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CÂMPUS DE JABOTICABAL

HIGH-RATE ANAEROBIC REACTORS TREATING COFFEE PROCESSING WASTEWATER AT DIFFERENT OLR AND POST-TREATMENTS: EFFECTS ON METHANE PRODUCTION, EFFLUENT QUALITY AND MICROBIAL POPULATIONS

Wilmar Alirio Botello Suárez
Engenheiro de produção biotecnológica

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*"Todo mundo quer viver em cima da montanha, sem saber que
a verdadeira felicidade está na forma de subir a escarpada"*

Gabriel Garcia Márquez

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RESUMO - Com o intuito de otimizar a geração de metano e reduzir a concentração de poluentes das águas residuárias do processamento de café (ARC), o presente estudo teve como objetivos gerais: (i) Avaliar o desempenho de reatores anaeróbios de alta taxa e analisar a microbiota associada a este processo sob diferentes condições operacionais, e (ii) Comparar a eficiência de sistemas de pós-tratamento na remoção de nitrogênio (N), fósforo (P), sólidos e compostos fenólicos presentes nas ARC biodigeridas. Para o desenvolvimento do primeiro objetivo, foi empregado um sistema UASB em dois estágios, e uma série de três reatores anaeróbios horizontais de leito fixo (RAHLF). Os sistemas foram submetidos a diferentes cargas orgânicas volumétricas (COV), de até 18,2 g DQO $(L\ d)^{-1}$ (UASB) e 9,5 g DQO $(L\ d)^{-1}$ (RAHLF), e operados de forma continua com recirculação parcial do efluente obtido. Os maiores valores médios de produção volumétrica de metano foram de 2,0 e 1,9 L CH_4 $(L\ d)^{-1}$ para os reatores UASB e RAHLF, respectivamente. Em condições de alta COV, a análise metagenômica da microbiota associada ao reator UASB de melhor desempenho evidenciou prevalência dos gêneros bacterianos sintróficos *Syntrophus* e *Candidatus* Cloacimonas assim como de arqueias hidrogenotrôficas e acetotrôficas dos géneros *Methanosaeta*, *Methanosarcina, Methanoculleus*, *Methanobacterium* e *Methanomassiliicoccus*. Em relação ao sistema RAHLF, análises de reação em cadeia da polimerase quantitativa (qPCR) dos principais grupos methanogênicos associados ao lodo dos reatores indicaram predominância de arqueias acetotróficas correspondentes à ordem Methanosarcinales. Os dois sistemas proporcionaram condições favoráveis para a remoção da matéria orgânica com simultânea produção de energia. Para a execução do segundo objetivo, o pós-tratamento biológico foi realizado em um reator em batelada sequencial (RBS) utilizando um ciclo de 24 h dividido em 4 fases: alimentação, reação (anaeróbia/aeróbia/anóxica), sedimentação e descarte. Operado sob curtos períodos de enchimento, este sistema permitiu atingir altos níveis de remoção de N (80%) e P (71%), apresentando altas concentrações de bactérias nitrificantes e desnitrificantes. O pós-tratamento químico foi baseado na reação de Fenton, e apresentou maiores níveis de remoção de sólidos totais (83%) e compostos fenólicos (99%) quando comparado com o RBS. A recirculação parcial do efluente do processo Fenton melhorou o desempenho de reatores anaeróbios tratando ARC, constituindo uma alternativa de reuso promissora. O sistema integrado (RBS seguido do processo Fenton), demostrou ser a melhor alternativa para atingir padrões de lançamento e disposição final.

Palavras-chave: arquéias metanogênicas, biogás, reatores UASB em dois estágios, reator anaeróbio horizontal de leito fixo, remoção biológica de nutrientes, processo Fenton

HIGH-RATE ANAEROBIC REACTORS TREATING COFFEE PROCESSING WASTEWATER AT DIFFERENT OLR AND POST-TREATMENTS: EFFECTS ON METHANE PRODUCTION, EFFLUENT QUALITY AND MICROBIAL POPULATIONS

ABSTRACT – In order to optimize the methane production and to reduce the pollutant concentrations from coffee processing wastewater (CPW), this study aimed: (i) To evaluate the performance of high-rate anaerobic reactors and to analyze the microbiota associated to this process at different operational conditions, and (ii) To compare the removal efficiency of post-treatment systems regarding nitrogen (N), phosphorus (P), solids and phenolic compounds contained in biodigested CPW. For the development of the first objective, a two-stage UASB system and a series of three horizontal-flow anaerobic immobilized biomass reactors (HAIB) were employed. The systems were submitted to different organic loading rates (OLRs), up to 18.2 g COD $(L\ d)^{-1}$ (UASB) and 9.5 g COD $(L\ d)^{-1}$ (HAIB), and operated continuously with partial effluent recirculation. The highest mean values of methane production rate were 2.2 and 1.9 L CH_4 $(L\ d)^{-1}$ for UASB and HAIB reactors, respectively. Under high OLR, the metagenomic analysis of the microbiota associated to the UASB reactor showed a high prevalence of the syntrophic genera *Syntrophus* and *Candidatus* Cloacimonas, as well as the hydrogenotrophic and acetotrophic methanogens *Methanosaeta*, *Methanosarcina,* *Methanoculleus*, *Methanobacterium* and *Methanomassiliicoccus*. Concerning the HAIB system, quantitative polymerase chain reaction (qPCR) analyses of the main methanogenic groups associated to the reactor sludge indicated the predominance of acetotrophic methanogens, corresponding to the order Methanosarcinales. Both systems provided favorable conditions for organic matter removal with simultaneous energy production. To achieve the second objective, the biological post-treatment was performed in a sequential batch reactor (SBR), using a 24-h cycle divided in 4 phases: feeding, reaction (anaerobic/aerobic/ anoxic), settle and decant. Operated at short inlet flow mode, this system showed high removal levels of N (80%) and P (71%), showing high concentrations of nitrifying and denitrifying bacteria. The Fenton reaction was used as chemical post-treatment, showing higher removal levels of total solids (83%) and phenolic compounds (99%) in comparison with the SBR option. The performance of anaerobic reactors treating CPW was improved by partial Fenton effluent recirculation, with which this activity represented a promising reuse alternative. The integrated system (SBR followed by Fenton process) was effective to meet with the discharge standards.

Keywords: biogas, biological nutrient removal, Fenton process, horizontal-flow anaerobic immobilized biomass reactor, methanogenic archaea, Two-stage UASB reactors

LIST OF ABBREVIATIONS

BOD:	Biological oxygen demand
COD:	Chemical oxygen demand
CPW:	Coffee processing wastewater
DCPW:	Digested coffee processing wastewater
DO:	Dissolved oxygen
HAIB:	Horizontal-flow anaerobic immobilized biomass
HRT:	Hydraulic retention time
IA:	Intermediate alkalinity
MLSS:	Mixed liquor suspended solids
MLVSS:	Mixed liquor volatile suspended solids
MPR:	Methane production rate
N-org:	Organic nitrogen
OLR:	Organic loading rate
OTUs	Operational taxonomic units
PA:	Partial alkalinity
PAOs:	Phosphate accumulating organisms
PHB:	Polihydroxybutyrate
qPCR:	Quantitative polymerase chain reaction
SBR:	Sequencing batch reactor
SLR:	Sludge loading rate
SMY:	Specific methane yield
SRT:	Sludge retention time
STP:	Standard temperature and pressure
SVI	Sludge volume index
TA:	Total alkalinity
TAN:	Total ammonia nitrogen
TDS:	Total dissolved solids
TKN:	Total Kjendal nitrogen
TN:	Total nitrogen
TP:	Total phosphorous
TPC:	Total phenol content
TS:	Total solids
TSS:	Total suspended solids
UASB:	Up-flow anaerobic sludge blanket
VFA:	Volatile fatty acids
VS:	Volatile solids

CHAPTER 1 - General considerations

1. INTRODUCTION

Coffee is one of the most commonly marketed beverages all over the world and the second largest commodity in trading volume, being surpassed only by oil (Alves et al., 2017). It is farmed in tropical development countries such as Brazil, Vietnam and Colombia, and is consumed mainly in Europe and in the United States (FAO, 2015). Brazil is by far the world's largest producer, with about a third of the total marketed worldwide (ICO, 2018). Following the several steps of production, the coffee industry generates huge amounts of agricultural waste and by-products, which usually ranged from 30% to 50% of the weight of the total coffee produced (Oliveira and Franca, 2014). Since these wastes and by-products constitute a serious environmental issue for hydric systems and lands around the production areas, its adequate disposal is a critical topic for all the producing countries.

Coffee processing wastewater (CPW) represents one of the more relevant wastes of the coffee industry. This effluent is produced during the application of the wet processing method in the post-harvest activities, and its generation can reach from 20 to 45 kg per kg of coffee beans (Schwan and Fleet, 2014). Arabica coffee is usually processed by these methods and accounts for approximately 62% of the world coffee market, which implies that most of the wastewater generated is from the production of quality coffees (Pires et al., 2017). In Brazil, Arabica coffee production corresponding to 76% of the total production (ICO, 2018). Consequently, given the adoption of the wet method as processing alternative, the generation of CPW has increased considerably the last years.

CPW presents a high concentration of organic pollutants which are released during coffee pulping and mucilage removal, thereby generating high levels (45 kg per ton of coffee beans) of chemical oxygen demand (COD) (Pires et al., 2017). Therefore, biological treatment systems can be applied for its stabilization, in order for meeting with the increasingly stringent regulatory discharging standards or for reuse purposes. This technology is additionally advantageous for its application in the producing regions, given that the favorable conditions of the tropics facilitate its implementation.

The processes based on anaerobic biotechnology, primarily through the use of high-rate bioreactors, constitute an exciting alternative for the effluent stabilization with simultaneous generation of renewable energy. This technology utilizes configurations that provide significant retention of active biomass, resulting in substantial differences between the sludge retention time (SRT) and the hydraulic retention time (HRT). Consequently, high removal efficiencies of organic matter and biogas production, and low sludge generation can be achieved if appropriate organic loading rates (OLRs) are employed (Grady et al., 2011). Considering these advantages and due to its high organic matter concentration, diverse studies have focused on the application of anaerobic digestion as strategy for the stabilization of CPW.

The main anaerobic technologies employed for CPW treatment apply suspended growth biomass systems like for example the upflow anaerobic sludge blanked (UASB) reactor in single or two stages (Jung et al., 2012; Guardia-Puebla et al., 2014) or the more recently developed expanded granular sludge blanket (EGSB) reactor (Cruz-Salomón et al., 2017). In addition, the performance of the attach biomass systems (i.e. the anaerobic fixed bed reactor (AFBR)) and hybrid bioreactors as the upflow anaerobic hybrid reactor (UAHR), which contain significant quantities of both suspended and attached biomass also has been evaluated (Selvamurugan et al., 2010a; Fia et al., 2012). However, some technical issues need to be addressed to optimize this technology. The high concentration of easily acidify organic matter contained in CPW, which generate significant amounts of fatty acids through partial degradation of substrate, can produce unfavorable conditions for the activity of the associated methanogenic microbiota and thereby alter the reactor performance (Guardia-Puebla et al., 2014; Villa-Montoya et al., 2017).

To overcome these drawbacks, most of the studies have focused on applying low OLRs (from 3 to 6 g COD $(L\ d)^{-1}$) in high rate systems such as the UASB reactor. Higher OLRs have been obtained only with the application of systems with adhered biomass (Oliveira and Bruno, 2013) or with hybrid reactors (Selvamurugan et al., 2010a) evidencing a COD removal efficiency between 46 and 79% (Table 1).

Regarding methane production, the best yields have been obtained only by applying high HRTs (Beyene et al., 2014) or by separating the acidogenic and methanogenic phases employing UASB systems in two stages (Jung et al., 2012). Therefore, there is still a need for addressed investigations to explore these aspects, aimed to define optimal operational conditions to enhance the methane production reaching higher removal efficiencies.

The knowledge of the structure of microbial communities associated to anaerobic treatment systems is a critical topic that can potentially offers solutions concerning the estimation of design parameters, operation and control of the process (Song et al., 2010). With the development of the high-throughput sequencing technologies and the increase of the data processing capacity, it has been possible the direct sequencing of environmental samples, which has allowed access to a large amount of information related to the structure of microbial communities associated with biological treatment systems (Fang and Zhang, 2015). Previous studies have used these approaches to investigate the microbial diversity and its interactions during the anaerobic digestion of different types of wastes, at taxonomic and functional level (Barros et al., 2017; Delforno et al., 2017a, 2017b), These works have reveled a high genetic potential in these systems, which open promising perspectives for biotechnological applications.

Despite its importance, information concerning to dynamic of the microbial community involved in the anaerobic digestion of CPW is scarce. In fact, only one study (Pires et al., 2017) has attempted to analyze the diversity of cultivable microbiota in a CPW treatment system. Thus, detailed studies on the effect of operational parameters on microbial consortia are required, and would enhance the levels of efficiency of this process, contributing to the development of more sustainable systems for the coffee industry.

Given the high organic strength of the CPW, not complete pollutant stabilization is obtained by using anaerobic technology, remaining amounts of solubilized organic matter, ammoniacal nitrogen, phosphorous and phenolic compounds in the effluent obtained (Villa-Montoya et al., 2017).

Table 1. Overview of works that have been done in the area of CPW treatment in recent years.

	HRT	OLR	T	Removal efficiency (%)					MPR	Reference
				COD	BOD	N	P	TPC		
Anaerobic systems										
EGSB	3-9	1.5-6	26	98	-	-	-	-	-	Cruz- Salomón et al., 2017
ABR	70	0.12	20-23	90	93	82	97	-	3.67	Beyene et al., 2014
UASB	0.7-0.9	3.6-4.1	35	77.2	-	-	-	-	0.44	Guardia-Puebla et al., 2014
Two stage UASB reactors										
Acidogenic stage	0.5	11	37	83.5	-	-	-	-	-	Guardia-Puebla et al., 2014
Methanogenic stage	2.6	2.6	37		-	-	-	-	0.19	
Acidogenic stage	0.3	-	55	98	-	-	-	-	-	Jung et al., 2012
Methanogenic stage	2	3.5	35		-	-	-	-	2.06	
UASB R1	4-6.2	3.6-5.8	21	55-94	-	-	-	72-90	0.42-0.66	Bruno and Oliveira, 2008
UASB R2	2-3.1	0.4-5.8		28-62	-	-	-		0.29-0.48	
HASBR + two HAFBR	3.8	3-12	21	79	-	-	-	52	1.71	Oliveira and Bruno, 2013
UAF	1.06	0.8-4.4	32.9	80	-	-	-	-	-	Fia et al., 2012
AFBR	1.3	1-5	16-19	80	-	-	-	-	-	Fia et al., 2010
UASB	-	-	-	-	70	-	-	-	-	Kondo et al., 2010
UAHR	0.3-1	7-28.4	-	46-70	71	-	-	-	0.26	Selvamurugan et al., 2010a
Aquatic-based systems										
Wetlands	12	-	18-25	87	84	-	-	-	-	Rossmann et al. 2013
Wetlands	12	-	-	-	-	69	72	72	-	Rossmann et al. 2012
Physicochemical process										
Fenton's oxidation	-	-	-	-	35	-	-	-	-	Kondo et al., 2010
Photo-Fenton's oxidation	-	-	-	-	65	-	-	-	-	
C/F process	-	-	-	67	-	-	-	-	-	Zayas et al., 2006
C/F + photo Fenton	-	-	-	86	-	-	-	-	-	
Integrated treatment systems										
Two stage UASB + SBR	3.75	3-6	24-25	94	-	-	-	94	0.75	Villa-Montoya et al., 2017
Two stage UASB + SBR	13.7	2.3-4.5	20-21	95	-	91	84	97	0.32	Bruno and Oliveira, 2013
UASB- UAFB	0.7	2.6	37	84.2	-	-	-	-	-	Guardia-Puebla et al., 2014
ECC + SBR	0.4	-	-	84	-	-	-	-	-	Maesh et al., 2014
UAHR + aeration + wetlands	1.8	9.6	-	97	98	-	-	-	-	Selvamurugan et al., 2010b
Photo-Fenton + UASB	-	-	-	-	95	-	-	-	-	Kondo et al., 2010

HRT: Hydraulic retention time (d), OLR: Organic loading rate (g COD $(L\ d)^{-1}$), T: Temperature (ºC), COD: Chemical oxygen demand, BOD: Biochemical oxygen demand, N: Total nitrogen, P: Total phosphorous, TPC: Total phenol content, MPR: Methane production rate ($L(L_{reactor}d)^{-1}$), EGSB: Expanded granular sludge bed reactor, ABR: Anaerobic batch reactor, UASB: Upflow anaerobic sludge bed reactor, HAS(F)BR:, Horizontal anaerobic sludge (fixed) bed reactor, UAFB: Upflow anaerobic fixed bed reactor AFBR: Anaerobic fixed bed reactor, UAHR: Upflow anaerobic hybrid reactor, C/F: Coagulation-flocculation, SBR: Sequential batch reactor, ECC: Electrochemical coagulation.

Therefore, to meet the effluent discharge standard the implementation of post-treatment systems is necessary. Thereby, in order to obtain an effluent of better characteristics, several experimental approaches have used integrated configurations of anaerobic-aerobic or biological and chemical processes with different degrees of success (Table 1). Selvamurugan et al. (2010b) used a hybrid reactor coupled to a wetland system to treat CPW previously aerated obtaining removal rates up to 97% for organic matter. Concerning the removal of nutrients (N, P) and phenolic compounds, the use of wetlands is highlighted as a plausible treatment strategy (Rossmann et al., 2012, 2013), with efficiency levels up to 69% and 72% for P and phenols, respectively.

The application of anaerobic-aerobic systems such as UASB followed by a sequential biological reactor (SBR) also has shown promising results (Bruno and Oliveira, 2013) (Table 1). In addition to these systems, post-treatment options can be established by application of (photo) Fenton and related advanced oxidation processes (AOPs), which involves reactions of peroxides (usually H_2O_2) with iron ions to produce oxygen species that oxidize organic or inorganic compounds when they are present (Pignatello et al., 2006). Other processes such as coagulation-flocculation, electrochemical coagulation and integrated treatment such as photo-Fenton plus UASB reactor have also been considered (Zayas et al., 2007; Kondo et al., 2010; Mahesh et al., 2014). Nevertheless, most of the research concerning CPW post-treatment has tended to focus on removal of organic matter becoming the lack of information about the scope of these technologies (in terms of stabilization of others critical pollutants and influence of operational variables) one of the main drawbacks to adopting it at real scale.

Therefore, considering the need to improve the performance of systems for treating CPW, and to generate fundamental knowledge that allows the optimization of processes for biological wastewater treatment, the aims of this thesis were:

(i) To evaluate the performance of high-rate anaerobic reactors in the treatment of CPW regarding methane production and organic load stabilization, and to determinate the diversity and structure of microbial populations involved in the anaerobic digestion in these systems.

In works independently realized by our research group (Bruno and Oliveira, 2013; Oliveira and Bruno, 2013; Villa-Montoya et al., 2017), the results showed that: (i) A two-stage UASB system can reach OLRs higher than 6 g total COD (L d)$^{-1}$ in stable conditions. Also (ii) It is possible to improve the methane production and COD removal of a series of horizontal-flow anaerobic immobilized biomass reactors (HAIBs). Therefore, we implemented the operation of these systems controlling the acidification employing effluent recirculation, stabilization of the substrate alkalinity and gradual increase of OLR. In Chapter 3 it will be shown that a two-stage UASB system submitted at different OLRs can obtain a high efficiency. In addition, a metagenomic analysis of the UASB reactor with the better performance is described. It will show the relevant microbial groups involved in the anaerobic treatment of CPW and as the microbial structure is affected in response to the organic loading variation. The results obtained contribute to the understanding of crucial microbiological and biochemical aspects for the development of optimization strategies for this process, and its potential for the application of others biotechnologies.

Chapter 4 presents the performance of a HAIB system composed of three reactors for CPW treatment and methane production, using an organic support material (corn cobs) as start-up strategy. Besides, the performances of HAIB and UASB reactors at similar operational conditions are compared. Otherwise, it will be shown the abundance of relevant methanogenic groups at different operational requirements (i.e. different OLRs and reactor configurations) by using microbiological and molecular techniques.

(ii) To compare the efficiency of biological (SBR reactor) and chemical (Fenton oxidation) processes as post-treatment strategies to improve the effluent quality generated by anaerobic processes.

Removal efficiencies obtained concerning nutrients (N, P) and phenolic compounds presents in CPW by using anaerobic reactors are not satisfactory. Despite the relative efficiency obtained by diverse post-treatment approaches, primary difficulty encountered is mainly associated with simultaneous pollutant removal and need by applying high HRTs. In Chapter 5 we aimed to assess two feeding regimes on an SBR treating anaerobically digested CPWs, to determine the most effective feeding strategy. The effects of this treatment process were additionally analyzed considering the performance of the SBR cycle (composed by an anaerobic/aerobic/anoxic scheme), abundance of cultivable heterotrophic, nitrifying and denitrifying bacteria and activated sludge proprieties. Besides, this section shows the application of the Fenton process as a strategy for digested CPW post-treatment, and an integrated option (SBR followed by Fenton process), aiming to meet with the standard regulations for effluent discharge. Finally, a possible method of reuse by partial Fenton effluent recirculation in anaerobic reactors is presented.

In the present chapter, the theoretical bases for the development of the proposed objectives are described.

3. REFERENCES

Ali Shah F, Mahmood Q, Maroof Shah M, Pervez A, Ahmad Asad S (2014) Microbial ecology of anaerobic digesters: The key players of anaerobiosis. **The Scientific World Journal** 2014:1-21.

Alves R, Rodrigues F, Nunes MA (2017) State of the Art in Coffee Processing By-Products. In: Galanakis, C. (Ed.). **Handbook of Coffee Processing By-Products**. London:Academic press, p. 1-22.

Amani T, Nosrati M, Sreekrishnan TR (2010) Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects – a review. **Environmental Reviews** 18:255-278.

Ayeche R (2012) Treatment by coagulation-flocculation of dairy wastewater with the residual lime of national Algerian industrial gases company (NIGC-Annaba). **Energy Procedia** 8:147-156.

Babuponnusami A, Muthukumar K (2014) A review on Fenton and improvements to the Fenton process for wastewater treatment. **Journal of Environmental Chemical Engineering** 2:557-572.

Badawy MI, Wahaab RA, El-Kalliny AS (2009) Fenton-biological treatment processes for the removal of some pharmaceuticals from industrial wastewater. **Journal of Hazardous Materials** 67:567-574.

Barana AC, Lopes DD, Martins TH, Pozzi E, Damianovic M, Del Nery V, Foresti E (2013) Nitrogen and organic matter removal in an intermittently aerated fixed-bed reactor for post-treatment of anaerobic effluent from a slaughterhouse wastewater treatment plant. **Journal of Environmental Chemical Engineering** 1:453-459.

Barros VG, Duda RM, Vantini S, Omori WP, Ferro MIT, Oliveira RA (2017) Improved methane production from sugarcane vinasse with filter cake in thermophilic UASB reactors, with predominance of Methanothermobacter and Methanosarcina archaea and Thermotogae bacteria. **Bioresource Technology** 244:371-381.

Benitez F, Acero J, Real F, Rubio F, Leal A (2001) The role of hydroxyl radicals for the decomposition of p-hydroxy phenylacetic acid in aqueous solutions. **Water Research** 35:1338-1343.

Beyene A, Yemane D, Addis T, Assayie AA, Triest L (2014) Experimental evaluation of anaerobic digestion for coffee wastewater treatment and its biomethane recovery potential. **International Journal of Environmental Science and Technology** 11: 1881-1886.

Bitton G, Wiley-Liss (Eds.) (2005) Wasterwater microbiology. New Jersey:John Wiley & sons, 746p.

Boone DR, Whitman WB, Rouvière P (1993) **Diversity and taxonomy of methanogens.** In: Methanogenesis. Springer, p. 35-80.

Bruno M, Oliveira R (2013) Performance of UASB reactors in two stages followed by post- treatment with activated sludge in wastewater batch of wet- processed coffee. **Engenharia Agrícola Jaboticabal** 33:808-819.

Cai M, Wilkins D, Chen J, Lu H, Jia Y, Lee PKH (2016) Metagenomic reconstruction of key anaerobic digestion pathways in municipal sludge and industrial wastewater biogas-producing systems. **Frontiers in Microbiology** 7:1-12.

Chan YJ, Chong MF, Law CL, Hassell DG (2009) A review on anaerobic-aerobic treatment of industrial and municipal wastewater. **Chemical Engineering Journal** 155:1-18.

Chatila S, Amparo MR, Carvalho LS, Penteado ED Tomita IN, Santos-Neto AJ, Lima Gomes PCF, Zaiat M (2016) Sulfamethoxazole and ciprofloxacin removal using a horizontal-flow anaerobic immobilized biomass reactor. **Environmental technology** 37:847-853.

Chernicharo CAL (2007) **Anaerobic reactors**. London: IWA, 188p.

Cruz-Salomón A, Ríos-Valdovinos E, Pola-Albores F, Lagunas-Rivera S, Meza-Gordillo R, Ruíz-Valdiviezo VM (2017) Evaluation of hydraulic retention time on treatment of coffee processing wastewater (CPWW) in EGSB bioreactor. **Sustainability (Switzerland)** 10:1-11.

Damianovic M, Godoi LAG, Saia FT, Foresti E (2018) Horizontal-flow anaerobic immobilized biomass (HAIB) reactor for organic matter and sulfate removal from paper recycling plant wastewater with simultaneous conversion of sulfide into elemental sulfur. **Chemical Engineering** 6: 964-969.

Damianovic M, Moraes EM, Zaiat M, Foresti E (2009) Pentachlorophenol (PCP) dechlorination in horizontal-flow anaerobic immobilized biomass (HAIB) reactors. **Bioresource technology** 100:4361-4367.

Delforno TP, Lacerda GV, Sierra-Garcia IN, Okada DY, Macedo TZ, Varesche MBA, Oliveira VM (2017a) Metagenomic analysis of the microbiome in three different bioreactor configurations applied to commercial laundry wastewater treatment. **Science of the Total Environment** 587:389–398.

Delforno TP, Lacerda GV, Noronha MF, Sakamoto IK, Varesche MBA, Oliveira VM (2017b) Microbial diversity of a full-scale UASB reactor applied to poultry slaughterhouse wastewater treatment: integration of 16S rRNA gene amplicon and shotgun metagenomic sequencing. **MicrobiologyOpen** 6:1-12.

Dias DFC, Passos RG, Rodrigues VAJ, Matos MP, Santos CRS, Von Sperling M (2018) Performance evaluation of a natural treatment system for small communities, composed of a UASB reactor, maturation ponds (baffled and unbaffled) and a granular rock filter in series. **Environmental technology** 39:490-502.

Drake HL, Küsel K, Matthies C (2006) Acetogenic Prokaryotes. In: **The Prokaryotes**. New York, NY: Springer New York, p. 354-420.

Duda RM, Vantini JDS, Martins LS, Varani ADM, Lemos MVF, Ferro MIT, Oliveira RA (2015) A balanced microbiota efficiently produces methane in a novel high-rate horizontal anaerobic reactor for the treatment of swine wastewater. **Bioresource Technology** 197:152-160.

Dutta A, Sarkar S (2015) Sequencing Batch Reactor for Wastewater Treatment : Recent Advances. **Curr Pollution Rep** 1:177:190.

Echeverria MC, Nuti M (2017) Valorisation of the Residues of Coffee Agro-industry : Perspectives. **The Open Waste Management Journal** 3:13-22.

Enzmann F, Mayer F, Rother M, Holtmann D (2018) Methanogens : biochemical background and biotechnological applications. **AMB Express** 8:1-22.

Fang HH, Zhang T (2015) **Anaerobic biotechnology: environmental protection and resource recovery**. London: Imperial college press, 400p.

FAO. **Statistical pocketbook coffee 2015** (2015) Rome:Food and Agriculture Organization of the United Nations.

Fia FRL, Matos AT, Borges AC, Fia R, Cecon PR (2012) Treatment of wastewater from coffee bean processing in anaerobic fixed bed reactors with different support materials: Performance and kinetic modeling. **Journal of Environmental Management** 108:14-21.

Fia FRL, Matos AT, Borges AC, Moreira DA, Fia R, Júnior EV (2010) Remoção de compostos fenólicos em reatores anaeróbios de leito fixo com diferentes materiais suporte. **Revista Brasileira de Engenharia Agrícola e Ambiental** 14:1079-1086.

Fia R, Matos A, Fia F (2013) Biological systems combined for the treatment of coffee processing wastewater: II - Removal of nutrients and phenolic compounds. **Acta Scientiarum. Technology** 35:451-456.

Fia R, Pereira EL, Fia FRL, Emboaba DG, Gomes EM (2016) Partida de reatores anaeróbios para tratamento de efluentes de abatedouro. **Engenharia Agrícola** 35.

Fontana A, Campanaro S, Treu L, Kougias PG, Cappa F, Morelli L, Angelidaki I (2018) Performance and genome-centric metagenomics of thermophilic single and two-stage anaerobic digesters treating cheese wastes. **Water Research** 134:181-191.

Gogate PR, Pandit AB (2004) A review of imperative technologies for wastewater treatment I: oxidation technologies at ambient conditions. **Advances in Environmental Research** 8:501-551.

Grady CPL, Daigger GT, Love NG, Filipe CDM (2011) Biological wastewater treatment. London:CRC press, 962p.

Guardia-Puebla Y, Rodríguez-Pérez S, Jiménez-Hernández J, Sánchez-Girón V Morgan-Sagastume J (2014) Experimental design technique is useful tool to compare anaerobic systems. **Renewable Bioresources** 2:1-12.

Guerreiro LF, Rodrigues CSD, Duda RM, Oliveira RA, Boaventura RAR, Madeira L M (2016) Treatment of sugarcane vinasse by combination of coagulation/flocculation and Fenton's oxidation. **Journal of Environmental Management** 181:237-248.

Hall, ER (1992) Anaerobic treatment of wastewaters in suspended growth and fixed film processes. **Design of anaerobic processes for the treatment of industrial and municipal wastes.** Technomic Publishing Co., Inc., Lancaster, Pa, 41:41-118.

Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, Hugenholtz P, Yuan Z, Tyson GW (2013) Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. **Nature** 500:567-570.

Hattori S. (2008) Syntrophic Acetate-Oxidizing Microbes in Methanogenic Environments. **Microbes and Environments** 23:118-127.

Hayatsu M, Tago K, Saito M (2008) Various players in the nitrogen cycle: diversity and functions of the microorganisms involved in nitrification and denitrification. **Soil Science & Plant Nutrition** 54:33-45.

ICO (2018). **Trade Statistics**. Available at: http://www.ico.org/. Accessed 13 January 2018.

Jung KW, Kim DH, Lee MY, Shin HS (2012) Two-stage UASB reactor converting coffee drink manufacturing wastewater to hydrogen and methane. **International Journal of Hydrogen Energy** 37:7473-7481.

Kanimozhi R, Vasudevan N (2013) Effect of organic loading rate on the performance of aerobic SBR treating anaerobically digested distillery wastewater. **Clean Techn Environ Policy** 16:467-476.

Khan AA, Gaur RZ (2013) Sustainable post treatment options of anaerobic effluent. In: Chamy R, Rosenkranz F (Ed.). **Biodegradation - Engineering and Technology**. InTech, p. 192–221.

Khanal SK (2008) Anaerobic biotechnology for bioenergy production. Oxford, UK: Wiley-Blackwell, 306p.

Kim YH, Yoo C, Lee IB (2008) Optimization of biological nutrient removal in a SBR using simulation-based iterative dynamic programming. **Chemical Engineering Journal** 139:11-19.

Kondo MM, Leite KU, Silva MRA, Reis ADP (2010) Fenton and Photo-Fenton Processes Coupled to UASB to Treat Coffee Pulping Wastewater. **Separation Science and Technology** 45:1506:1511.

Leng L, Yang P et al. (2018) A review on the bioenergetics of anaerobic microbial metabolism close to the thermodynamic limits and its implications for digestion applications. **Bioresource Technology** 247:1095-1106.

Lessner DJ (2009) Methanogenesis Biochemistry. **Encyclopedia of Life Sciences (ELS)**, p. 1-11.

Lettinga G, Van Velsen AFM, Hobma SW, Zeeuw W, Klapwijk A (1980) Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment, especially for anaerobic treatment. **Biotechnology and Bioengineering** 22:699–734.

Li D, Lv Y, Zeng H, Zhang J (2016) Enhanced biological phosphorus removal using granules in continuous-flow reactor. **Chemical Engineering Journal** 298:107-116.

Liu Y, Whitman WB. (2008) Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. **Annals of the New York Academy of Sciences** 1125:171-189.

Lucas MS, Peres JA (2009) Treatment of olive mill wastewater by a combined process: fenton's reagent and chemical coagulation. **Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering** 44:198-205.

Mahesh S, Srikantha H, Lobo AL (2014) Performance evaluation of two batch operations using electrochemical coagulation followed by sequential batch reactor in treating coffee wastewater. **International Journal of ChemTech Research** 6:339-346.

Makwana AR, Ahammed MM (2017) Electrocoagulation process for the post-treatment of anaerobically treated urban wastewater. **Separation Science and Technology** 52:1412-1422.

Martins S, Ferreira BO et al. (2016) Metagenomic analysis and performance of a mesophilic anaerobic reactor treating food waste at various load rates. **Environmental Technology** 0:1-11.

Mazareli RC, Duda RM, Leite VD, Oliveira RA (2016) Anaerobic co-digestion of vegetable waste and swine wastewater in high-rate horizontal reactors with fixed bed. **Waste Management** 52:112-121.

Mchugh S, Carton M, Collins G, O'flaherty V (2004) Reactor performance and microbial community dynamics during anaerobic biological treatment of wastewaters. **FEMS Microbiology Ecology** 48:369-378.

Metcalf and Eddy (2003) **Wastewater engineering: treatment and reuse**. 4. ed. Boston: McGraw Hill.

Nieto LM, Hodaifa G, Rodríguez S, Giménez JA, Ochando J (2011) Degradation of organic matter in olive-oil mill wastewater through homogeneous Fenton-like reaction. **Chemical Engineering Journal** 173:503-510.

Novaes RFV (1986) Microbiology of anaerobic digestion. **Water Science and Technology** 18:1-14.

Oliveira LS, Franca AS (2014) **An overview of the potential uses for coffee husks**. Elsevier Inc., p. 283-291.

Oliveira RA, Bruno NMN (2013) Start-Up of Horizontal Anaerobic Reactors With Sludge Blanket and Fixed Bed for Wastewater Treatment From Coffee Processing By Wet Method. **Engenharia Agricola** 33:353-366.

Pajares S, Bohannan BJM (2016) Ecology of Nitrogen Fixing , Nitrifying , and Denitrifying Microorganisms in Tropical Forest Soils. **Frontiers in Microbiology** 7:1-20.

Pignatello JJ, Oliveros E, Mackay A (2006) Advanced Oxidation Processes for Organic Contaminant Destruction Based on the Fenton Reaction and Related Chemistry. **Critical Reviews in Environmental Science and Technology** 36:1-84.

Pires JF, Cardoso LS, Schwan RF, Silva CF (2017) Diversity of microbiota found in coffee processing wastewater treatment plant. **World Journal of Microbiology and Biotechnology** 33:1-12.

Rattan S, Parande AK, Nagaraju VD, Ghiwari GK (2015) A comprehensive review on utilization of wastewater from coffee processing. **Environmental Science and Pollution Research** 22:6461-6472.

Rodrigues CSD, NETO AR et al. (2017) Combination of chemical coagulation, photo-Fenton oxidation and biodegradation for the treatment of vinasse from sugar cane ethanol distillery. **Journal of cleaner production** 142:3634-3644.

Rodríguez D, Pino N, Peñuela G (2011) Monitoring the removal of nitrogen by applying a nitrification-denitrification process in a Sequencing Batch Reactor (SBR). **Bioresource Technology** 102:2316-2321.

Rossmann M, Matos AT, Abreu EC, Silva FF, Borges AC (2012) Performance of constructed wetlands in the treatment of aerated coffee processing wastewater: Removal of nutrients and phenolic compounds. **Ecological Engineering** 49:264-269.

Rossmann M, Matos AT, Abreu EC, Silva FF, Borges AC (2013) Effect of influent aeration on removal of organic matter from coffee processing wastewater in constructed wetlands. **Journal of Environmental Management** 128:912-919.

Rotaru AE, Shrestha PM (2014) A new model for electron flow during anaerobic digestion: direct interspecies electron transfer to Methanosaeta for the reduction of carbon dioxide to methane. **Energy Environ. Sci.** 7:408-415.

Schwan RF, Fleet GH (2014) **Cocoa and coffee fermentations**. Boca Ratón: CRC Press.

Selvamurugan M, Doraisamy P, Maheswari M, Nandakumar N (2010a) High Rate Anaerobic Treatment of Coffee Processing. **Methods** 7:129-136.

Selvamurugan M, Doraisamy P, Maheswari M (2010b) An integrated treatment system for coffee processing wastewater using anaerobic and aerobic process. **Ecological Engineering** 36:1686-1690.

Shuizhou K, Zhou S, Fang HHP (2005) Applications of two-phase anaerobic digestion for industrial wastewater treatment.pdf. **International Journal of Environmental and Pollution** 23:65-80.

Silva-Teira A, Sánchez A, Buntner D, Rodríguez-Hernández L, Garrido JM (2017) Removal of dissolved methane and nitrogen from anaerobically treated effluents at low temperature by MBR post-treatment. **Chemical Engineering Journal** 326:970-979.

Stams AJM, Sousa DZ, Kleerebezem R, Plugge CM (2012) Role of syntrophic microbial communities in high-rate methanogenic bioreactors. **Water Science & Technology** 66:352-362.

Sundberg C, Al-Soud, WA (2013) 454 Pyrosequencing Analyses of Bacterial and Archaeal Richness in 21 Full-Scale Biogas Digesters. **FEMS Microbiology Ecology**, 85:612-626.

Sychev AY, Isak VG (1995) Iron compounds and the mechanisms of the homogeneous catalysis of the activation of O_2 and H_2O_2 and of the oxidation of organic substrates. **Russian Chemical Reviews** 64:1105-1129.

Venkiteshwaran K, Bocher B, Maki J, Zitomer D (2015) Relating Anaerobic Digestion Microbial Community and Process Function: Supplementary Issue: Water Microbiology. **Microbiology insights** 8:37-44.

Villa-Montoya AC, Ferro MIT, Oliveira RA (2017) Removal of phenols and methane production with coffee processing wastewater supplemented with phosphorous. **International Journal of Environmental Science and Technology** 14:61-74.

Von Sperling M (2007) **Activated sludge and aerobic biofilm reactors**. V ed. London: IWA Publishing, 322p.

Wang DB, Li X et al. (2008) Biological phosphorus removal in sequencing batch reactor with single-stage oxic process. **Bioresource Technology** 99:5466–5473.

Yalfani MS, Contreras S, Medina F, Sueiras J (2009) Phenol degradation by Fenton's process using catalytic in situ generated hydrogen peroxide. **Applied Catalysis B: Environmental** 89:519-526.

Yan X, Geng, A, Zhang J et al. (2013) Discovery of (hemi-) cellulase genes in a metagenomic library from a biogas digester using 454 pyrosequencing. **Applied microbiology and biotechnology** 97:8173-8182.

Yang Y, Shi X, Ballent W, Mayer BK (2017) Biological Phosphorus Recovery: Review of Current Progress and Future Needs. **Water Environment Research** 89:2122-2135.

Yang Y, Yu K et al. (2014) Metagenomic analysis of sludge from full-scale anaerobic digesters operated in municipal wastewater treatment plants. **Applied microbiology and biotechnology** 98:5709–-5718.

Zaiat M, Cabral A, Foresti E (1994) Horizontal-flow anaerobic immobilized sludge reactor for wastewater treatment: conception and performance evaluation. **Brazilian Journal of Chemical Engineering** 11:33-42.

Zaiat M, Passig FH, Foresti E (2000) Treatment of domestic sewage in horizontal-flow anaerobic immobilized biomass (HAIB) reactor. **Environmental Technology (United Kingdom)** 21:1139-1145.

Zayas T, Geissler G, Hernandez F (2007) Chemical oxygen demand reduction in coffee wastewater through chemical flocculation and advanced oxidation processes. **Journal of Environmental Sciences** 19:300-305.

Zhu G, Peng Y, LI B, Guo J, Yang Q, Shuying W (2008) Biological Removal of Nitrogen from Wastewater. In: WHITACRE, D. M. (Ed.). **Reviews of Environmental Contamination and Toxicology**. New York:Springer New York p.159-195.