSS, VSS) were measured in the influent and effluent following the Standard Methods for Examination of Water and Wastewater (APHA et al., 2005).

COD concentrations and COD removal efficiency, among the different HRT operated, were tested for normality using the Kolmogorov-Smirnov test (K-S). Outliers and extremes were not found among the data. The parameters were statically analyzed using the One way-ANOVA, followed by the Tuckey HSD (honestly significant difference), verify whether the HRT had a significant statistical effect on COD concentrations and COD removal efficiency at a 90% confidence level, using the software Statistica 10 (Stat Soft, Inc., Tulsa, USA).

4.2.3.2 Specific methanogenic activity (SMA)

At the end of the reactor operation, the biomass's specific methanogenic activity (SMA) developed attached to the polyurethane foam was evaluated. A batch assay was performed using 100 ml glass flasks, kept in a chamber under controlled mesophilic temperature (32.6 ± 0.4°C), in triplicate for 25 days until biogas production stabilization. The foam sticks with biomass attached were cut (1.6 cm approximately) and added to ensure a concentration of 0.08 ± 0.21 mgTVS L^{-1} in each bottle. The substrate was DS influent of the ASTBR system, added in each bottle at a concentration of 356 mgCOD L^{-1}.

The working volume of the flasks were 58 ml, with a headspace of 42 ml. Before the assay start, the flasks were flushed for 3 min with N_2 : CO_2 (70:30%) to ensure anaerobic conditions.

The pressure of each bottle was monitored once a day, using a TPR-18 pressure transducer coupled to a BS 2200 interface (Desing Instruments, Barcelona, ESP), and gas composition was analyzed using gas chromatography (GC) (GC 2014, Shimadzu®, Kyoto, Japan), through samples of the headspace. The accumulated volume of CH_4 (ml) was obtained from the pressure and composition of the biogas data.

The modified Gompertz equation (equation 1) was adjusted to the CH_4 accumulated volume (mL) raw data versus time using Statistica 10 software (Stat Soft, Inc., Tulsa, USA). Therefore, obtaining the value of the maximum velocity of biogas production (R_{max} – mLCH_4 d^{-1}), maximum potential biogas production (P – mLCH_4), and lag phase (L – d) (Zwietering et al., 1990).

$$B = P * Exp\left\{-Exp\left[\frac{R_{max} * e}{P} * (L - t) + 1\right]\right\} \quad (1)$$

Hence, the SMA (mgCOD-CH_4 $gTVS^{-1}$ d^{-1}) values were obtained from the fitted R_{max} using Equation (2) from Zinatizadeh et al. (2006).

$$SMA = R_{max} * fcstp/C_{TVS} \quad (2)$$

where:

fc_{stp} = conversion factor to convert the volume of CH_4 (L) at standard temperature and pressure (0°C and 1atm) to COD-CH_4 (2.3595 gCOD-CH_4 L-CH_4^{-1}).

C_{TVS} = concentration of sludge (gTVS L^{-1}) added to the bottles as biomass seed per working volume.

4.2.3.3 Operational stability measurements

The stability of an anaerobic reactor may be assessed by monitoring parameters such as the temperature (liquid and ambient), pH, alkalinity, total volatile fatty acids (VFA) and biogas (Chernicharo, 2007).

For pH measurements, a calibrated pH meter (DM 20 - DIGIMED) was used, according to the Standard Methods for Examination of Water and Wastewater (APHA et al., 2012). For alkalinity, the titration method was used to determine total, partial, and intermediate alkalinity (TA, PA, and IA) and the IA/AP ratio (Ripley et al., 1986). For VFA, the titration method was used, according to Dilallo and Albertson (1961).

The biogas composition (CO_2, CH_4, and H_2S) was monitored by gas chromatography (GC 2014, Shimadzu®, Kyoto, Japan) equipped with a thermal conductivity detector (GC-TCD) using hydrogen as the carrier gas and an HP- PLOT/Q-column (30 m,0,53 mm, 40 μm) (Adorno et al., 2014).

The theoretical CH_4 production (LCH_4 d^{-1}) was calculated according to Chernicharo (2007) and the estimated CH_4 production (LCH_4 d^{-1}) was calculated considering the COD load removal values ($CODL_{rem}$ - gCOD d^{-1}) of the continuous reactor and the P(LCH_4) and the COD removal ($CODB_{rem}$- gCOD) obtained for the reactor's biomassa in the SMA batch assay, according to equation 3.

$$CH_4\ production = CODL_{rem}\frac{P}{CODB_{rem}} \quad (3)$$

4.2.3.4 Metals assessment in the ASTBR

The metals concentration in the clean foam, in samples of inoculated foam before startup procedure and at the end of the operation, and in DS (42 samples of influent and effluent) were assessed. DS samples were divided into the wet and dry seasons. It was considered wet season from August to December 2017 (HRT of 12 hours) and September to December of 2018 (HRT of 6 hours). The dry season was from March to August of 2018 (HRT of 8 hours) and February to April 2019 (HRT of 5 hours). These classifications were based on São Carlos's forecast and average precipitation.(World Weather & Climate Information, 2018). The metals in each season were compared statistically between them by T-test, at a 95% confidence level, using the software Statistica 10 (Stat Soft, Inc., Tulsa, USA).

For carrying out the sludge metal analysis, the samples were submitted to a pre-treatment, previously to the microwave digestion, as follows: 0.25 g of foam of each sampling point was kept in soak for 30 hours in a solution of 7.5 ml of HNO_3 16N and 2.5 H_2O_2 30%, according to Dillner et al., (2007).

All samples for metals analysis were digested in a microwave (Four Speed, Berghof GmbH, Germany) to remove any residual solids, filtered (Whatman® 589/1, Sigma-Aldrich, MO, USA), and diluted to 25 mL in volumetric flasks. After, the samples were analyzed by Inductively coupled plasma optical emission spectrometry - ICP/OES (Optima 8000, Perkin Elmer, USA) (Braga et al., 2017). An internal standard (yttrium at 1 mg L^{-1}) was added as an internal standard (Y 324.227 axial) and the TMs analyzed were: Al (308.215 nm radial), Ba (233.527 nm axial), Cd (226.502 nm axial), Co (228.616 nm axial), Cr (267.716 nm axial), Cu (324.752 nm axial), Fe (238.204 nm radial), Mn (257.610 nm axial), Ni (231.604 nm axial), Pb (217.000 nm axial), Se (196.026 nm axial), Zn (206.205 nm axial), plus K (766.49 nm radial) and Mg (280.271 nm radial).The digestion and ICP analysis were based on Braga et al. (2017).

The concentration of metals in the sludge was calculated considering the foam mass in the reactor and total solids of the biomass attached, expressed in mg gTS^{-1}. Metal loading rate (mg L^{-1} d^{-1}) calculations took into account the ASTBR volume, influent flow and metal concentration, and the percentage of retention was calculated from the difference between the influent and effluent metal concentration.

4.2.3.5 ASTBR hydrodynamic assays

Hydrodynamic assays were carried out to assess the flow patterns, and the real HRT applied to the reactor at the end of system operation when the biomass had accumulated within the structured beds. For the assays, the reactor was continuously fed DS supplied with NaCl (10 mg L^{-1}) for 15 h, equivalent to 3 times the theoretical HRT (5 h). NaCl concentrations were monitored online using a conductivity probe (Model CON-BTA, Vernier Software & Technology, Beaverton, OR, USA) coupled to an interface for data acquisition (Model CBL 2, Texas Instruments Inc., Dallas, TX). Experimental data were stored by the software LoggerLite 1.9.4. for further analysis.

The stored data were analyzed through residence time distribution curves, using the dispersion coefficient model, based on Levenspiel, (1999). Data were processed using R Studio version 1.3.1093.

At the end of the operation, Fe was supplemented (120 mgFe L^{-1}) for 15 h (period equivalent to 3 times the final HRT operated - 3 x 5 hours) to investigate the hydrodynamic behavior of the ASTBR regarding TMs. Fe was selected based on Silva et al., (2021).The authors found that among seven TM (Ba, Co, Cu, Fe, Mn, Ni and Se), Fe had a positive statistically significant effect on the maximum CH_4 production from DS using sewage sludge as inoculum, both from the same source as the present study. Within six different concentrations evaluated (0, 40, 80, 120, 160 and 200 mg L^{-1}), 120 mg L^{-1} showed statistically improved AD. During the supplementation assay, samples were collected every 75 min during the first 15 hours, then 3 hours after the end of the experiment and then 36 hours later. The samples were analyzed following the procedures described in item 2.2.3 to verify the Fe concentration over time.

4.3 RESULTS AND DISCUSSION

4.3.1 Treatment efficiency

In general, even with the substantial variation in COD and TSS influent concentrations, the ASTBR maintained steady concentrations in the effluent during most of the operation time at different OLR (Fig 2 A, B and D).

However, an abrupt increase in effluent TSS concentrations occurred during the 77º to 87º day (HRT of 12 h), from an average value of 35 ± 19 mg L^{-1} (1º to 76ºday) to 104 ± 26 mg L^{-1} (Fig. 2 B). There was no increase in the TSS concentrations in the influent during the same period.

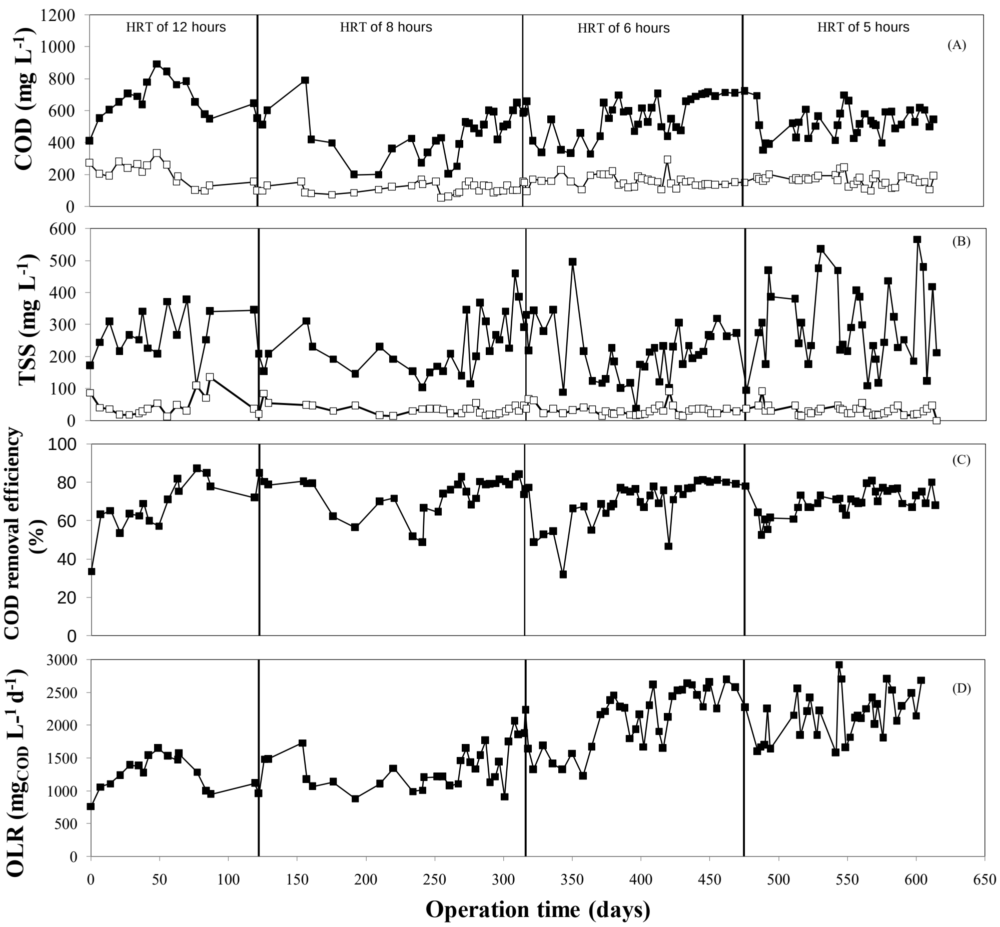

Figure 2. COD (A), TSS (B), COD removal efficiency (C) temporal profiles for influent and effluent samples of the ASTBR. (D) OLR applied to ASTBR at different HRTs. Legend: ▪ Influent; ▫ Effluent.

According to Moussavi et al.(2010), inadequate and high upward velocities cause detachment of particles, especially during startup when biomass is on adaptation. The upward velocities applied to the ASTBR in each HRT was 0.07 m h^{-1} (HRT of 12 h); 0.10 m h^{-1} (HRT of 8 h); 0.14 m h^{-1} (HRT of 6 h); 0.17 m h^{-1} (HRT of 5 h), with no oscillation due to the constant flow rate control. Mockaitis et al. (2014) operated with an upward velocity of 0.06 m h^{-1}, with no washout of solids. Moussavi et al. (2010) pointed out that particles whose settling velocities exceed the upward velocity will be retained in the reactor but increasing the influent's upward velocity into the reactor can expand and disrupt the sludge bed in addition to suspension and subsequence washout of small solids and colloids. However, the upward velocity applied in the HRT of 12 h (0.07 m h^{-1}) agrees with Mockaitis et. al. (2014), and even at higher values (up to 0.17 mh^{-1}), it was not verified washout of biomass. In this case, the upward velocity was not considered as the originator of the described washout of solid.

During the startup period (HRT of 12 h), the COD removal efficiency remained under 60% most of the time, increasing to 71% after 55 days of operation (Fig.2 C). The lower COD removal efficiency in the operation beginning is expected. The microbial consortium coming from the inoculum was adapting to the new carbon source, with a COD concentration much lower than a poultry slaughterhouse wastewater. This may had originated the washout of solids during days 77 to 87. In addition, the substantial change of substrate, with much lower concentration and different characteristics, collaborate for the adaptation of the microbial consortium.

Pareboom, et al., (1994) reported that large microbial consortium releases particles (microorganisms) during adaptation and new community formation. These suspended microorganisms attach themselves and form new community or may be lost in washouts. According to Ike et al., (2010), the startup may be considered as the most critical step in the operation of anaerobic digestion processes. critical point. The inoculation and start-up phase leads the development the an active microbial biomass (Silva et al., 2017), adapted to the new conditions.

After the HRT reduction of 12 h to 8 h, the reactor performance decreased (126th to 250th day), with no TSS effluent concentration increases. The COD removal efficiency dropped from 85% (day 122) to an average value of 68±11% (days 126 to 250). After this period, during the 256^{o} to 315^{o} days, the reactor recovered stability, and the COD removal efficiency reached an average value of 78±4%. The same behavior occurred during changes in HRT 6 and 5. After reducing HRT to 6 h, the ASTBR presented an average COD removal efficiency of 54 ± 13%, increasing to 75 ± 4% after day 387 (Fig. 2C). After the HRT change to 5 h, the COD removal efficiency decreased from 78% (day 476) to 64% (day 485), increasing to 73 % in day 516, remaining with an average COD removal efficiency of 72 ± 4 % until the end of the operation.

Based on the statistics, comparing the COD removal efficiency among the HRTs operated, the normally distributed data showed a significant difference between the HRTs of 8 and 6 h, and 6 and 5 h, using the One way-ANOVA/Tuckey test with unequal variances. After each HRT change, the reactor showed the capacity to recover the COD removal efficiency, showing stable values again between days 56 to 122 (HRT of 12 h), 256 to 315 (HRT of 8 h),388 to 425 (HRT of 6 h), and 521 to 555 (HRT of 5 h).

Mockaitis et al. (2014) evaluated the ASTBR performance and kinetics treating synthetic DS with low solid content. The reactor operated by the authors achieved an average organic matter removal of 78 ± 5% (HRT of 12 h) with an influent COD concentration of 404.4 mg L^{-1}, in a bench-scale anaerobic fixed structure bed reactor, with a working volume of 4.77 L. The ASTBR did not show instability during the operation, and they concluded that the ASTBR is a developing and suitable alternative technology for complex wastewater treatment. As the authors had used synthetic wastewater with a low concentration of solids, they emphasize the necessity of additional studies with this configuration, using different conditions and actual wastewater. According to the present work results, the ASTBR had fulfilled its role in the treatment of DS.

The volume of CH_4 generated by DS treatment systems has great importance since it is considered an option for the conversion of organic residues to renewable energy, and DS treatment plants are influencing the development towards energy sustainability (Bachmann, 2015), attracting interest, as it is a low-cost fuel for power (Udaeta et al., 2019).

4.3.2 Evaluation of the sludge SMA

The SMA is a considerable tool that determines the methane-producing capability of sludge for a specific substrate at a concentration level where substrate availability is not a limiting factor (Hussain and Dubey, 2017). The amount of active methanogenic population in an anaerobic reactor is critical in achieving efficient wastewater treatment. Thus, the SMA test can outline the system's operating condition and a parameter to assess the system performance by better understanding the system performance and stability.

The batch assay carried out using the sludge attached to foam from ASTBR at the end operation showed a final COD concentration of 90.0 ± 0.7 mg L^{-1}, with a 77.5 ± 0.1% removal efficiency. Gompertz modified Equation was fitted to the CH_4 accumulated production measured, and it was observed a maximum SMA of the sludge of 122.2 ± 6.8 mgCH_4-COD $gTVS^{-1}d^{-1}$, requiring 13.4 days to reach it, with an R^2 of 0.9972.

Souza et al. (2016) studied the SMA of sewage sludge from the UASB reactor under different linear alkylbenzene sulfonate (LAS) concentrations. Six anaerobic flasks reactors with 1 L of total volume were inoculated with anaerobic sludge (2 $gVSS^{-1}$) and assayed for 42 days using volatile fatty acids and nutrients as substrate, corresponding to a COD of 3g L^{-1}. The control group (0 mg L^{-1} of LAS) showed a maximum SMA of the sludge of 70 mgCH_4-COD $gVSS^{-1}$ d^{-1}, being approximately 60% less of the sludge in this study.

Braga et al. (2017) evaluated six different samples of UASB sludge obtained in different full-scale sewage treatment plants (STPs), located in the states of São Paulo and Minas Gerais, in Brazil. The authors used acetic acid (1 g L^{-1}) and macronutrients (N, P, and K) as the substrate for the sludge SMA tests, which reached maximum values ranging from 33 to 103 mgCOD-CH_4 $gTVS^{-1}$ d^{-1}. The authors attributed the different values obtained to factors such as substrate adaptation and inoculum composition. In particular, the sludge from STP3, reached a maximum SMA of 103 mgCOD-CH_4gTVS^{-1}d^{-1}.

Silva et al., (2021) evaluated the methane production from DS (335 mgCOD L^{-1}) digestion, adding six different concentrations of Fe in a biomethane potential test, using UASB sludge as biomass. The SMA of the sludge for the control group (0 mg L^{-1}ofFe), was 56 mgCOD-CH_4 gTVS^{-1} d$^{-1.}$

The SMA values found in the present study shows the capacity of the ASTBR to promote the methane-forming bacteria, resulting in values comparable or higher in comparison with Souza et al. (2016), Braga et al. (2017) and Silva et al., (2021).

4.3.3 ASTBR operational stability assessment

The ASTBR showed stable influent pH values, without critical oscillations, during the 614 days of operation with an average of 7.16 ± 0.16 in the influent and an average effluent of 7.23 ± 0.16. During HRT increasing, the reactor also did not show drastic changes in pH values, remaining stable.

Concerning VFA values during the startup period, at the beginning of HRT of 12 h (days 18 to 48), the influent VFA values increased from 34.1 to 78.2 mg L^{-1} (Fig.3). In the same period, the pH value decreased from 7.22 to 6.75. The VFA values from the effluent in this period (days 18 to 48) remained stable, showing values between 13.3 and 22.4 mg L^{-1}. This behavior demonstrated an adaptation of the reactor in the startup period. Even with a fluctuation in the influent VFA values, at the HRT of 12 h, the system buffered, with pH stable values within the operation time, which is very important to the methanogenic consortia.

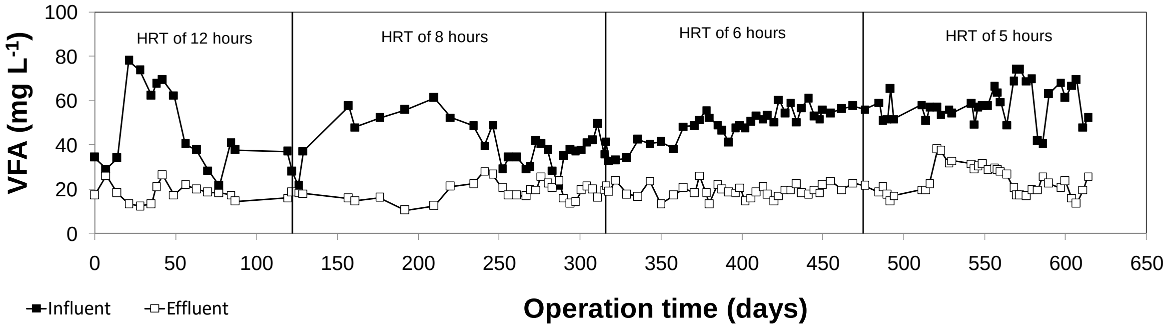

Figure 3. Temporal profiles of VFA (Influent and effluent) in the ASTBR.

The IA/PA ratio data reinforces the stability of the process. The IA/PA ratio for the operation of the ASTBR is presented in Fig. 4. According to Ripley et al. (1986), the ratio IA/PA with values greater than 0.3 indicates the occurrence of disturbances in the process of anaerobic digestion. However, Foresti (1994) says that the closer to 0.3 the ratio is, the more stable the system is, but the system's stability can occur even for values higher than 0.3 if the variations are discreet during the operation. The average of the IA/PA ratio at the effluent in each HRT was 0.22 ± 0.05 (HRT of 12 h), 0.24 ± 0.08 (HRT of 8 h), 0.21 ± 0.08 (HRT of 6 h), and 0.23 ± 0.09 (HRT of 5 h). Thus, the ratio IA/PA average remained close to or below the value indicated by the literature. The observed values above 0.3 in HRT of 8 h (days 246 to 308) and HRT of 8 h (days 364 to 371) did not represent destabilization of the system.

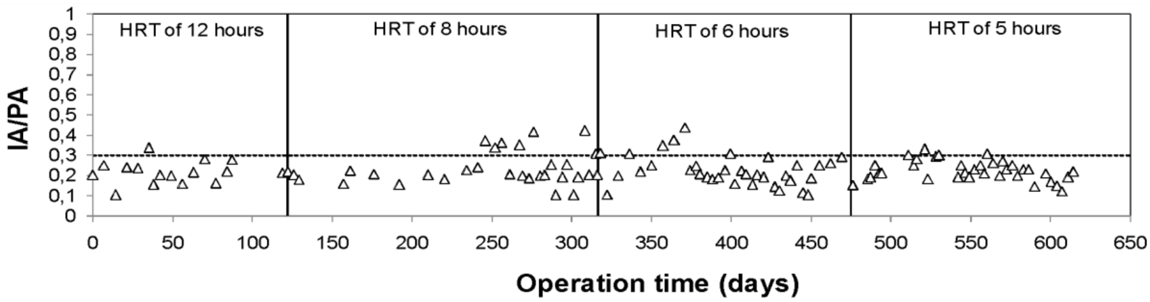

Figure 4. IA/PA ratio for the ASTBR effluent samples. Legend: Δ effluent IA/PA ratio; ....Ideal IA/PA ratio suggested by Ripley et al., (1986) and Foresti, (1994)

The volume of CH_4 generated by DS treatment systems has great importance since it is considered an option for the conversion of organic residues to renewable energy, and

DS treatment plants are influencing the development towards energy sustainability (Bachmann, 2015), attracting interest, as it is a low-cost fuel for power (Udaeta et al., 2019).

The percentage of CH_4 in biogas reached in this study was 38 ± 9% (HRT of 12 h), 62 ± 8% (HRT of 8 h), 65 ± 6% (HRT of 6 h), and 69 ± 4% (HRT of 5 h). The theoretical CH_4 volume during the ASTBR oscillated between 0.4 to 4.1 $LCH_4\ d^{-1}$, with an average value for each HRT of 1.4 ± 0.3 (12 h), 1.6 ± 0.6 (8 h), 2.5 ± 0.8 (6 h), and 2.8 ± 0.6 (5 h) LCH_4d^{-1}, respectively. The estimated CH_4 volume per HRT were: 1.0 ± 0.2 (12 h), 1.1 ± 0.6 (8 h), 1.4 ± 0.5 (6 h), and 1.7 ± 0.2 (5 h) LCH_4d^{-1}. The estimated results are lower than the theoretical, however this it is expected. The theoretical CH_4 calculated for an anaerobic reactor is usually higher than the actual/estimated production. The difference may be around 50% (Agrawal et al., 1997) due to the loss of gas through the effluent. In the present study, the CH_4 dissolved in the liquid phase of the bottles on the SMA assay might lead to underestimating the CH_4 production.

The SMA assay carried out using the sludge attached to foam from ASTBR at the end operation showed a final COD concentration of 90.0 ± 0.7 mg L^{-1}, with a 77.5 ± 0.1% removal efficiency. The SMA of the sludge was 122.2 ± 6.8 $mgCH_4$-COD $gTVS^{-1}d^{-1}$, requiring 13.4 days to reach it, with an R^2 of 0.9972.

Braga et al. (2017) evaluated six different samples of UASB sludge obtained in different full-scale sewage treatment plants (STPs), located in the states of São Paulo and Minas Gerais, in Brazil. The authors used acetic acid (1 g L^{-1}) and macronutrients (N, P, and K) as the substrate for the sludge SMA tests, which reached maximum values ranging from 33 to 103 mgCOD-CH_4 $gTVS^{-1}$ d^{-1}. The authors attributed the different values obtained to factors such as substrate adaptation and inoculum composition. In particular, the sludge from STP3, reached a maximum SMA of 103 mgCOD-$CH_4gTVS^{-1}d^{-1}$.

Silva et al., (2021) evaluated the methane production from DS (335 mgCOD L^{-1}) digestion, adding six different concentrations of Fe in a biomethane potential test, using UASB sludge as biomass. The SMA of the sludge for the control group (0 mg L^{-1}ofFe), was 56 mgCOD-CH_4 gTVS^{-1} d^{-1}.

The SMA values found in the present study shows the capacity of the ASTBR to promote the methane-forming bacteria, resulting in values comparable or higher in comparison with Souza et al. (2016), Braga et al. (2017) and Silva et al., (2021).

The CH_4 yield (LCH_4 gCOD$_{remov}^{-1}$) calculated for the biomass of the ASTBR in the SMA assay was 0.21 LCH_4 gCOD$_{remov}^{-1}$. Carneiro et al., (2019) monitored the CH_4 production of an ASTBR with a working volume of 2.69 L. When feeding the reactor with DS (approximately 450 mgCOD L^{-1}) and operating at HRT of 12 h, the authors obtained an average CH_4 yield of 0.15 LCH_4 gCOD$_{remov}^{-1}$. In this case, it may infer that the ASTBR showed adequate CH_4 yield.

4.3.4 Metals monitoration

4.3.4.1 Concentration in the DS

The metals concentrations found in the DS were divided into the dry season and wet season (Table 2). Therefore, it was possible to evaluate the change in the concentration of metals in the influent considering rainfall variation.

Table 2. Metals average concentrations in DS in the present study compared different cities of São Paulo State/Brazil.

Metals	**Concentration (mg L^{-1})** São Paulo Carlos (present study)		**Ribeirão Preto(Oliveira et al., 2007)**	**Campinas (Souza et al., 2014)**		**Jaguariuna (Souza et al., 2014)**		**Bauru (Candeia) Pipi et al., (2018)**	**Bauru (Tibiriça) Pipi et al., (2018)**
	Dry season	**Wet season**	**-**	**Dry season**	**Wet season**	**Dry season**	**Wet season**	**-**	**-**
Al	1.14 ± 0.79	0.83 ± 0.65	-	-	-	-	-	3.503 ± 0.004	2.501 ± 0.004
Ba	0.06± 0.02	0.06±0.03	-	-	-	-	-	0.127 ± 0.013	0.057 ± 0.013
Cd	*nd	*nd	0.15± 0.21	-	-	-	-	-	-
Cr	*nd	*nd	6.87 ± 3.73	0.403 ± 0.009	0.227 ± 0.009	0.427± 0.013	0.474 ± 0.013	-	-
Cu	*nd	*nd	17.21 4.93	0.343 ± 0.005	0.510 ± 0.005	0.546 ± 0.005	0.509 ± 0.005	0.052 ± 0.002	0.039 ± 0.002
Fe	1.05 ± 0.81	0.89 ± 0.49	-	-	-	-	-	1.722 ± 0.005	1.428 ± 0.005
K	13.35 ± 7.89	7.10 ± 3.41	-	-	-	-	-	-	-
Mn	0.09 ± 0.07	0.05 ± 0.02	52.53± 4.06	-	-	-	-	0.032 ± 0.004	0.041 ± 0.004
Mg	4.11 ± 2.70	2.95 ± 1.98	-	-	-	-	-	-	-
Ni	*nd	*nd	-	0.22 ± 0.005	0.322 ± 0.005	0.104 ± 0.004	0.065 ± 0.004	-	-
Pb	0.04 ± 0.03	0.05 ± 0.02	37.42 ± 59.67	0.011 ± 0.001	0.013 ± 0.001	0.067 ± 0.001	0.066 ± 0.001	-	-
Zn	0.09 ± 0.13	0.18 ± 0.05	79.20 ± 41.06	1.468 ± 0.008	1.100 ± 0.008	1.554 ± 0.010	1.562 ± 0.010	-	-

*nd: Not detected

From 25 samples in the wet season and 26 in the dry season analyzed, Cd, Co, Cr, Cu, Ni, and Se were not detected. The highest concentrations of metals found were K and Mg. A relevant point observed was the higher concentrations of Zn and Pb in the wet season. However, the statistical analysis showed a significant difference between wet and dry seasons only for Zn, with *p-value* = 0.01 and *t value* = 2.66. Usually, the contaminants are more concentrated during the dry season. They may increase due to irregular industrial discharges, slag wastes, mine tailings, discharge of chemical products.

Oliveira et al. (2007) in their study, investigated Cd, Cr, Cu, Mn, Pb and Zn concentrations in DS of a wastewater treatment plant located in Ribeirão Preto – São Paulo State/Brazil. The authors did not consider seasonality in the study. Compared with the present study, it is possible to notice that the authors found concentrations of investigated TMs much higher in the DS (Table 2).

Souza et al. (2014) investigated the Cr, Ni, Cu, Zn and Pb concentrations in DS of two wastewater treatment plants located in large urban cities in São Paulo State/Brazil, Campinas and Jaguariuna. The authors also divided the samples into the dry and wet seasons. Unlike the present study, Souza et al.(2014) found Cr, Ni, and Cu concentrations in the DS of both studied cities. Regarding Zn, analyzing the DS for the city of Campinas, showed the same behavior of the concentration found in this study. The concentration in the wet season (1.468 ± 0.008 mg L^{-1}) was higher compared to the dry season (1.100 ± 0.008 mg L^{-1}). The authors also related this to the prevalence of illegal rainwater connections that facilitate the drag of materials from streets and rooftops during the rainy season or possible illegal industrial. Pb concentrations in Jaguariuna were similar to the present study and lower than the city of Campinas for both seasons.

Similar concentrations to the present study were found by Pipi et al. (2018). The authors analyzed samples of raw and treated DS, from two treatment plants, Candeia (Bauru, São Paulo, Brazil) and Tibiriça (Tibiriça, São Paulo, Brazil), with no season divisions. The authors investigated the presence of Ba, Mn, Cu, Fe and Al, and in both cities this TMs were found in the DS.

These results (Table 2) illustrate how quite variable the TMs concentrations on DS may be. TMs in DS will vary depending on the size of the city, the economic activities in operation, such as industries, agriculture, cattle raising, and if the environmental laws are observed.

4.3.4.2 Metals concentration in the ASTBR effluent and sludge - Within the scope of environmental discharge

Table 3 shows the average metal concentration found in the inoculation foam (foam with sludge + clean foam) and in the end of operation foam (foam with sludge). It can also be seen, the average concentrations found in the effluent. Both results are compared with the current Brazilian resolutions for discharge.

Table 3. Average total metal content in the inoculation foam (foam with sludge + clean foam) and end of operation foam, from ASTBR treating DS.

Metals	Inoculation foam (mg gTS^{-1})	Sewage sludge (End of operation) (mg gTS^{-1})	BR resolution no. 498, 2020** (mg gTS^{-1})	Effluent (mg L^{-1})	BR resolution no. 430, 2011*** (mg L^{-1})
Al	36.63 ± 1.81	27.63 ± 1.80		1.47 ± 1.31	---
As	n.d.*	n.d.*	0.04	n.d.*	0.5
Ba	1.13± 0.122	0.88 ± 0.12	1.3	0.04 ± 0.04	5.0
Cd	n.d.*	n.d.*	0.03	n.d.*	0.2
Co	0.005 ± 0.002	0.002 ± 0.001	---	n.d.*	---
Cr	0.06 ± 0.05	0.05 ± 0.05	1.0	n.d.*	1.0
Cu	5.87 ± 0.32	4.63 ± 0.12	1.5	n.d.*	---
Fe	49.94 ± 2.0	34.42 ± 2.01	---	1.33 ± 1.00	15
K	17.36 ± 0.90	15.95 ± 0.90	---	7.67 ± 5.76	---
Mg	9.88 ± 1.91	7.51 ± 1.91	---	2.61 ± 2.05	---
Mn	0.97 ± 0.04	0.78 ± 0.02	---	0.05 ± 0.02	1.0
Ni	0.09 ± 0.03	0.06 ± 0.03	0.4	n.d.*	2.0
Pb	0.11 ± 0.09	0.11 ± 0.09	0.3	0.01 ± 0.01	0.5
Se	0.02 ± 0.04	0.02 ± 0.04	0.03	n.d.*	0.3
Zn	6.22 ± 2.51	4.33 ± 2.51	2.8	0.07 ± 0.03	5.0

*n.d.: not - detected; *Brazilian Resolution (BR) - Environmental Sanitation Company no. 498, 2020 - maximum permitted values in biosolids for use in soils; ***Brazilian Resolution (BR) - Environmental Sanitation Company no. 430, 2011 - maximum values allowed for effluent discharge

Considering the effluent concentrations, the values of Ba, Fe, Mn, Pb and Zn are in agreement with the Brazilian Resolution CONAMA no. 430, 2011 (National Environmental Council, 2011), which sets standards maximum metals values allowed for effluent discharge, which means in relation to these metals, the effluent treated by the ASTBR are allowed to be discharge in water bodies.

Anaerobic digestion produces much less sludge compared to aerobic digestion, but it still requires proper management of its disposal and possible uses. There are opportunities and challenges about the sustainable treatment and reuse of sewage sludge.

The common employed routes for the safe treatment and disposal of sewage sludge includes incineration, pyrolysis(Raheem et al., 2018), substrate for soil fertilization and remediation.

The use of sewage sludge as fertilizer has the potential to reducing greenhouse gas emissions and improve soil conditions. Also, has economic potential, with low costs compared to traditional fertilization. Sewage sludge reuse requires careful management, not only the removal of pathogens and other substances, but also inorganic and contaminants(Kacprzak et al., 2017).

The Brazilian Resolution (BR) CONAMA - Environmental Sanitation Company no. 498, 2020(National Environmental council, 2020)standards maximum metals values allowed for sewage sludge disposal in the soil. According to the results presented in this research (Table 4), the metals concentrations found in the inoculum and the sludge of the end of operation are in agreement, being available for reuse, analyzing only metals content.

4.3.4.3Metals loading rate in the ASTBR influent and effluent

The analysis of the total metal from samples of the liquid phase of the ASTBR, operated in the long term, allowed the determination of the dynamics of the TM found. The TM loading rate found in the ASTBR influent and effluent is present in Figure 6. As, Cd, Co, Cr, Cu, Ni, and Se were not detected on the collected samples. Observing the TM concentration in the HRT of 12 h, all the eight metals detected showed higher concentrations in the effluent than the influent, decreasing throughout the operation and remaining similar to the influent concentrations (Fig. 5). The higher values in the effluent can be related to the loss of metals present in the sludge used for inoculation, as the values of total metal content in the sample of the sludge attached to the foam at the end of the reactor operation are lower than the initial values (Table 4).

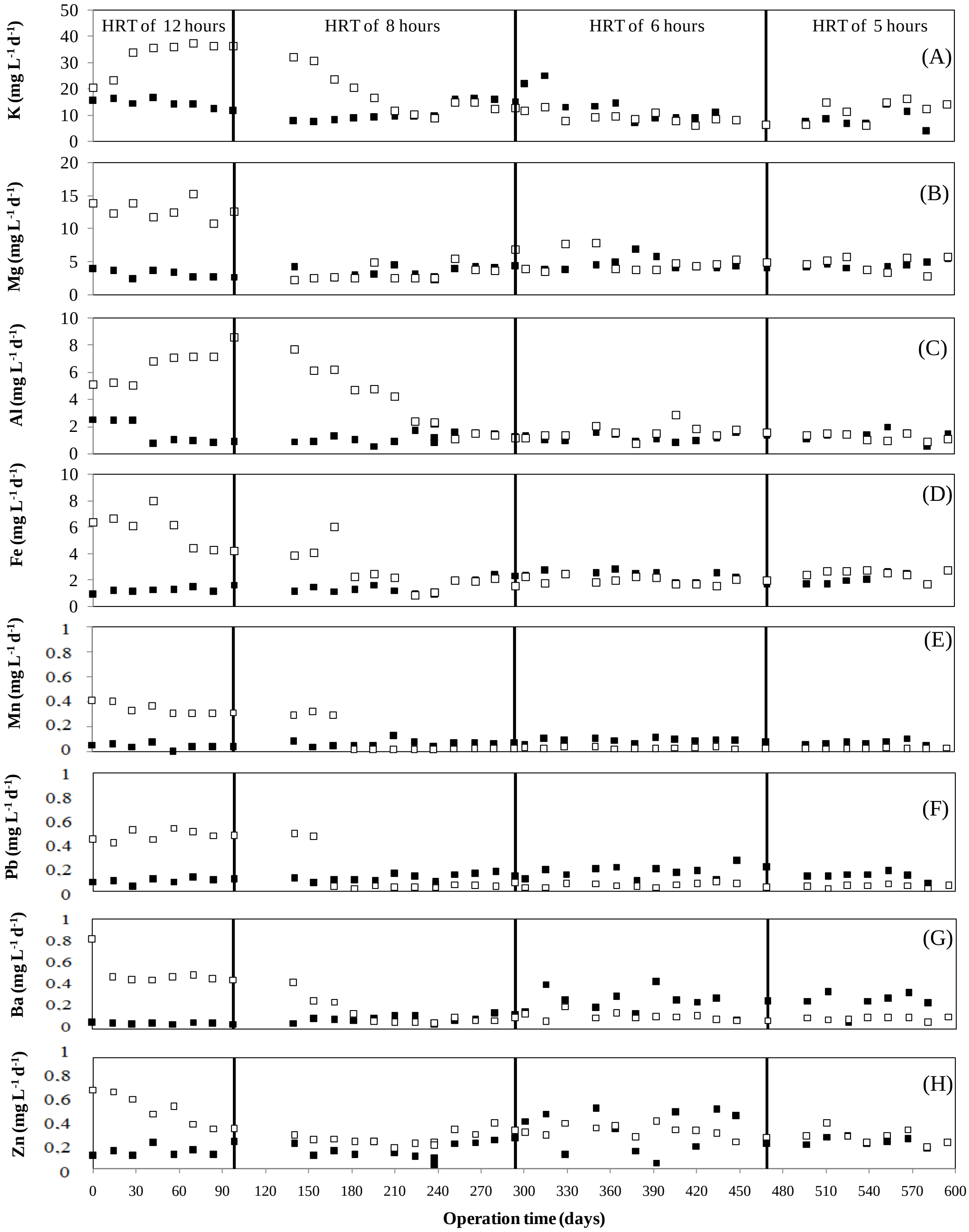

Figure 5. K (A), Mg (B), Al (C), Fe (D), Mn (E), Pb (F), Ba (G), Zn (H) loading rate (mg $L^{-1}d^{-1}$) of the influent and effluent, along the ASTBR operation. Legend: ■Influent; □ Effluent.

It is also possible to observe that (Fig. 6), that the effluent concentration decreases after a certain period, more specifically, Mg and Zn around the 100^0 day of operation, Fe around the 200^0, Mn, Pb, and Ba around the 170^0 day and K and Al around the 240^0 day.

This may indicate that the metals present in the affluent leave the reactor without being absorbed by the biomass. The results which show that the concentrations in the sludge at the end of operation are lower than the sludge from the inoculum corroborate with the fact of no metals absorbency by the biomass. It can be inferred that the simple addition of metals thought the influent may have no impact on reactor performance.

Zitomer et al., (2008) elucidate that even with total metal content being provided in sufficient concentrations, the metals may not be present in a bioavailable form that can be utilized by the microorganisms.

The metals content in the substrate have to be retained by the sludge in order to prevent losses with the effluent and to be available for the biomass (Zandvoort et al., 2006). In this case, the supplementation of metals using appropriate strategies such pulse or constant addition, with a bioavailability study should be verified.

4.3.5 Hydrodynamic assays

The exit age distribution (E), or residence time distribution (RTD) curve of a tracer represents the distribution of the times for the fluid output of the ASTBR (Fig 6). The results showed that the concentration peak of the tracer occurred at 300 min (5h). Thus, the expected theoretical HRT coincided with the actual HRT obtained by the hydrodynamic assay at the end of the operation, which indicates the absence of anomalies in the flow pattern of these reactor configurations with polyurethane foam. Fitting the dispersion model presented a coefficient of 1.5 x 10^{-5}, with correlation to the model equals to 0.995, demonstrating the flow pattern throught the reactor is plug-flow. Anzola-Rojas and Zaiat (2016) and Carneiro et al. (2019) considered the flow pattern was plug-flow using ASTBR with polyurethane foam, corroborating the results of the present work.

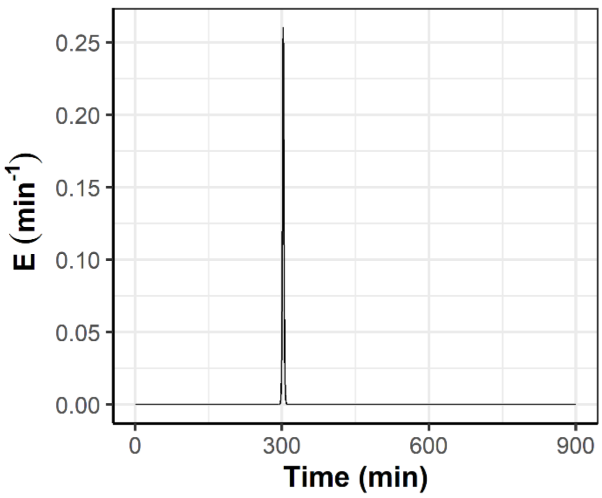

Figure 6. Exit age distribution (E), or residence time distribution (RTD), curve of tracer leaving the ASTBR.

Figure 7 shows the exit age distribution (E), or residence time distribution (RTD), of FeCl solution (120 mgFe L^{-1}) leaving the ASTBR.

The results presented shows the concentration peak of the tracer at 231 min (3.85 h). In this case, the expected theoretical HRT of 5 hours did not coincided with the actual HRT obtained by the hydrodynamic assay using Fe Cl solution, which indicates the presence of anomalies in the flow pattern. Fitting the dispersion model presented a coefficient of 4.1×10^{-3}, with correlation to the model equals to 0.995. This coefficient is still considered as plug-flow according to Levenspiel, (1999) (plug-flow - dipersion coefficient <0.01).The anomaly found in the hydrodynamic assay using FeCl solution, may be related to stagnant areas or preferred paths inside the reactor caused perhaps by the characteristics of the tracer.

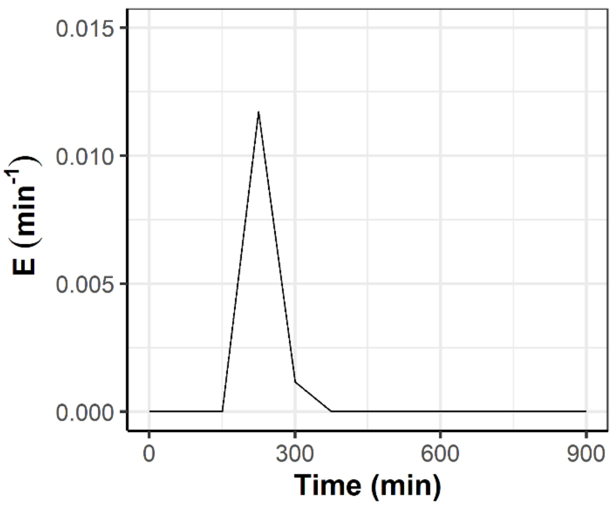

Figure 7. Exit age distribution (E), or residence time distribution (RTD), of FeCl solution (120 mgFe L^{-1}) leaving the ASTBR.

Regarding the reactor's performance, the addition of Fe to the reactor during the hydrodynamic assay, resulted in a discreet improvement in the COD removal efficiency (Tab. 4), even for a short period of exposition of the metal. This improvement is also reported in Silva et al., (2021), which corroborates to the improvements founded in removal.

Table 4. COD results (concentration in the influent, effluent and removal efficiency) for the last days of operation and during the Hydrodinamic assay for Fe

ASTBR COD concentrations					
End of operation			**Hydrodinamic assay for Fe**		
Influent (mg L^{-1})	**Effluent (mg L^{-1})**	**COD removal effiency (%)**	**Influent (mg L^{-1})**	**Effluent (mg L^{-1})**	**COD removal effiency (%)**
465,0	139,5	70,00	654,5	159,5	75,6
514,5	159,0	69,10	604,0	151,0	75,0
582,0	178,5	69,33	633,0	135,0	78,7
534,5	110,5	79,33	639,5	146,0	77,2
514,0	99,0	80,74	655,0	140,5	78,5
506,5	176,5	75,00	663,0	132,0	80,1
399,5	198,5	70,00	627,0	131,0	79,1
594,0	136,0	77,10	576,5	131,0	77,3
592,5	145,5	75,44	542,0	118,5	78,1
487,0	115,5	76,28	551,0	113,0	79,5
512,0	119,5	76,66	599,0	114,0	81,0
606,0	187,5	69,06	634,5	116,5	81,6
527,0	174,0	66,98	649,0	113,5	82,5

4. 4 CONCLUSIONS

With respect to the global performance, the results obtained demonstrated the feasibility of applying the ASTBR to the treatment of domestic sewage. The immobilized cell growth led to a peak organic matter removal value of 81.6% during the HRT of 8 hours, in a methanogenic based on a stable long-term operation (614 days).

Focusing in the different HRT operated, even with the decrease in the COD removal efficiency in each period of change, the ASTBR was capable of recovering the efficiency, with the adaptation of the biomass and showing stable values again along the operation

Concerning about the metal concentrations in the raw domestic sewage and in the treated domestic sewage were detected Al, Ba, Fe, K, Mn, Mg, Pb, and Zn. In the inoculum and sludge in the end of operation were detected Al, Ba, Co, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Se and Zn. For the metals standardized in the Brazilian legislation (sewage: Ba, Cr, Cu, Ni, Pb, Se and Zn; effluent: Ba, Cr, Fe, Mn, Ni, Pb, Se and Zn) both liquid phase and solid phase of the samples of the ASTBR are in accordance with the limits allowed for discharge and use in agriculture or soil disposal.

Regarding to the metals loading rate, the fact that outwardly, the metals were not absorbed by the biomass, maybe the simple addition of metals through the influent may have no impact on reactor performance. Thus, the supplementation of metals using appropriate dosing strategies such pulse or constant addition, with a bioavailability study for each applied condition, using metals fractionation straction should be encouraged to make sure that there is a sufficient amount of metals present in the bioavailable fraction for microbial uptake.

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Chapter 5

GENERAL CONCLUSIONS

The main objective of this thesis was to analyze different operational conditions applied to two anaerobic reactors treating DS, in terms of operational stability and trace metals profile. This thesis also aims to answer the following questions: “What is the statically significant effect of TMs on maximum methane production from DS? Considering these, it was concluded:

- Both anaerobic reactors, The UASB and the ASTBR were capable of treat the DS applied as substrate, showing COD removal efficiencies above 68%. Considering the ASTBR as an innovative anaerobic reactor treating DS, the reactor was considered as feasible.
- Both reactors maintain a steady operation, with neutral pH in the effluents.
- The Placket-Burman fractional factorial design (PB) was useful to select the statistically significant metals in CH_4 production, resulting in two statistically significant metals in CH_4 production, Fe and Se, being Fe the only with positive effect, influencing an improvement of 33% in CH_4 production at a concentration of 120 mg L^{-1}.
- In the operations of the UASB and the ASTBR, the metals detected in th effluent were above the Brazilian legislation for waterbodies discharge.
- For further research, metals bioavailability and dosing assessments in the reactor may contributed for understanding complexity of metals in anaerobic digestion, to ensure maxi mum substrate conversion rates, to prevent disturbances in reactor performance using a minimum amount of metals.