



“Gheorghe Asachi” Technical University of Iasi, Romania



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## ENHANCED REMOVAL OF ORGANIC MATTER AND NUTRIENTS BY SEQUENTIAL BATCH REACTORS

Jorge Alonso Cortes-Esquivel<sup>1</sup>, Germán Giacomán-Vallejos<sup>1\*</sup>,  
Roger Méndez-Novelo<sup>1</sup>, Carmen Ponce-Caballero<sup>1</sup>, Icela Dagmar Barceló-Quintal<sup>2</sup>,  
Gladys Vidal<sup>3</sup>, Pascale Champagne<sup>4</sup>

<sup>1</sup>Faculty of Engineering, Autonomous University of Yucatan, Mérida, Yucatan, Mexico

<sup>2</sup>Department of Basic Sciences, Autonomous Metropolitan University, Azcapotzalco Unit, Mexico

<sup>3</sup>Group of Engineering and Environmental Biotechnology, Faculty of Environmental Sciences and EULA-Chile Center,  
University of Concepcion - Chile

<sup>4</sup>Department of Civil Engineering and Department of Chemical Engineering, Queen's University, Kingston, ON, Canada

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### Abstract

The aim of this research was to improve the removal of organic matter and nutrients from wastewater from pig farms in a combined batch reactor performed as anaerobic-aerobic sequential. For this purpose, it was suggested to include recirculation cycles of wastewater in a sequentially pulsed manner in the anaerobic treatment followed by intermittent aeration in the aerobic-anoxic treatment. This novelty implemented in both sequential reactors was studied under pilot scale conditions. The process of pulsed intermittent recirculation allowed better contact between the microorganisms and organic matter, and intermittent aeration improved the removal of nutrients, primarily nitrogen (nitrification and denitrification), total phosphorous and organic matter. The best configuration tested for the combined system was the one consisting of pulsed intermittent recirculation with 1 hour of recirculation and 3 hours rest in the anaerobic step and 2 hours of aeration and 1 hour without aeration cycle in the aerobic step. The removals achieved were  $98 \pm 1\%$  of total organic matter,  $86 \pm 5\%$  of soluble organic matter,  $96 \pm 1\%$  of total phosphorous and  $55 \pm 18\%$  of total nitrogen. Hence, it was demonstrated that a combined system with pulsed intermittent recirculation in the anaerobic stage and intermittent aeration in the aerobic stage could enhance the overall treatment of swine wastewater.

*Key words:* intermittent aeration, pulsed recirculation, sequencing batch reactor, swine wastewater

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### 1. Introduction

Swine wastewater contains high concentrations of organic matter ( $>10000$  mg TCOD/L), nutrients ( $>1000$  mg/L in the form of nitrogen and phosphorous), pathogens ( $>1 \cdot 10^{+9}$  NMP), heavy metals (Cu  $>3$  mg/L, Zn  $>54$  mg/L) and suspended solids ( $>15000$  mg/L) (González, 2012), as well as compounds that are not readily biodegradable (long-chain fatty acids, C8 - C22) (Aragón, 2012). The direct discharge of this untreated wastewater to receiving

environments could have serious detrimental effects on the quality of soils, surface waters and groundwater. This problem is more significant in regions with karst soils (limestone with fractures and fissures, such as those found in the Yucatan Peninsula, Mexico) that typically have high permeability, rendering aquifers particularly vulnerable to contamination.

Different biological technologies have been applied worldwide to mitigate the effects of pollutants discharged from swine farms, including: two-stage

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\* Author to whom all correspondence should be addressed: e-mail: [giacomán@correo.uady.mx](mailto:giacomán@correo.uady.mx), [giacoviera@hotmail.com](mailto:giacoviera@hotmail.com); Phone: +52 999 9300550 ext. 1058; Fax: +52 999 9300559

upflow anaerobic reactors (UASB) followed by a sequential batch reactors (SRB) (Oliveira and Santana, 2011; Urbinati and Oliveira, 2014), submerged membrane bioreactors (MBR) (Gupta et al., 2008), anaerobic upflow bed filters (AUBF) (Shin et al., 2005), anaerobic sequential batch reactors (ASBRs) (Massé et al., 2003; Ndegwa et al., 2008), and constructed wetlands (Cortes et al., 2012). However, most studies have been conducted at the laboratory scale, where a higher control of operating parameters is possible. In recent years, a number of factors that could affect the performance of ASBRs have been investigated including; mixing conditions (Farias de Novaes et al., 2010), feeding strategies (length of time) during the reactor filling phase (Cheong and Hansen, 2008), temperature (Massé and Mase, 2001; Massé et al., 2003; Ndegwa et al., 2008), Organic Low Rates (OLRs) (Cheong and Hansen, 2008; Ndon and Dague, 1997), relation of the concentrations of substrate and biomass and geometric characteristics of the reactor (Cheong and Hansen, 2008). However, to date, the best performances reported using ASBRs have failed to lower swine wastewater constituent concentrations (organic matter < 600 mg COD/L and nutrients TN and TP < 100 mg/L) to meet allowable wastewater effluent discharge standards set by the Official Mexican Standard for wastewater (NOM-001-SEMARNAT-1996, 2003; González, 2012). Therefore, the implementation of a post-treatment step to improve effluent quality is required.

According to the literature, anaerobic-aerobic systems have been successfully employed for the treatment of different types of wastewaters (Bernet et al., 2000; Deng et al., 2007; Novak et al., 2011; Saucedo-Terán et al., 2017; Yang et al., 2016; Zonoozi et al., 2018), anaerobic/anoxic/oxic systems have been applied for nutrient removal from municipal wastewater (Leyva-Díaz et al., 2016; Wang et al., 2018; Zhang et al., 2016) and integrated anaerobic/aerobic sequencing batch reactors to treat poultry slaughterhouse wastewater (Rajab et al., 2017). Working with a conventional combined treatment system (anaerobic-aerobic) for the treatment of domestic wastewater, Novak et al. (2011) reported better solids reduction, improved sludge dewatering properties and nitrogen reductions. Deng et al. (2007) evaluated the combination of an UASB reactor with a capacity of 6000 m<sup>3</sup> and a sequential batch reactor (SBR) consisting of 4 reactors with a capacity of 1880 m<sup>3</sup> to treat swine wastewater. They also tested the SBR alone for the treatment of the same wastewater. The integrated anaerobic/aerobic sequencing batch reactor (IAASBR) treating poultry slaughterhouse wastewater exhibited high organic and ammonia nitrogen removal efficiencies, with a relatively consistent performance and tolerance to shock loading.

In recent years, it has been demonstrated that the integration of aerobic treatment following anaerobic treatment allows for an increase in the biological removal of phosphorus (Robeiro da Silva et al., 2018; Kim and Pagilla, 2000; Lin et al., 2003). It has been considered that with this combined process

some bacterial strains, such as *Acinetobacter* and *Pseudomonas* referred to as phosphate accumulating organisms (POAs), are able to accumulate phosphate, facilitating the removal of phosphorus in activated sludge processes. Under aerobic conditions, phosphorous removal by the POAs is enhanced through the preferential degradation of volatile fatty acids (VFA) (as a carbon source) readily formed under anaerobic conditions. These are rapidly assimilated and accumulated within the cells as poly-β-hydroxyalkanoates (PHAs). To obtain the energy for PHA synthesis, the POAs degrade intracellular polyphosphate (POLYP) granulates to orthophosphate forms and release them to the bulk liquid (Mino et al., 1998). Under the subsequent aerobic conditions, the POAs form POLYP in excess of the concentrations normally required to satisfy the metabolic demand. The POAs must then use the stored PHAs as their carbon and energy sources (Mino et al., 1998). Finally, the phosphorus is incorporated in the PAO cells in large quantities and is removed from the system through the elimination of excess biological sludge (Kim and Pagilla, 2000; Lin et al., 2003).

The aim of this research was to assess a combination of biological processes to enhance the treatment of swine wastewater. A pulsed sequential intermittent recirculation of wastewater from the upper part to the bottom was used in the ASBR and sequential intermittent aeration in the SBR, after a short discharge - feeding process in each of the reactors on a daily basis. This combined system (ASBR-SBR) was selected because limiting nutrient, particularly nitrogen, can be expected under anaerobic conditions alone, since the removal of NH<sub>3</sub>-N is through nitrification involving the oxidation of ammonia to nitrite, nitrate; followed by denitrification where these products are reduced to gaseous nitrogen. As such, the ASBR was selected to reduce the high organic loading. The process aerobic-anoxic (realized in SBR) was subsequently selected to enhance the removal of ammonia nitrogen and phosphorous.

The addition of pulsed sequential intermittent recirculation in the ASBR stage allows for an increased biomass suspension and increased contact between biomass (POAs and other microorganisms), organic matter (VFA as carbon source for POAs) and nutrients. Hence, a higher assimilation and accumulation of PHA, as well as organic matter removal can be expected. Similarly, the operation of the SBR stage with intermittent aeration allows for a higher dissolved oxygen concentration, which leads to higher POLYPs formation in the biomass, and the oxidation of NH<sub>3</sub>-N to nitrate, while the intermittent periods without aeration permit denitrification (NO<sub>3</sub><sup>-</sup> to N<sub>2</sub>) and more effective biomass sedimentation.

## 2. Material and methods

The experimental unit was located at the agricultural research and production farm at the Technological Institute of Conkal, Yucatan, Mexico (N21°5'7.52", W89°32'17.99"). The animal

population during the one-year period of study ranged from 195 to 214 heads of boars, sows, gilts, barrows and piglets.

### 2.1. Swine manure characteristics and treated water quality analysis

The swine manure characterization was conducted using 18 samples, collected in 1 L plastic bottles. For treated water quality analysis, samples (2 L) were collected at the influent and effluent of the ASBR and at the effluent of the SBR for each experimental configuration (Table 1). Both type of samples were preserved with sulfuric acid (2 mL/L) and stored at a temperature of 4°C according to Standard Methods (APHA, 2005) and transported to the laboratory where they were immediately analyzed. The water quality parameters monitored during characterization and operation of all planned configurations for the system were analyzed according to the following analytical techniques: total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) were determined by closed reflux digestion using the HACH method 8000 (APHA, 2005; Jirka and Carter, 1975); total phosphorus (TP) was determined by the molybdovanadate method with persulfate acid digestion (HACH method 10127); total nitrogen (TN) was determined according to the persulfate digestion method (HACH method 10072); Cu and Zn using atomic absorption spectroscopy; total solids (TS), volatile total solids (VTS), total suspended solids (TSS), volatile total suspended solids (VTSS) and alkalinity were determined according to Standard Methods (APHA, 2005).

### 2.2. Experimental treatment systems

The treatment train consisted of mechanical separation of coarse solids through a drum screen (6.35 mm) and Imhoff-type settler (Fig. 1). The secondary biological treatment consisted of a combination of two sequential reactors in series, a pulsed intermittent recirculation into the anaerobic process (ASBR) followed by an intermittent aeration in the aerobic-anoxic (SBR) as shown in Fig. 1.

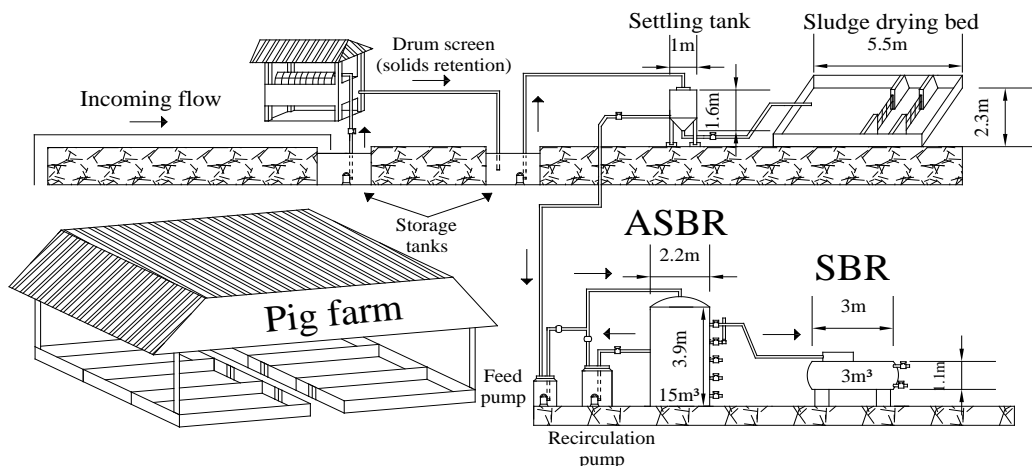


Fig. 1. Schematic from combined system ASBR-SBR and pretreatment

#### 2.2.1. Anaerobic sequencing reactor ASBR

The anaerobic reactor (ASBR) consisted of cylindrical high-density polyethylene reactor with a diameter of 2.2 m, height of 3.9 m and total volumetric capacity of 15,000 L. Initially, it was seeded with sludge obtained from settling tank sediments (swine farm) and ruminal liquid (cattle) collected from the slaughter house at the Faculty of Veterinary Medicine and Zootechnique at the Autonomous University of Yucatan (N20°51'57.91", W89°37'27.57").

The acclimatization period for the microorganisms in the ASBR was approximately of four months. During the acclimatization and the experimental periods, the ASBR was fed on a daily basis with 500 L of wastewater obtained from the settling tank located prior to the ASBR process (Fig. 1), for a period of approximately 2 minutes, using a submersible pump (Franklin 10S-CIM, 0.5 hp) at a flow rate of 320 L/min. The feed and recirculation pumps shown in Fig. 1, as well as the solenoid valves, which allowed for the inflow and outflow of water for all the ASBR-SBR system, were controlled by an automatic on-off programmable logic controller (PLC-Crouzet Millennium 3).

The experiments conducted in the ASBR employed a working volume of 13,750 L (allowing for a headspace of 1,250 L to contain the generated biogas). The feed volume per day for all experiments (Table 1) was of 500 L, providing a hydraulic retention time (HRT) 27.5 days for this reactor. At this HRT an average OLR of  $0.91 \pm 0.27$  kg COD  $m^{-3}d^{-1}$  was computed for the different experiments.

#### 2.2.2. Aerobic sequencing reactor SBR

The aerobic reactor (SBR) was constructed of high-density polyethylene with a capacity of 3,000 L and a working volume of 2,500 L. The dimensions of the SBR were as follow: 1.1 m diameter and 3 m length (Fig. 1). Initially, the reactor was inoculated with 400 L of activated sludge, obtained from a municipal sewage treatment plant (N20°59'45.72", W89°38'58.54"). The acclimatization period for the microorganism in the SBR was the same as that of the ASBR (four months).

During the acclimatization and experimental periods (Table 1) this reactor was fed with the effluent (500 L) from the ASBR. This feeding was performed by gravity through an on-off solenoid valve in a short time (5 minutes a day). The HRT in the SBR was 5 days. The intermittent aeration was provided using a blower (FPZ 10DL) with a maximum flow of 3.6 m<sup>3</sup>/h (60 Hz), which was controlled automatically by the on-off programmable logic controller (PLC-Crouzet Millennium 3). The air was conveyed through a distribution tube to a disc covered by a porous membrane in order to generate fine bubbles, which allowed for an increased surface area and, as such, adequate oxygen transfer to the system. The main function of the aerobic system was to reduce the nutrient (nitrogen and phosphorus) and organic loads from the anaerobic system.

### 2.3. Operating conditions of the combined ASBR-SBR system

The pulsed intermittent recirculation ASBR and intermittent aeration SBR was operated in a sequential manner. Four independent and unique configurations for each system were randomly combined (according to the established experimental plan presented in Table 1). However, these combinations were based in the condition that, whichever operational sequence, a quantity of wastewater that comes from the ASBR remained 24 h in order to allow for the maximum organic matter reduction. Subsequently, the effluent would flow into the SBR, where it was retained for 24 h, under one specified condition (with and without aeration cycle), in order to removal nutrients, especially to reach the maximum removal of ammonia nitrogen.

The operating conditions for each combined experimental configuration takes over an 8-day monitoring period. Once a programmed operational configuration was completed, the systems were allowed to stabilize for a 21-day period (González, 2012).

#### 2.3.1. Discharging and feeding of the pulsed intermittent recirculation ASBR and the intermittent aeration SBR systems

Reactor feeding and discharging was conducted over of a period of two hours on a daily basis, in the first one-hour period; the system (ASBR-SBR) was at rest to allow for sedimentation to take

place in each reactor. The subsequent one-hour period was dedicated to the discharging and feeding of the system, which was performed in three stages. In the first stage, the electro valve installed at the effluent of the SBR was opened and the SBR reactor was allowed to discharge 500 L into a receptor tank. This operation was carried out for approximately five minutes and the SBR effluent electro valve was then turned off. In the second stage (ten minutes later), the electro valve for the effluent of the ASBR was opened, discharging 500 L to feed the SBR reactor. This operation was allowed to proceed over a five-minute interval.

The electro valve was then closed, completing the discharging and feeding cycle of the SBR. Next, the third stage was initiated during which a ten-minute rest period was allowed prior to the feeding of the ASBR (500 L) (Fig. 1). This feeding was conducted over a period of approximately 2 minutes, using a submersible pump (Franklin 10S-CIM, 0.5 hp) at a flow rate of 320 L/min. After the feeding of the ASBR, the system was maintained at rest for the remainder of the 1-hour period (established for discharging and feeding). Finally, the process of recirculation in the ASBR and aeration in the SBR was initiated as detailed below.

#### 2.3.2. Operating conditions of the pulsed intermittent recirculation ASBR

For the first stage (ASBR), the recycle ratio was alternated sequentially providing 1 hour of recirculation followed by 1 hour without recirculation over a period of 22 hours for the first configuration tested (Table 1). During this recirculation time (1 hour), the flow was pulsed using a submersible recirculation pump (Franklin 9S-CIM, 0.4 hp) intermittently operated at a flow of 200 L/min at 3-minute intervals (3 minutes of recirculation and 3 minutes of rest). Hence, for each hour of recirculation, the pump was activated 10 times. To complete the 24 hours cycle, 2 hours per day were used to allow for sedimentation and for the discharging and feeding of ASBR-SBR systems as previously described. Under these operational conditions, the ASBR was subjected to 11 recirculation (1 hour) cycles daily (TWR) and 13 cycles without recirculation (TWR) (Table 1). The second and third testing configurations differed from the first simply in the time delay between recirculation events (Table 1). The fourth configuration corresponded to 0 hours of recirculation (22 hours rest).

**Table 1.** Operating conditions for the intermittent recirculation of the ASBR and intermittent aeration of the SBR

C <sup>a</sup>	Anaerobic Digestion, ASBR-Reactor Intermittent recirculation				Aerobic Digestion, SBR-Reactor Intermittent aeration			
	Hourly operational condition		Daily cycle operational condition		Hourly operational condition		Daily cycle operational condition	
	TWR <sup>b</sup> (h)	TWOR <sup>c</sup> (h)	TWR (h/cycle)	TWOR (h/cycle)	TWA <sup>d</sup> (h)	TWOA <sup>e</sup> (h)	TWA (h/cycle)	TWOA (h/cycle)
1	1	1	11	13	1	1	11	13
2	1	3	6	18	2	1	14	10
3	1	2	8	16	3	1	15	9
4	0	24	0	24	1	2	8	16

<sup>a</sup> Configuration, <sup>b</sup> Time with recirculation, <sup>c</sup> Time without recirculation, <sup>d</sup> Time with aeration, <sup>e</sup> Time without aeration

### 2.3.3. Operating conditions of the intermittent aeration SBR

For the second stage (SBR), the operating configurations were realized as noted in Table 1: Initially, aeration was provided for 1 hour, followed by a 1 hour period without aeration for a total of 22 hours (daily cycle), A 2 hour period was also required each day to settle, feed and waste the system. The first SBR operating configuration consisted of 11 aeration cycles (1 hour) (TWA) and 13 cycles without aeration (TWOA) (daily cycle) (Table 1). The second, third and fourth operating configurations differed from the first in the length of periods with and without aeration (Table 1).

### 2.4. Parameter analysis

Since the data collected naturally exhibited a high degree of variability, a one-way analysis of variance (ANOVA) at a 95% significance level was applied to determine significant differences among the parameter means of all operational configurations. In order to better understand the contribution of a particular operational condition to the complete performance of the combined system (ASBR-SBR), the data from each reactor (ASBR, SBR) were evaluated, first individually and then combined. The statistical difference of means was performed via multiple range tests.

In the event that the data was not normally distributed, a non-parametric Kruskal-Wallis test was also applied. The statistical analyses were conducted using Statgraphics Centurion XVI.I.

## 3. Results and discussion

### 3.1. Swine manure characteristics

The pollutant concentrations and the quantity of swine wastewater generated (4,500-5,000 m<sup>3</sup>) were variable and were generally found to be dependent on the swine population at the farm. The objective of the characterization was to quantify the variability of the organic matter and nutrient effluent concentrations to the system and is provided in Table 2.

### 3.2. Performance of the combined ASBR-SBR system under the different operating conditions

In order to assess the effect of each operational condition (pulsed intermittent recirculation ASBR and intermittent aeration SBR, Table 1) on the overall performance of the system, each reactor was also monitored individually throughout the testing. A sample was collected at the ASBR effluent, as well as the SBR effluent.

#### 3.2.1. Assessment of the pulsed intermittent recirculation ASBR system performance

Constituent removals observed under different pulsed intermittent recirculation operating conditions in the ASBR are illustrated in Fig. 2. The TCOD removal under the different recirculation conditions was generally greater than 95%, with the exception of configuration 3 (1 hour pulsed recirculation and 2 hours rest), where a removal of 88 ± 6% was obtained.

**Table 2.** Swine wastewater characteristics generated in experimental farm (Technological Institute of Conkal, Yucatan, Mexico)

Parameter	Unit	Minimum	Maximum	Average (mean ± SD <sup>a</sup> )
pH	-	6.7	7.9	7.3 ± 0.5
OD	mg/L	0.05	0.22	0.15 ± 0.05
Conductivity	mS/cm	2.4	5.0	3.5 ± 0.9
T	°C	26.4	30.6	27.8 ± 1.3
TCOD	mg/L	2,770	19,000	8,680 ± 4,892
SCOD	mg/L	797	6,085	2,086 ± 1,208
TN	mg/L	240	1,770	589 ± 349
NO <sub>3</sub>	mg/L	86	156	110 ± 31
NH <sub>3</sub> -N	mg/L	139	675	321 ± 154
TP	mg/L	202	1,624	576 ± 353
Alkalinity	mg/L	990	2,178	1,514 ± 396
TS	mg/L	3,221	23,047	9,393 ± 5,076
VTS	mg/L	2,044	18,130	6,889 ± 4,178
TSS	mg/L	2,806	22,200	6,893 ± 4,961
VTSS	mg/L	2,333	18,216	6,185 ± 4,455
Zn	mg/L	0.51	327.57	54.02 ± 104.78
Cu	mg/L	0.26	5.14	3.10 ± 2.13

<sup>a</sup> "±" refers to one standard deviation (SD) from the mean (n = 18) with the exception of NO<sub>3</sub> (n = 4). pH = potential of hydrogen, OD = Dissolved Oxygen, T = Temperature, TCOD = Total chemical oxygen demand, SCOD = Soluble chemical oxygen demand, TN = Total nitrogen, NO<sub>3</sub> = nitrate, NH<sub>3</sub>-N = ammonia nitrogen, TP = Total phosphorus, TS = Total solids, VTS = Volatile total solids, TSS = Total suspended solids, VTSS = Volatile suspended solids. Zn = Zinc, Cu = Copper

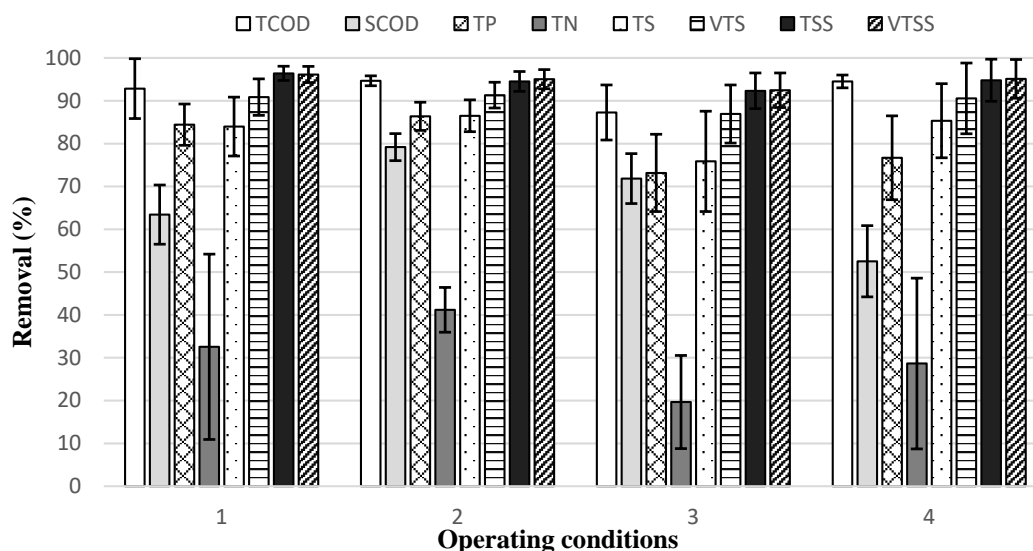


Fig. 2. Constituent removals observed under different pulsed intermittent recirculation operating conditions in the ASBR, [n = 6]

The highest removal of SCOD observed in the ASBR was under operating configuration 2 (1 hour pulsed recirculation and 3 hours rest), for which removals of 73-82% were achieved. For all other recirculation configurations, removals were less than 79% (Fig. 2). These results were consistent with the 82-90% TCOD and 80-94% SCOD removals reported by Massé et al. (2003). They measured the effects of temperature and OLRs on treatment performance using biogas recirculation to mix reactor liquor for a 14-day HRT and OLRs between 1.2-1.4 g L<sup>-1</sup>d<sup>-1</sup> at 20°C, with an average influent TCOD concentration of 48,770 mg/L. Ndegwa et al. (2008) compared the operational efficiency of ASBRs at two different temperatures (20 and 35°C) and two different operating cycles per day (1 and 3 cycles/day). It was found that approximately 87% TCOD removals could be achieved at a temperature of 20°C and one long reaction phase (1 cycle/day).

In contrast, Bernet et al. (2000) investigated the effectiveness of a bench-scale combined ASBR-SBR system to promote denitrification in the anaerobic stage (ASBR). The ASBR exhibited much lower TOC removals (as indicator of organic matter removal) of 40.5% when fed with raw wastewater and recycled treated wastewater enriched with nitrate from the SBR. The lower efficiency reported is done by the anoxic conditions in the ASBR produced by the oxygen that comes from the NO<sub>3</sub><sup>-</sup>, which generates carbon oxidation instead of its reduction that normally occurs in an anaerobic condition. Oxygen presence inhibits carbon removal in anaerobic process.

The pulsed recirculation applied in this study to the anaerobic reactor (ASBR) ensured an active microbial biomass by allowing better contact between the substrate and the microorganisms through the application of short mixing cycles, which also reduces the suspension of solids due to the release of trapped gas bubbles contained therein. This in fact prevents the formation of flocculent solids and, instead, promotes sedimentation of dense material, thereby generating a

granular sludge (Demirer and Cheng, 2005; Massé et al., 2003; Ndegwa et al., 2008). Moreover, as suggested by Demirer and Cheng (2005) and Ndegwa et al. (2008) mixing process into the ASBR improves mass transfer fluxes during the reaction phase.

The statistical analysis (ANOVA) indicated that in the case of TCOD, the data was not normally distributed; therefore, a non-parametric Kruskal-Wallis test was applied. This test showed that there was no statistically significant difference among the configurations investigated ( $P = 0.2059 > 0.05$ ). For the SCOD, the data was normally distributed and the statistical analysis noted a significant difference among the configuration studied, where the mean values obtained for each of configurations 1-2, 1-3, 1-4, 2-4 and 3-4 were statistically different ( $P = 0.0000$ ).

Although the main objective of this study was primarily to investigate the effects of pulsed intermittent recirculation in the anaerobic stage (ASBR) in the enhancement of organic matter removal; high removals of TP were also achieved. As can be seen from Fig. 2, the removals observed in all ASBR recirculation configurations were in the range of 73-86% for TP, with the highest removal efficiencies  $86 \pm 3\%$  noted in configuration 2 (1 hour pulsed recirculation and 3 hours rest).

Conversely, Ndegwa et al. (2008) reported that TP concentrations in the ASBR effluent (~70 mg/L) were not significantly reduced from influent ( $TP 74 \pm 3$  mg/L) concentrations, concluding that nutrients such as TP were not attenuated significantly in the treatment of low-strength manure. Nevertheless, according to the literature, there are two main forms of phosphorus removal, chemical and the biological (Baetens, 2000; Caravelli et al., 2010). The addition of chemicals such as iron salts (ferric chloride), aluminum or calcium oxide (lime) can lead to chemical precipitation, yielding 70 to 90% removals in phosphorus. The primary disadvantages in using chemical phosphorus removal are the large quantities of sludge produced, the cost of the precipitant and the

negative ecological effects of high iron and aluminum concentrations on receiving environments.

Biological phosphorus removal can be achieved via one of two phosphate accumulating organisms (PAOs). One group is capable of utilizing only oxygen as the final electron acceptor under a classic anaerobic and aerobic sequence. According to Kuba et al. (1994), there is another group of microorganisms, known as denitrifying phosphate accumulating organisms (DNPAOs), capable of accumulating phosphorus using nitrate/nitrite as electron acceptor instead of oxygen. This mechanism support the results observed in the ASBR in this study. As there was no oxygen present in the anaerobic reactor, nitrification process was not likely. Hence, TP removal by PAOs through the nitrification process was considered to be negligible. However, the nitrate concentrations in the ASBR influent promote TP removal. Under the different operating configurations for the ASBR investigated in this study, the average nitrate concentrations in the influent were  $110 \pm 31$  mg/L, while in the effluent were  $23 \pm 4$  mg/L, which suggest the occurrence of nitrate removal in the reactor (Bernet et al., 2000; Kuba et al., 1994; Wanner et al., 1992). Therefore, the results of this research show that the TP removal observed in the ASBR was due to the denitrification process. The ANOVA indicated a statistically significant difference ( $P = 0.03$ ) between the means of the TP removal efficiencies under different operating conditions.

TN removals under all ASBR recirculation configurations were in the range of 20-41%. The highest removal efficiencies observed for TN were  $41 \pm 5\%$  in configuration 2. It is generally accepted that TN is represented by the addition of total organic nitrogen (TON) and total ammonia nitrogen (TAN), measured from TKN and nitrate. With respect to TN removal, in this study, only nitrate exhibited a notable change, while TON and TAN did not change significantly. These results are consistent with those reported by Ndegwa et al. (2008), where for none of the ASBR operational conditions investigated (number of cycles/day or temperature) were observed to affect TAN and TKN concentrations significantly. Similarly, Bernet et al. (2000), reported 2.7% TKN, 5.1%  $\text{NH}_3\text{-N}$ , 28.7% TN and 100% nitrate removals in their ASBR study, which was consistent with the high nitrate and low TN removals observed in this study. It should be noted, however, that the TN removals observed in this study, did not satisfactorily meet discharge objectives stipulated by Official Mexican Standard (NOM-001-SEMARNAT-1996, 2003). The ANOVA established that the removal efficiencies observed among the operating configurations investigated were not statistically significantly different ( $P = 0.3632 > 0.05$ ), which would indicate that the pulsed intermittent recirculation operation did not significantly affect TN removal.

Total solids removals in the ASBR were observed to be on the order of  $86 \pm 4\%$  TS, and  $94 \pm 2\%$  for TSS for configuration 2. According to the ANOVA, the removal efficiencies noted between the

operating conditions were not considered to be statistically significantly different ( $P = 0.2523 > 0.05$ ), which would suggest that recirculation configuration did not significantly affect the removal of solids. According to Ndegwa et al. (2008), better solids sedimentation or lower entrained solids could be achieved in ASBR systems operated with longer cycles/day of reaction phase, since this allows better solids agglomeration. Introducing rest times between reactions phases (that in the case of the present research achieved through pulsation time) gives advantages, because a better mixing of reactor constituents occurs. This was consistent with the results obtained this study (Fig. 2). From the results it can be inferred that the pulsed intermittent recirculation produced a positive effect in TP and organic matter removal, which corresponded to configuration 2 (1 h recirculation and 3 h rest) where the best performance was observed. Therefore, one of the project objectives was achieved; improve organic matter removal in the anaerobic process. In addition, high removals of TP were obtained.

### 3.2.2. Assessment of the intermittent aeration SBR system performance

Constituent removals observed under different intermittent aeration operating conditions in the SBR are shown in Fig. 3. The removal efficiencies were determined based on the concentrations entering the SBR (ASBR effluent) and those of SBR effluent. The highest TCOD removal ( $79 \pm 7\%$ ) was observed in the SBR under operating configuration 2 (2 hours with aeration and 1 hour without aeration).

In the case of SCOD, reductions ranged from 0-38%, for the entire aeration configuration conditions investigated. Higher removals were also observed for configuration 2, which yielded removal efficiencies of  $38 \pm 26\%$ . The low SCOD removals in the SBR could likely be attribute to the consumption of the more readily biodegradable compounds in the first anaerobic stage (ASBR), leaving more complex or recalcitrant compounds in the second aerobic stage (SBR). This is consistent with results presented by Bernet et al. (2000), although TOC was used as an indicator of organic matter removal. Bernet et al. (2000) obtained TOC removals of 53.6% at pH value of about 8, and indicated that the relatively low removal efficiency in the SBR was likely due to the presence of low biodegradable substances.

The ANOVA indicated that the TCOD data was not normally distributed, therefore a non-parametric Kruskal-Wallis test was applied, which showed that there was no statistically significant difference among the configuration studied ( $P = 0.05564 > 0.05$ ). On the other hand, the SCOD removals were found to be statistically significantly different ( $P = 0.000972 < 0.05$ ) amongst the configuration studied, where configuration 2 was found to be the most effective for organic matter removal. With respect to TP concentrations, SBR, configuration 2 also yielded the highest removals (as in the ASBR), with efficiencies of  $71 \pm 10\%$  (Fig. 3).

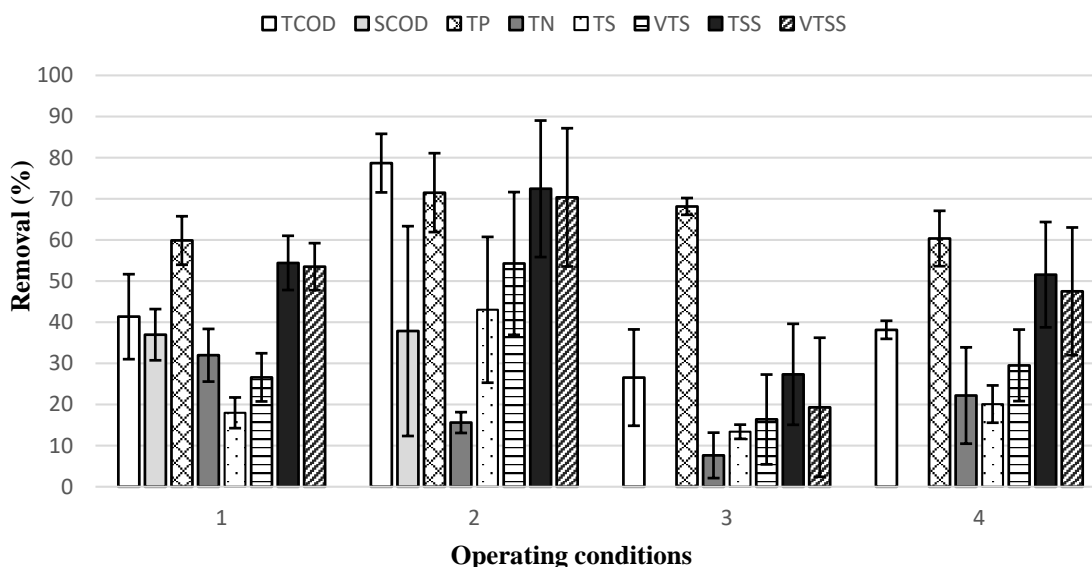


Fig. 3. Constituent removals observed under different intermittent aeration operating conditions in the SBR, [n = 6]

As observed in the ASBR, TP removal in the SBR was associated with nitrate removal. Statistical analysis also showed that there was a significant difference among the configuration studied ( $P = 0.0251 < 0.05$ ) indicating that intermittent aeration had a significant impact on the removal of TP, where configuration 2 operated in the aerobic-anoxic process (SBR) exhibited the best performance.

In the case of TN, the highest removal ( $32 \pm 6\%$ ) was also observed in configuration 2. This value was consistent with the findings of Bernet et al. (2000), where TN removals of only 25.7% were reported. These results suggested that in the SBR system, nitrogen conversion from organic nitrogen to ammonia nitrogen and then nitrate was likely taking place. In addition, measurements supporting nitrogen oxidation in the SBR (TKN 53.3% and  $\text{NH}_3\text{-N}$  of 81.5%) were observed (Bernet et al., 2000). In this study, the ANOVA also indicated that there was a statistically significant difference ( $P = 0.0385 < 0.05$ ) among the aeration configurations and hence that intermittent aeration has a significant impact in the TN removal, where configuration 2 showed the best removals.

It has been reported by Zhang et al. (2019) that the optimum pH for partial nitrification in an SBR is between 7.2 and 8.0, which corroborates the results found in this research, since the pH determined in the SBR was on average 7.6 and without significant changes in the alkalinity that remained around 1,130 mg/L.

In general, the solids removals observed in the SBR were not as high as those noted in the ASBR. However, the TSS removal was generally higher than TS removal. Configuration 2 was also found to exhibit the best removal efficiencies:  $43 \pm 18\%$  of TS,  $54 \pm 17\%$  of VTS,  $72 \pm 17\%$  of TSS and  $70 \pm 17\%$  of VTSS (Fig. 3).

Ndegwa et al. (2008) noted that a longer reaction phase in the SBR system negatively affected the degree of settling, therefore would be beneficial to consider longer settling periods prior to discharge. In this study, configuration 2 resulted seven intermittent aeration cycles over a 21 h period, with 1 hour in which the system was not operated and 2 hours for feeding and discharging as previously noted. The additional hour in the rest cycle allowed for a further hour of sedimentation prior to discharge, which appeared to be significant.

From the observed performance of the individual SBR, it can be concluded that significant  $\text{NH}_3\text{-N}$  and TP removals are possible, indicating that an aerobic-anoxic process could lead to considerable nutrient removals, which was the main objective in incorporating the SBR reactor after the ASBR reactor. For all the constituents and removal efficiencies analyzed, the best results were reported for configuration 2 (2 h with aeration and 1 h without aeration).

### 3.2.3. Assessment of the combined pulsed intermittent recirculation ASBR and intermittent aeration SBR system performance

The data considered to evaluate the performance of the combined (ASBR-SBR) were collected from ASBR-influent and SBR-effluent, taking into account the two feeding and discharge cycles, this allowed for a total retention time of 48 h for a quantity of wastewater entering and leaving the combined system.

The combined pulsed intermittent recirculation ASBR and intermittent aeration SBR system demonstrated that removal efficiencies of  $98 \pm 1\%$  TCOD,  $86 \pm 5\%$  of SCOD could be achieved (Fig. 4). These values corresponded to operating configuration 2 in each of the respective two stages consisting of 1 h



pulsed recirculation and 3 h rest in the ASBR, followed by 2 h of aeration and 1 h without aeration in the SBR. The results were consistent with Deng et al. (2007), where 95.2% TCOD removal was observed, Rajab et al. (2017), with  $97\% \pm 2\%$  TCOD and  $95\% \pm 3\%$  SCOD removals, reported, and with Bernet et al. (2000), with 88.7% organic matter removal, expressed as TOC. Rajagopal et al. (2011) reported low TCOD removals (38-52%), but the SCOD was 79-88% consistent with this paper.

The ANOVA analysis of the TCOD data ( $P = 0.01227 < 0.05$ ) indicated that there was a statistically significant difference among the configuration studied, specifically for the means of configurations 3-1, 3-2, 3-4 and 2-4. An ANOVA of the SCOD data also demonstrated that there was a significant difference among the configuration studied ( $P = 0.014$ ), with the means of configuration 1-2, 1-4, 2-3 and 2-4 showing statistically significantly different performances.

TN and TP removals in the combined system exhibited better results in configuration 2, with removals of  $55 \pm 18\%$  of TN and  $96 \pm 1\%$  of TP. From these results it was evident that the system could achieve limited nitrogen removal, but that a high phosphorus removal was possible. Hence, the nitrate concentrations in the system influent and the anaerobic conditions in the ASBR, followed by the intermittent aeration in the SBR, likely promoted denitrification in both stages of the treatment process (anaerobic/aerobic-anoxic). Similar results in a combined anaerobic-aerobic system for TN removal were noted by Bernet et al. (2000) and Rajagopal et al. (2011), who achieved 66.2% and 66-75%, respectively.

Conversely, higher TN removals were reported by Deng et al. (2007). (96.1%), as well as Rajagopal et al. (2011) and Rajab et al. (2017) achieving  $98\% \pm 1.3\%$  and 98-99%  $\text{NH}_3\text{-N}$  removals, respectively. However they did not report TP removals.

The ANOVA for TP in this study showed that there was a statistically significant difference among the configuration studied ( $P = 0.03994 < 0.05$ ). For the TN, the ANOVA also demonstrated that there was a significant difference among the configuration studied ( $P = 0.0258 < 0.05$ ), particularly 1-3 and 2-3 were statistically significantly different. For both parameters (TP and TN) the best performance was achieved in configuration 2 too. Table 3 presents a summary of the percentages obtained for the main parameters in the best configuration used in the individual evaluations of the reactors as well as the combination of both (ASBR-SRB) demonstrating the positive effect of pulsed intermittent recirculation (ASBR) and intermittent aeration (SBR). With respect to solids removals in the combined system, higher TS ( $92 \pm 3\%$ ), VTS ( $96 \pm 1\%$ ), TSS ( $98 \pm 1\%$ ) and VTSS ( $98 \pm 1\%$ ) removals were observed for configuration 2 (Table 1, Fig. 4). These removals were found to be higher than those reported by Novak et al. (2011), where 62% VTS and 54% TS were noted in a combined system anaerobic-aerobic. Nevertheless, the results were consistent with Rajab et al. (2017), which reported  $96\% \pm 3\%$  TSS removal.

#### 4. Conclusions

The combination of pulsed intermittent recirculation (ASBR) and intermittent aeration (SBR) was shown to improve the removal of organic matter and nutrients from swine wastewater. In particular, a significant positive effect was noted on the removal of TP ( $86 \pm 3\%$ ) and SCOD ( $80 \pm 3\%$ ) due to pulsed intermittent recirculation in the ASBR, since there was a significant difference among the removal efficiencies under the different configurations studied.

For the SBR, the intermittent aeration allowed for a significant increase in the removal of TP ( $71 \pm 10\%$ ) due to the denitrification process ( $\text{NO}_3^-$  removal of  $54 \pm 4\%$ ) and a statistically significant difference among the configurations employed was noted.

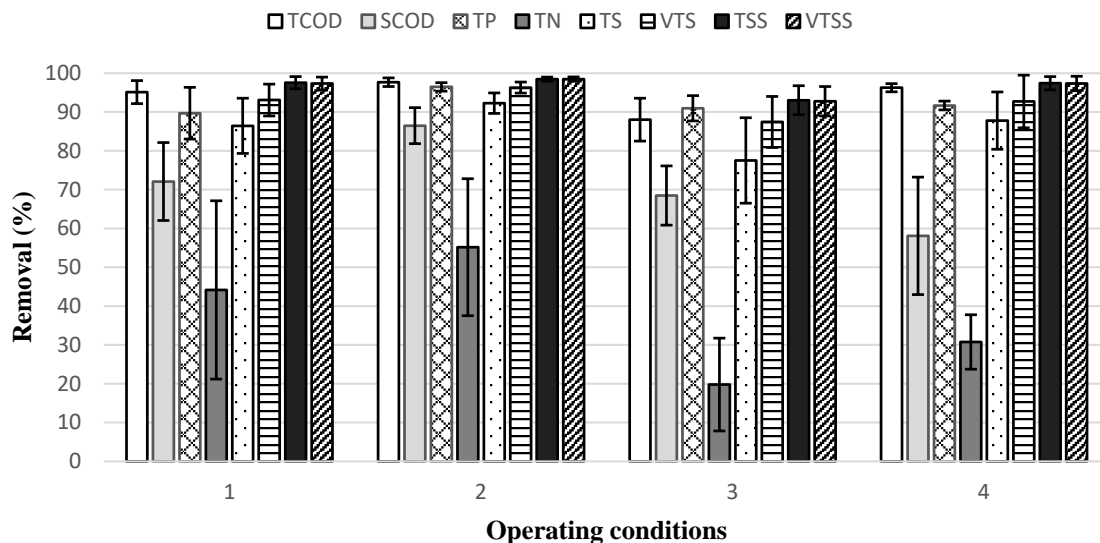


Fig. 4. Constituent removals observed for the combined ASBR-SBR system with different pulsed intermittent recirculation (ASBR) and intermittent aeration (SBR) operating conditions, [n = 6]

**Table 3.** Removals of the main parameters reached in the optimal configuration analyzed in this research

<i>Process</i>		<i>ASBR</i>						
Parameter → Configuration ↓	pH	TCOD Removal %	SCOD Removal %	TP Removal %	TN Removal %	NH <sub>4</sub> <sup>+</sup> Removal %	NO <sub>3</sub> <sup>-</sup> Removal %	Alkalinity mg/L
2	7.4	95 ± 2	80 ± 3	86 ± 3	41 ± 5	8 ± 2	82 ± 8	1,390
<i>Process</i>		<i>SBR</i>						
Parameter → Configuration ↓	pH	TCOD Removal %	SCOD Removal %	TP Removal %	TN Removal %	NH <sub>4</sub> <sup>+</sup> Removal %	NO <sub>3</sub> <sup>-</sup> Removal %	Alkalinity mg/L
2	7.6	79 ± 7	38 ± 26	71 ± 10	32 ± 6	74 ± 7	54 ± 4	1,130
<i>Process</i>		<i>ASBR-SBR</i>						
Parameter → Configuration ↓	pH	TCOD Removal %	SCOD Removal %	TP Removal %	TN Removal %	NH <sub>4</sub> <sup>+</sup> Removal %	NO <sub>3</sub> <sup>-</sup> Removal %	Alkalinity mg/L
2	7.6	98 ± 1	86 ± 5	96 ± 1	55 ± 18	76 ± 1	60 ± 9	1.130

Finally, the results demonstrated that a sequential system (the pulsed intermittent recirculation ASBR and the intermittent aeration SBR reactors) achieved a better performance for removal of TCOD (98 ± 1 %), SCOD (86 ± 5 %), TP (96 ± 1 %) and represent an important alternative for the treatment of swine wastewater.

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