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ANAEROBIC, AEROBIC AND ANOXIC TREATMENT OF DAIRY CATTLE WASTEWATER

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ALEXANDRA ELIZABETH MOYA MALDONADO – Filha de Fausto Moya e Amparo Maldonado, nascida em Quito, Equador, no dia 3 de janeiro de 1992. Graduada em Microbiologia pela Pontificia Universidad Católica del Ecuador em julho de 2016. No mês de março de 2017, iniciou o Curso de Pós-graduação em Microbiologia Agropecuária, em nível de Mestrado na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista, situada em Jaboticabal - São Paulo.

Que la vida te sorprenda con compasión y con crueldad, con humildad y egoísmo, con intolerancia y libertad; para que puedas discernir por cual camino andar sin sentir opresión, ni patrones que llenar.

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<< Resiliencia >>

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CONTENTS

TRATAMENTO ANAERÓBICO, AERÓBIO E ANÓXICO DE ÁGUAS RESIDUÁRIAS DE BOVINOCULTURA DE LEITE

RESUMO - A digestão anaeróbia é uma alternativa de baixo custo para o tratamento de águas residuárias de bovinocultura de leite. Neste estudo utilizou-se um sistema de tratamento, em escala piloto, composto primeiramente por um reator anaeróbio compartimentado (ABR) com duas câmaras (ABRc1 e ABRc2 com 451,9 L), seguido de um filtro aerado submerso (SAF de 160 L) e um reator de fluxo ascendente com manta de lodo anóxico (USB_{nox} de 120 L) finalizando o tratamento de efluentes da bovinocultura leitera.O estudo foi dividido em três fases onde o sistema foi operado sob diferentes condições: TDH de 93,7 h e COV de 8,0 g DQO_{total}/(L·d) no ABR na fase I. Na fase II foi incluído o pós-tratamento (SAF + USBnox), o TDH aplicado no sistema foi de 121,6 e 91,3 h com COV de 15 e 23 gDQO_{total}/(L·d) na fase II e III, respectivamente. Nas três fases, o ABR demonstrou condições de estabilidade e na fase I obteve maiores remoções de DQO, N e P com TDH de 93.7 h. O sistema de tratamento (ABR-SAF-USB_{nox}) obteve maior remoção de DQO, nitrogênio e fósforo na fase II, média de 81, 68 e 68%, respectivamente. As maiores remoções de nutrientes (Fe, Na, K, Ca, Mg, Zn, Cu, Mn) ocorreram na fase II, utilizando o pós-tratamento (SAF + USBnox). O sistema anaeróbico, aeróbio e anóxico apresentou uma estratégia eficaz para o tratamento de águas residuárias de bovinocultura de leite com alta concentração de matéria orgânica, nitrogênio, fósforo e micronutrientes, obtendo altas eficiências de remoção.

Palavras-chave: digestão anaeróbia, águas residuárias de bovinocultura leiteira, remoção biológica de nutrientes, pós-tratamento aeróbio/anóxico, carga orgânica volumétrica

ANAEROBIC, AEROBIC AND ANOXIC TREATMENT OF DAIRY CATTLE WASTEWATER

ABSTRACT - Anaerobic digestion is a low-cost alternative for the treatment of wastewater from dairy cattle. In this study, a pilot scale treatment system was used; consisting of a two-chamber compartmentalized anaerobic reactor (ABRc1 and ABRc2 with 451.9 L), a submerged aerated filter (SAF of 160 L) and an anoxic upflow sludge blanket (USB_{nox} of 120 L) for dairy cattle wastewater treatment. The study was divided into three phases where the anaerobic system was operated under TDH of 93.7 and OLR of 8.0 g $COD_{total}/(L \cdot d)$ in phase I. Phase II and phase III included post-treatment (SAF + USBnox) and the HRT applied was 121.6 h and 91.3 h with OLR of 15 and 23 g $COD_{total}/(L \cdot d)$, respectively. In the three phases, the ABR demonstrated stability conditions and in Phase I, it obtained greater removals of COD, N and P with HRT of 93.7 h. The treatment system (ABR + SAF + USB_{nox}) obtained higher removal of COD, nitrogen and phosphorus in phase II, with a mean of 81, 68 and 68%, respectively. The highest removals of micronutrients (Fe, Na, K, Ca, Mn, Zn, Cu, Mg) occurred in phase II, using post-treatment (SAF + USBnox). The anaerobic, aerobic and anoxic system presented a versatile strategy for the treatment of dairy cattle wastewater with high concentration of organic matter, nitrogen, phosphorus and micronutrients, obtaining high removal efficiencies.

Keywords: Anaerobic digestion, Dairy cattle wastewater, Biological nutrient removal, Aerobic/anoxic post-treatment, Organic loading rate.

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LIST OF ABBREVIATIONS

ABR - Anaerobic Baffled Reator
ABRc1 – ABR's compartment one
ABRc2 – ABR's compartment two
AD – Anaerobic digestion
AF – Anaerobic filter
AN/OX – anoxic /aerobic
AOA – anaerobic/oxic/anoxic
AOB – ammonium-oxidizer bacteria
AOA-SBR – anaerobic/aerobic/anoxic sequencing batch reactor
As – Arsenic
ASBR – anaerobic sequencing batch reactor
AT – anaerobic treatment
BAF – biological aerated filter
C – Carbon
Ca – Calcium
CAMBR – ABR-MBR process
Cd – Cadmium
CH_4^+ - methane
CO_2 – carbon dioxide
CODs – soluble chemical oxygen demand
CODtot– total chemical oxygen demand
CPW – coffe processing wastewater
Cr – Chromium
Cu – Cooper
DHS – down-flow hanging sponge
DMI – dry matter intake
DNPAOs – denitrifiyng phosphate accumulating organisms
DOC– dissolved oxygen concentration
Fe – Iron
GAOs – glycogen accumulating organisms
Hg - Mercury
HM – heavy metals
HRT – hydraulic retention time
IA – intermediary alkalinity
K – Potassium
LSM – liquid swine manure
Mg – Magnesium
Mn – manganese
MNP – most probable number
N – Nitrogen
N_2 – molecular nitrogen
Na – sodium
N_2O – nitrous oxide
NH_4^+ - ammonium
NH_3 – free ammonia
Ni – nickel

NO_2^- - nitrite
NO_3^- - nitrate
NOB – nitrite-oxidizer bacteria
OM – organic matter
ORL – organic loading rate
P - Phosphorus
PA – partial alkalinity
PAOs – polyphosphate accumulating organisms
Pb - lead
PHA – polyhydroxyalkanoates
PVC – polyvinyl chloride
PO_4^{3-} - orthophosphate
Poly-P – polyphosphate
SAF – submerged aerated filter
SBR – sequencing batch reactor
SDNR – specific denitrification rate
SND – simultaneous nitrification and denitrification
SRT – solid retention time
SS – suspended solids
SSM – solid swine manure
TA – total alkalinity
THI – temperature-humidity index
TKN – total Kjedahl nitrogen
TN – total nitrogen
TP – total phosphorus
TS – total solids
TSS – total suspended solids
U-PABF – upflow partially aerated biological filter
UASB – upflow anaerobic sludge blanket
USB_{nox} – anoxic upflow sludge blanket
UBR-A – anoxic-upflow bioreactor and aerobic system
UNESP – Universidade Estatual Paulista
VFA – volatile fatty acids
VS – volatile solids
VSS – volatile suspended solids
WWTP – wastewater treatment plant
Zn – Zinc

I INTRODUCTION

The worldwide population growth promotes the necessity to produce more animal and vegetal food. Nowadays, livestock is one of the principal activities around the world with 1'474 526.581 millions of cattle approximately. India, Brazil and China lead the list of dairy cow population worldwide (FAOSFAT, 2014). Dairy livestock is an important Brazilian activity. In 2016, Brazil approximately had 19 678 817 lactating cows (FAO, 2016).

Dairy cattle industries impulse intensive confinement and the accumulation of manure in small places; a lactating cattle with 400 kg of mean weight produces 38 to 50 kg of waste where 28 to 32 kg is manure and the rest is urine (Matos, 2005). Daily cattle manure contains high concentration of organic matter, nitrogen, phosphorus and other components such as heavy metals, considered as macro and micro nutrients (FAO, 2018; Stowe et al., 2015; Tao et al., 2016). In addition, liquid waste resulted of processing milk and clean practice must be consider as a problem because it depends of management style than could consume from 40 to 600 L of water per lactating cow (Matos, 2005). The management of dairy cattle wastewater is the major problem in intensive confinement (Ricardo, 2016).

High-rate anaerobic reactors represent a versatile strategy to process dairy cattle wastewater. AT has been performed in systems with different configurations. ABR is a common model which has a series of high rate anaerobic reactors with a single design, without moving parts, no special gas or sludge separation required, stable performance to hydraulic shock loads, high solids retention times, low HRT, etc. (Barber e Stuckey, 1999).

AT of wastewater is commonly used because of its low energy consumption, capability to reduce biological solids, biogas generation, low building and operating price, reduction of N, and P compounds, odor control and decrease of greenhouse gases emissions (Chernicharro, 2016; Holly et al., 2017; Pelaz et al., 2018; Stowe et al., 2015).

Regardless of AT advantages, biological removal process known as post-treatment is indispensable and the developing of a compact system which can process wastewater achieving organic matter, nitrogen and phosphorus removal with low energy consumption, plus less sludge formation is the principal objective of the majority activities which generate effluent (Santos, 2011).

Aerated post-treatment is used worldwide to achieve a good biological nutrient removal; SAF offers a high nitrification and phosphorus removal with consumption of organic matter achieving removal efficiencies of 95 to 99%, but insufficient total nitrogen removal as N_2 (Garzón-Zuñiga et al., 2005).

To complete nutrient and organic matter removal, an alternative aerobic-anoxic post-treatment can be established. An USB_{nox} reactor performs the denitrification step (under anoxic conditions) which needs a high organic matter effluent to complete biological nitrogen removal (Pelaz et al., 2018). Aerobic-anoxic configuration offers a viable economic technique to treat dairy cattle wastewater. Finally, the remaining effluent of the system may be used as a soil fertilizer due to its content of ammonia and orthophosphate, directly available to crops (Toumi et al., 2015).

The high-rate anaerobic reactor joined to aerobic-anoxic post-treatment system was evaluated under HRT decrease and OLR increase, in order to obtain a greater organic matter, nitrogen and phosphorus removal, taking advantages of dairy cattle wastewater produced in dairy industries.

1.1 Objective

Evaluate the high-rate anaerobic, aerobic and anoxic system under HRT decrease and OLR increase, to organic matter, macronutrients and micronutrients removal of dairy cattle wastewater.

II LITERATURE REVIEW

2.1. MILK PRODUCTION

The human population is rising each year worldwide and at the same time, human alimentation has been increasing too. So to cope with this fact, animal growth, feeding and production operations are expanding day by day, having as a good example the dairy industry. Annual dairy products consumption will increase a 27%, going from 87 kg to 119 kg per person by the year 2067. To reach this consumption, it will be needed 600 billion kilograms of milk more than the current production, approximately (Britt et al., 2018). India, Brazil and China lead the list of dairy cow population worldwide (FAOSTAT, 2014). Dairy livestock is an important Brazilian activity. In 2016, Brazil approximately had 19 678 817 lactating cows (FAO, 2016) and industrialized 6 251 035 millions of liters of milk only between October – December, 2018 (IBGEa, 2018). In the year 2017, Brazil had 17.06 million of lactating cows, 5.18 million were in southeast region and 3.40 million of animals were in Minas Gerais state (GEPEC/COAGRO, 2018).

Intensive confinement of daily cattle generates large amounts of manure which contains high concentration of organic matter, nitrogen, phosphorus and other components such as heavy metals, considered as macro and micro nutrients (FAO, 2018; Stowe et al., 2015; Tao et al., 2016). Irshad et al. (2013) analyzed the concentration of heavy metals in five different types of manure (chicken, cow, goat, sheep and ostrich). Cow manure presented approximately 26 mg/kg of Cd, 46 mg/kg of Ni, 8.5 mg/kg of Hg), 35 mg/kg of Pb, 200 mg/kg of soluble phosphorus and 41% of total carbon.

Manure applied to soils, enriches and contributes to the build-up of soil organic matter (FAO, 2018), but its excessive application, directly or after treatment in anaerobic reactors or lagoons, has resulted in over-pollution of fields (Tao et al., 2016). Crohn (2004) emphasize that soil organic N concentration from manure will mineralize until a solid steady-state be reached and the excess of N could leach into the groundwater. Mattias et al. (2010) evaluated the accumulation of metals in the soil under the systematic application of swine manure. It showed an increase in the availability of Cu, Zn and Mn. Qian et al. (2018) applied dry-cleaned and squeezed swine manure to paddy field after a month of composting during the period of 2014-2017. Results indicated the field accumulation of Cu, Zn, Hg, Mn, As and Cr, while a model-simulated accumulation of Cu, Zn and Cd could happen in the next 10-50 years.

Furthermore, animal manure contributes to greenhouses effect problem because volatilization of NH_3, CO_2 and N_2O happens (FAO, 2018; Holly et al., 2017). Raw manure came from a farm where it was storaged during 182 d and then applied directly to field in the USDA Dairy Forage Research Station, USA. The experiment showed emission of 14 mg N_2O /(kg raw manure) and 12 g CO_2 /(kg raw manure) during 126 consecutive days. Brazil, the biggest country in South America, registered CO_2 emissions since 1990. Equivalent CO_2 emissions or CO_2 (eq) is a standard measure for comparing emissions of different greenhouse gases, but does not imply the same climate change responses. Figure 1 shows the percentage of CO_2 (eq) from different sources (FAOSTATa, 2019). The average between 1990 and 2016 of manure left on pasture and applied to soils were 26.1%.

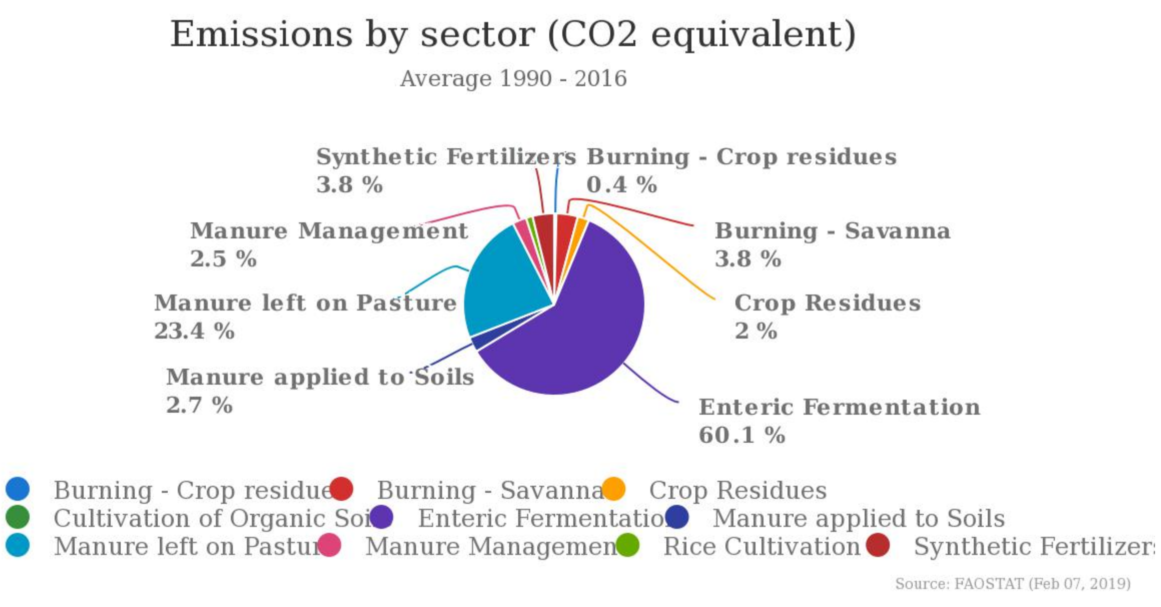


Figure 1 CO_2 (eq) emissions percentage of several Brazilian sectors since 1990 to 2016 (FAOSTATa, 2019).

In addition, Brazil is the first country in the top 10 of carbon dioxide (CO_2 (eq)) emitters corresponding to cattle dairy-manure applied to soils, generating an average of 5374.58 Gg of CO_2 (eq) during the years 2010 to 2016. The cattle dairy contribution of CO_2 was 44.8% (2010 - 2016 period) in a group of different Brazilian animals as buffaloes, non-dairy cattle, goats, etc. (FAOSTATb, 2019). The mitigation of greenhouse gases is a worldwide purpose which involves many countries contribution. Finally, daily livestock sector has been implementing different technologies to upgrade manure management in order to reduce the greenhouse effect and soil contamination. Anaerobic digestion or/and aerobic treatment are options for manure management (FAO, 2018).

2.2 ANAEROBIC TREATMENT OF DAIRY CATTLE WASTEWATER

AD represents a versatile strategy to process dairy cattle manure. This option has several benefits such as the reduction of organic material, N and P, odor control and decrease of greenhouse gases emissions (Holly et al., 2017; Stowe et al., 2015). Hydrolysis is the first step in an anaerobic digestion, where complex organic matter is dissolved to small compounds; those ones will become available for microorganisms. Consequently, fermentation is the intermediate stage of the process and as a result, CH_4 and CO_2 are emitted. Complex nitrogen compounds are mineralized to NH_4^+ and phosphorus is converted into inorganic orthophosphates; both are used for microorganisms growth (Chernicharro, 2016; Tao et al., 2016).

AD has been performed in systems with different configurations in order to treat waste and/or wastewater from numerous origins. There are many examples of this, some of them are mentioned below.

Zeb et al. (2017) tested fresh dairy manure in a series of batch anaerobic digester under mesophilic temperature (37 ± 1 ^oC) to: a) determine the effects of salinity and total ammonia nitrogen accumulation due to recycling of separated liquid-effluent on methane production and b) present mass balances of water, solids and nutrients for a typical dairy farm with an effluent recycle system. The effluent recycle system was an alternative to reduce volume and nutrients of manure in dairy farmers.

Page et al. (2014) used two lab-scale reactors with dairy barn manure (raw) to evaluate volatile fatty acids (formic, acetic, propionic, butyric, and 2-methylbutyric) production during three-months of storage in order to simulate manure storing farm conditions at 20 ^oC, results of odor and gas emissions also were present.

Toumi et al. (2015) used four laboratory-scale ASBR, each one of two liters, to treat anaerobically cattle manure + dairy wastewater (manure + water of washing) at a 35 ^oC controlled temperature. Crop application of anaerobic sludge from ASBR was done and agronomic benefits were the aim of this research.

Intensive confinement produced effluents with high concentration of suspended solids. This situation made difficult the use of common digesters; enlarge them inside farms/industries is not feasible within establishment (Oliveira, 1997). Many dairy farms are seeking technologies where bacteria activity increases to obtain high rate contact between microbiota and substrate (Faisa e Unno, 2001). High-rate reactors are operated with low HRT and high SRT, resulting in less volume reactor used and microorganism retention inside reactor (Vazoller, 1995). Researches using high-rate reactors such as UASB, AF or ABR to treat swine wastewater exist; unfortunately, dairy cattle wastewater treatment has not been studied using high-rate reactors.

2.2.1 Anaerobic baffled reactor

A frequent AT configuration is the ABR, created by Bachmann, Beard and McCarty (1985) at Stanford University, is considered a series of high rate anaerobic reactors with a single design, without moving parts, no special gas or sludge separation required, stable performance to hydraulic shock loads, high solids retention times, low HRT, etc (Barber e Stuckey, 1999; Chen et al., 2016; Ferraz et al., 2009; Yu et al., 2015;).

Common ABR was designed with series of baffles (four or five) to force organic contaminated influent passes from the input to the output flowing under and through the baffles. Bacteria rise and settle with gas production allowing the contact of active biological mass with wastewater (Bachmann et al.,1985). Many modifications were built in the ABR to enhance the solid retention in order to allow a better substrate accessibility to methanogens, encourage cell retention in upflow chambers, ease and control the gas measurement (Barber e Stuckey et al., 1999).

Additionally, the ABR works with high OLR, being this fact the reason of variable size in worldwide experiments. The ABR configuration of this research has not been studied treating dairy cattle manure.

Ferraz et al. (2009) evaluated the performance of ABR in treatment of cassava wastewater came from a manioc flour industry in São Paulo, Brazil. The ABR divided in four compartments (1 L each one) showed a 92% of organic matter removal with OLR between 2 to 5 mg/L and HRT of 3.5 d. The same author presented a decrease a 9% of removal when OLR increase to 7 mg/L.

Santos (2011) evaluated the removal of organic matter, nutrients and thermotolerant coliforms of swine wastewater in a two stage ABR followed by SAF and anoxic upflow sludge blanked. The OLR increased from 35.9 to 93.0 g COD/(L·d) and its removal ranged between 94 to 99% into combined system. The removal efficiency were 99.99% for thermotolerant coliforms, 98% for dissolved COD, 99% for TSS, 91% for TN, 88% for TP, 99% for Cu and 94% for Zn.

Rodrigues (2013) treated swine wastewater into a combined system (two stages ABR, SAF, USB anoxic) with six essays under different HRT and OLR. Analyzed only two ABR stages, the first ABR camera had a high OLR between 4.8 to 37 g COD/(L·d) and had non limitation on its removal efficiency, showing a 71% as the best organic matter removal. In addition, the experiment showed the highest total COD removal in the fifth phase with a COD influent of 10.45 mg/L and COD effluent of 2.57 mg/L.

Hahn e Figueroa (2015) treated raw municipal wastewater at psychrophilic temperature (≤ 20^{o}C) in an ABR consisted of four sequential cells. Under a HRT of 12 h during 730 d, an average OLR of 1.3 g COD/(L·d) and an influent COD of 760 mg/L, this study obtained 43% of COD removal, 83% of TSS removal and an overall CH_4^+ production of 0.24 L/g $COD_{removed}$.

Baker yeast wastewater was treated in a laboratory scale ABR (14.5 L total volume). Any type of chemical substrate was added and the whole experiment was performed in a short period of time with a HRT of 2-6 d. Despite this wastewater had high-strength organic matter, the system achieved a relatively complete digestion exhibiting a 95% of COD removal. It showed that the organic matter removal efficiency increased when HRT increased too (Pirsaheb et al., 2015).

Gulhane et al. (2016) used an ABR system of four chambers (total volume 60 L) to produce biomethane from a vegetable market slurry and tap water mixture. The system worked one year at different recirculation ratio, a stable OLR (0.5 g VS/L/d) and HRT of 30 d. The study evidenced the high potential of biomethanation of this mixture, the recirculation as a good option for stabilization and extra carbon source, and the possibility to increase OLR and apply shorter HRTs.

Li et al. (2016) used an ABR system with four compartments to treat brown sugar liquid (95% sucrose, 97% carbohydrate and 0.7% protein) under HRT of 24 h, an OLR of 2 g COD/(L·d) and influent COD of 4000 mg/L. This research displayed a COD removal of 44, 22, 14 and 11% in compartment 1, 2, 3 and 4, respectively and a percentage of CH_4^+ in biogas production of 5, 3, 2, 0.8 L/d in compartment 1, 2, 3 and 4, respectively.

Fujihira et al. (2018) treated solid/lipid-rich wastewater using a high-rate ABR and DHS. The research was divided in three phases: applying four compartments (phase one), six compartments (phase two) and four compartments (phase three); where the first one was bigger than the other three. Each phase had a working volume of 6.8 L, 10.2 L and 15.6 L, respectively. Inlet OLR were 4.8, 2.7 and 5.9 g COD/(L·d) corresponding to phase one, two and three, respectively. Only ABR reactors obtained a COD removal of 95.7% in phase one, 95.7% in phase two and 92.7% in the last phase.

Anaerobic treatment is a versatile strategy to process and take advantage of dairy cattle wastewater in dairy industry. In addition, ABR system is a common configuration to treat different wastewater aiming a high OLR application, a decrease of HRT and removal of different pollutants.

2.3 WASTEWATER POST-TREATMENT

In general, AT of wastewater is commonly used because of its low energy consumption, capability to reduce biological solids and generate biogas (Pelaz et al., 2018). Regardless of these advantages, post treatments offer complementary removal for biological nutrient removal success in order to achieve discharge standards. Biological removal of nitrogen involves nitrification and denitrification. Nitrification process occurs under aerobic conditions; ammonium (NH_4^+) is converted to nitrite (NO_2^-) (Eq. 1), then nitrite is oxidized to nitrate (NO_3^-) (Eq. 2). Stoichiometric chemical reactions are described below:

(Eq. 1) Nitrite generation

$NH_4^+ + (3/2)O_2 \rightarrow NO_2^- + H_2O + 2H^+$

(Eq. 2) Nitrate generation

$NO_2^- + (1/2)O_2 \rightarrow NO_3^-$

On the other hand, denitrification is an anoxic process in which organic matter is used as electron donor and nitrate is reduced into nitrite and molecular nitrogen gas (N_2) (Eq. 3) (Alzate et al., 2016; Pelaz et al., 2018). Stoichiometric chemical reaction below:

(Eq. 3) Denitrification with glucose as carbon source

$5C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 30CO_2 + 42H_2O + 12N_2 + \text{energy}$

Biological removal of phosphorus is performed by PAOs under anaerobic-aerobic/anoxic conditions. In anaerobic conditions, volatile fatty acids are consumed and storage as polyhydroxy-butyrate, while energy is provided by reduction of internally stored poly-P and glycogen. In aerobic/anoxic circumstances released phosphate is taken back into the cell and stored as poly-P like an energy source (Brdjanovic et al., 1998).

2.3.1 Aerobic and anoxic post-treatment

SAF is a kind of up-flow biofilter with internal air system, internal support structure and packed of support media (random support media as plastic rings, wool, etc). The media provide a large surface area on which the biofilm attach themselves to grow; the aeration produces a homogeneous solution in full contact with the entire microbial population present in media bed (Holloway e Soares, 2018; Hu et al., 2011).

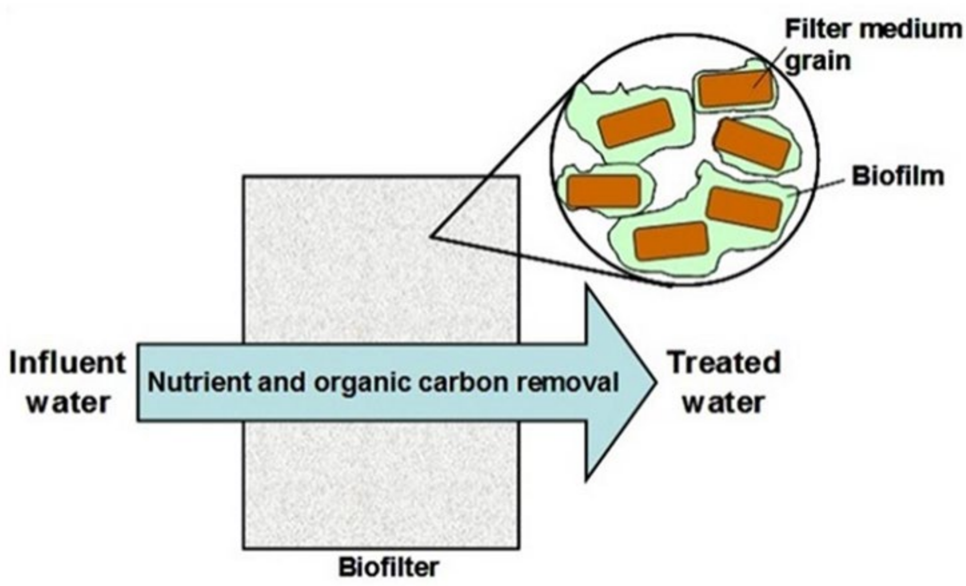


Figure 2 Filter medium, biofilm and pollutants removal from wastewater (Sehar e Naz, 2016).

The filter media should meet certain characteristics such as durability, insolubility, chemical resistant, low cost, providing high specific surface area and porosity (ventilation). This solid media provide a surface where biofilm grows (Figure 2), as a result, microbial concentration increase as well as its rates of pollutants degradation by removal mechanism as biodegradation, bioaccumulation, biosorption or biomineralization. Biofilm development is affected principally by changes in pH and temperature. Acid pH interrupts normal polysaccharides and exopolymeric substances production, consequently disrupting in biofilm stability or formation; meanwhile abrupt changes in temperature affects bacterial healthy growth or normal enzyme activities (Sehar e Naz, 2016).

USB_{nox} reactor does not require sophisticated equipment; influent enters at the bottom creating an upflow environment. Inside, high nitrate concentrations are used as electron acceptors to conclude nitrogen removal process until molecular nitrogen (Eq. 3). This reactor is widely used in high-rate wastewater treatment systems that focus in denitrification (Letelier-Gordo e Martin, 2019).

Worldwide, research groups have been developing different wastewater treatment systems to attain the basic removal of organic matter and nutrients during the initial process, followed by a post-treatment to obtain greater nitrogen and phosphorus removal.

Farhan et al. (2018) treated dairy farm wastewater (teat-wash, yard-wash, milking residuals and cleaning chemicals) in a laboratory-scale UBR-A system with a total working volume of 127 L. The system operation was of 334 d, with different recirculation ratios of 2, 3 and 4. SRT of 140-150, 65-75 and 30-40 d was applied in R of 3. The research showed a nitrification efficiency >90% and COD removal >80% under all SRT conditions and lower denitrification efficiencies at R > 3. Nitrification and denitrification rates increased at lower SRT of 30-40 d.

Post-endogenous denitrification and phosphorus removal was evaluated by Zhao et al. (2018). This author used an AOA system to treat low carbon/nitrogen domestic wastewater for simultaneous removal of COD, N and P without external carbon sources. The research was divided in three phases, the first one only used anaerobic/oxic conditions. Aerobic zone showed dissolved oxygen of 1-2 mg/L, this condition promoted SND. Strengthen intracellular carbon storage and endogenous nitrification was obtained with an extended anaerobic and anoxic stage. The system displayed 92.15 and 92.67% of nitrogen and phosphorus removal efficiencies under stable state.

Municipal wastewater with low C/N ratio was treated in AOA-SBR using sludge fermentation products as carbon source to solve the dual problem of insufficient carbon source and sludge reduction. Liu et al. (2017) operated the system during 145 d and the average removal efficiencies of TN, PO_4^{3-} and COD were 88.8, 99.3 and 81.2%, respectively. TN removal by SND was 34.4% and by denitrification was 57.5%. Sludge reduction rate reached 44.1 - 52.1%.

Alzate et al. (2016) evaluated nitrification and aerobic denitrification in AN/OX sequencing batch reactor. The study was divided in three experiments applying different: AN/OX ratio, cycle duration, HRT, pH, DOC and organic load. The highest inorganic N removal (close to 70%) was obtained at pH 7.5, at 440 mg COD/(L·d) (low organic load), high aeration (12 h cycle), AN/OX ratio = 0.5:1.0 and DOC higher than 4.0 mgO_2/L. Nitrification followed by aerobic denitrification happened in aerobic phase.

Tao et al. (2016) treated synthetic domestic wastewater in U-PABF, using ceramic and zeolite as a filling material. Different performances were observed and application of different HRT (5.2 and 2.6 h) only was done with ceramic material that showed better performance. Ammonium removal was attributed to filling material absorption, at HRT of 5.2 h, ceramic U-PABF achieved NH_4^+, TN and COD removal efficiencies of 99.08, 72.83 and 89.38%, respectively.

Santos (2011) worked with two ABR compartments, one SAF and one anoxic upflow sludge blanket reactor to treat swine wastewater. The in-series high-rate anaerobic/aerobic/anoxic system applied HRT of 41 h and 20.4 h. The SAF reactor was filled with bamboo and plastic rings and used an intermittent and continuous aeration, this reactor contributed with 29-76% and 6-32% of TN and TP, respectively. Anoxic reactor contributed with 15-62% and 15-78% of TN and TP, respectively. The highest mean removals were 99% for dissolve COD, 91% for TN and 88% for TP.

Rodrigues (2013) treated swine wastewater using an ABR with two cells, one SAF and one anoxic up-flow sludge blanket under a HRT were of 40.6 to 10.6 h, and OLR from 4.8 to 37 g COD/ (L·d). This author operated the system for 365 d and divided the research in six assays with different operation times; intermittent and continuous aeration was applied to SAF reactor and it was filled with plastic rings. Aerobic/anoxic post-treatment showed TN removal efficiencies between 21-38%. SAF and anoxic reactor contributed in TP removal with 27-71% and 27-70%, respectively. The better removal efficiencies were 85 % for COD and 77 % for TP.

The use of aerobic-anoxic reactors as a post-treatment after an anaerobic treatment offers to effluent a complement removal of organic matter and different nutrients as nitrogen or phosphorus. In-series anaerobic-aerobic-anoxic reactors are an interesting alternative to eliminate effluent transportation inside wastewater treatment place.

III MATERIAL AND METHODS

3.1 Experimental location

The experiment took place inside the Department of Rural Engineering facilities in the School of Agricultural and Veterinarian Science of UNESP. Its geographic coordinates are: 21° 15´22¨ S, 48° 18´58¨ W latitude and 575m altitude.

3.2 Experimental setup

The system was composed of an influent tank, an anaerobic baffled reactor with two compartments in-series (ABRc1 and ABRc2), a SAF, an USB_{nox} and an effluent tank (Figure 3).

ABR reactor was composed of two compartments in-series (ABRc1 and ABRc2). Each one was built with PVC and a total height of 1.8 m. The first compartment had a diameter of 0.4 m and the second 0.3 m. The empty bed liquid volume was 289.4 L and 162.5 L, respectively. Each compartment had a conical bottom connected to a 0.032 m pipe (inflow) and a closing dome connected to 0.015 m pipe (gas output). Conical bottom and closing dome were fixed by flange to guarantee easy assembling and maintenance. Each chamber has five sludge collection points. Adapted gasometers were fabricated with fiberglass as it is described by Fernandes and Oliveira, 2006, to measure the gas production.

Submerged aerated filter was fabricated with PVC and it had 60 L of working volume, internal diameter of 0.3 m and a length of 2.9 m, filled in 62.5% of its volume with corrugated PVC rings (400 mm length, 190 mm internal diameter, specific surface area of 135 m^2/m^3 and 87% of void volume) (Figure 4). A porous thin-membrane capsule was fixed internally at the bottom in order to generate thin-bubbles; a perforated stainless steel plate was fixed upper it (to hold the PVC rings) (Figure 5). The aeration was performed by common air compressor.

Anoxic upflow sludge blanket reactor was used to complement the post-treatment system. USB_{nox} diameter is 0.25 m, a height of 2.6 m, total volume of 120 L and useful volume of 70 L.

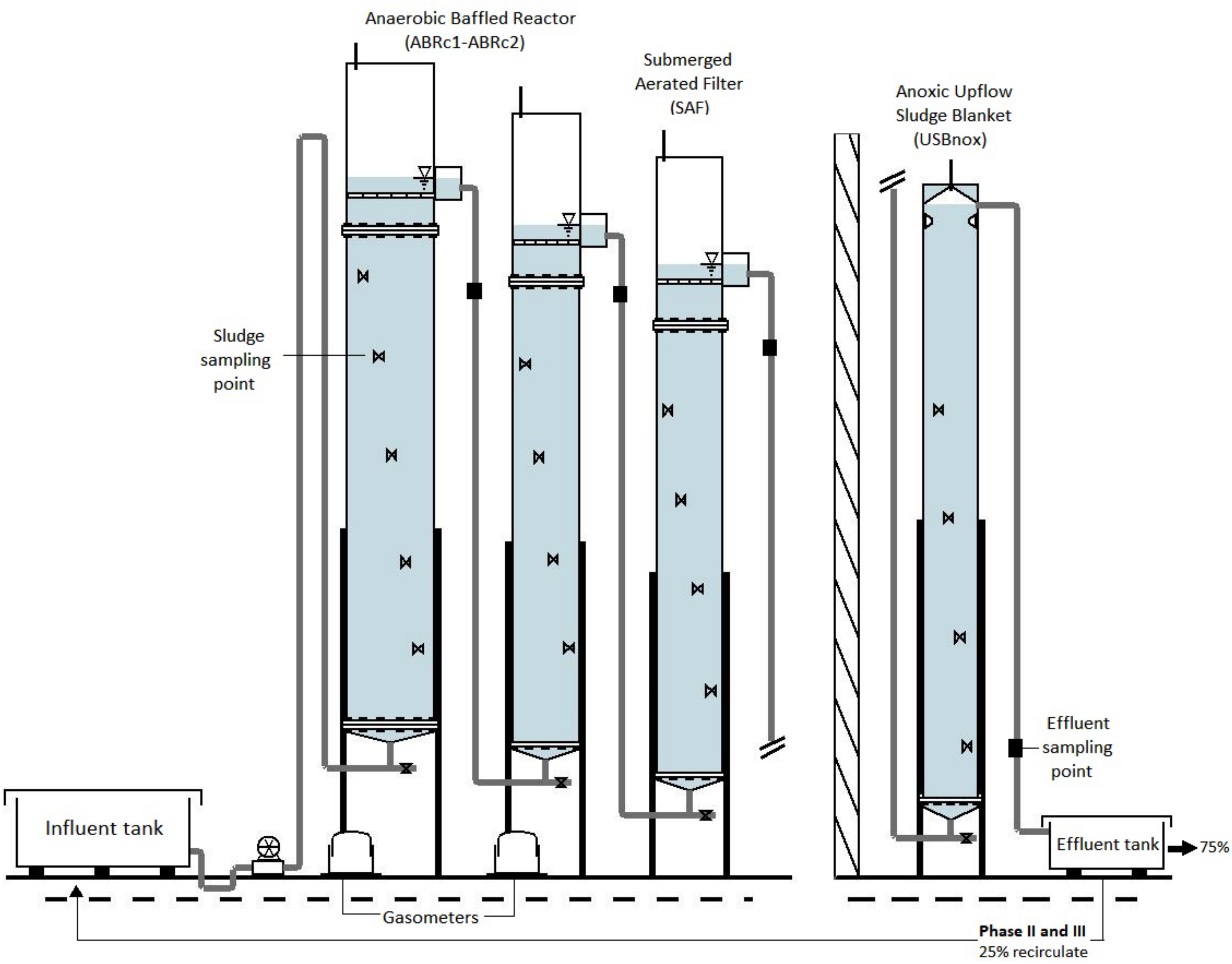


Figure 3 Schematic representation of the in-series high-rate reactor system comprising an anaerobic baffled reactor with two chambers (ABRc1 and ABRc2), a submerged aerated filter (SAF) and an anoxic upflow sludge blanket (USB_{nox}) reactor.

3.3 Inoculum sludge

The sludge inoculated amount was 30% of the total volume for each reactor. Both, ABR compartmets and USB_{nox} reactor were inoculated with anaerobic sludge from swine wastewater treatment. SAF reactor was inoculated with aerobic sludge from WWTPm“Cia. Matonense de Saneamento”, Matão, São Paulo, Brazil. Sampling of inoculum sludge was done under appropriate biosecurity for wastewater management of each place.

Figure 4 Corrugated PVC rings with 400 mm length, 190 mm internal diameter, specific surface area of 135 m^2/m^3 and 87% of void volume.

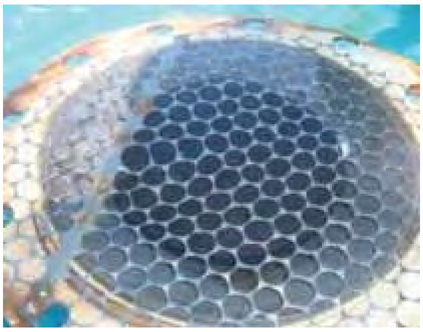
A  B 

Figure 5 Air diffuser membrane (A) and perforated stainless steel plate support (B).

3.4 Start-up

Anaerobic treatment had two starts up: the first at the beginning of Phase I in July, 2017 and the second one at the beginning of Phase III in May, 2018. Both of them with 65 days of operation to achieve steady-state conditions. SAF had two stars up: the first at the beginning of Phase II in September, 2017 and the last at the beginning of Phase III in July, 2018. Both of them with 5 days to achieve steady-state conditions.

3.5 Influent

Three times per week dairy manure was collected from milking sector of the School of Agricultural and Veterinarian Science of UNESP, Jaboticabal, São Paulo, Brazil. The manure was diluted with tap water in a proportion (1:5) (manure : water) three times per week; then solid fraction was separated with a static 2 mm mesh and a mobile 1 mm mesh. A helicoidal pump was used to push influent from influent tank to ABRc1 (Figure 1).

Table 1 Average values and variation coefficient (v.c.) of physicochemical characteristics of dairy cattle wastewater used as a influents system during phase I, II and III.

Parameter	Phase I		Phase II		Phase III	
		v.c.		v.c.		v.c.
pH	6.06	7	6.4	7	6.23	6
PA (mg $CaCO_3$/L)	669.2	58	821.6	57	319.7	55
VFA (mg/L)	903.7	42	1002.1	44	707.3	28
TS (mg/L)	5993.5	56	12724.4	63	11113.3	23
VS (mg/L)	4409.4	70	8906.5	77	9261.2	26
COD_{tot} (mg/L)	15025.03	57	14530.6	48	13850.4	38
NH_4^+(mg/L)	85.8	39	142.6	36	70.9	31
TKN (mg/L)	338.8	46	301.3	47	427.2	40
TP (mg/L)	33.4	60	45.3	37	148.3	17

v.c.: (standard deviation/mean)*100.

3.5.1 Livestock feeding

Milking sector of UNESP has a semi-confinement feeding operation with grass, silage and feed diet. Lactatings cows are feeding with 60% of silage and *Panicum maximum*, Mombasa grass. Feed diet represents 40 % of feeding and it is composed by ground corn, ground soy, wheat, soy beans, soy flour, cotton, dicalcium phosphate and premix diet (Table 2).

Table 2 Dairy cattle premix diet supplied in milking sector of UNESP.

Value for 100g of formulate diet	
Macrominerals	g/day
Calcium (Ca)	14.0/17.6
Phosphorus (P)	4.0
Sodium (Na)	16.0
Magnesium (Mg)	0.5
Microminerals	mg/day
Cooper (Cu)	94.5
Manganese (Mn)	73.0
Zinc (Zn)	350.0

3.6 Sampling

Influent and effluent samples were collected in the inlet tube of the compartmentalized reactor (ABR) and in the outlet of each of the compartments, in the outlet tubes of the SAF and in the USB_{nox} reactor. Morning sampling was performed between 7:30 a.m to 13:30 p.m, twice a week. Each hour, it was collected 400 mL of sample, forming a compound final sample.

3.7 Operating conditions of system

The experiment was divided in three phases (phase I, phase II and phase III). Simple anaerobic system, ABRc1 and ABRc2, was operated in phase I. phase II and phase III included the post-treatment SAF-USB_{nox} where effluent of USB_{nox} was recirculated in a proportion of 4:1 (dairy cattle wastewater:effluent recirculate). The operating conditions such as operation days, HRT of each reactor, OLR and SAF aeration time are described in Table 3 and Table 4.

3.8 Determination and examination of influent, effluents, sludge and biogas.

Physical analysis (Table 5), organic and inorganic constituents determination (Table 6) were done in influent, effluent and sludge collected samples, respectively. Methodologies and bibliographical references are listed in each table.

Biogas production was determinated with the measurement volume produced daily by gasometers constructed of fiberglass. The vertical gasometer displacement was measured and multiplied with inner transversal section area, then each gasometer was emptied (Oliveira, 1997). The volume was corrected for the standard conditions of temperature and pressure (STP, 273 K and 1 atm) for the calculation of methane production. The daily gas temperature was measured with a digital thermometer. The biogas composition was determined monthly by a FININGAN 6C-9001 gas chromatograph with a thermal conductivity detector, ‘‘Poropac Q’’ (3 m 1/800) columns and a molecular sieve, according to the methodology described by APHA et al. (1998).

Table 3 Operational conditions of in-series anaerobic baffled reactors (ABRc1 and ABRc2), a submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) as a post-treatment during three phases. Proportion (v:v) of dairy cattle wastewater (DW) and effluent recirculate (R).

Parameters	Phase (Proportion DW:R)									
	I (4:0)		II (4:1)				III (4:1)			
Time of operation (d)	77		82				95			
	ABRc1	ABRc2	ABRc1	ABRc2	SAF	USB_{nox}	ABRc1	ABRc2	SAF	USB_{nox}
HRT (h)	60.0	33.7	48.0	27.0	26.6	20.0	36.0	20.3	20.0	15.0
OLR (g CODtot/(L·d))	5.9	1.7	7.3	3.6	1.8	2.2	9.3	7.4	2.7	3.5
Aeration (h)					13.5				13.5	

Table 4 Intermittent aeration cycle used in Submerged Aerated Filter (SAF); colored spaces show the time when aeration happens.

Hour	15min	15min	15min	15min	Hour	15min	15min	15min	15min
00					**12**		■	■	■
1		■	■		**13**	■	■		
2					**14**				
3	■	■	■	■	**15**	■	■		
4	■				**16**				
5		■	■	■	**17**	■	■		
6					**18**				
7					**19**				
8					**20**	■	■		
9		■	■		**21**				
10					**22**			■	■
11		■	■		**23**				

Table 5 Physical analysis of influent and ABRc1, ABRc2, SAF and USB_{nox} effluents, frequency of analysis and bibliographical reference.

Physical analysis Influent, ABRc1, ABRc2, SAF, USB_{nox}	Frequency	Bibliographical reference
pH	Twice a week	APHA, AWWA, WPCF (1998)
Total (CODtot), soluble (CODso) and suspended (CODsu) chemical oxygen demand	Twice a week	APHA, AWWA, WPCF (1998); ELMITWALLI et al. (2000); CHERNICHARRO (2016)
Total (TA), partial (PA) and intermediary (IA) alkalinity	Twice a week	APHA, AWWA, WPCF (1998) JENKINS et al. (1983)
Total (TSS), volatile (VSS) and fixed (FSS) suspend solids	Twice a week	APHA, AWWA, WPCF (1998)
Total volatile acids (VFA)	Twice a week	DILALLO and ALBERSTON (1961)
BIOGAS		
Production	Daily	OLIVEIRA (1997) (gasometers)
Composition	Monthly	APHA, AWWA, WPCF (1998)

Table 6 Organic and inorganic constituents determination of influent and ABRc1, ABRc2, SAF and USB_{nox} effluents, frequency of analysis and bibliographical reference.

Organic and inorganic constituents determination Influent, ABRc1, ABRc2, SAF, USB_{nox}	Frequency	Bibliographical reference
Total ammonia nitrogen (NH_4^+)	Twice a week	APHA, AWWA, WPCF (1998)
Total Kjedahl nitrogen (TKN)	Once a week	APHA, AWWA, WPCF (1998)
Total phosphorus (TP)	Once a week	APHA, AWWA, WPCF, 1998
Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Copper (Cu), Iron (Fe), Manganesium (Mg) and Zinc (Zn).	Once a week	APHA, AWWA, WPCF (1998) (1) atomic absorption spectrophotometer
SAF and USB_{nox}		
Nitrite (NO_2^-)	Twice a week	APHA (Method 4500-NO_2-B), AWWA, WPCF (1998) (1) UV spectrophotometer (220nm); (2) UV spectrophotometer (543nm)
Nitrate (NO_3^-)	Twice a week	APHA (Method 4500-NO_3-B), AWWA, WPCF (1998) (1) UV spectrophotometer (220nm); (2) UV spectrophotometer (543nm)

3.9 Estimation of microorganism concentrations

Methanogenic archaea were studied in sludge samples from anaerobic reactors in phases II. Nitrifying, denitrifying and heterotrophic bacteria were studied in sludge samples from post-treatment reactors at final of phases II and III. Total and thermotolerant coliforms were investigated of influent and effluents of each reactor during phase II.

3.9.1 Methanogenic archaea

The MPN of methanogenic archaea was based in Wagner et al. (2012) research; this methodology was adapted to laboratory conditions by Environmental Sanitation Laboratory team (specifications below). In this research, sludge of ABRc1 and ABRc2 samples were analyzed.

Sampling: took 20 mL of sludge sampling point 1, 3 and 5 of each reactor, to create the “principal sample”, in sterile flasks.

Material preparation: autoclave one 96-well plates (MPN plates), 80 tips (20µl), 30 tips (1 mL) and 2 volumetric pipettes (10 mL and 1 mL). One flask for each sludge sampling point with 45 mL of Na-acetate culture medium, one flask for each sludge sampling point with 45 mL of Na-formate culture medium and one flask for each decimal dilution with 4.5 mL Milli-Q water. These autoclaved flasks were allowed to cool under aseptic conditions, close to Bunsen burner.

Culture media: Specification of Na-acetate culture medium and Na-formate culture medium are in Table 7 and Table 8. Media were autoclaved and allowed to cool under aseptic conditions, close to Bunsen burner.

Principal sample dilution: for each sludge sampling point, add 5 mL of principal sample to flask with 45 mL of medium.

Sludge sampling point decimal dilution preparation: under aseptic conditions, close to Bunsen burner, were done the decimal dilutions. First, add 0.5 mL of principal sample dilution in one sterile flask with 4.5 mL of Milli-Q water. Homogenized, resulted in 10^{-1} dilution. Then, took 0.5 mL of dilution 10^{-1} and add to another sterile flask with 4.5 mL of Milli-Q water, resulted in 10^{-2}. Same process was done until got 10^{-8} dilution.

Table 7 Culture media specifications to most probable number (MPN) of methanogenic archaea by Wagner et al. (2012).

Chemical compounds	Concentration of stock solution (1000mL Milli-Q water)	
	Na-acetate culture media	Na-formate culture media
KH_2PO_4	0.50 g	
$MgSO_4.7H_2O$	0.40 g	
NH_4Cl	0.40 g	
NaCl	0.40 g	
$CaCl_2.2H_2O$	0.05 g	
$FeSO_4.7H_2O$	0.002 g	
Na-acetate	1.00 g	-
Na-formate	-	2.00 g
Resazurin	0.001 g	
Cystein	0.50 g	
Trace element	1 mL	

Table 8 Trace element specifications. Information took of Wagner et al. (2010).

Chemical compounds	Concentration of chemical compounds (1000 mL Milli-Q water)
$Na_2MoO_4.\ 2H_2O$	0.036 g
$NiCl_2.\ 6H_2O$	0.024 g
$MnCl_2.\ 4H_2O$	0.1 g
$ZnCl_2$	0.070 g
$FeCl_2.\ 2H_2O$	1.5 g
$CuCl_2.\ 2H_2O$	0.002 g
$CoCl_2.\ 6H_2O$	0.19 g
H_3BO_3	0.006 g
25%-HCl	10mL

Well plates: for each sludge sampling point decimal dilution, fill well of the MPN plates with 180 µL of Na-acetate culture medium and Na-formate culture medium.

Well plates inoculation: inoculate 20 µL of each dilution in corresponding well. After, MPN plates were sealed with foil.

Chamber preparation: disinfect the principal box with hydrogen peroxide 10% and alcohol 70% with cleaning clothing.

Incubation: introduce MPN plates to chamber and close it. Fill the chamber with 70% H_2 (gas introduced for 5 minutes) to ensure anaerobic conditions, using inlet port. Incubate for fifteen days to early interpretation, and 30 days to final interpretation.

Interpretation: results were achieved according to Wagner et al. (2012), and the MPN values were calculated using the MPN Calculator (http://www.i2workout.com/mcuriale/mpn/).

3.9.2 Ammonium and nitrite oxidizer bacteria

The ammonium-oxidizer bacteria and nitrite-oxidizer bacteria were analyzed by MPN methodology based in Schmidt e Belser (1984); this was adapted to sample wastewater analysis by Mendonça (2002). In this research, sludge SAF and sludge USB_{nox} samples were analyzed.

Sampling: in a sterile flask, took 20 mL of each sludge sampling point of each reactor to create the “principal sample”.

Dilution water: mix 2 mL of K_2HPO_4 solution (3.48 g/100 mL) and 0.5 mL de KH_2PO_4 (2.72 g/ 100 mL) in 500 mL of Milli-Q water.

Dilution tubes preparation: add 18 mL of dilution water in each tube. Each tube was closed with cotton stoppers wrapped in gauze and autoclaved at 120 ^{o}C for 20 min and 1 atm and allowed to cool under aseptic conditions, close to Bunsen burner.

Decimal dilutions: under aseptic conditions, close to Bunsen burner. First, add 2 mL of principal sample in one sterile dilution tube. Homogenized, resulted in 10^{-1} dilution. Then, took 2 mL of dilution 10^{-1} and add to another sterile dilution tube, resulted in 10^{-2}. Same process was done until got 10^{-12} dilution.

Culture media tubes: each culture media was prepared separately according to specifications of Table 9. Add 9 mL of culture media in a tube, for ammonium oxidizer bacteria add 1 g of $CaCO_3$ in each tube. Each tube was closed with cotton stoppers wrapped in gauze, sterilized in autoclave (20 min/1 atm/120 ^{o}C) and allowed to cool under aseptic conditions, close to Bunsen burner. Inoculation: under aseptic conditions, close to Bunsen burner, the inoculation was done. Add 1 mL of dilution 10^{-1} in a culture media tube (five tubes for each sample dilution) until inoculated dilution 10^{-12}.

Incubation: thirty days under 30 ^{o}C.

Interpretation of results: Table 10 explained last step instruction and interpretation of different oxidizer bacteria culture.

Table 9 Ammonium oxidizer and nitrite oxidize bacteria culture media specifications

Chemical compounds	Concentration of stock solution (g /100mL Milli-Q water)	Stock solution volume for 250mL of culture media (mL)	
		Ammonium oxidizer	Nitrite oxidizer
$(NH_4)_2SO_4$	10.000	1.00	-
Bromothymol blue	0.040	0.70	-
$NaNO_2$	0.680	-	0.25
K_2HPO_4	3.480	-	1.0
$CaCl_2.H_2O$	1.340	0.25	0.25
$MgSO_4.7H_2O$	4.000	0.25	1.25
KH_2PO_4	2.720	1.88	0.25
EDTA-iron chelate	-	0.25	0.25
$FeSO_4.7H_2O$	0.246	-	-
EDTA disodium	0.331	-	-
Trace elements	-	0.25	0.25
$NaMoO_4.2H_20$	0.010	-	-
$MnCl_2$	0.020	-	-
$CoCl_2.6H_2O$	0.0002	-	-
$ZnSO_4.7H_2O$	0.010	-	-
$CuSO_4.5H_2O$	0.002	-	-

Table 10 Last step instructions and interpretation of ammonium-oxidizer bacteria (AOB) and nitrite-oxidizer bacteria (NOB) most probable number results (MPN).

Ammonium oxidizer bacteria	Nitrite oxidizer bacteria
-Add three drops of sulfanilamide solution (dissolve 0.5 g of sulfanilamide in 100 mL of hydrochloric acid 2.4 N). -Immediately, add three drops of naphthyl-ethylenediamine hydrochloric solution (dissolve 0.3 g of naphthyl-ethylenediamine in 100 mL of hydrochloric acid 0.12 N)	
Pink-reddish coloration (nitrite presence) showed the activity of bacteria that oxidize ammonium to nitrite.	Absence of pink-reddish coloration showed the activity of bacteria that oxidize nitrite to nitrate. Pink-reddish coloration = negative
Alexander's table (ANEXO A) was used to give a value expressed in MPN g/VS of existing most probable number bacteria within principal sample.	

3.9.3 Denitrifying and heterotrophic bacteria

The denitrifying bacteria and heterotrophic bacteria were analyzed by MPN methodology based in Tiedje (1984); this was adapted to sample wastewater analysis by Mendonça (2002). In this research, sludge SAF and sludge USB_{nox} samples were analyzed.

Sampling: took 20 mL of each sludge sampling point of each reactor to create the "principal sample", in a sterile flask.

Dilution water: mix 2 mL of K_2HPO_4 solution (3.48 g/ 100 mL) and 0.5 mL de KH_2PO_4 (2.72 g/ 100 mL) in 500 mL of Milli-Q water.

Dilution tubes preparation: add 18 mL of dilution water in each tube. Tubes for heterotrophic bacteria were closed with cotton stoppers wrapped in gauze and denitrifying tubes were closed with screw cap for denitrifying bacteria. Tubes were autoclaved at 120 ^{o}C/20 min/1atm and allowed to cool under aseptic conditions (close to Bunsen burner).

Decimal dilutions: under aseptic conditions, close to Bunsen burner, the decimal dilutions were done. First, add 2 mL of principal sample in one sterile dilution tube, with 18 mL of dilution water. Homogenized, resulted in 10^{-1} dilution. Then, took 2 mL of dilution 10^{-1} and add to another sterile dilution tube, resulted in 10^{-2}. Same process was done until got 10^{-12} dilution.

Culture media tubes: mix 5 g of peptone and 3 g of meat extract in 500 mL of Milli-Q water. Separate 250 mL of culture media and add 0.107 g of $NaNO_3$ that was used as culture media to heterotrophic bacteria. Add 4.5 mL of culture media in each tube, tubes for heterotrophic bacteria were closed with cotton stoppers wrapped in gauze and tubes for denitrifying bacteria were closed with screw caps. Tubes were autoclaved at 120 ^{o}C/20 min/1atm and allowed to cool under aseptic conditions (close to Bunsen burner).

Inoculation: under aseptic conditions, close to Bunsen burner, the inoculation was done. Add 0.5 mL of sample dilution 10^{-1} in a culture media tube (five tubes for each sample dilution) until inoculate dilution 10^{-12}. Close denitrifying bacteria tubes tightly to maintain tight conditions and prevent oxygen entry.

Incubation: thirty days under 30 ^{o}C.

Results interpretation:

- Heterotrophic bacteria: after incubation time, turbidity in tubes was interpreted as positive to heterotrophic bacteria.
- Denitrifying bacteria: after incubation time, add three drops of diphenylamine solution (0.2 g of diphenylamine in 100 mL of sulfuric acid). Non-color reaction was interpreted as the consumption of nitrate and denitrifying bacteria presence. Blue coloration is interpreted as negative reaction.
- Alexander’s table (ANEXO A) was used to give a value expressed in MPN g/VS of existing most probable number bacteria within principal sample.

3.9.4 Total and fecal coliforms

The total and fecal coliforms analyzed by MPN methodology based in the practical guide written by Bartram and Pedley (1996).

Sampling: in a sterile flask, took 100 mL of effluent of each reactor to create the "principal sample".

Dilution water: mix 15 g of peptone water (free flowing powder) in 1000 mL of Milli-Q water.

Dilution tubes preparation: add 9 mL of dilution water in each tube. Each tube was closed with cotton stoppers wrapped in gauze and autoclaved at 120 ^oC for 20 min and 1 atm and allowed to cool under aseptic conditions, close to Bunsen burner.

Decimal dilutions: under aseptic conditions, close to Bunsen burner. First, add 1 mL of principal sample in one sterile dilution tube. Homogenized, resulted in 10^{-1} dilution. Then, took 1 mL of dilution 10^{-1} and add to another sterile dilution tube, resulted in 10^{-2}. Same process was done until got 10^{-15} dilution.

Culture media: follow specifications in the bottle.

1. Isolation media

Lauryl tryptose (lactose) broth

2. Confirmatory media:

Brilliant green lactose bile broth (BGLB broth)

EC medium

Culture media tubes:

*Note 1: the volume of each culture media must be adequate for quantity of samples.

*Note 2: for dairy cattle wastewater, 3 repetition tubes of each confirmatory media for each dilution were done.

1. Isolation media (total coliforms): add 9 mL of culture media in a tube and introduce a Durham tube too. Close each tube with cotton stoppers wrapped in gauze and sterilize in autoclave (20 min/1 atm/120 ^oC) and allow to cool under aseptic conditions, close to Bunsen burner.

2. Confirmatory media (thermotolerant coliforms): add 5 mL of culture media in a tube and introduce a Durham tube too. Close each tube with cotton stoppers wrapped in gauze and sterilize in autoclave (20 min/1 atm/120 ^oC) and allow to cool under aseptic conditions, close to Bunsen burner.

For total coliforms

Inoculation:

Under aseptic conditions, close to Bunsen burner, the inoculation was done. Add 1 mL of dilution 10^{-1} in a culture media tube until inoculated dilution 10^{-15}.

Incubation:

48 hours at 35 °C for total coliforms and 24 hours at 44 °C for thermotolerant coliforms.

Interpretation:

Tube with gas visible in Durham tube plus turbidity of the medium is considered positive. ANEXO A was used to give a value expressed in MPN of existing most probable number bacteria within principal sample.

For thermotolerant coliforms

Inoculation:

Prepare the required number of tubes of confirmation culture medium (BGLB broth for total coliforms and EC medium for fecal coliforms). Using a sterile wire loop, transfer inoculum from positive tubes (+ tubes of total coliforms) into the confirmation medium, sterilize the loop between successive transfers by heating in a flame until it is red hot.

Incubation:

Incubate them for 48 hours at 35 °C (BGLB broth) and 24 hours at 44.5 °C (EC medium).

Interpretation:

Tube with gas visible in the Durham tube plus turbidity of the medium is considered positive. ANEXO A was used to give a value expressed in MPN of existing most probable number bacteria within principal sample.

3.10 SAF effluent temperature and dissolved oxygen concentration

Mean, maximum and minimum air temperature during each period phase was obtained by agro-climatologic station of Exact Science Department of FCAV/UNESP, campus Jaboticabal. SAF effluent temperature and dissolved oxygen were measure between 8 to 9 am daily with a dissolved oxygen meter.

3.11 Specific denitrifying rate formula

SNDR is the radio between the removed nitrite and the amount of biomass in the denitrification reactor and was determined as shown in Eq. (4) described by Jena et al. (2016).

(Eq. 4) $$SDNR = \frac{S_{NO3-1} - S_{NO-2}}{t\ x\ VSS}$$

S_{NO3-1} and S_{NO3-2}: initial and final concentration of nitrate, respectively, under anoxic condition.

t: duration of anoxic condition,

VSS: Volatile Suspended Solid in g/L

3.12 Statistical analysis

For each parameter evaluated in the samples collected during all phases were calculated standard deviation (±), variation coefficient (v.c.) with Microsoft Excel ® 2010; Test F and Tukey (5%) for media comparation with InfoStat 2013.

IV. RESULTS AND DISCUSSION

4.1 General Aspects

4.1.1 Temperature

Table 11 shows the average air temperature during the three experimental phases. Figure 6 displays maximum, mean and minimum temperature registered by Climatological Station of UNESP-Campus Jaboticabal. All phases were under mesophilic conditions, between 20 ^oC and 40 ^oC. The highest mean temperature was in phase II, from September to December 2017, and the lowest was in phase I, from July to September, 2017.

Table 11 Average values and variation coefficient (v.c.) of air temperature and submerged aerated filter (SAF) effluent.

Parameter (^oC)	Phase I	v.c.	Phase II	v.c.	Phase III	v.c.	Test F
Air mean temperature	21.0a	15	24.3c	9	22.3b	13	28.0**
SAF effluent temperature	-	-	24.8a	20	23.8a	17	0.6ns

-: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability ($p<0.05$); ** Significant with 1% probability ($p<0.01$); ns: Not significant.

Different air mean temperature registered between phases were statistically significant ($p<0.05$), it means that fluctuations in these mean temperatures could affect the results of removal components in the system.

Fujihira et al. (2018) tested a laboratory scale ABR + DHSR reactor to treat solid/lipid-rich wastewater. All three phases were placed in a controlled temperature room at 30 ^oC. At this temperature, the system achieved more than 95% COD removal at OLR of 6.5 g COD/(L·d) demonstrating that the increase in temperature improves different conditions such as organic matter removal or methane production. System temperature was directly influenced by air temperature around it, because the inside cellular temperature, growth rate, and microbial metabolism are determined by external temperature (Chernicharro, 2016). The same author mentioned that outside cells temperature helps in different reactions and thermodynamic reaction alterations and metabolic imbalance happens due to drastic changes in temperature.

Treating swine wastewater in a high-rate anaerobic baffled reactor with post-treatment, Rodrigues (2013) showed an average air mean temperature between 19.8 and 24.5 ^{o}C among its six assays. The average CODtot removal was 77% and better removal occurred in essay 2 (24.5 ^{o}C) and assay 5 (19.8 ^{o}C) indicating system versatility. In addition, the same author obtained an average SAF effluent temperature of 27.4 ± 2 ^{o}C during all operation time. Something similar occurred in this research, the best CODtot removal was in phase II (80 ± 16% and 24.3 ºC) and phase III (84 ± 10% and 22.3 ºC) and the average SAF effluent temperature were 24.76 and 23.84 in phase II and III, respectively.

To better understand the effects of fluctuating environmental conditions on the treatment performance, Dolejs et al. (2017) evaluated the impact of short- term temperature. Under psychrophilic conditions, more than 80% of the influent COD was accumulated in the reactor (compared to 39% under mesophilic conditions). According to the authors, an abrupt and short-term temperature decrease from 35 to 15 ºC can largely be absorbed by system with no negative effect on effluent quality. This is corroborated by the present study, since short-term decreases were observed, which mean values were lower in phase III (15.2 ºC day 175 and 14.7 ºC day 200) (Figure 6); however the treatment system managed to maintain stability.

Mean temperature SAF effluent was measured between 8 a.m to 10 a.m, average SAF effluent temperature values are displayed in Table 11. Phase II (Figure 7) and Phase III showed average temperature range between 24.8 and 23.8 °C, respectively. Nitrification is sensitive to low temperatures, below 8 °C this biological process ceases (Hurse e Connor, 1999). The temperature of effluents in all phases was higher than mean air temperature values registered by the climatological station, corroborating stable temperature to harbor microbial reactions.

SAF mean effluent temperature registered between phases II and III were not statistically significant ($p>0.05$) (Table 11), it means that some temperature fluctuations could not affect biological process inside reactor.

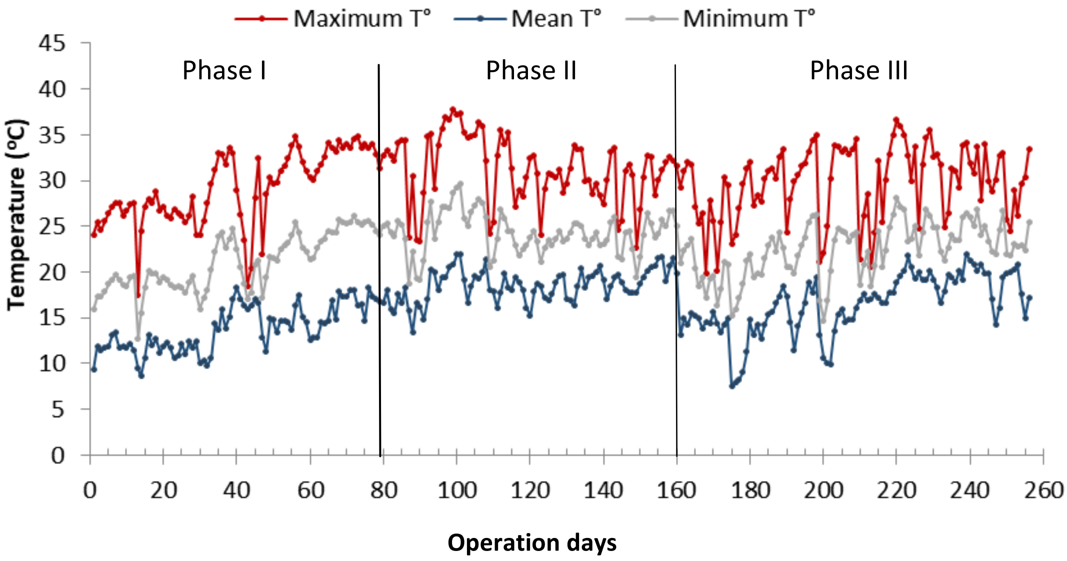


Figure 6 Minimum, mean and maximum values of air temperature during phase I, II and III. Values provided by Climatological station UNESP-Campus Jaboticabal.

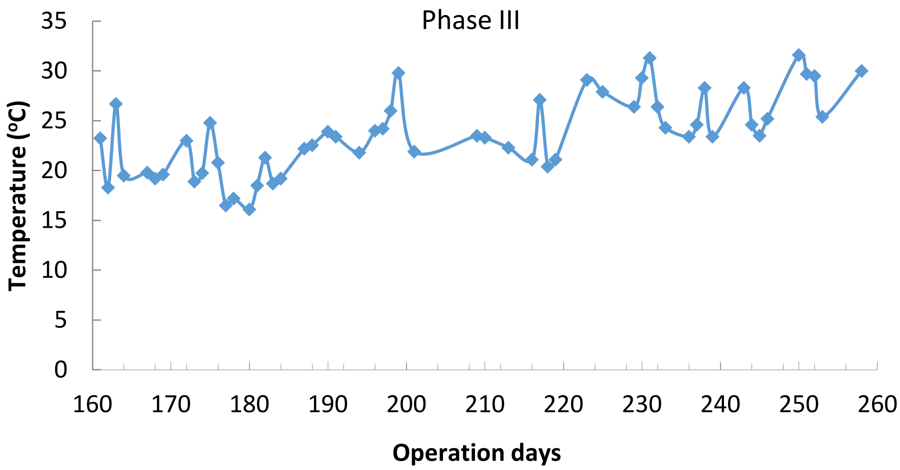


Figure 7 Temperature values of submerged aerated filter (SAF) effluent during phase III.

4.1.2 pH, alkalinity and volatile fatty acids.

The pH values in the effluent of ABRc1 and ABRc2, increased relative to the influent in all the treatment phases, indicating adequate buffer conditions as a consequence of VFA removal and increased PA. Figure 8, figure 9, table 12 and table 13 represent mean values of pH, total alkalinity (TA), partial alkalinity (PA), intermediate alkalinity (IA), IA/PA relation and volatile fatty acids (VFA) of influent and effluents from ABRc1, ABRc2, SAF and USB_{nox} during phase I, II, and III.

pH variations may occur as a function of VFA concentrations, alkalinity, bicarbonate concentrations and CO_2 in the reactor (Liu et al. 2008). Influent pH values ranged between 5.60 and 6.81 in phase I; 5.68 and 7.01 in phase II; 5.80 and 6.81 in phase III. However, the pH values in the effluent of the ABR compartments 1 and 2 ranged from 6.05 to 7.07 (ABRc1) and from 6.02 to 7.08 (ABRc2) during the three phases and were statistically significant ($p<0.05$). It is widely known that maximal biogas yield in anaerobic digestion occurs under a pH of 6.5-7.5 (Liu et al., 2008).

Liu et al. (2008) treated anaerobically, through model simulation, the organic fraction of municipal solid waste in an ABR and observed that pH 7.20 was the optimal temperature under mesophilic conditions (35 ± 2 °C) to cumulative methane production. According Chernicharro (2016), optimal pH for methanogenic microorganism growth is 6.6–7.4. Rodrigues (2013) treated swine wastewater in an ABR system obtaining a methane volumetric production ranged between 0.265 and 0.843 m^3 $CH_4/(m^3 reactor \cdot d)$ under pH ranged from 6.9 to 7.5 and the highest production was performed in assay 6 under pH 6.9.

PA values increased from the influent to the effluent of the ABRc1 and ABRc2 compartments, evidencing the capacity of buffering by bicarbonate ions in the ABR compartments during all phases and significant results of $p<0.05$ and $p<0.01$. In general, anaerobic treatment effluents achieve higher alkalinity than influent by buffering capacity of reactors improving system stability and methanogenic archaea metabolism (Mazareli et al., 2016).

IA/PA ratio is a significant indicator of process stability, when ratio values are greater than 0.3 it is interpreted as a turbulence process in anaerobic digestion of domestic wastewater (Ripley et al., 1986). The IA/PA ratios obtained in phase I and phase II were higher than the value proposed by Ripley et al. but hydrolyses of organic matter (decrase in COD concentration) and methane production were parameters that prove an stable anaerobic digestion inside ABRc1 and ABRc2; did not show statistical significance ($p>0.05$) between results were demonstrated (Table 8). Mazareli et al., (2016) observed a satisfactory buffering capability in the anaerobic treatment of swine wastewater showing IA/PA values between 0.21 and 0.36 in the final effluent system.

Throughout the ABR reactor compartments, the VFA concentrations of the influent in phases I, II and III decreased from 904 to 204 mg/L; from 1002 to 270 mg/L and from 707 to 304 mg/L, respectively (Table 12 and Figure 10). VFA mean values of ABRc2 were statistically significant ($p<0.05$). Santos (2013) treating swine wastewater using ABR with two chambers, also observed reduction of VFA along the ABR reactor chambers, the VFA concentration of the influent decreased from 598 to 211 mg/L value of the tributary, in trials 1, 3 and 4, decreased from 402 to 297 mg/L; from 443 to 388 mg/L and from 598 to 211 mg/L.

Table 12 Mean values and variation coefficients (v.c.) of pH, total alkalinity (TA), partialalkalinity (PA), intermediate alkalinity (IA), IA/PA relation, volatile fatty acids (VFA) and VFA/TA relation of influent and effluents from ABRc1 and ABRc2 during phase I, II and III.

Parameter	Reactor	Phase I	Phase II	Phase III	v.c.	Test F
pH	Influent	6.06	6.39	6.23	3	2.71ns
	ABRc1	7.07b	7.13b	6.85a	2	16.09**
	ABRc2	7.20c	7.08b	6.84a	3	24.92**
TA (mg/ L $CaCO_3$)	Influent	826a	1238b	692a	142	6.84**
	ABRc1	1746	1780	1001	29	9.30ns
	ABRc2	1886b	1878b	1043a	30	11.91**
IA (mg/ L $CaCO_3$)	Influent	156a	416b	373b	44	5.38**
	ABRc1	777b	616b	104a	70	13.66**
	ABRc2	822b	637b	96a	73	15.58**
PA (mg/ L $CaCO_3$)	Influent	669b	822b	320a	43	10.58**
	ABRc1	969ab	1164b	897a	14	4.42*
	ABRc2	1064ab	1240b	946a	14	5.64**
IA/PA	ABRc1	0.71a	0.49b	0.12c	16	2,59*
	ABRc2	0.70a	0.48b	0.10c	20	2.11*
VFA (mg/ L CH_3COOH)	Influent	904ab	1002b	707a	17	3.80*
	ABRc1	243	248	296	11	1.88ns
	ABRc2	204a	270ab	304b	19	4.37*
VFA/TA	ABRc1	0.14a	0.14a	0.30b	47	15.65**
	ABRc2	0.11a	0.14a	0.29b	53	24.76**

Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability ($p<0.05$); ** Significant with 1% probability ($p<0.01$); ns: Not significant.

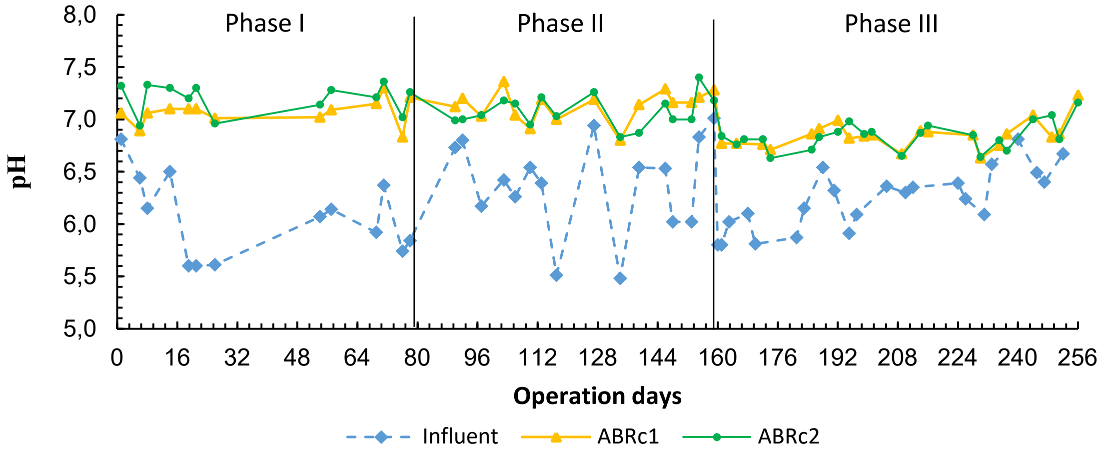


Figure 8 Influent and effluents pH values of the two compartments of anaerobic baffled reactor (ABRc1 and ABRc2) during phases I, II and III.

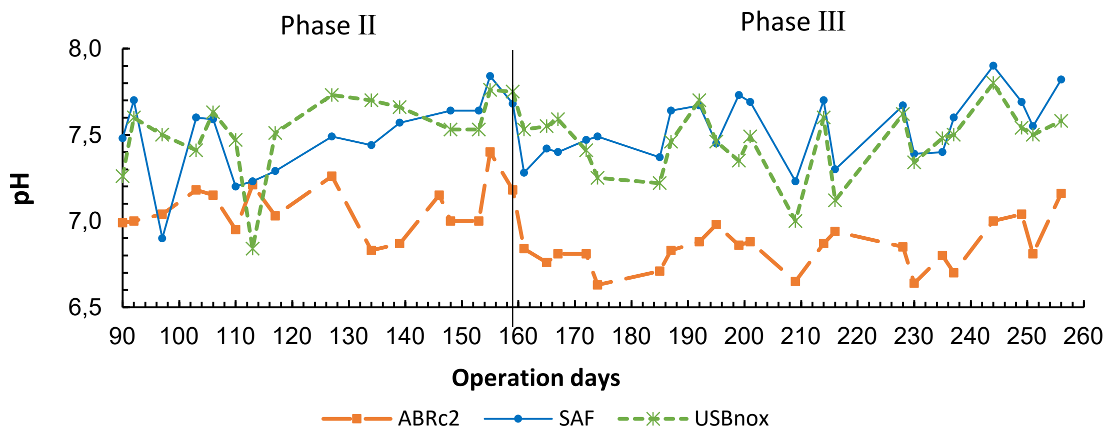


Figure 9 pH values of second compartment of ABR (ABRc2), submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents during phases II and III.

The relation between VFA and bicarbonate concentrations has an essential role in anaerobic systems monitoring. Zhao and Viraraghavan (2004) mentioned that VFT/TA ratios higher than 0.8 can indicate inhibition of methanogenic archaea. This study displayed VFA/TA ratios ranged from 0.14 to 0.30 (ABRc1) and from 0.11 to 0.29 (ABRc2) in all phases and showed statistical significance ($p<0.01$). Phase III showed the highest ratios inside reactors (ABRc1-ABRc2) with OLR increased to 16 g CODtot/(L·d) (Table 12).

Despite high OLR in phase III, the volatile acids concentrations of ABRc1 and ABRc2 remained at stable conditions. The VFA reductions in all phases indicated efficient interactions between acetogenic bacteria and methanogenic archaea inside anaerobic reactors (ABRc1 and ABRc2) preventing accumulation of hydrogen or excessive acidification even with OLR increase.

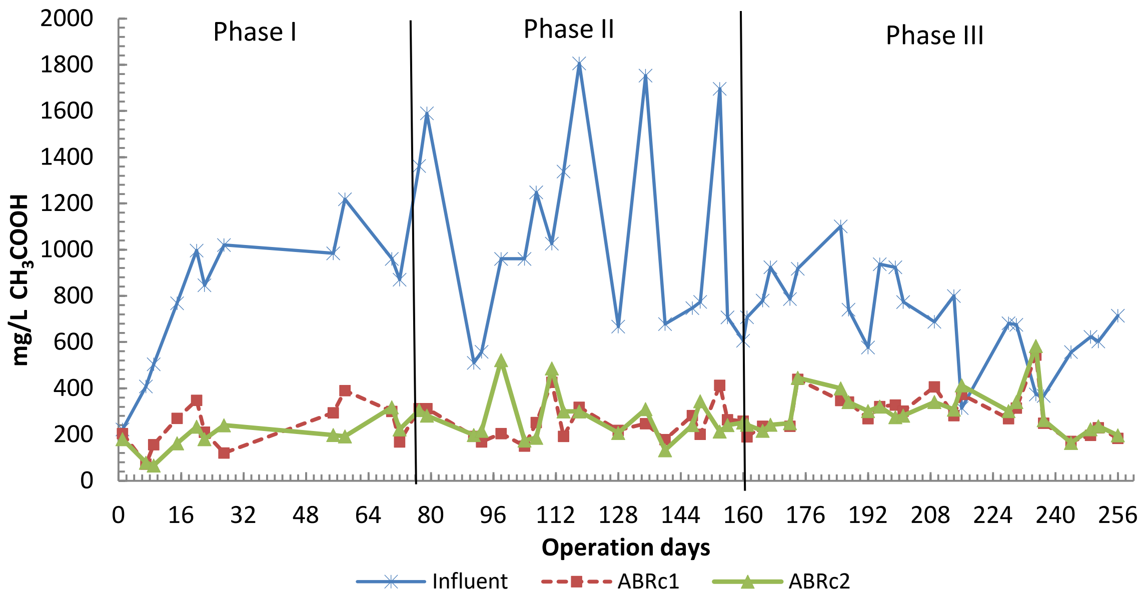


Figure 10 Volatile fatty acids (VFA) values of influent and effluents of the two compartments of anaerobic baffled reactor (ABRc1 and ABRc2) during phase I, II and III.

In the SAF effluent, the pH was within the optimum range for nitrification, 7.49 (Phase I) and 7.54 (Phase II). Normal rate nitrification takes place at pH between 7.2 and 8.0 (Downing, 1978). Normally, nitrification reduces buffering capability because the conversion of ammonium to nitrite, generating H^+ release (Eq. 1) (Von sperling, 2016). The preservation of alkalinity is an important parameter in post-treatment systems because nitrification is carried out in alkaline conditions (recommended at least 50-60 mg/L CH_3COOH) (Metcalf e Eddy, 2014). Total, partial and intermediate alkalinity of this study are represented in Table 13, showing alkalinity reduction due to consumption of alkalinity for the nitrification process and, at the same time, good alkaline condition for nitrification to happen with statistical significance of $p<0.05$ in TA and IA in all phases.

Denitrification happens in a pH range of 6.0 to 9.0 and optimal pH ranged of 6.5 to 7 (Surampally et al., 1997). This study exhibited pH values between 6.84 and 7.54 in both phases without statistical significance ($p>0.05$) (Table 13). Total alkalinity average values of USB_{nox} effluent in both phases were higher than SAF effluent with a statistical significance ($p<0.05$). Half of the alkalinity is consumed when total denitrification happens, in contrast to nitrification which consumes 7.1 mg/L of alkalinity for each 1 g NH_4^+/L (Von Sperling, 2016). Less consume of alkalinity is evidenced in this research (Table 13) demonstrating that denitrification took place in USB_{nox}. Phase II and III showed decrease in average VFA concentrations indicating stability in SAF and USB_{nox} (Table 13 and Figure 11).

Table 13 Mean values and variation coefficients (v.c.) of pH, total alkalinity (TA), partial alkalinity (PA), intermediate alkalinity (IA) and volatile fatty acids (VFA) of ABRc2, SAF and USB_{nox} effluents during phase II and III.

Parameter	Reactor	Phase II	Phase III	v.c.	Test F
pH	ABRc2	7.08b	6.84a	2	24.92**
	SAF	7.49	7.54	0	0.58ns
	USB_{nox}	7.53	7.46	1	0.92ns
TA (mg/L $CaCO_3$)	ABRc2	1878b	1043b	40	11.91**
	SAF	1455b	818a	40	10.32**
	USB_{nox}	1222a	906b	21	5.36*
IA (mg/L $CaCO_3$)	ABRc2	637b	96a	104	15.58**
	SAF	56b	60a	6	18.30**
	USB_{nox}	422b	77a	98	13.07**
PA (mg/L$CaCO_3$)	ABRc2	1240b	946a	19	5.64**
	SAF	899	757	12	2.10ns
	USB_{nox}	801	829	2	0.23ns
VFA (mg/L CH_3COOH)	ABRc2	270ab	304b	8	4.37*
	SAF	180	190	4	0.11ns
	USB_{nox}	128a	195b	29	5.18*

Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability ($p<0.05$); ** Significant with 1% probability ($p<0.01$); ns: Not significant.

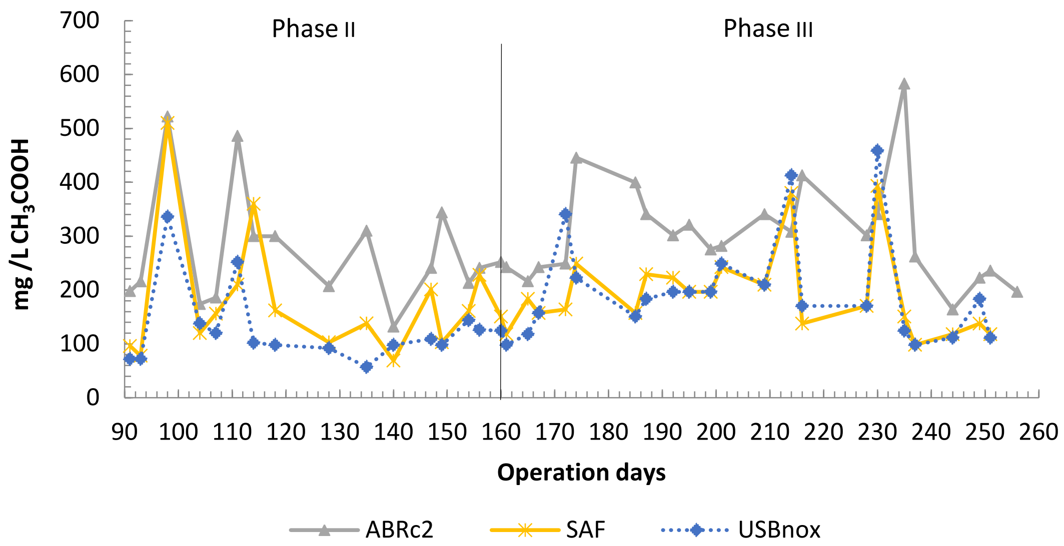


Figure 11 Volatile fatty acids (VFA) values of second compartment of anaerobic baffled reactor (ABRc2), submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents during phase II and III.

4.2. Organic matter and macronutrients

4.2.1. Chemical oxygen demand

The average values of OLR applied in the ABR reactor compartments are presented in Table 14, which were 6, 7, and 9 (ABRc1) and 2, 4, and 7 (ABRc2) in phases I, II and III, respectively. The first compartment was operated with higher OLR, and the highest OLR were obtained throughout both compartments in phase III.

High removal efficiency of organic matter was obtained throughout both compppartments. The average values of influent CODtot were 15025, 14531, and 13850 mg/L in phases I, II, III, respectively and CODso were 3650, 1969 and 2312 mg/L in phase I, II and III, respectively. The effluent CODtot were 2430 mg/L, 4000 mg/L and 7625 mg/L corresponding to phase I, II and III in ABRc2, respectively. The first phase shows a better CODtot removal than the rest of the phases due to higher HRT and lower OLR. The overall average efficiency of the first, second and third phase was 84%, 72%, and 45%, respectively; showing that it is possible to reduce from global HRT of 93.7 h (phase I) to 74.6 h (phase II) in ABR reactors and get similar CODtot removal efficiencies at similar OLR and temperature (Table 3).

Reduction of HRT in an ABR system for treating raw municipal wastewater demonstrated the same pattern; the highest achievable CODtot removal efficiency happened in high HRT (48 h) and that removal efficiency decreased when HRT was reduced to 24 h. The same behavior occurs in CODs removal, 82 % in HRT of 48 h decreased to 70% in HRT of 24 h (Aqaneghad and Moussavi, 2016). Fujihira et al. (2018) used an ABR system to treat solid/lipid-rich wastewater getting a high CODtot removal efficiency (95.7 %) in HRTs of 5.6 d and 12.1 d and a perceptible decrease in removal capability (92.7%) when HRT was reduced to 2.9 d.

Duda et al. (2015) treated swine wastewater using four in-series high-rate horizontal anaerobic reactors with influent CODtot of 5868 mg/L and HRT of 24 h, attaining a CODtot and CODs removal efficiency of 68 and 41%, respectively. Gulhane et al. (2017) treated vegetable waste slurry with a global removal efficiency of 72% on the ABR system under HRT of 192 h and OLR of 0.5 g VS/(L· d). Despite the use of high-rate ABR with two compartments and 50% lesser HRT and twelve-fold OLR, removal efficiency results in this investigation are similar than those mentioned, evidencing the system sturdiness to treat dairy cattle manure.

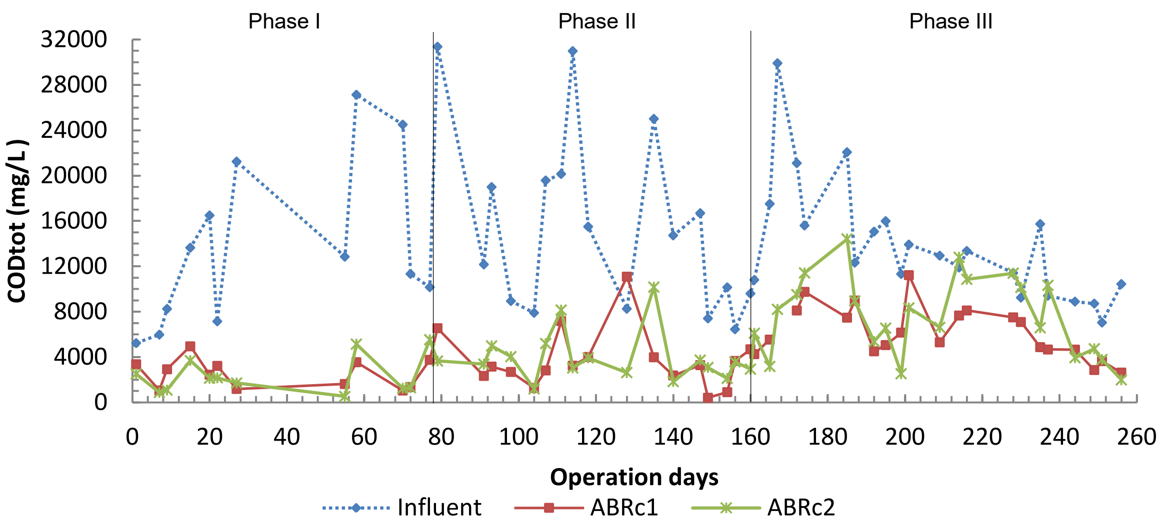


Figure 12 Total chemical oxygen demand (CODtot) values of influent and effluents of the two compartments of anaerobic baffled reactor (ABRc1 and ABRc2) during phase I, II and III.

Otherwise, the phase III of this study had a lesser HRT (55.3 h) and an increased OLR of 16 g CODtot/(L·d). This was reflected in the removal efficiency of

CODtot, which decreased to 45 % from 72% obtained in Phase II. Instead of their longer HRT (68 h), vinasse treatment in UASB reactors showed a removal efficiency decrease when OLR increased to 7.5 g CODtot /(L·d) from 5 g CODtot/(L·d) (Barros et al., 2016). Longer HRTs are directly associated with high organic matter and different inorganic compounds removal efficiencies, depending on the waste composition (Fazli et al., 2018; Kim et al., 2015; Sangeetha et al., 2017).

Table 14 Average values and variation coefficient (v.c.) of total (CODtot), soluble (CODso) and suspended (CODsu) chemical oxygen demand of influent, ABRc1, ABRc2, SAF and USB_{nox} effluents and CODtot removal efficiency of each parameter (%R) in phase I, II and III.

Parameter	Reactor	Phase I	v.c.	Phase II	v.c.	Phase III	v.c.	Test F
CODtot (mg/L)	Influent	15025	57	14531	55	13850	38	0.13ns
	ABRc1	2849	58	3570	72	6194	37	10.77ns
	ABRc2	2430a	66	4000a	57	7625b	49	14.41**
	SAF	-	-	1965	69	2203	37	0.49ns
	USB_{nox}	-	-	1743	75	2138	68	0.57ns
%R	ABRc1	76b	23	74b	33	49a	40	9.95**
	ABRc2	23	106	8	258	9	191	2.47ns
	ABR	80b	20	71b	18	44a	61	14.77**
	SAF	-	-	44a	78	67b	21	7.70**
	USB_{nox}	-	-	29	107	22	89	0.76ns
	System	-	-	81	18	83	12	0.07ns
CODso (mg/L)	Influent	3650b	11	1969a	39	2312a	52	5.87**
	ABRc1	1393	34	750	41	1316	41	2.39ns
	ABRc2	1514	16	800	61	1269	56	1.43ns
	SAF	-	-	430a	28	1077b	48	5.73*
	USB_{nox}	-	-	341a	65	1028b	48	6.93*
%R	ABRc1	62b	23	54ab	55	38a	38	4.64*
	ABRc2	3	160	30	160	14	120	1.85ns
	ABR	58	11	66	30	42	50	2.70ns
	SAF	-	-	42	87	17	128	2.88ns
	USB_{nox}	-	-	31	97	16	166	1.01ns
	System	-	-	73	44	46	55	3.37ns
CODsu (mg/L)	Influent	11375	-	12562	-	11538	-	-
	ABRc1	1456	-	2820	-	4878	-	-
	ABRc2	916	-	3200	-	6356	-	-
	SAF	-	-	1535	-	1126	-	-
	USB_{nox}	-	-	1402	-	1110	-	-

-: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability ($p<0.05$); ** Significant with 1% probability ($p<0.01$); ns: Not significant.

This fact is achieved due to the normal microbial activity; more contact time between the compounds and microorganisms maximizes the microbial removal efficiencies of pollutants (Li et al., 2013).

Organic matter, as a COD source, is one of the main issues of wastewater treatment because its reduction in BAF is given by the heterotrophic microorganisms that need carbon. In this post-treatment, SAF reactor removed a large portion of the organic matter content in ABRc2's effluent. The average CODtot inlet in phase II was 4000 ± 2296 mg/L and its average CODtot outlet was 1965 ± 1363 mg/L; the average CODtot inlet in phase III was 7625 ± 3497 mg/L and its average CODtot outlet was 2202 ± 819 mg/L (Table 10). Despite HRT variation in both phases, CODtot removal efficiency did not show huge fluctuations.

Denitrification occurred at USB_{nox} reactor and the nitrogen consumption was carried out through three pathways: use of polyhydroxyalkanoates, use of exogenous carbon source and sludge hydrolysis (Liu et al., 2017). USB_{nox} reactor contributed with consumption of exogenous carbon during phase II and III, showing a lower decrease of CODtot than SAF reactor. The CODtot reduction in phase II was 221.5 mg/L and in phase III was 64.7 mg/L (Table 10 and Figure 13). This reactor contributed with 11.28% and 2.92% of CODtot removal efficiency in phase II and III, respectively.

However, it is possible to observe that most of the organic matter removal occurred in the ABR reactor, a promising alternative for the treatment of wastewater with high concentrations of COD. The use of the submerged aerated filter was fundamental for good results of nitrification occurrence (next items). The USB_{nox} reactor was important in maintaining stable treatment system removal efficiencies in response to aeration, sludge stabilization, and mitigation of possible organic shock conditions.

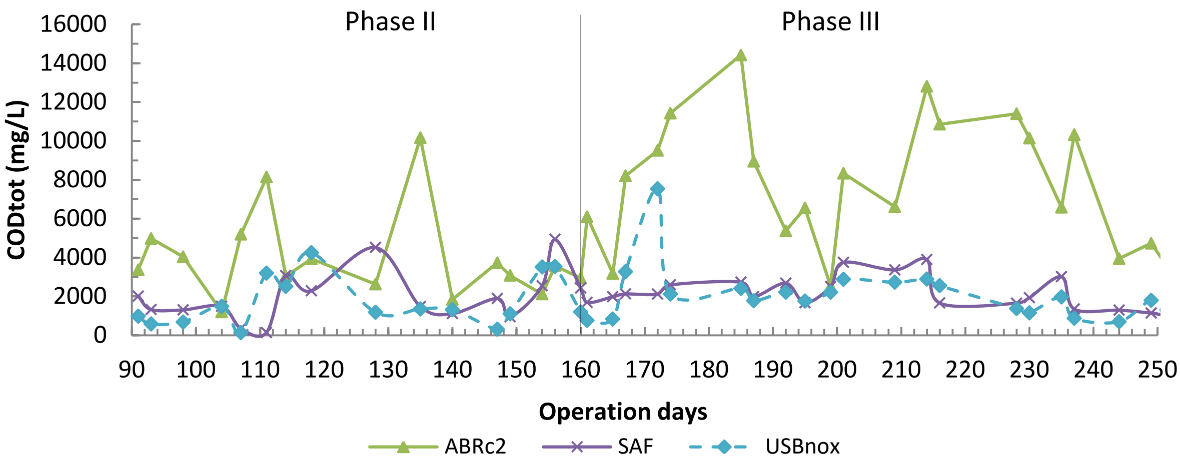


Figure 13 Total chemical oxygen demand (CODtot) values of second compartment of anaerobic baffled reactor (ABRc2), submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents during phase II and III.

4.2.2. Total suspended solids and volatile suspended solids of influent and effluents.

In influent, the highest concentration of TSS happened in phase II and the highest concentration of VSS was in phase III. The mean values of the concentrations of TSS and VSS in the influent from chamber 1 of the ABR ranged from 3565 to 7426 mg/L and from 2805 to 6432 mg/L, respectively (Table 11 and Figure 14). VSS values represented 79, 77 and 97% of TSS during three phases; these results show that solid suspended of dairy cattle wastewater was composed mainly of organic matter (Table 11).

The average concentrations of TSS and VSS in the effluent from ABRc2 ranged from 1177 to 5018 mg/L and from 824 to 4521 mg/L, respectively. The highest concentrations of TSS and VSS in effluent from ABRc2 were observed in phase III, which can be due to application of smaller HRT (greater liquid velocity) and lower temperature. According to Oliveira e Foresti (2004), the increase of HRT and higher temperatures allow greater solubilization of suspended organic matter.

Table 15 Average values and variation coefficient (v.c.) of total suspended solids (TSS), volatiles suspended solids (VSS) and fixed suspended solids (FSS) of influent, ABRc1 and ABRc2 effluents and removal efficiency (%R) in phase I, II and III.

Parameter	Reactor	Phase I	Phase II	Phase III	v.c.	Test F
HRT (h)	ABRc1	60.0	48.0	36.0	-	-
	ABRc2	33.7	27.0	20.3	-	-
OLR (g CODtot/(L·d))	ABRc1	5.9	7.3	9.3	48	3.96*
	ABRc2	1.7	3.6	7.4	54	29.21**
TSS (mg/L)	Influent	3565a	7427b	6632ab	35	4.50*
	ABRc1	1301a	2881ab	4270b	53	5.95**
	ABRc2	1177a	2765a	5018b	65	13.00**
%R	ABRc1	63	61	36	28	0.60ns
	ABRc2	10	4	nr	61	0.61ns
	ABR	67	63	24	46	12.3ns
VSS (mg/L)	Influent	2805a	5747ab	6433b	39	4.46*
	ABRc1	818	2272	3551	62	2.51ns
	ABRc2	824a	2124a	4521b	75	12.94**
%R	ABRc1	71	60	45	22	0.34ns
	ABRc2	nr	7	nr	-	0.11ns
	ABR	71	63	30	40	6.06ns
FSS (mg/L)	Influent	760	1680	199	54	-
	ABRc1	483	609	720	33	-
	ABRc2	354	641	497	25	-

nr: Negative result; -: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability ($p<0.05$); ** Significant with 1% probability ($p<0.01$); ns: Not significant.

The same behavior was observed by Santos (2011) and Rodrigues (2013) treating wastewater with higher TSS and VSS concentrations. Santos (2011) treated swine wastewater in two-chamber ABR reactor, which VSS concentration in test 4 represented 77% of TSS. Rodrigues (2013) treated swine wastewater in ABR system with two chambers (C1 and C2), the ABR (C2) showed more solid suspended concentration in assays 1 and 3, compared with ABR (C1). The author applied an OLR of 9 g CODtot/ (L·d) with a HRT of 40.6 h (test 1); an OLR of 13.9 g CODtot/ (L·d) with a HRT of 21.2 h (test 4), obtaining a TSS removal of 45 and 72%, respectively. This study applied 8 g CODtot/ (L·d) in phase I and 11 g CODtot/ (L·d) in phase II, observing a TSS removal of 67 and 63%, respectively (Table 15).

Fujihira et al. (2018) treated solid/lipid-rich wastewater with 6500 mg TSS/L using a high-rate system formed by an ABR with two compartments and a DHS reactor. The phase 1 under a HRT of 157 h and 4.8 g COD/(L·d) showed a final effluent with 210 mg TSS/L. Removal efficiencies results in this investigation are similar than those mentioned above, evidencing the system hardiness to treat dairy cattle wastewater. Duda et al. (2015) treated swine wastewater using four in-series high-rate horizontal anaerobic reactors (R1-R4) with an influent CODtot of 5.868 mg/L under a HRT of 24 h attaining a removal efficiency of 50% TSS and 48% VSS in R1 only and efficiencies 72 and 75% of TSS and VSS, respectively, for the entire system (R1-R4).

In the post-treatment system, composed by SAF and anoxic USB, the average concentrations of TSS and VSS were greatly reduced. TSS and VSS are the indicators of the operational behavior of any biological wastewater treatment plant (Metcalf e Eddy, 2014). Table 16 demonstrates the average values of TSS and VSS of SAF and USB_{nox} effluents and their removal efficiencies. Fig. 15 and 16 show TSS and VSS values during different operation time in phase II and III.

The consumption of sludge was evidenced between ABRc2 and SAF reactor in phase II; the reduction was of 685 mg TSS/L and 513 mg VSS/L, but the greatest TSS and VSS removal contribution was inside USB_{nox} reactor.

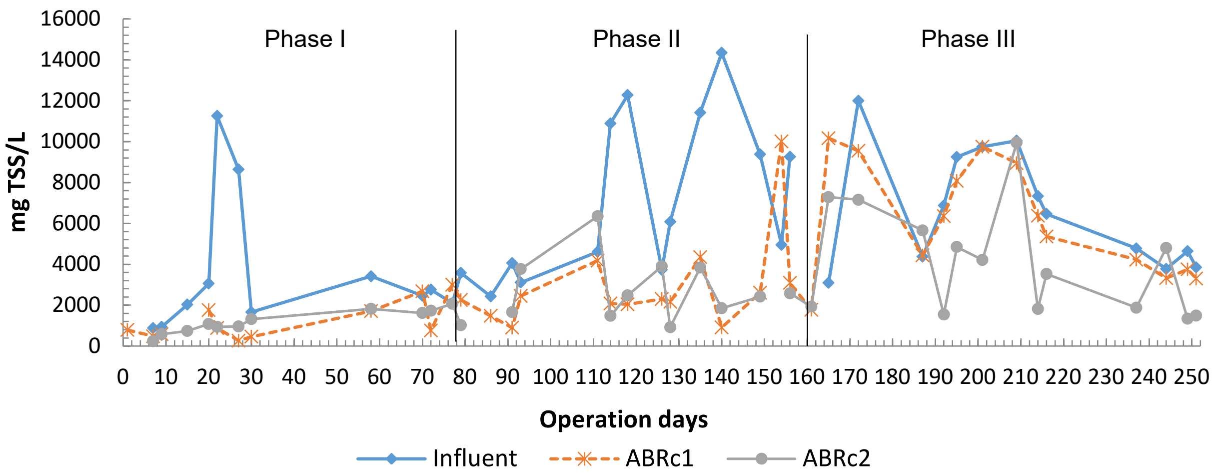


Figure 14 Average values of total suspended solids (TSS) of influent and effluents of the two compartments (ABRc1 and ABRc2) during phases I, II and III.

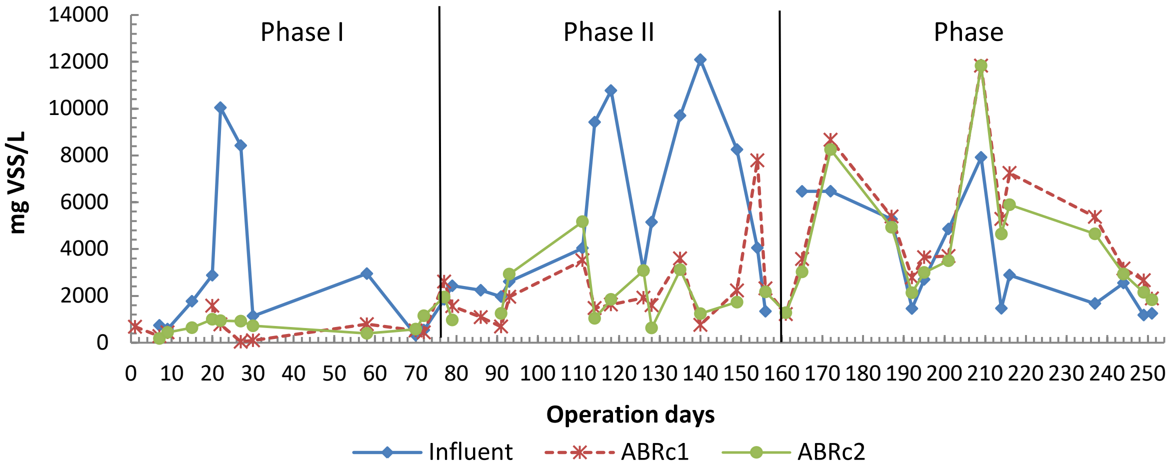


Figure 15 Average values of volatile suspended solids (VSS) of influent and effluents of the two compartments (ABRc1 and ABRc2) during phases I, II and III.

In phase III, sludge reduction in USB_{nox} reactor was observed, showing the best TSS and VSS removal efficiencies when compared with Phase I. SAF reactor contributed with the greatest TSS and VSS removal in Phase III post-treatment. In this research, the biomass was consumed as organic matter, for this reason, sludge diminishing was evidenced. Phase II take 64% of TSS and phase III 37% of TSS.

Jena et al. (2016) displayed TSS and VSS effluent concentrations of 510-690 mg/L at the end of the long anoxic phase in an anoxic-aerobic SBR system treating nitrate and COD high-strength wastewater. Santos (2011), with the inclusion of the post-treatment system composed by the SAF and anoxic USB, observed concentrations of TSS and VSS reduction from 3068 mg/L to 288 mg/L and from 2680 mg/L to 227 mg/L, respectively.

The results of SST and SSV removal in this study indicate that with the anaerobic treatment system, composed of ABR reactor, followed by post-treatment, with SAF and USB_{nox}, it was possible to obtain high values of efficiencies of solid removal, confirming the possibility of using this configurations in the treatment of dairy cattle wastewater with high concentrations of suspended solids.

Table 16 Average values and variation coefficient (v.c.) of total suspended solids (TSS), volatiles suspended solids (VSS) and fixed suspended solids (FSS) of submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents and removal efficiencies (%R) in phase II and III.

Parameter	Reactor	Phase II	Phase III	v.c.	Test F
HRT (h)	SAF	26.6	20.0	-	-
	USB_{nox}	20.0	15.0	-	-
OLR (g CODtot/(L·d))	SAF	1.8	2.7	53	4.95*
	USB_{nox}	2.2	3.5	72	3.32ns
TSS (mg/L)	ABRc2	2765a	5018b	65	13.00**
	SAF	2080	1079	45	1.99ns
	USB_{nox}	749	681	7	0.23ns
%R	SAF	25	78	73	0.04ns
	USB_{nox}	64b	37a	38	33.22**
	System	73	86	12	0.26ns
VSS (mg/L)	ABRc2	2124a	4521b	75	12.94**
	SAF	1611	952	36	1.33ns
	USB_{nox}	619	569	6	0.19ns
%R	SAF	24	79	76	0.02ns
	USB_{nox}	62b	40a	31	23.97**
	System	71	87	14	0.11ns
FSS (mg/L)	SAF	469	127	66	-
	USB_{nox}	130	112	42	-

-: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability ($p<0.05$); ** Significant with 1% probability ($p<0.01$); ns: Not significant.

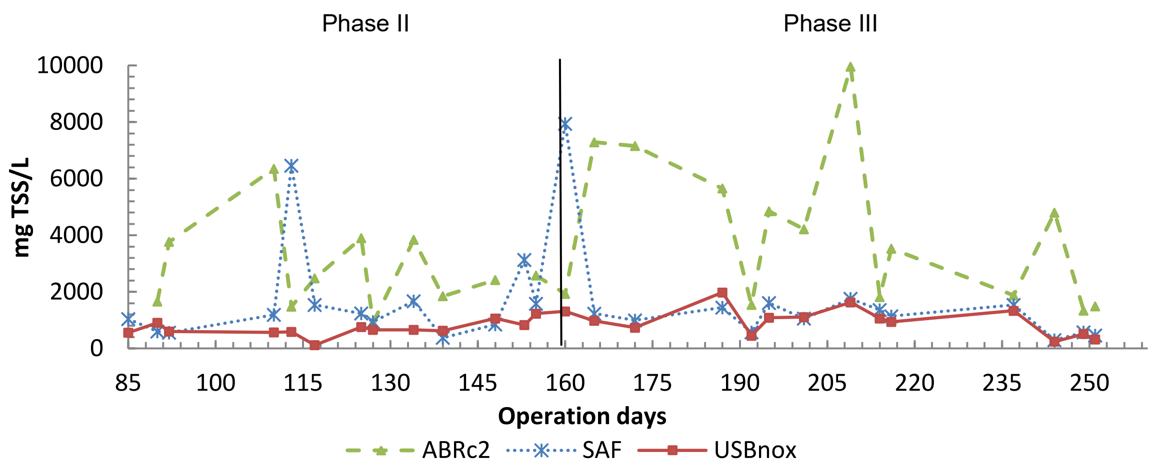


Figure 16 Average values of total suspended solids (TSS) of submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents during phases II and III.

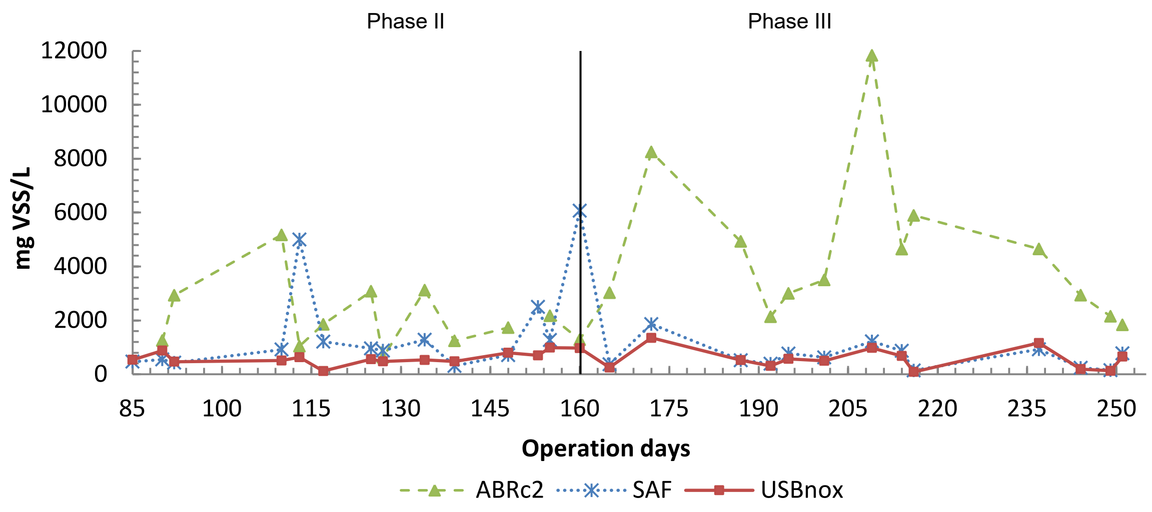


Figure 17 Average values of volatile suspended solids (VSS) of submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents during phases II and III.

4.2.3 Nitrogen

Total nitrogen and total nitrogen ammonia concentration in influent and ABR's compartments are shown in Figure 18 and Table 17. The average TKN concentrations in the influent were 338.8, 313.3 and 427.2 mg/L in phase I, II and III, respectively. The first phase has a decrease of 50% in the total nitrogen detected in ABRc1 effluent. In contrast, there is an increase in ABRc2 from 170 to 195 mg N/L.

Phase II shows the same pattern, ABRc1 could remove nutrient but ABRc2 has a 22% more concentration than ABRc1. The total nitrogen increased in ABRc2, during the two phases, this could be due to the wastewater composition. For example, sewage sludge, swine and cattle manure are nitrogen-rich organic wastes. In fact, cattle manure has proteins (approximately 19% of crude protein) that after anaerobic hydrolysis are converted into inorganic ammonia (Tao et al., 2016). TKN concentration in phase I, II and III did not show a statistical significance ($p>0.05$) when were compared amoung them.

In addition, the decrease of HRT in the second phase could be another reason for high nitrogen concentration, especially in the last chamber. Phase III shows the highest influent TKN of all phases with 427.19 mg N/L (Figure 18). This fact could be associated with the lower HRT applied, triggered by an inlet substrate increment and the parameters associated with the environment of dairy cattle, animals breeding such as feeding and genetics which can be directly associated with the animals' food digestibility. West (2003) mentioned that air temperature and THI increment resulted in a decrease of DMI of dairy cattle; the optimal temperature in production zones for lactating dairy cows is estimated to be from 0.5 to 20 ^{o}C and critical air temperature is 25 to 26 ^{o}C. DMI exhibited significant declines under minimum, mean and maximum THI of 64, 72 and 76%, respectively (Igono et al., 1992). Phase III reported an air temperature of 22.28 ^{o}C between July – October, 2018, assuming a better DMI than other phases in this study. Air relative humidity in this phase was between 52.6 and 70.3%. In contrast, phase II showed a high range of THI between 58.7 and 74.2% under an average air mean temperature of 24.3 ^{o}C at September 29th to December 12th, 2017.

In all phases, the mineralization of organic nitrogen through the anaerobic baffled reactor (ABRc1 and ABRc2) was observed (Figure 18). A remarkable ammonification is obtained in Phase I and Phase II, from 85.8 mg NH_4^+/L in influent to 146.5 mg NH_4^+/L in ABRc2 and from 142.6 mg NH_4^+/L in influent to 302.4 mg NH_4^+/L, respectively. Phase III showed mineralization but lesser than phase I and II (Figure 18). Statistical significance of $p<0.01$ were observed between all phases in ABRc1 and ABRc2. Similar behavior was observed in Singh et al. (2009) research; high-strength wastewater was treated in an anaerobic baffled reactor with a HRT of 28.8 h. In this case, the inlet concentration was 142 mg and ABR effluent was 209 mg for NH_4^+/L. The same author affirms that NH_4^+ increase is result of organic N ammonification. Swine manure and vegetable waste, treated in three in-series high-rate HARFB, reported high concentrations of NH_4^+ in its four phases tested while OLR increased. This happened due to the high concentration of protein compounds, showing ammonification rise (Mazareli et al., 2016).

Decrease of NH_4^+ was visible in both, SAF and USB_{nox} reactors, during phase II and III (Table 17 and Figure 20). The average ammonium removal efficiency of the overall post-treatment process was 56 % in phase II, which decreased from 302.4 ± 178 to 103.9 ± 77 mg NH_4^+/L. Pelaz et al. (2018) tested a denitrification/nitrification pilot plant which previously received effluent of a raw municipal wastewater anaerobic treatment. The authors obtained NH_4^+ removal of 86.1% in case 1 and

73.6% in case 2 and observed a similar simultaneous utilization of organic matter and ammonium. Contrary to expectations, phase III has a sharply decrease in overall ammonium removal achievement, only 31%, from 108.5 ± 24 to 75.3 ± 33 mg NH_4^+/L. The utilization of organic matter simultaneously with ammonium removal profile shows an analogy with COD removal (Pelaz et al., 2018).

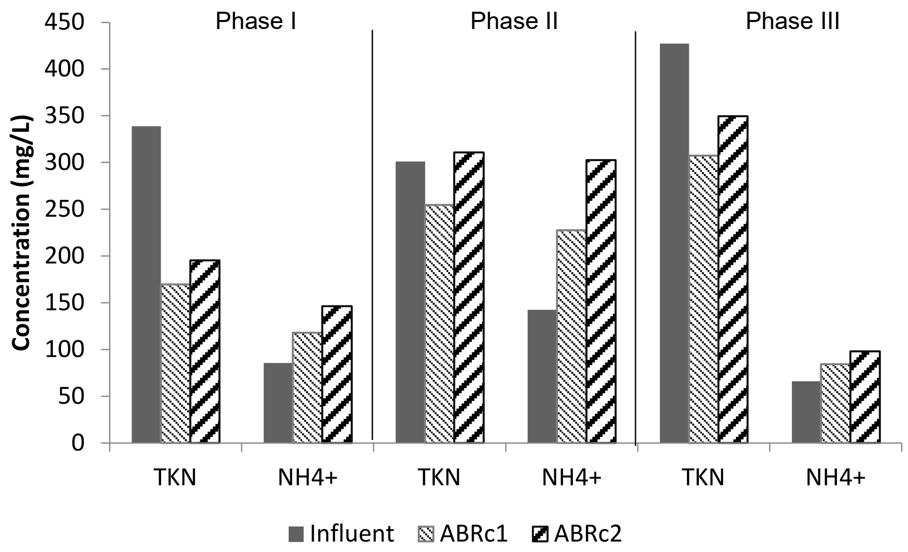


Figure 18 Mean values concentration of total Kjeldahl nitrogen (TKN) and total ammonia nitrogen (NH_4^+) of influent and effluents of two compartments of the anaerobic baffled reactor (ABRc1 and ABRc2) in phase I, II and III.

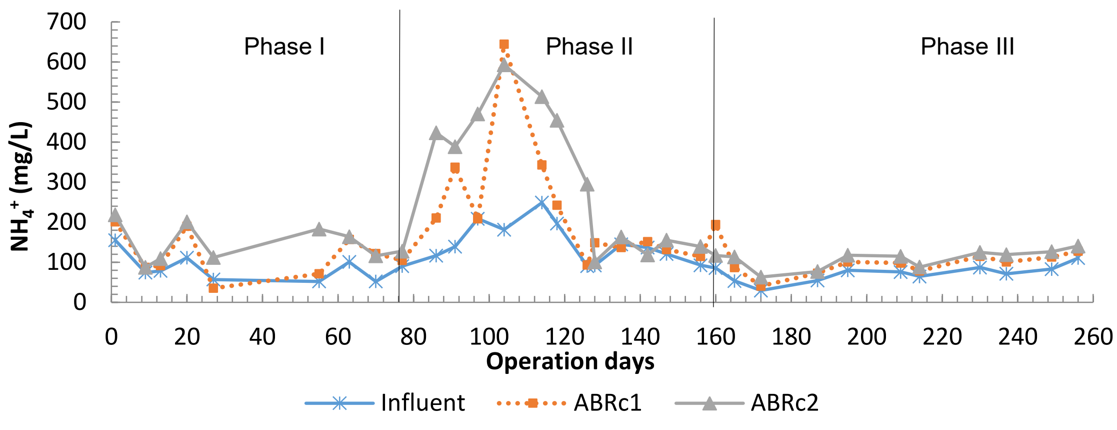


Figure 19 Total ammonia nitrogen (NH_4^+) values of influent and effluents of the two compartments of anaerobic baffled reactor (ABRc1 and ABRc2) during phase I, II and III.

In addition, heterotrophic biomass was detected in this research, phase II had 9.2 x 10^{14} MPN g/VS and phase III showed a smaller population, 2.4 x 10^{12} MPN g/VS (Table 19) in SAF reactor. Hence, NH_4^+ uptake was reflected in this period. The active heterotrophic biomass is present in aerobic and anoxic process, and uses nitrogen transformed in ammonium and organic matter for synthesis (Von Sperling, 2016).

Table 17 Average values and variation coefficient (v.c.) of total ammonia nitrogen (NH_4^+), total Kjeldahl nitrogen (TKN) and TKN removal efficiencies (%R) of influent, two compartments anaerobic baffled reactor (ABRc1-ABRc2), submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents in phase I, II and III.

Parameter	Reactor	Phase I	v.c.	Phase II	v.c.	Phase III	v.c.	Test F
OLR (g CODtot/(L·d))	ABRc1	6a	57	7ab	50	9b	38	3.96*
	ABRc2	2a	66	4a	59	7b	37	29.21**
	SAF	-	-	2a	69	4b	37	4.95*
	USB_{nox}	-	-	2	75	3	68	3.32ns
NH_4^+ (mg/L)	Influent	85.8a	39	142.6b	36	70.9a	31	10.57**
	ABRc1	117.8a	47	227.5ab	65	93.4a	28	5.91**
	ABRc2	146.5a	32	302.4b	59	108.5a	23	8.89**
	SAF	-	-	105.4	98	66.2	48	1.34ns
	USB_{nox}	-	-	103.9	74	75.3	44	0.72ns
TKN (mg/L)	Influent	338.8	46	313.3	40	427.2	40	1.65ns
	ABRc1	169.7	38	254.6	64	307.3	37	2.81ns
	ABRc2	195.2	39	310.8	61	349.6	35	2.90ns
	SAF	-	-	142.1	78	165.5	45	0.33ns
	USB_{nox}	-	-	136.9	62	160.8	59	0.41ns
%R	ABRc1	49.9	-	18.7	-	28.1	-	1.86ns
	ABRc2	nr	-	nr	-	nr	-	0.26ns
	ABR	42.4	-	0.8	-	18.2	-	11.2ns
	SAF	-	-	54.3	-	52.7	-	0.07ns
	USB_{nox}	-	-	3.7	-	2.8	-	0.63ns
	System	-	-	68.1a	-	62.4a	-	7.01ns

nr: Negative result; -: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability ($p<0.05$); ** Significant with 1% probability ($p<0.01$); ns: Not significant.

Inside SAF reactor, aerobic system, the nitrification and the consumption of organic N can be observed in both phases (Figure 21) but when all phases were analized statistically did not demonstrate significance ($p>0.05$) (Table 17). The ammonium oxidation to nitrite is done by AOB which are little sensitive to low DOC and has slow developing; the nitrite oxidation to nitrate is carried out for NOB which are more sensitive to low DOC and has a rapid development, helping to the non-accumulation of nitrites inside the system (Liu et al., 2017; Von Sperling, 2016).

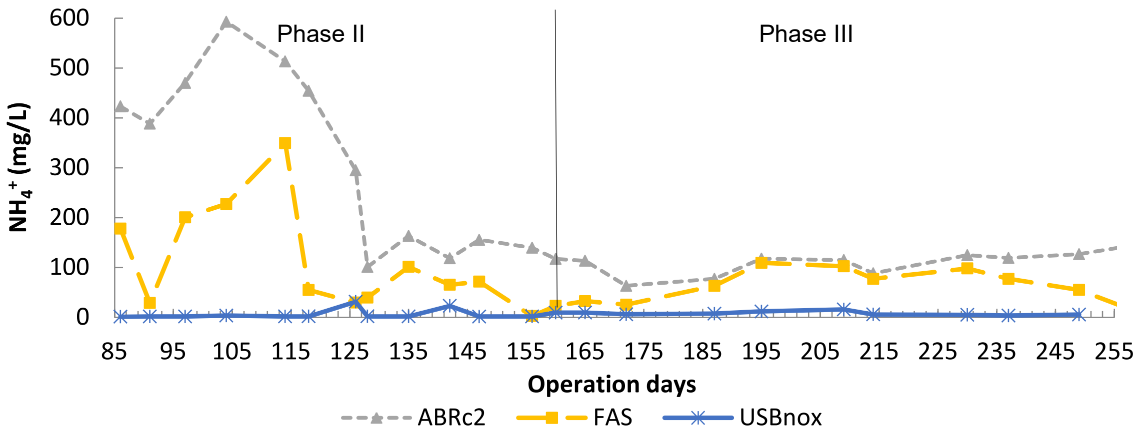


Figure 20 Total ammonia nitrogen (NH_4^+) values of submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents during phase I, II and III.

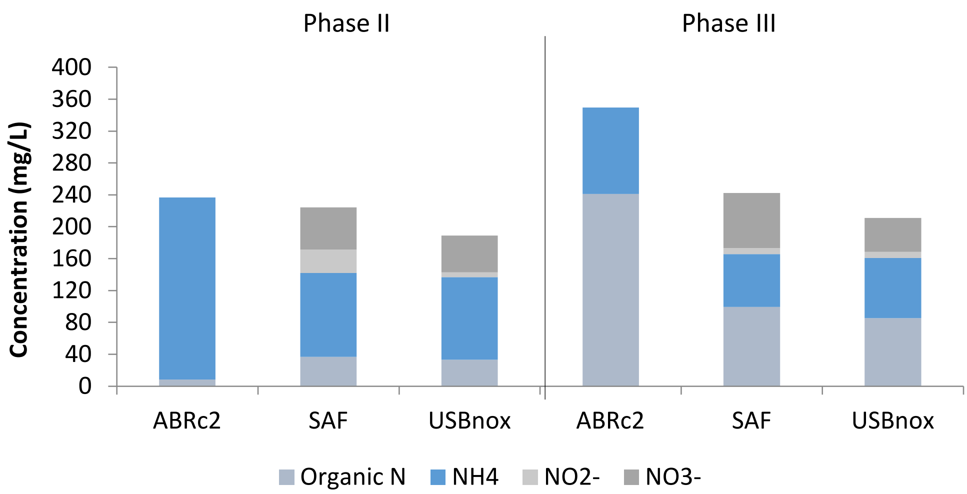


Figure 21 Formation of different nitrogen compounds in ABRc2 and submerged aerated filter and anoxic upflow sludge blanket (SAF/USB_{nox}) post-treatment during the phases II and III

In this research, nitrite concentration average was 29 ± 41 mg NO_2^-/L in phase II and 8 ± 3 mg NO_2^-/L in phase III (Table 18), showing the activity of AOB. The AOB population of SAF reactor was 4.1 x 10^{13} MPN g/VS in phase II and 7.2 x 10^{13} MPN g/VS in phase III (Table 19).

Nitrate concentrations in SAF reactor were 52.7 ± 22 mg/L in phase II and 77.9 ± 20 mg/L in phase III (Table 18). NOB population in this reactor was 3.5 x 10^7 MPN g/VS in phase II under a HRT of 26.6 h with 2 g COD/(L·d) and 4.3 x 10^9 MPN g/VS in phase III under HRT of 20 with 3 g COD/(L·d) (Table 19).

Rodrigues (2013) evidenced NOB population in the submerged aerated filter of in-series anaerobic-aerobic-anoxic reactors system treating swine wastewater; assay 2, under a HRT of 6.6 h with OLR of 13.2 g COD/(L·d), had NOB population less than 10^6 and assay 3, under a HRT of 3.3 h with OLR of 14.3 g COD/(L·d), had a population less than 10^8. At an AOA-SBR system, treating municipal wastewater + sludge fermentation products, operated by Liu et al. (2017), it was found *Nitrosomonas* as the AOB dominant genera. In contrast, Zhang et al. (2016) identified *Nitrosomonas*, the most typical AOB, and *Nitrospira*, a common genus of NOB, in sludge samples of the A^2/O system (anaerobic-anoxic-oxic) treating domestic wastewater.

Table 18 Average values concentration and variation coefficient (v.c.) of pH, dissolved oxygen concentration (DOC), total nitrogen ammonia (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) of submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) during phase II and III.

Parameter	Reactor	Phase II	v.c.	Phase III	v.c.	Test F
pH	ABRc2	7.1b	2	7.0a	2	24.92**
	SAF	7.5	3	8.0	2	0.58ns
	USB_{nox}	7.5	3	7.5	3	0.92ns
DOC (mg/L)	FAS	2.2	59	2.0	64	1.15ns
NH_4^+ (mg/L)	ABRc2	302.4b	59	108.5a	23	8.89**
	SAF	105.4	98	66.2	48	1.34ns
	USB_{nox}	103.9	74	75.3	44	0.72ns
NO_2^- (mg/L)	SAF	28.9	140	8.0	36	2.64ns
	USB_{nox}	6.0	167	7.8	47	0.30ns
NO_3^- (mg/L)	SAF	52.7a	41	77.9b	42	7.63*
	USB_{nox}	46.0	26	45.0	57	0.01ns

Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability (p<0.05); ** Significant with 1% probability (p<0.01); ns: Not significant.

Table 19 Most probable number (MPN) estimation of ammonium oxidizer, nitrite oxidizer, denitrifying and heterotrophic bacteria of submerged aerated filter (SAF) and upflow sludge blanket (USB_{nox}) from sludge samples at the final of phase II and III.

Phase	Reactor	Ammonium oxidizer bacteria	Nitrite oxidizer bacteria	Denitrifying bacteria	Heterotrophic bacteria
		MPN (g/VS)			
II	SAF	4.1×10^{13}	3.5×10^{7}	1.7×10^{5}	9.2×10^{14}
	USB_{nox}	2.8×10^{7}	6.2×10^{7}	4.1×10^{9}	3.2×10^{13}
III	SAF	7.2×10^{13}	4.3×10^{9}	2.6×10^{4}	2.4×10^{12}
	USB_{nox}	9.5×10^{8}	16×10^{7}	7×10^{9}	2.2×10^{11}

The last part of the post-treatment was performed in an USB_{nox} reactor. Nitrate removal efficiency was different between the two phases (Figure 21 and Table 18). In phase II nitrate removal has an average of 13 ± 20% and in phase III displayed a better mean removal efficiency, 43 ± 26%. Analyzing the SDNR, a mean value of 4.04 ± 11 mg NO_3^-/(gVSS·h) was observed in phase II, explaining a lower denitrifying activity. Otherwise, SNDR in phase III was 7.8 ± 9 mg NO_3^-/(gVSS·h), showing a nitrate elimination average of 36 mg NO_3^-. Smaller values were observed by Winkler et al. (2011) using an anaerobic-oxic-anoxic SBR treating screened and de-gritted raw wastewater, 0.47 and 0.80 mg NO_3^-/(gVSS·h) in reactors O and C, respectively.

Denitrifying microbiota in USB_{nox} reactor was 4.1×10^9 MPN g/VS in phase II under a HRT of 26.6 h with 2 g COD/(L·d) and 7×10^9 MPN g/VS in phase III under HRT of 20 with 3 g COD/(L·d) (Table 19). The anoxic up-flow sludge blanket reactor of in-series anaerobic-aerobic-anoxic reactors treating swine wastewater showed denitrifying bacteria in their six tests. Assay 3 under a HRT of 2.8 h with OLR of 12.4 g COD/(L·d) had a population greater than 10^{12}; assay 4 under HRT of 5.6 h with 9.7 g COD/(L·d) had a bacteria population higher than 7.8×10^{10} (Rodrigues, 2013).

GAOs are one of the microbial populations that are capable of denitrification. Different GAO subgroups, under anoxic conditions, could use their glycogen reserves for maintenance and survival but not for nitrate reduction. Consequently, excess endogenous consumption happens (Liu et al., 2013; Winkler et al., 2011; Zhao et al., 2018). Based on this interpretation, phases II and III exhibit this microbial behavior, supporting the high consumption of VSS (Table 16) and the non-completely nitrate uptake into USB_{nox} reactor (Table 18).

Another reason to experiment a non-completely nitrate consumption is the effluent recirculation, which increase the inlet nitrate concentration in the system. Wu et al. (2013) increased the recirculation rate and decreased HRT, triggering a lower nitrate removal in CAMBR system.

The results observed in this study show that nitrifying and denitrifying activity occurred during the SAF and USB_{nox} reactor phases, confirmed by the marked reduction in TKN, NH_4^+ and alkalinity values in the submerged aerated filter due to the conversion of NH_4^+.

4.2.4 Phosphorus

The averages of the total phosphorus concentration in the influent ranged from 148.3 to 219.7 mg/L and in the effluents from compartments 1 and 2 of ABR reactor, the concentrations decreased to 84.2 to 129.2 mg/L and 66.4 to 133.6 mg/L, respectively (Table 20). TP concentration of influents and ABR's effluents demonstrated statistical significance ($p<0.05$ and $p<0.01$) when were compared amoung all phases. TP removal showed statistical significance ($p<0.01$) when were compared amoung ABR of phase I, II and III; system removal (specifically SAF removal) were statistical significance ($p<0.05$). Total phosphorus removal was notable in Phase I. ABRc1 became the chamber which removes more phosphorus, 45%. ABRc2 brings to the anaerobic treatment, in this phase, a global removal efficiency of 56% with a HRT of 93.7 h (Table 20). A different result is observed in the treatment of CPW in a two-stage UASB during Phase 1. That phase without recirculation, OLR of 6.1 g COD/(L·d) and HRT of 60 h achieved 91.1% P removal in reactor 1 and 41.3% P removal in reactor 2 (Botello Suárez et al., 2018).

Better removal efficiency could be achieved due to the different composition of wastewater used CPW is rich in organic matter, mainly by easily fermentable sugars (Botello Suárez et al., 2018). In contrast, cattle manure is rich in biodegradable (high fiber content such as lignin) and non-biodegradable organic matter (Appuhamy et al., 2018), in which, probably, microbial degradation time must be longer.

Table 20 Average values and variation coefficient (v.c.) of total phosphorus (TP) and removal efficiencies (%R) of influent, two compartmets anaerobic baffled reactor (ABRc1-ABRc2), submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents in phase I, II and III. Average values of organic loading rate (OLR) and dissolved oxygen concentration (DOC) were included in table.

Parameter	Reactor	Phase I	v.c.	Phase II	v.c.	Phase III	v.c.	Test F
OLR (g CODtot/(L·d))	ABRc1	6a	57	7a	50	9b	38	3.96*
	ABRc2	2a	66	4ab	59	7b	37	29.21**
	SAF	-	-	2a	69	4b	37	4.95*
	USB_{nox}	-	-	2	75	3	68	3.32ns
DOC (mg/L)	SAF	-	-	2.3	56	2.0	64	1.15ns
TP (mg/L)	Influent	152.7a	58	219.7a	34	148.3a	17	3.81*
	ABRc1	84.2a	25	111.4ab	17	129.2b	26	7.69**
	ABRc2	66.4a	47	87.9a	28	133.6b	29	13.42**
	SAF	-	-	87.9	32	69.0	43	2.64ns
	USB_{nox}	-	-	63.1	16	65.8	35	0.07ns
%R	ABRc1	44.9ab	-	49.3b	-	12.8a	-	4.20*
	ABRc2	21.1a	-	21.1a	-	nr a	-	1.46ns
	ABR	56.5a	-	60.0b	-	9.9a	-	10.95**
	SAF	-	-	nr	-	48.4	-	19.25**
	USB_{nox}	-	-	28.2	-	4.6	-	1.02ns
	System	-	-	71.3b	-	55.6a	-	4.93*

nr: Negative result; -: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *Significant with 5% probability ($p<0.05$); ** Significant with 1% probability ($p<0.01$); ns: Not significant.

Phase II showed a higher influent P concentration due to less HRT and recirculation applied (4:1). However, this phase achieved a better phosphorus removal in ABRc1 with 49%. ABRc2 brings to the anaerobic treatment global phosphorus removal efficiency of 60% when HRT was 75.0 h and air temperature was higher than other phases (Table 20). Anaerobic zones produce an excellent environment to P-release by the PAOs, the recirculation cause dilution by the recirculated stream and PAOs return. Similar behavior was observed in the synthetic municipal wastewater treatment in CAMBR. The system had a TP removal efficiency of 64% when working with HRT of 10 h, a 2 mg/L DO inlet and a recirculation of 100%. Recirculation increment to 300% improves the removal efficiency to 87%, obtaining a significant TP decrease, going from 1.4 mg/L to 0.5 mg/L. The author suggests that in a higher recirculation ratio, large amount of PAOs are recirculated to the anaerobic zone and participate in anaerobic phosphorus release and aerobic phosphorus uptake, promoting consumption and consequently TP decrease (Wu et al., 2013).

Phase III, with a ratio recirculation 4:1, demonstrate an insufficient phosphorus removal and a slightly accumulation in ABRc2 (Table 20). Analogous results are obtained in Singh et al. (2009) research who could not achieve a P removal in primary ABR treatment of high-strength wastewater. The raw wastewater had an average P concentration of 24.4 mg P/L and ABR effluent got 28.4 mg P/L. The authors mentioned that it could have happened because a lower HRT was applied (28.8 h). Wu et al. (2013) mentioned that a shorter HRT with recirculation resulted in insufficient phosphorus release because a nitrate recirculation to the anaerobic zone could have happened. In this case, most of the organic material was prioritized for denitrification, resulting in incomplete anaerobic phosphorus release and subsequent phosphorus uptake.

Botello Suárez et al. (2018) showed the reduction of phosphorus removal capability pattern during their experiment under different conditions. Phase 1 began with less ORL (6.1 g COD/(L·d)), HRT of 60 h, and non-recirculation; and the last phase, Phase 5, with high ORL (18.2 g COD/(L·d)), HRT of 30 h and recirculation of 3:1 (CPW:effluent) showed the reduction of phosphorus removal in the two-stage UASB from 91.1% to 54.7% only in reactor 1. In different systems, treating several wastewaters, the reduction of HRT and the application of higher OLR at the same time diminish the capability to remove phosphorus.

SAF-USB$_{nox}$ post-treatment worked well for the phosphorus removal. Phase III showed a better global performance than phase II, with 53% of phosphorus removal efficiency (Table 20). The aerobic environment, SAF reactor, stimulates the oxidation

of PHA, storaged in anaerobic conditions in PAOs as energy and carbon source to maintenance, growth, and glycogen storing (Luo et al., 2018; Winkler et al., 2011). In this research, phosphorus removal in phase II did not occur. Phase III demonstrated higher phosphorus consume, insinuating the high prevalence and activity of PAOs inside SAF reactor.

Inside USB_{nox}, different microbiota reside, which consumes phosphate and nitrate. An anoxic phosphorus uptake by DNPAOs could prevail, because under excess nitrate load, denitrifying potential of other heterotrophic cease and DNPAOs rise (Kim et al., 2013). On the other hand, GAOs are incapable to assimilate phosphate. Phase II exhibit a phosphorus removal only in the anoxic zone, implying that DNPAOs exist inside this reactor. DNPAOs utilize their internally stored PHA to uptake phosphate and use nitrate as an electron acceptor. A lesser population of denitrifying bacteria in USB_{nox} reactor could support this interpretation (4.1 x 10^9 MPN g/VS) (Table 19).

Therefore, the lower phosphorus consumption at USB_{nox} in Phase III could imply that few DNPAOs were present using nitrate in order to intake phosphate. At the same time, the prevalent population in the reactor could be GAOs (based in the interpretation of 4.2.3 section) or another general heterotrophic microbiota with denitrifying activity (2.2 x 10^{11} MPN g/VS) (Table 19). Jena et al. (2016) attained an 87% of phosphate uptake by DNPAOs in their initial anoxic conditions but when effluent pass to aerobic phase the removal efficiency decrease to 76%, indicating a phosphate release. This author identified PAOs (*Betaproteobacteria*, *Rhodocycus*, *Actinobactor*) and DNPAOs (*Azoarocous*, *Azonexus*, *Thaurea*) in anoxic-aerobic SBR system.

4.2.5 System performance

Anaerobic treatment in high-rate ABR (ABRc1 and ABRc2), had good removal efficiencies in all parameters analized, especially CODtot, which displayed the best removal efficiency if compared with phase II and III. In this study, it is remarkable that anaerobic removal efficiency decreased when HRT reduced (Nonexistent boxes – Figure 22) and aerobic-anoxic post-treatment (biological nutrient removal) constituted a sustainable technique to reduce pollulants.

In addition, phase II throughout the ABRc1-ABRc2-SAF-USBnox system, achieved a 83% of CODtot removal, an average total nitrogen concentration reduction to 136.9 ± 85 from 313.3 ± 124 g/L, and total phosphorus removal of 68%.

On the other hand, under less HRT of 91.3 h and high OLR of 23 g CODtot/(L·d), phase III showed a similar CODtot removal than phase II, 84% in 95 days of operation. This phase exhibited better total nitrogen removal where average concentration was from 427.2 ± 169 to 160.8 ± 95 g/L, and a notorious total phosphorus removal decrease, 56% (Table 20 and Figure 22). Despite of less nutrient removal, this phase reduced much of organic matter and TSS, showing a compact and robust system that can be used in dairy cattle wastewater treatment with lower HRT and high OLR.

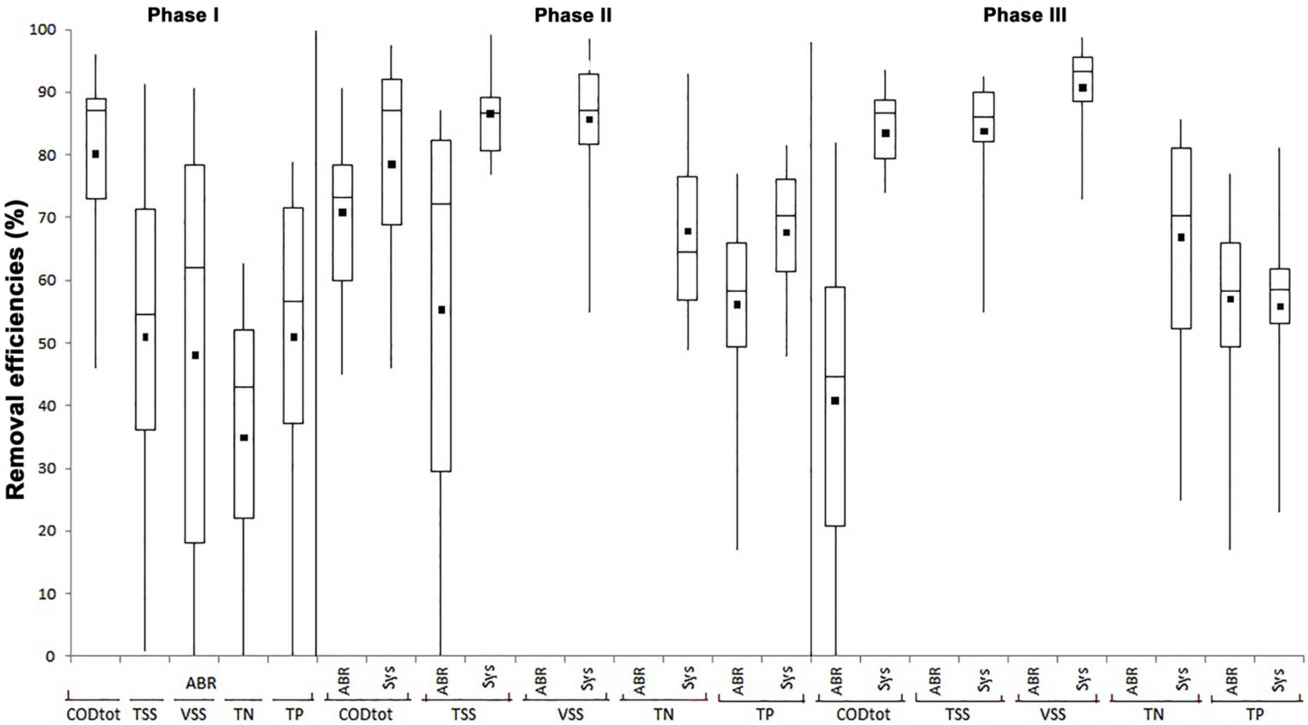


Figure 22 Box and whiskers plot showing the mean removal efficiencies of organic matter from dairy cattle wastewater by an in-series high-rate system comprised an anaerobic baffled reactor (ABR) with two chambers connected to a submerged aerated filter (SAF) and an anoxic upflow sludge blanket (USB_{nox}) reactor. Phase I relates to the efficiency of the ABR reactor alone, whereas phases II and III compare the efficiencies of the ABR and of the whole system (Sys = ABR + SAF + UASB). Abbreviations: CODtot, total chemical oxygen demand; TKN, total Kjeldahl nitrogen; TP, total phosphorus; TSS, total suspended solids; and VSS, volatile suspended solids. On each box, the horizontal cross line represents the median, the central square represents the mean, the lower and upper ends represent the 25th and 75th percentiles, respectively, and the whiskers represent the ranges for the lower and upper quartiles.

There was a notable utilization of TSS and VSS at the final effluent in phase II and III (Figure 22), especially due to the post-treatment, where aerobic conditions

(SAF) promote fast intake of sludge and an anoxic environment (USB_{nox}) where normal metabolic activity of denitrifying microbiota consume their own organic matter (Table 16).

4.3. Biogas production and composition

Figure 23 shows the biogas production by anaerobic treatment along all phases. Higher biogas production was at the beginning of phase II, correlated with the higher mean temperature registered (Table 11). In phase II and III, ABRc2 showed stable biogas production suggesting a steady microbiota and, at the same time, more biodegradable inlet substrate (Mazareli et al., 2016). ABRc1 in phase I and II displayed an important contribution for biogas concentration in each phase. On the other hand, ABRc1 in phase III did not show the same pattern, it might have resulted from low HRT and increase in average OLR to 9 CODtot/(L·d).

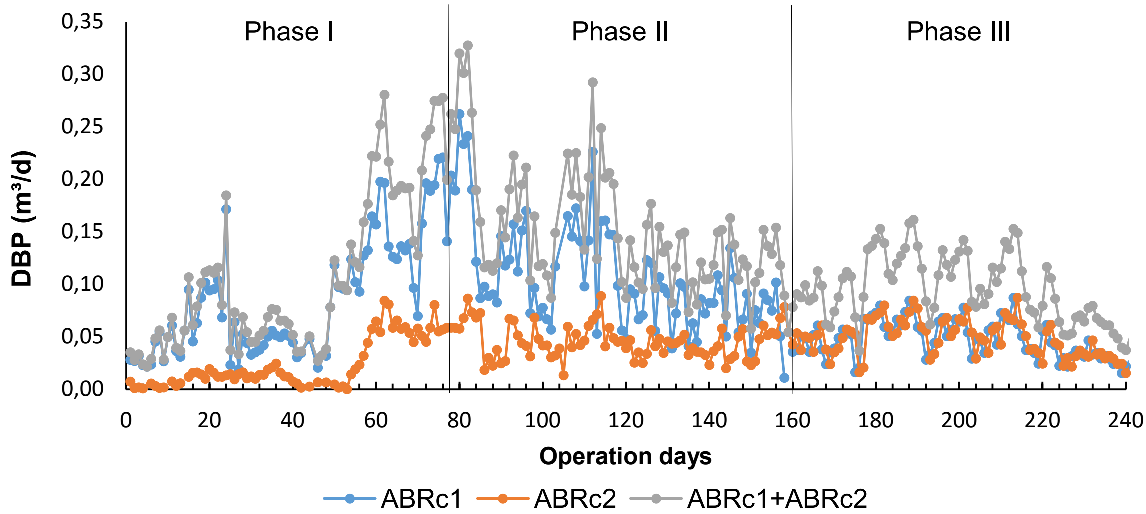


Figure 23 Average values of daily biogas production (DBP) of ABRc1 and ABRc2 reactors during phases I, II and III.

Table 21 shows mean values of methane percentage in biogas, volumetric and specific methane production in ABRc1 and ABRc2 during phaseI, II and III. ABRc1 in phase I showed a CH_4 percentage in biogas ranged between 66.1 – 75.4% and in ABRc2 between 63.3 - 73.3%. Phase II showed a CH_4 percentage in biogas of 66.1% and 63.3% in ABRc2. Nevertheless, CH_4 percentage in phase III ranged between 42.6 - 53.7% in ABRc1 and 19.6 and 55.9% in ABRc2. Phase III displayed the higher VFA/TA ratio, 0.30 in both ABRc1-ABRc2, and pointed out the decrease in methane production but not its total inhibition (Table 12).

Phase I showed similar CH_4 volumetric production than second phase; phase II shows higher CH_4 volumetric production (Figure 24) than phase III due to less HRT applied in the last phase. CH_4 volumetric production of ABRc1 and ABRc2 demonstrated statistical significance ($p<0.01$) amoung phases. In phase II, ABRc1 contributed with the greater volumetric production; in contrast, ABRc2 in phase III displayed the greater volumetric production evidencing the importance of this reactor to non-degraded organic matter (Table 21). Rodrigues (2013) treated swine wastewater in anaerobic-aerobic-anoxic system and used an ABR with two compartments (C1 and C2) to anaerobic treatment. Assay 4 under 13.9 g COD/(L·d) and HRT of 21.2 h displayed similar CH_4 volumetric production than this study, 0.265 and 0.270 in C1 and C2, respectively. Essay 6 demonstrated a better contribution of C2 than C1.

Table 21 Mean values and variation coefficients (v.c.) of methane percentage in biogas, volumetric and specific methane production in ABRc1 and ABRc2 during phase II and III.

Parameter	Reactor	Phase I	v.c.	Phase II	v.c.	Phase III	v.c.	Test F
OLR (g CODtot/(L·d))	ABRc1	5.9a	57	7ab	50	9b	38	3.96*
	ABRc2	1.7a	66	4a	59	7b	37	29.21**
CH_4 (%)	ABRc1	69.3	-	62.2	-	48.3	-	-
	ABRc2	66.6	-	68.3	-	38.9	-	-
CH_4 volumetric production (m^3 CH_4/(m^3reactor·d))	ABRc1	0.26b	55	0.26b	47	0.07a	40	78.27**
	ABRc2	0.13a	75	0.18b	36	0.11a	45	18.52**
CH_4 specific production* (m^3 CH_4/kg$COD_{tot\ added}$)	ABRc1	0.04b	16	0.03b	47	0.008a	37	60.26**
	ABRc2	0.68b	121	0.88b	110	0.14a	52	26.90**
CH_4 specific production* (m^3 CH_4/kg$COD_{tot\ removed}$)	ABRc1	0.08b	39	0.07b	149	0.02a	63	11.57**
	ABRc2	8.46	240	12.05	155	9.81	183	0.65ns

-: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *:Significant with 5% probability ($p<0.05$); **:Significant with 1% probability ($p<0.01$); ns: Not significant.

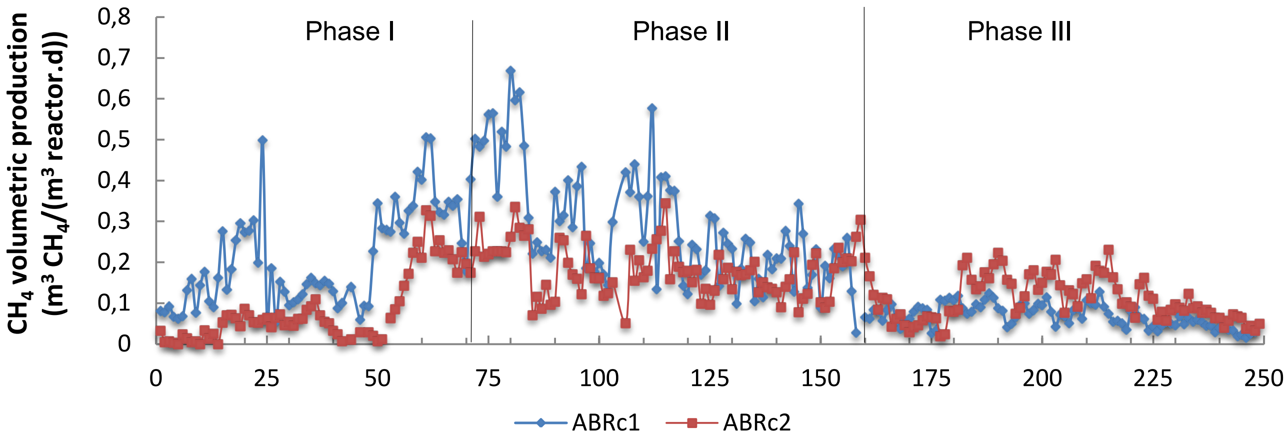


Figure 24 Values of methane volumetric production in ABRc1 and ABRc2 during phase I,II and III.

CH_4 specific production by kg COD_{tot} added is represented in Table 21, the results evidenced contribution of each anaerobic compartment of ABR and values were statistically significant ($p<0.01$) amoung phase I, II and III. Phase I demonstrated high specific production in ABRc1 when compared with other phases, specially in response of high HRT applied in this phase, this condition improved microbial contact with wastewater resulting in more CODtot consumption. In phase II, ABRc1 and ABRc2 had better contribution than phase III, this decrease could be attributed to less HRT applied in the last phase. Santos (2011) displayed less CH_4 specific production than this study. The author used an anaerobic-aerobic-anoxic system reactor to treat swine wastewater and divided the study in four tests. In the anaerobic part was employed an ABR with two chambers (C1 and C2). C1 under all tests achieved less CH_4 specific production than this study. C2 in test 3 under 48 OLR and test 4 under 120.4 OLR achieved similar CH_4 specific production than this study, stand out that phase II and III applied an OLR only of 11 and 16 in ABR, respectively.

Figure 24 and Table 21 show the CH_4 specific production by kg COD_{tot} removed in phase II and III. In this study, only ABRc2 in phase II and III could achieve higher methane production, evidencing the prevalence of methanogenic archaea in this reactor and indicating that OLR increase improved conversion of

CODtot removed in methane. On the other hand, values demonstrated a statistical significance ($p<0.01$) in CH_4 specific production of ABRc1.

Hahn e Figueroa (2015) treated raw municipal wastewater in a pilot scale ABR of 1000 L, applying a HRT of 12 h, average annual air temperature of 18 °C and average TSS and COD influent of 510 and 760 m/L, respectively. The four-cell ABR displayed a methane production of 0.24 L CH_4/g total COD removal, where each cell contributed at least 20-35% of total methane production.

Mazareli et al. (2016) treated swine wastewater and vegetable waste in and in-series high-rate HARFB with three reactors (R1, R2 and R3) evidenced in test 1 an increased methane specific production across all reactors. In test 1 treated only swine wastewater under a HRT of 312 h and an total OLR of 6.5 g CODtotal/ (L·d), R1, R2 and R3 displayed methane specific production of 0.15, 0.21 and 0.24 L CH_4/g COD_{total} removed, respectively.

4.3.1 Methanogenic archaea

MPN analysis identified acetotrophic and formatotrophic methanogens inside ABRc1 and ABRc2 reactors during phase II. MPN quantification (in MPN/gVS) showed a prevalence of acetotrophic methanogens in reactors, obtained a population estimate of 10^2, 10^6 and 10^3 in 1, 3 and 5 ABRc1's sampling sludge points and 10^2, 10^5 and 10^4 in 1, 3 and 5 ABRc2's sampling sludge point, respectively. Population estimate of formatotrophic methanogens were 10^2, 10^3 and 10^2 in 1, 3 and 5 ABRc1's sampling sludge points and 10^3, 10^4 and 10^2 in 1, 3 and 5 ABRc2's sampling sludge point, respectively.

4.4 Nutrients

Nutrient supplementation in dairy cow alimentation is necessary to avoid health problems, often reduction of milk production or reproductive efficiency reduction. Overfeeding minerals could result in greater feces excretion, urine, and consequently negative environmental effects. Several minerals have been identified as potential negative effects to crop yields and environment: Cd, Cu, Fe, Hg, P, K, Na, and Zn (Castillo et al., 2013). Formulate diet of milking sector of UNESP is represented in Table 2.

4.4.1 Secondary macronutrients - Potassium, Calcium and Magnesium

The average values and variation coefficient (v.c.) of K, Ca and Mg of influent and effluents are presented in table 22.

Meng et al. (2018) analyzed, during two years, nutrients in cattle manure which was applied directly in corn crop cultivation. This author obtained 14.8, 9.4, 4.1 g/kg of K, Ca and Mg, respectively. This study displayed high mean values of Mg, the concentration in phase I and II were higher than Meng et al. (2018) research. Phase II and III showed higher removal efficiencies system than phase I, 40.1% and 42.4%, respectively. Reduction of Mg concentration in effluents was significant ($p<0.01$) and SAF removal performance demonstrate a statistical significance ($p<0.01$) amoung all phases.

Rodrigues (2013) treated swine wastewater in an anaerobic-aerobic-anoxic system, applying an inlet K concentration of 89.3 mg/L under HRT of 16.7 h and an OLR of 99.4 Anaerobic treatment removed 15%; while all system achieved 79% of K removal. In this study, the inlet K concentration was lesser in all phases and the best system removal was evidenced in phase II with 40.6% with an ABR removal performance statistically significant ($p<0.05$).

Organic fertilizer Brazilian regulation (Normative Instruction No.25 of July 23rd, 2009) classified to effluent system reactor as Class B, due to the use of raw material from industrial or agroindustrial activities containing heavy metals, elements or organic synthetic compounds potentially toxics in the obtaining process. According this normative, effluent as fertirrigation must have secondary macronutrients (soluble in water) at least 300 mg/L of Ca and Mg. In this study, the values of Ca and Mg were lower (Table 22), so it could be applied as fertilizer without dilution or could be applied with more frequency until reach optimal crop concentration.

Table 22 Average values (mg/L) and variation coefficient (v.c.) of potassium (K), calcium (Ca) and manganese (Mg) of influent, two compartments anaerobic baffled reactor (ABRc1-ABRc2), submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents in phase I, II and III. Removal efficiencies (%R) of ABR and system (ABR-SAF-USB_{nox}) were included.

Parameter	Reactor	Phase I	v.c.	Phase II	v.c.	Phase III	v.c.	Test F
K	Influent	5.6a	81	10.9b	19	5.5a	17	9.43*
	ABRc1	5.2a	68	8.4b	19	4.5a	19	7.01**
	ABRc2	4.7a	66	8.0b	20	5.5a	15	6.73**
	SAF	-	-	8.1a	23	3.8b	42	29.00**
	USB_{nox}	-	-	6.4a	25	3.7b	39	16.34**
%R	ABRc1	11.8	124	23.1	48	14.9	85	1.30ns
	ABRc2	16.9	105	4.8	156	nr	-	3.25ns
	ABR	18.2ab	84	21.8	58	nr	-	8.61**
	SAF	-	-	nr	-	35.0	56	1.71ns
	USB_{nox}	-	-	24.5	79	16.7	108	0.01ns
	System	-	-	40.6	43	38.7	55	0.10ns
Ca	Influent	8.1	90	10.7	31	5.0	19	3.09ns
	ABRc1	4.2	50	5.3	27	3.6	27	2.79ns
	ABRc2	4.8ab	58	6.1b	21	4.0a	21	3.77*
	SAF	-	-	4.0a	36	2.5b	32	9.21**
	USB_{nox}	-	-	3.0	37	2.3	19	3.84ns
%R	ABRc1	37.2ab	74	46.7b	36	26.9a	57	3.51*
	ABRc2	nr	-	nr	-	nr	-	1.14ns
	ABR	35.1	87	39.1	37	21.9	70	2.77ns
	SAF	-	-	33.8	58	34.6	62	0.08ns
	USB_{nox}	-	-	24.2	91	13.8	152	2.25ns
	System	-	-	66.8b	23	53.9a	17	6.75*
Mg	Influent	6.5a	121	7.4b	63	2.8a	21	8.01**
	ABRc1	2.0a	44	2.8b	36	2.1a	36	10.92**
	ABRc2	2.7a	122	3.8b	37	2.8a	35	11.65**
	SAF	-	-	2.7a	22	1.0b	36	60.94**
	USB_{nox}	-	-	2.0a	32	1.0b	25	90.40**
%R	ABRc1	25.2ab	75	30.7b	43	16.7a	93	3.63*
	ABRc2	nr	-	nr	-	nr	-	1.83ns
	ABR	25.9ab	102	26.6	55	nr	-	5.71**
	SAF	-	-	10.3a	127	35.8b	56	9.55**
	USB_{nox}	-	-	14.1	156	nr	-	0.07ns
	System	-	-	40.1	50	42.4	38	0.08ns

nr: Negative result; -: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *:Significant with 5% probability ($p<0.05$); **:Significant with 1% probability ($p<0.01$); ns: Not significant.

In general, an anaerobic-aerobic-anoxic system achieves better removal of K, Ca and Mg than only an anaerobic treatment. SAF performance was better in phase

II attributed by HRT of 121.6 h applied, evidencing less removal contribution in phase III with low HRT. In contrast, USBnox in phase III increased its removal performance due to the less contribution of SAF removal. In conclusion, dairy cattle wastewater with elevated concentration of K, Ca and Mg could be treated by an anaerobic-aerobic-anoxic system under HRT of 121.6 h and OLR of 15 gCODtot/(L·d) (phase II) achieving good secondary macronutrients removal.

4.4.2 Micronutrients

The average values and variation coefficient (v.c.) of Mn, Cu, Zn, Fe and Na of influent and effluents are presented in Table 20 and 21.

This study displayed high mean values of Na, especially associated with dairy cow feeding (Table 2). Na removal was evidence in Table 23, phase II achieved better removal than phase I considering the high inlet Na concentration, and phase III showed the best removal efficiency system (46%) and a statistical significance of $p<0.01$ inside SAF in the same phase. Meng et al. (2018) analyzed, during two years, nutrients in cattle manure which was applied directly in corn crop cultivation and obtained a concentration of 5 g/kg.

Micronutrients as Fe plays an important role to accelerate the anaerobic digestion specially increasing biogas production yield due to its correlation with the activation of methanogens and being enzymatic cofactor for several enzymes under anaerobic, aerobic or anoxic conditions (Yun et al., 2019). This study shows a large consumption of Fe in all phases, emphasizing the phase II where methane production was higher; in adition, Fe removal demonstrated statistical significance ($p<0.01$) between phase I and II.

In addition, Fe removal in SAF during phase II was larger than the other phases, achieving 66.6% of removal (Table 23). Mouchet (1992) mentioned that optimal DOC for Fe oxidizing bacteria is 0-1 mg. In this study, Fe removal was achieved despite of the DOC average concentration was few higher than the stipulated by Mouchet.

In section 4.5, it was mentioned that commercial feeds are often enriched with essential elements such as Cu, Mn or Zn; these HM have antimicrobial properties or are used as growth stimulants; normally are excreted by the animals. HM soil inputs to agricultural soils include sewage sludge, animal manures, atmospheric deposition and inorganic fertilizers (Nicholson et al., 1999).

Table 23 Average values (mg/L) and variation coefficient (v.c.) of iron (Fe) and sodium (Na) of influent, two chamber anaerobic baffled reactor (ABRc1-ABRc2), submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents in phase I, II and III. Removal efficiencies (%R) of ABR and system (ABR-SAF-USB_{nox}) were included.

Parameter	Reactor	Phase I	v.c.	Phase II	v.c.	Phase III	v.c.	Test F
Fe	Influent	34.6a	124	86.1b	60	86.1b	24	4.54*
	ABRc1	9.8a	54	25.5a	128	49.5b	23	10.37**
	ABRc2	7.1a	74	35.2b	78	59.8b	37	13.42
	SAF	-	-	9.8a	68	31.9b	52	12.68**
	USB_{nox}	-	-	6.1a	86	30.9b	44	29.49**
%R	ABRc1	45.1	92	71.4	42	39.2	36	2.68ns
	ABRc2	34.5b	89	nr a	-	nr a	-	5.65*
	ABR	61.6	52	48.0	71	32.0	63	2.20ns
	SAF	-	-	66.6	52	48.0	45	2.04ns
	USB_{nox}	-	-	48.8a	71	8.5b	181	10.92**
	System	-	-	87.0b	19	67.4a	18	7.95 **
Na	Influent	174.0b	45	202.5b	12	98.1a	7	23.72**
	ABRc1	114.3b	28	119.5b	16	59.4a	10	22.60**
	ABRc2	111.1b	29	125.1b	14	62.9a	10	23.75**
	SAF	-	-	133.4a	13	54b	14	139.97**
	USB_{nox}	-	-	128.9	14	54	16	33.1**
%R	ABRc1	37.4	39	41.0	12	38.4	13	0.32ns
	ABRc2	11.0	137	nr	-	nr	-	2.54ns
	ABR	38.9	50	38.5	25	36.8	9	0.07ns
	SAF	-	-	nr	-	14.9	63	11.97**
	USB_{nox}	-	-	7.1	178	nr	-	0.56ns
	System	-	-	37.8	23	45.9	15	4.38ns

nr: Negative result; -: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *:Significant with 5% probability ($p<0.05$); **:Significant with 1% probability ($p<0.01$); ns: Not significant.

Qian et al. (2018) monitored the accumulation of HM such as Cu, Zn, and Mn with land application of swine manure for four years, and estimate accumulation risk of this HM. The author applied SSM compost and LSM anaerobically digested as a fertilizer in wheat crops and paddy, respectively. Consequently, increase concentration of HM was evidenced from January-2014 to November-2017;

predictions to exceed HM China standards to Cu and Zn were 15 and 10 years, respectively.

As the swine manure, application of dairy cattle manure in agricultural soil could be dangerous and to protect farmland, different alternatives should be studied. This research showed a good strategy to reduce the concentration of HM of dairy cattle manure applying an anaerobic-aerobic-anoxic treatment. Removal efficiencies of Cu, Zn and Mn, and wastewater initial concentration (manure : water) are represented in table 20.

Santos (2011) treated swine wastewater in an anaerobic-aerobic-anoxic system (two-chamber ABR-SAF-anoxicUSB), divided the research in four tests where the test 3 had an OLR of 58 g CODtot/(L·d) under HRT 40.9 h. Swine wastewater in this test obtained concentration of 1.9, 2.7 and 10.8 mg/L of Cu, Mn and Zn, respectively. The greater removals of HM were performed in ABR and aerobic-anoxic treatment contributed with huge portion of removal to achieve 99, 74 and 64 % Cu, Mn and Zn removal, respectively.

Organic fertilizer Brazilian regulation (Normative Instruction No.25 of July 23rd, 2009) mentioned that effluent system reactor could be used in fertirrigation with, at least (soluble in water), 20 mg/L of Fe, 200 mg/L of Cu and Mn, and 500 mg/L of Zn. In this study, the values of these micronutrients were lower (Table 20), so it could be applied as fertilizer without dilution or could be applied with more frequency until reach optimal crop concentration.

Phase II displayed the highest HM concentration and the best removal efficiencies, 91.0, 87.4 and 60.2 to Mn, Cu, Zn, respectively. The aerobic and anoxic treatment of this study was fundamental to improve the anaerobic removal, concluding that OLR of 15 g CODtot/(L·d) and HRT of 121.6 h were the best conditions to obtain a good treatment of dairy cattle wastewater in an ABR-SAF-USB_{nox} system.

Table 24 Average values (mg/L) and variation coefficient (v.c.) of manganese (Mn), copper (Cu) and zinc (Zn) of influent, two chamber anaerobic baffled reactor (ABRc1-ABRc2), submerged aerated filter (SAF) and anoxic upflow sludge blanket (USB_{nox}) effluents in phase I, II and III. Removal efficiencies (%R) of ABR and system ($ABR\text{-}SAF\text{-}USB_{nox}$) were included.

Parameter	Reactor	Phase I	v.c.	Phase II	v.c.	Phase III	v.c.	Test F
Mn	Influent	6.5	122	7.4	63	2.8	21	1.80ns
	ABRc1	2.0	44	2.8	36	2.1	36	2.46ns
	ABRc2	2.7	122	3.8	37	2.8	35	0.89ns
	SAF	-	-	1.5	86	0.9	100	1.15ns
	USB_{nox}	-	-	0.5	42	0.8	48	3.95ns
%R	ABRc1	45.5	58	40.0	65	15.1	150	1.52ns
	ABRc2	nr	-	nr	-	nr	-	3.47ns
	ABR	45.5	58	40.0	65	15.1	150	1.52ns
	SAF	-	-	62.6	45	67.5	34	0.14ns
	USB_{nox}	-	-	57.0a	47	17.8b	145	9.87**
	System	-	-	91.0b	6	74.a0	17	12.52**
Cu	Influent	5.1	158	2.2	50	1.7	101	1.27ns
	ABRc1	0,6	45	0.8	36	0.6	53	1.74ns
	ABRc2	1.7	205	1.4	41	0.9	49	0.38ns
	SAF	-	-	0.5	83	0.3	67	3.34ns
	USB_{nox}	-	-	0.2	56	0.2	48	2.35ns
%R	ABRc1	58.2	61	39.4	63	31.6	115	2.04ns
	ABRc2	nr	-	nr	-	nr	-	3.72ns
	ABR	58.2	61	39.4	63	31.6	115	2.94ns
	SAF	-	-	62.0	46	68.2	31	0.27ns
	USB_{nox}	-	-	45.4	63	33.0	87	0.80ns
	System	-	-	87.4	8	85.9	9	0.14ns
Zn	Influent	40.3	75	26.5	66	20.0	115	1.62ns
	ABRc1	5.8a	67	18.2b	76	7.6a	76	5.17*
	ABRc2	16.4	144	14.8	75	10.6	78	0.36ns
	SAF	-	-	13.6	66	11.0	164	0.14ns
	USB_{nox}	-	-	12.5	91	9.2	75	0.61ns
%R	ABRc1	72.9	40	36.9	103	53.2	65	2.18ns
	ABRc2	nr	-	21.4	149	nr	-	0.10ns
	ABR	56.9	69	41.5	65	35.6	98	1.91ns
	SAF	-	-	29.6	85	53.1	72	2.04ns
	USB_{nox}	-	-	21.7	186	19.8	175	1.49ns
	System	-	-	60.2	50	39.8	99	0.10ns

nr: Negative result; -: Not determined; Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *:Significant with 5% probability ($p<0.05$); **:Significant with 1% probability ($p<0.01$); ns: Not significant.

4.5 Total and volatile solids of sludge

The average values of TS and VS solids of sludge, VS/TS relation of each sludge sampling point in each reactor under different phases are represented in Table 25, 26, 27 and 28.

Table 25 Average values and variation coefficient (v.c.) of total solids (TS) (g/L) and volatile solids (VS) (g/L) of the first compartment of ABR reactor (ABRc1) during phases I, II and III. SV/ST relation also is represented.

Sampling point	Parameter	Phase I	v.c.	Phase II	Phase III	v.c.	Test F
0	TS	65.47	50	56.19	41.37	2	1.18ns
	VS	39.65	47	39.66	29.80	13	0.65ns
	VS/TS	0.63	15	0.71	0.72	12	0.98ns
1	TS	28.46	107	35.57	49,94	4	1.08ns
	VS	21.16	95	26.76	40.93	5	2.14ns
	VS/TS	0.76	28	0.75	0.82	4	0.24ns
2	TS	35.01	38	64.66	39.71	15	3.71ns
	VS	25.13a	33	52.49b	32.71a	13	7.19*
	VS/TS	0.73a	4	0.81b	0.83b	2	16.07**
3	TS	22.64	64	18.96	40.14	17	3.09ns
	VS	16.64a	62	13.86a	34.24a	11	6.56*
	VS/TS	0.74	2	0.73	0.90	7	5.73ns
4	TS	5.11a	22	16.89b	34.38c	10	95.41**
	VS	3.35a	27	11.08b	29.95c	2	1200**
	VS/TS	0.65a	7	0.66a	0.88b	9	10.76*
5	TS	4.46a	25	21.00b	35.64c	10	113.7**
	VS	3.37a	43	13.59b	32.41c	4	386.1
	VS/TS	0.74	29	0.65	0.92	13	1.59ns

Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *: Significant with 5% probability ($p<0.05$); **: Significant with 1% probability ($p<0.01$); ns: Not significant.

In ABRc1, TS decreased from bottom to the top in phase I and II. In contrast, phase III evidenced an irregular reduction in TS in response to low HRT applied. High VS concentration was evidenced in all phases, especially into button sludge sampling point. Phase II did not show decrease in VS despite of decrease HRT; in this phase, sludge sampling point number 3 suffered an abrupt increase but it decreased in upper points. On the other hand, VS in phase III had a decrease in bottom point but high concentrations in the rest of reactor interpreted as accumulation in biodegradable organic matter.

Table 26 Average values and variation coefficient (v.c.) of total solids (TS) (g/L) and volatile solids (VS) (g/L) of the second compartment of ABR reactor (ABRc2) during phases I, II and III. SV/ST relation also is represented.

Sampling point	Parameter	Phase I	v.c.	Phase II	Phase III	v.c.	Test F
0	TS	19.73a	58	23.21a	52.85b	13	14.27**
	VS	13.01a	53	15.56a	41.84b	13	26.54**
	VS/TS	0.68a	8	0.67a	0.79b	3	10.09**
1	TS	30.62	50	53.00	53.23	23	2.63ns
	VS	20.16	51	53.73	41.22	22	4.36ns
	VS/TS	0.66b	3	0.58a	0.78c	11	110.3**
2	TS	18.48a	48	56.74c	42.21b	3	33.54**
	VS	11.96a	45	59.07b	32.92b	2	39.47**
	VS/TS	0.66a	5	0.65a	0.78b	2	29.19**
3	TS	8.14a	70	52.90b	42.32b	4	69.16**
	VS	5.40a	81	35.81b	33.62c	4	79.19**
	VS/TS	0.63a	12	0.68ab	0.79b	2	10.77*
4	TS	2.68a	86	50.40b	34.86b	7	174.6**
	VS	1.49a	87	34.69b	29.51c	10	108.8**
	VS/TS	0.53a	9	0.69b	0.85c	4	57.28**
5	TS	3.77a	18	45.62b	29.93b	21	30.04**
	VS	2.27a	16	33.49a	24.27b	19	32.13**
	VS/TS	0.60a	8	0.73b	0.81b	2	36.46**

Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *: Significant with 5% probability ($p<0.05$); **: Significant with 1% probability ($p<0.01$); ns: Not significant.

TS and VS during phase I and III in ABRc2 decreased from bottom to the top as a result of the stratification provided by the mixture from the upflow. In contrast, higher concentration were observed in phase III due to the long working period (256 d) in this study, and insinuating also sludge drug for low HRT applied in this phase from ABRc1 to ABRc2.

In phase II, SAF reactor also showed sludge reduction from the bottom to the top, zero sampling point showed elevated sludge but immediately was reduced in upper zones (Table 27). In this study, aerobic reactor reduced more sludge than anaerobic ABR reactors due to the normal developing of nitrifying biomass which is slower than other microorganism. In addition, Pang et al. (2018) declared that micro-aerobic environment was favorable to sludge reduction than anaerobic and aerobic conditions. Niu et al. (2016) used a sludge process reduction activated sludge system and found that under a DO level of 0.5 mg/L the sludge reduction efficiency increased 25.4% than a DO of 2.5 mg/L. In this study, it was applied an intermittent aeration (Table 4), achieving, in different periods of time, micro-aerobic environment until aeration was applied, this could be a reason to obtain a notable sludge reduction. On the other hand, phase III showed slight sludge reduction possibly by low HRT applied in this phase.

Table 27 Average values and variation coefficient (v.c.) of total solids (TS) (g/L) and volatile solids (VS) (g/L) of Submerged Aerated Filter (SAF) during phases I, II and III. SV/ST relation also is represented.

Sampling point	Parameter	Phase II	Phase III	v.c.	Test F
0	TS	55.41	38.30	12	3.60ns
	VS	38.20a	31.53b	6	52.30**
	VS/TS	0.69	0.83	15	1.05ns
1	TS	17.65a	29.98b	6	83.60**
	VS	11.63a	21.37b	11	35.98**
	VS/TS	0.66	0.72	16	0.21ns
2	TS	13.78a	26.74b	6	102.8**
	VS	8.30a	19.51b	2	927.6**
	VS/TS	0.60a	0.73b	4	13.80*
3	TS	9.60a	28.47b	3	552**
	VS	5.94a	21.90b	1	30.02**
	VS/TS	0.62a	0.77b	3	34.26**
4	TS	15.04	15.41	21	2.85ns
	VS	9.30	10.86	17	6.30ns
	VS/TS	0.62	0.71	7	3.02ns
5	TS	3.16a	16.44b	9	81.65**
	VS	2.24	8.94	39	4.19ns
	VS/TS	0.71	0.55	43	0.35ns

Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *: Significant with 5% probability ($p<0.05$); **: Significant with 1% probability ($p<0.01$); ns: Not significant.

Inside USB_{nox} reactor, phase II showed more TS and VS concentration than phase III (Table 28). This fact could be in response of low HRT applied in the last phase provoked high fluidity of wastewater, less sedimentation in USB_{nox}, and sludge drug evidenced in parameters as COD and SS which demonstrated high concentration in effluent samples. On the other hand, similar than other reactors, TS and VS decreased from bottom to the top.

Table 28 Average values and variation coefficient (v.c.) of total solids (TS) (g/L) and volatile solids (VS) (g/L) of Anoxic Upflow Sludge Blanket (USB_{nox}) during phases I, II and III. SV/ST relation also is represented.

Sampling point	Parameter	Phase II	Phase III	v.c.	Test F
	TS	88.39	41.41	11	2.43ns
0	VS	49.64	32.47	8	4.03ns
	VS/TS	0.56a	0.79b	4	37.26**
	TS	22.60a	39.13b	9	42.03**
1	VS	12.67a	31.35b	11	29.49**
	VS/TS	0.56a	0.8b	6	19.19*
	TS	82.47b	25.67a	22	23.23*
2	VS	56.07	19.06	15	5.29ns
	VS/TS	0.68	0.75	7	1.52ns
	TS	53.88b	19.72a	10	67.59**
3	VS	37.07	15.79	10	0.37ns
	VS/TS	0.69	0.80	5	5.32ns
	TS	63.70b	18.44a	11	131.1**
4	VS	44.23	15.54	13	2.72ns
	VS/TS	0.69	0.84	7	5.93ns

Different lowercase letters in the same row are differently significant for Tukey's test at 5%; *: Significant with 5% probability ($p<0.05$); **: Significant with 1% probability ($p<0.01$); ns: Not significant.

In general, municipal sludge or derived products used in agriculture must show a SV/ST relation lesser than 0.70 to be consider stable (CONAMA n^{o} 375, 2006). Anaerobic reactors (ABRc1 and ABRc2) evidenced stability in phase I. Phase II evidenced sludge stability compared with phase III in all reactors. In phase II, ABRc1 was stable in 4 and 5 sludge sampling point, ABRc2 was stable in all points except the last one, in SAF reactor occurred the same of ABRc2, and USB_{nox} showed stability in all sludge sampling points. On the other hand, the application of HRT of 91.3 h in phase III provoked that sludge of all reactors did not achieve the stable SV/ST relation that Brazilian normative 375 established.

4.6 Total and thermotolerant coliforms

Table 29 represented the results of the estimation of total and thermotolerant coliforms in influent and effluents of each compartment of ABR, SAF and USBnox reactors in phase II. The influent during this phase showed similar MPN of coliforms than Rodrigues (2013) which treated swine wastewater in a same system of reactors. This study demonstrated high microbial population in ABRc2 but the post-treatment removed between 92.2% and 99.9%.

Table 29 Values of most probable number (MPN/100mL) of total coliforms and thermotolerant coliforms in influent and effluents of ABRc1, ABRc2 SAF and USB_{nox} in phase II. Removal efficiency (%R) also is represented.

		Total coliforms		Thermotolerant coliforms	
		MPN/100mL	%R	MPN/100mL	%R
Phase II	Influent	7.8×10^9	-	3.9×10^9	-
	ABRc1	1.3×10^8	98.3%	9.1×10^7	97.6%
	ABRc2	8.1×10^{13}	-	3.8×10^{10}	-
	SAF	2.2×10^{10}	99.9%	4.1×10^7	99.9%
	USB_{nox}	1.7×10^9	92.2%	1.3×10^6	96.8%
	System	-	3.5%	-	99.9%

-:Negative result

V CONCLUSIONS

In general, the anaerobic-aerobic-anoxic system formed by an anaerobic baffled reactor with two compartments (ABRc1 and ABRc2), a submerged aerated filter (packed with corrugated PVC rings) and an anoxic upflow sludge blanket (USB_{nox}) under different operational conditions, achieved high efficiencies removal of COD, suspended solids, total nitrogen, total phosphorus, micronutrients and heavy metals, treating dairy cattle wastewater.

In addition, the greater removal of different parameters was performed under high HRT, especially in the first chamber of ABR (ABRc1). When HRT decrease and OLR increase, the incorporation of submerged aerated filter (SAF) and anoxic reactor installed in-series promoted the removal complementation of COD, suspended solids, nitrogen and phosphorus.

In this study, the specific results were:

The optimal HRT only for high-rate ABR (ABRc1-ABRc2) treatment was of 93.7 hours, where highest COD, N and P removal were achieved; 72, 42 and 56 %, respectively. Less OLR applied and a high HRT provided enough microbiota and a longer time to microorganism oxidize the substrate.

A remarkable mineralization of organic nitrogen in ABRc1 and ABRc2 was present in phase I and II under HRT of 121.6 and 91.3, respectively. This fact demonstrated that ABR should not consider a settler, because under different conditions and wastewater (in this case dairy cattle wastewater) microbial ammonification achieved high levels.

Aerobic-anoxic post-treatment were tied for complementary removal of COD, N and P removal. Whereas for anaerobic-aerobic-anoxic as a complete system, the optimal global HRT for removal of nutrients and organic matter was 121.6 h at OLR of 15 g COD_{total}/L obtaining an average removal of 83, 68 and 68 % to COD, N and P, respectively.

There was a notable utilization of TSS and VSS in post-treatment during phase II and III, where aerobic conditions (SAF) promote fast intake of sludge and an anoxic environment (USBnox), where normal metabolic activity of denitrifying microbiota consume their own organic matter.

Intermittent aeration (13.5 h) applied in SAF reactor does not interrupts nitrification rates neither phosphorus removal, when global HRT was 121.6 h and OLR of 15 g COD_{total}/ (L·d). On the other hand, when HRT decrease to 91.3 and OLR increased to 23 g COD_{total}/ (L·d) (phase III), nitrification and phosphorus removal were affected.

The anaerobic-aerobic-anoxic system under an OLR of 15 g CODtot/(L·d) and HRT of 121.6 h achieved a better removal of micronutrients (Fe, Na, K, Ca and Mg) and HM (Cu, Zn, Mn) than only an anaerobic treatment. Micronutrient removal in SAF under this HRT and OLR was better than anoxic reactor. In contrast, when HRT decrease and OLR increase (phase III) USB_{nox} increased its removal performance. On the other hand, HM removal under an OLR of 15 g CODtot/(L·d) and HRT of 121.6 h (phase II) were performed by SAF and anoxic reactor; but when conditions changed (phase III) the greater HM removal was performed only by SAF reactor.

The anaerobic-aerobic-anoxic system showed a versatile strategy to treat dairy cattle wastewater with high organic matter, nitrogen, phosphorus and heavy metals concentration, achieving high removal efficiencies.

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ANEXO A- Alexander's table to most probable number of five tubes of dilutions (Alexander, 1984)

		Número mais provável para a indicação do p_3.					
p_1	p_2	0	1	2	3	4	5
0	0	–	0.018	0.036	0.054	0.072	0.090
0	1	0.018	0.036	0.055	0.073	0.091	0.11
0	2	0.037	0.055	0.074	0.092	0.11	0.13
0	3	0.056	0.074	0.093	0.11	0.13	0.15
0	4	0.075	0.094	0.11	0.13	0.15	0.17
0	5	0.094	0.11	0.13	0.15	0.17	0.19
1	0	0.020	0.040	0.060	0.080	0.10	0.12
1	1	0.040	0.061	0.081	0.10	0.12	0.14
1	2	0.061	0.082	0.10	0.12	0.15	0.17
1	3	0.083	0.10	0.13	0.15	0.17	0.19
1	4	0.11	0.13	0.15	0.17	0.19	0.22
1	5	0.13	0.15	0.17	0.19	0.22	0.24
2	0	0.045	0.068	0.091	0.12	0.14	0.16
2	1	0.068	0.092	0.12	0.14	0.17	0.19
2	2	0.093	0.12	0.14	0.17	0.19	0.22
2	3	0.12	0.14	0.17	0.20	0.22	0.25
2	4	0.15	0.17	0.20	0.23	0.25	0.28
2	5	0.17	0.20	0.23	0.26	0.29	0.32
3	0	0.078	0.11	0.13	0.16	0.20	0.23
3	1	0.11	0.14	0.17	0.20	0.23	0.27
3	2	0.14	0.17	0.20	0.24	0.27	0.31
3	3	0.17	0.21	0.24	0.28	0.31	0.35
3	4	0.21	0.24	0.28	0.32	0.36	0.40
3	5	0.25	0.29	0.32	0.37	0.41	0.45
4	0	0.13	0.17	0.21	0.25	0.30	0.36
4	1	0.17	0.21	0.26	0.31	0.36	0.42
4	2	0.22	0.26	0.32	0.38	0.44	0.50
4	3	0.27	0.33	0.39	0.45	0.52	0.59
4	4	0.34	0.40	0.47	0.54	0.62	0.69
4	5	0.41	0.48	0.56	0.64	0.72	0.81
5	0	0.23	0.31	0.43	0.58	0.76	0.95
5	1	0.33	0.46	0.64	0.84	1.1	1.3
5	2	0.49	0.70	0.95	1.2	1.5	1.8
5	3	0.79	1.1	1.4	1.8	2.1	2.5
5	4	1.3	1.7	2.2	2.8	3.5	4.3
5	5	2.4	3.5	5.4	9.2	16	–