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DAIRY MANURE WASTEWATER IN SERIAL UASB REACTORS FOR ENERGY RECOVERY AND POTENTIAL EFFLUENT REUSE

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Abstract – This manuscript addresses the use of dairy manure wastewater (DMW) as a substrate for methane production in a high rate system – two serial UASB reactors – and the characterization of the effluent in the different phases of the treatment. Two experimental conditions were applied. In the first, the organic load rate (OLR) was 6.2 $gCOD_{total}$ L^{-1} d^{-1} and the hydraulic retention time (HRT) was 12 days, whereas in experiment 2 they were 14.2 $gCOD_{total}$ L^{-1} d^{-1} and 7.5 days, respectively. The reactors showed a high capacity of removal of COD and solids, i.e., above 71%, which led to bioenergy productivities of 0.55 and 0.73 L CH_4 L^{-1} d^{-1} in experiments 1 and 2, respectively. The methane yield in UASB1 was 0.19 and 0.08 L CH_4g COD_{rem}^{-1}, respectively, in experiments 1 and 2. The methane yield in UASB1 was greater than UASB2, with approximately 70% of methane content in the biogas in this reactor.The effluent characterization showed micro and macronutrients - some in high concentrations, as nitrogen, phosphorous, K, and Fe between 12.1 and 521.4 mg L^{-1}, and others in low concentrations, as Ca, Mg, Zn and Mn between 0.6 and 13 mg L^{-1}. Na was not removed from the UASB systems and its concentration in the effluent was raised to 23.5% in comparison with the substrate.

Keywords: Livestock waste, Bio-energy, High-rate reactors, Nutrients.

INTRODUCTION

Milk is one of the most consumed foods worldwide, due to its variety of important nutrients for metabolism and physiologically functional compounds, such as bioactive peptides, antioxidants, and omega-3 (Yao *et al.,* 2016). Because of the increase in its demand, animal confinement systems have been an alternative for productivity enhancement and reductions in management costs (Silva and Roston, 2010). However, the disadvantage of such systems is that they generate a high amount of polluting waste (Puget *et al.,* 2004) - on average, 37 kg cow^{-1} d^{-1} in manure and urine forms (Tabatabaei *et al.,* 2010). As a result, the effluent generation is 4.7 higher than the milk production (Silva and Roston, 2010).

Due to increased demands and rising prices for fossil fuels, livestock waste has been converted into renewable energy as a sustainable alternative. The biogas production is one of the several tools that can alleviate the problems of global warming, energy security and livestock waste generation. According to Appels et al. (2011), anaerobic digestion is an efficient alternative technology for the

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treatment of wastewater and sludge stabilization and offers advantages, such as production of biogas, agricultural recycling of organic matter and recovery of nutrients that remain in the effluents of anaerobic reactors.

Such agroindustrial wastes are characterized by the presence of salts, toxic elements, pathogens, high concentrations of nutrients and COD (Adhikari *et al.,* 2015; Witarsa and Lansing, 2015), which make the DMW an adequate substrate for biogas production (mainly methane, CH_4) and stable sludge (Witarsa and Lansing, 2015).

Arikan *et al.* (2015) obtained methane production values of 5.0 m^3 $week^{-1}$, 0.37 $m^3 kg^{-1}$ SV methane yield and a 44% reduction in volatile solids (VS). The authors operated a field-scale anaerobic digester at 35°C fed with scraped cow manure of 1.4 kg VS m^3 d^{-1} OLR. They also reported a higher methane production of 8.6 m^3 $week^{-1}$ and 65% VS removal, and OLR increase to 2.6 kg VS m^3 d^{-1}. Similarly, Rico et al. (2011a), reached a methane production of 10.3 L CH_4 L^{-1} d^{-1} in a UASB reactor operating at 35°C and OLR of 40.8 g COD L^{-1} d^{-1}, whose liquid fraction was separated by flocculation and screening.

In general, conventional systems such as digesters have been applied for the treatment of DMW in rural areas and exhibit high HRT, low nutrient removal and low methane production (Nikolaeva *et al.,* 2013; Ogejo and Li, 2010). For improvements in their performance, high-rate anaerobic systems, such as UASB reactors (*Upflow Anaerobic Sludge Blanket)* are a suitable alternative for the treatment of effluents with high organic solids concentrations, such as DMW, since cattle confinement systems are characterized by a high manure generation in small areas (Liu and Haynes, 2011). Such a configuration can enable high OLR applications, and provide lower HRT, continuous operation and a higher biodegradation rate, due to the retention of biomass of high metabolic capacity (Powar *et al.,* 2013).

Additionally, a serial UASB configuration is adequate for the treatment of such residues, because the second reactor removes the remaining organic matter from the first reactor, which increases the amount of methane produced and water quality in comparison with an individual system.

Due to the limitation of the DMW treatment in UASB reactors, few studies on the characteristics of the effluent have been developed, in comparison with those on bio-digester systems, mainly regarding micro- and macronutrients. The effluent characterization is important, due to the potential negative effects, such as water eutrophication and soil salinization caused when the effluent is not correctly disposed. However, the potential use of the effluent in soil fertilizing or irrigation must also be taken into consideration.

In consequence, the exploitation of DMW, the application of high-rate reactors with wastes of high organic solids concentration, and potential re-use of bioenergy and an effluent in the agricultural sector are interesting topics to be more deeply studied.

This article addresses the evaluation of the methane production capacity of a two serial UASB reactors that treat DMW and the characterization of effluents in relation to macro- and micronutrients and organic matter. The focus is on the effect of the increase in the OLR and reduction in the HRT over the reactor's performance.

MATERIALS AND METHODS

Dairy manure wastewater

The DMW influent used for feeding the reactors was simulated by a dairy manure dilution. The manure was collected weekly by a confinement system located in the dairy cattle sector at São Paulo State University, in Jaboticabal. It was diluted in proportions of 500 and 800 g for 1 L of water in experiments 1 and 2, respectively. The mixture was then sieved in a 0.2 mm mesh (Nikolaeva *et al.,* 2013).

Serial UASB reactors

The experimental unit consisted of two serial UASB reactors of 20 L (UASB1) and 40 L (UASB2) volumes (Figure 1) with four sludge collection points distributed along their horizontal axes.

Inoculum. The reactors were inoculated with 30% granular sludge from a UASB reactor that treats swine wastewater. The total solids (TS) were 4077 and 19230 mg L^{-1} and volatile solids (VS) were 2866 and 12157 mg L^{-1} for UASB1 and UASB2, respectively.

Operational conditions. The reactors were monitored for 90 days, from October to December/2013 (Table 1), and operated at ambient temperature (between 20 and 30°C). The temperature data were collected daily at an agrometeorological station located at São Paulo State University, in Jaboticabal, close to the experimental laboratory.

Organic loading rate and hydraulic retention time. The OLR applied increased from 6.2 (experiment 1) to 14.2 g COD_{total} L^{-1} d^{-1} (experiment 2) and the HRT decreased from 12 (experiment 1) to 7.5 days (experiment 2) (Table 1).

Sampling and analytical methods

Influent and effluent. Composite samples of influents and effluents of the reactors were collected twice a week and physical and chemical tests evaluated their pH, chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), Total Kjeldahl Nitrogen (TKN) and ammonia nitrogen (N-am.) according to a standard procedure (APHA, 2005). Total alkalinity (TA) and Volatile fatty acids (VFA) were determined by

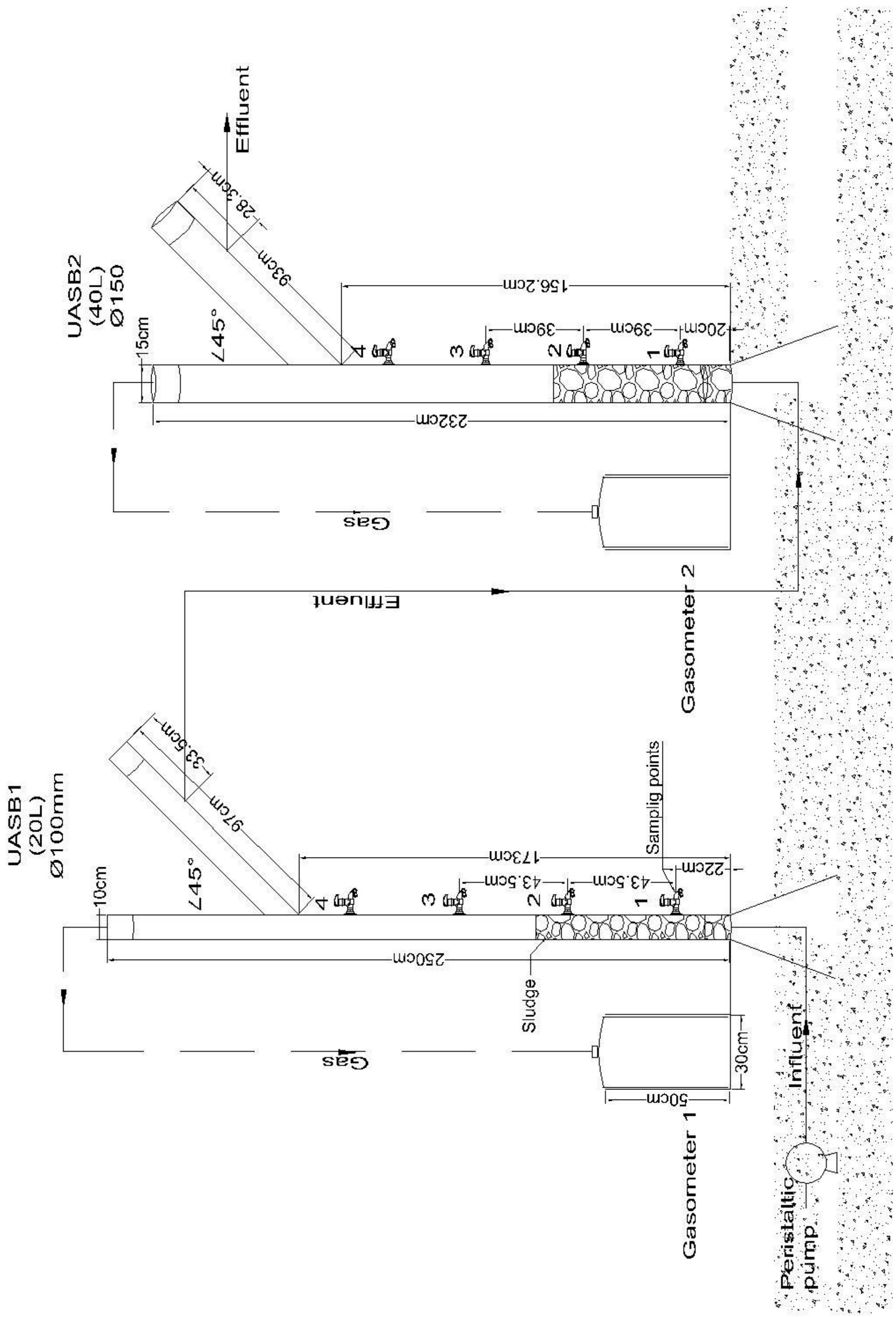

Figure 1. Serial UASB reactors system.

Jenkins *et al.* (1983) and Dilallo and Albertson (1961). Non-filtered samples of influent and effluent were digested by nitric perchloric acid and the total phosphorus (TP) was analyzed by the vanadomolybdophosphoric acid colorimetric method. Elements, such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were quantified in the nitric perchloric acid digested by atomic absorption (APHA, 2005).TA and VFA were used for the calculationof bicarbonate alkalinity (BA) as BA = TA – (0,85)(0.83)(VFA) (McCarty, 1964).

Sludge. Sludge samples were collected monthly from different sludge sampling points (Figure 1). Total solids (TS) and volatile solids (VS) were determined according to a standard procedure (APHA, 2005).

Biogas. The biogas production was measured daily by fiberglass floating dome-type gasometers. The biogas composition was determined fortnightly by a gas chromatograph (FININGAN 6C-9001) equipped with a thermal conductivity detector, "Porapak Q" column (3 m 1/8 ") and a molecular sieve, according to the gas chromatographic method (APHA, 2005). The volume was corrected to Standard Temperature and Pressure conditions (STP, 273 K and 1 atm) for the calculation of the methane production.

Statistical Analysis

The mean values and confidence limits (95%) were calculated by Tukey test for multiple comparisons (α = 0.5) and *Statgraphics Centurium XV* version 15.1. Two treatments corresponding to two experiments with different replicates were evaluated according to the sampling frequency.

RESULTS AND DISCUSSION

Methane production from dairy manure wastewater

OLRs of 6.3 and 14.2 g COD_{total} L^{-1} d^{-1} in UASB1 were achieved through the feeding of the reactors with more concentrated substrates (Table 2).

The reduction in the organic matter concentration in UASB1 was 62 - 65%. Therefore, the removal of the remaining organic matter content from the UASB1 effluent was enhanced in UASB2, which improved the potential for the methane conversion.

The performance of the two serial UASB reactors was evaluated according to pH, BA, and VFA (Table 2). Initially, the pH values of the influent were 6.8 and 6.4 for experiments 1 and 2, respectively, which are below the optimal range for the growth of methanogenic microorganisms. Nevertheless, the addition of a chemical alkalizer was not necessary, as the BA production in UASB1 (1447 and 2254 mg $CaCO_3$ L^{-1} in experiments 1 and 2, respectively) was enough for raising the pH to values higher than neutrality.

The minimal pH in both reactors was 7.0. According to Sakar *et al.* (2009), the pH required for methanogens ranges between 6.5 and 7.8 and 5.0 and 6.0 for the acid-producing bacteria. The pH observed in both UASB systems was adequate for methanogenic archaeas and avoided the separation of acidogenic and methanogenic phases in different reactors.

Influents with high VFA concentrations, i.e., 2297 and 3039 mg L^{-1}, were obtained in experiments 1 and 2, respectively. During experiment 2, the OLR was superior

Table 1. Operational conditions of the two serial UASB reactors.

Experiment	OLR (g COD L^{-1} d^{-1})	Duration (d)	HRT (d) UASB1	HRT (d) UASB2	Substrate concentration (mg COD L^{-1})
1	6.2	63	4.0	8.0	25117
2	14.2	35	2.5	5.0	35548

Table 2. VFA, BA and pH values and methane production of two serial UASB reactors.

Experiment	Temp. (°C)	Reactor	OLR (g CODL^{-1} d^{-1})	HRT (d)	pH	VFA (mg L^{-1})	BA (mg L^{-1})	CH_4 (%)	Bioenergy productivity (L CH_4 L^{-1} d^{-1})	Methane yield (L CH_4 g $COD_{rem.}$)	Methane yield (L CH_4 g CODadd)
1	22.9 ±0.6^{b} (64)	UASB1	6.3 ±1.5^{b} (16)	4.0	7.0 ±0.1^{a} (16)	841 ±142^{a} (16)	2062 ±325^{b} (16)	71.3 ±1.0^{a} (58)	0.50 ±0.04^{b} (58)	0.19 ±0.02^{a} (25)	0.10 ±0.01^{a}(58)
		UASB2	2.4 ±1.7^{A} (16)	8.0	7.4 ±0.1^{B} (16)	803 ±143^{A} (16)	1981 ±325^{A} (16)	69.4 ±1.2^{A} (41)	0.05 ±0.04^{A} (41)	0.08 ±0.02^{A} (40)	0.01 ±0.01^{A}(41)
2	24.2 ±0.8^{a} (34)	UASB1	14.2 ±2.0^{a} (11)	2.5	7.2 ±0.1^{a} (11)	696 ±172^{a} (11)	3368 ±412^{a} (11)	71.7 ±1.3^{a} (35)	0.61 ±0.05^{a} (35)	0.08 ±0.02^{b} (21)	0.05 ±0.01^{b}(34)
		UASB2	5.0 ±2.0^{A} (11)	5.0	7.6 ±0.1^{A} (11)	501 ±180^{A} (10)	2345 ±412^{A} (10)	67.6 ±1.4^{A}(31)	0.12 ±0.06^{A} (31)	0.08 ±0.02^{A} (31)	0.02 ±0.01^{A}(30)

Different letters (Tukey test, $p<0.05$) mean statistical difference.Comparisons between UASB1 (lowercase) and UASB2 (uppercase). (X) Number of samples for the calculation of the average.

and the HTR was inferior. Nonetheless, such operating changes exerted no toxic effect on the microbial biomass, because the buffer capacity was maintained in both UASB reactors.

The minimal VFA consumptions were 1454 mg L^{-1} in UASB1 and 38 mg L^{-1} in UASB2, which revealed both UASB reactors were methanogenic, because VFA was consumed for the maintenance of the cellular metabolism and conversion of intermediate products (H_2, CO_2 and acetate) to methane.

UASB1 was responsible for at least 83.6% of the total methane production in the system. Such performance was satisfactory, as it demonstrated the microorganism's adaptability to the application of a high OLR. On the other hand, UASB2 showed lower VFA consumption and an effluent with minimal 501 mg VFA L^{-1}. Some VFAs, such as propionate and butyrate, might be present in the influent of UASB2. The reactions of such acids are thermodynamically unfavorable for their transformation into acetate (ΔG^o (kj) = +76.1 from propionate and +48.1 from butyrate to acetate) (Harper and Pohland, 1986), which may produce low methane yields. Similarly, methanogenic archaea do not use propionate and butyrate for methane production; therefore, a syntrophic association with acetogenic bacteria is required.

The association between methanogenic and acetogenic microorganisms was observed in UASB1 and UASB2, as a consequence of the application of a HRT higher than the time of growth of methanogenic organisms, such as *Methanothrix* of 6.9 h (maximum growth rate, μ_{max}=0.1 h^{-1}) (Harper and Pohland, 1986). The metabolic phases could be separated through the kinetic control and the washing of methanogenic microorganisms (Pohland and Ghosh, 1971). However, the conditions applied increased the HRT for a higher hydrolysis of the polymeric material present in the DMW and the metabolic phases were not separated.

Because of the metanogenenic activity in both UASB reactors, the methane production in the biogas was higher than 67% (Figure 2), which is significant (α = 0.5) for experiments 1 and 2, even with an HRT decrease from 12 to 7.5 d (Table 2 and Figure 2). A maximum 4% difference between UASB1 and UASB2 demonstrated their ability to produce biogas with high methane content for energy reuse.

Likewise, the amount of methane produced increased in experiment 2 (Table 2 and Figure 3) with bioenergy productivities of 0.61 L CH_4 L^{-1} d^{-1} in UASB1 and 0.12 L CH_4 L^{-1} d^{-1} in UASB2. Such a result was due to the adaptation of the system to the increase in the OLR, which led to the elevation of BA, reduction in VFA and consumption of COD. This adaptation was significant, because it did not affect the quality and amount of methane produced.

Therefore, HRT can be reduced to 7.5 days without affecting the methane production. The HRT reduction in UASB reactors is more advantageous than the high HRT commonly applied to biodigesters for the anaerobic treatment of DMW.

The methane yield was compared to the stoichiometric amount of 0.35 L CH_4 g^{-1} COD. However, the conversion of the organic matter to methane in the reactors achieved maximum efficiencies of 54.3% for UASB1 and 22.8% for UASB2, which were lower in relation to the organic matter added (Table 2). The low conversion of COD to methane might be due to the lignocellulosic material present in the animal feed. DMW contains fiber and polymers, which hamper its anaerobic degradation. Under such conditions, the hydrolytic phase is the limiting step (Ogejo and Li, 2010; Rico *et al.,* 2011a).

Similarly, a low VFA conversion to methane might be a consequence of thermodynamic inhibition, with a possible accumulation of propionate and butyrate. The separation of hydrolytic/acidogenic and methanogenic phases in different

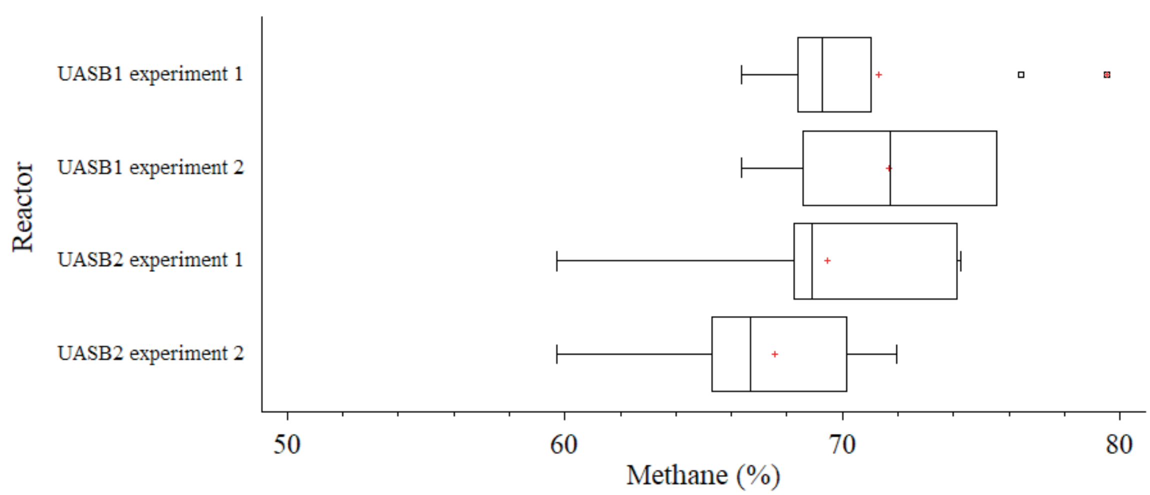

Figure 2. Methane production (%) by a two serial UASB reactors.

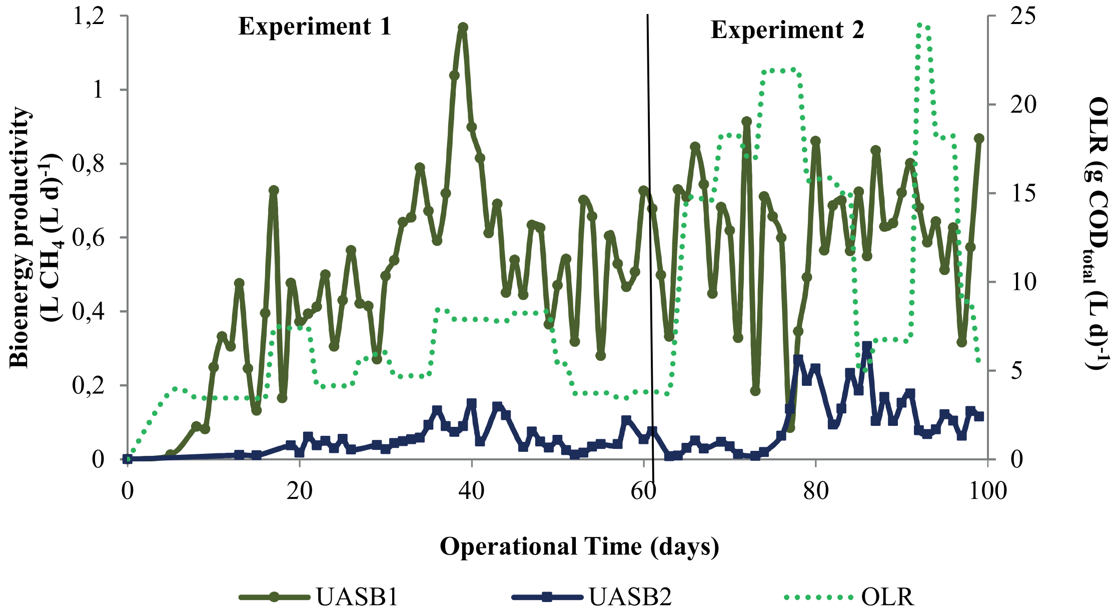

Figure 3. Bioenergy productivity and OLR during the operation of two serial UASB reactors.

reactors (two-stage configuration) could enable superior methane productions. During the physical separation of metabolism, the growth kinetics are favored, according to the nutritional conditions and velocity of duplication of each microbial group (Zoetemeyer *et al.,*1982).

Harper and Pohland (1986) described the anaerobic digestion as symbiotic associations between different groups of bacteria, in which the reaction is maintained through the continual removal of some products, such as hydrogen. Hydrogen can be removed through the separation of the reactor into two phases, so that the acetic acid pathway becomes favorable. Consequently, superior hydrolysis and acetogenesis are promoted, and, with an increased acetate production, the methane yield can be higher.

Demirer and Chen (2005) compared a single stage with two-stage UASB reactors. The conditions applied were unscreened dairy manure, mesophilic temperature, HRT of 10 days and OLR between 5 and 6 kg VS m^{-3} d^{-1}. The authors obtained a 50 to 67% higher biogas production with a two-stage configuration (Demier and Chen, 2005).

In experiment 2, the methane yield was reduced to 0.08 L CH_4 g $COD_{rem.}^{-1}$, which represents half of that obtained in experiment 1 (Table 1). The same effect was observed by Arikan *et al.* (2015), who achieved a 0.26 to 0.16 m^3 kg^{-1} VS decrease in the methane yield when the OLR increased from 1.4 to 2.6 kg VS m^{-3} d^{-1} in a biodigester operating at 22°C and 17 days HRT. A large quantity of fibers was detected in the solid fraction, which is more difficult to degrade and be converted to methane (Arikan *et al.,* 2015 and Moset *et al.,* 2015).

For an increase in the substrate biodegradability, the DMW must be pretreated through sieving, so that the particle size is reduced and the fibrous material is retained (Rico *et al.,* 2011a). The pretreatment technique alone is not enough in comparison with the flocculation and sieving adopted by Rico *et al.* (2011a). The authors operated the UASB reactor with the liquid fraction and obtained superior methane yield results of 0.28 L CH_4 g $COD_{add.}^{-1}$ and bioenergy productivity of 14 L CH_4 L^{-1} d^{-1}. Although the physical-chemical pretreatment must be applied for improvements, the costs, technical expertise, and operational stages must also be considered. Therefore, the development of simple technologies is fundamental for the maximum exploitation of the raw residue and a high methane yield.

Witarsa and Lansing (2015) reported a superior methane yield, i.e.,0.27 and 0.42 L CH_4 g $COD_{add.}^{-1}$, but at a higher HRT, i.e.,216 days. Nevertheless, the two serial UASB system showed a favorable conversion of organic matter to methane when the DWM was sieved, even with low HRT (7.5 days), high OLR (14.2 g $CODL^{-1}$ d^{-1}) and no temperature control during the operation, which highlighted the ability of high-rate systems to achieve superior performance and time optimization.

Removal of organic material from dairy manure wastewater

Organic matter, such as chemical oxygen demand (COD), total suspended solids (TSS) and volatile suspended solids (SSV) are the elements found in high concentrations in the DMW; therefore, their removal in the UASB reactors is desirable. Table 3 and Figure 4 show the organic matter concentration in both influent and effluent.

Table 3. Removal of organic material in the two serial UASB reactors.

	Experiment 1				Experiment 2			
	Influent	Effluent UASB1	UASB2	Removal UASB1 + UASB2	Influent	Effluent UASB1	UASB2	Removal UASB1 + UASB2
	mg L^{-1}			(%)	mg L^{-1}			(%)
COD	25117 ±4355^{a}(19)	19314 ±4745^{A}(16)	6790 ±4901$^{\underline{a}}$(15)	72.2 ±7.9$^{\underline{A}}$(15)	35548 ±5723^{a}(11)	25006 ±5723^{A}(11)	9839 ±5723$^{\underline{a}}$(11)	73.3 ±9.6$^{\underline{A}}$(10)
TSS	16568 ±24497^{a}(15)	155453 ±24497^{A}(15)	3558 ±25356$^{\underline{a}}$(14)	74.5 ±11.7$^{\underline{A}}$(14)	23834 ±28606^{a}(11)	84011 ±28606^{B}(11)	5303 ±28606$^{\underline{a}}$(11)	71.2 ±13.2$^{\underline{A}}$(11)
VSS	14040 ±18304^{a}(115)	115473 ±18305^{A}(15)	2595 ±18304$^{\underline{a}}$(11)	78.6 ±10.8$^{\underline{A}}$(14)	19203 ±21374^{a}(11)	64583 ±21374^{A}(11)	3808 ±21374$^{\underline{a}}$(11)	74.2 ±12.6$^{\underline{A}}$(11)

Different letters (Tukey test, $p<0.05$) mean statistical difference.Comparisons between influents (lowercase), UASB1 (uppercase) and UASB2 (underline lowercase). (X) Number of samples for the calculation of the average.

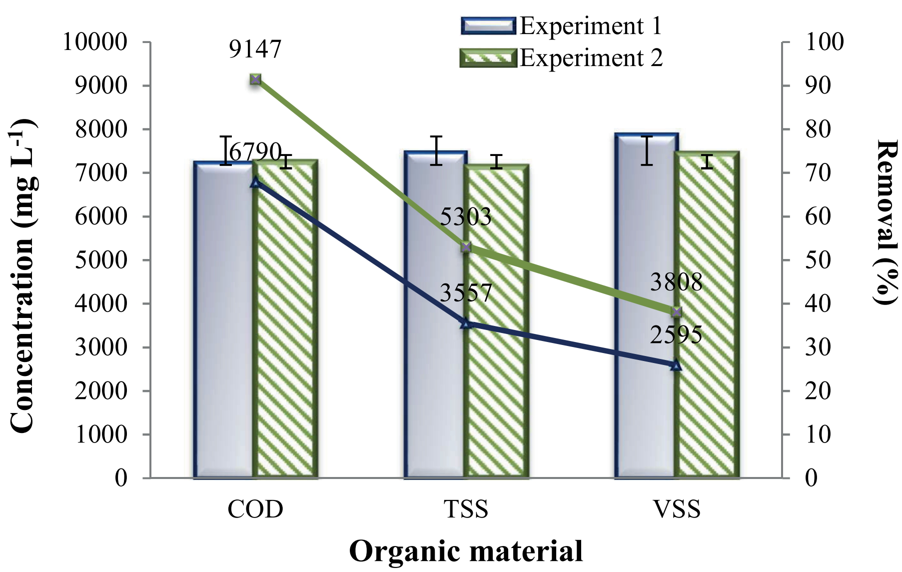

Figure 4. Performance of the two serial UASB reactors in the removal and organic materials (COD, TSS and VSS).

TSS values of 16568 and 23834 mg L^{-1} and VSS of 14040 and 19302 mg L^{-1} were obtained in the effluents for experiments 1 and 2, respectively, which indicates over 80% are volatile organic matter;therefore, they are more easily degraded and converted to methane. Approximately 66% of COD consists of suspended solids, which implies hydrolysis is the limiting phase for the supply of dissolved material available for the microorganism consumption during anaerobic digestion.

Under such substrate conditions, the COD, TSS and VSS removals were higher than 71% and showed no significant statistical difference between experiments 1 and 2. Therefore, either the reactor scale can be decreased 1.6 times, or the OLR can be increased up to 2.2 times without affecting the UASB reactor performance during the organic matter removal, as observed in experiment 2. UASB2 contributed to such efficiencies, as it retained the excess sludge washed out from UASB1 and improved the quality of the effluents. Therefore, the use of serial high-rate reactors is recommended for the treatment of highly concentrated substrates.

The DMW characteristics are highly variable, as the COD values range from 10220 to 55900 mg L^{-1}(Nikolaeva *et al.,* 2013, Rico *et al.,* 2011a). Such a variation depends on the number of animals per space unit, amount of water used for cleaning the barn, washing techniques and influent pretreatment.

The removals of COD by a treatment system composed of two serial UASB reactors and wetland achieved 86% and 81%, respectively, which demonstrates that high-rate reactors offer technological advantages, because they provide higher efficiencies with low HRT (7.5 days), in comparison to wetland (22.5 days) (Adhikari *et al.,* 2015; Rico *et al.,* 2011a).

The effluent from the two serial UASB system showed organic matter concentrations between 6790 and 9839 mg L^{-1} of COD and neutral pH (between 7.4 and 7.6), which could be explored for irrigation (Möller and Müller, 2012) or reduction in the water consumption during forage growth.

Characterization of dairy manure wastewater after anaerobic treatment

The characterization of the effluent from UASB reactors was evaluated according to important elements recognized as nutrients for the agricultural sector (Table 4 and Figure 5) that show the effluent can potentially fertilize plants. During experiment 2, the concentration of most nutrients was high, due to the superior solids application in the reactors (Sakar *et al.,* 2009).

The concentrations of macro- and micronutrients in the influent varied in comparison to other studies (Adhikari *et al.,* 2015; Page *et al.,* 2015; Rico *et al.,* 2011b), because of differences in the composition of the animal food, productivity, cattle age, management of waste from bedding and the cleaning technique of the confinement area (Cai*et al.,* 2013; Rico *et al.,* 2011b).The use of a raw substrate may have caused the differences in the nutrient concentrations in the effluents, as observed during experiments 1 and 2 and substrates with similar COD.

High concentrations of TKN and N-am, i.e., 642 and 1004 mg L^{-1} and 153 and 306 mg L^{-1}, respectively, are also present in DMW. The removals of TKN by the UASB1+ UASB2 system achieved 23.9% in experiment 1 and 49.1% in experiment 2. Nevertheless, the concentration of TKN increased in UASB1 due to sludge washout, the ammonification phenomenon and an increased concentration of N-am in the effluents, with values of 304 and 522 mg L^{-1} in experiments 1 and 2, respectively.

UASB2 removed 23.9% and 49.1% of TKN in experiments 1 and 2. Consequently, the effluent showed a higher nitrogen concentration in the soluble form of N-am, which could be interesting for plants, since the aim is the use of the effluent for fertigation (Prieto *et al.,* 2013). The nitrogen removal was justified by the minimal use of nitrogen for cellular biosynthesis during the anaerobic digestion (Möller and Müller, 2012).

TP showed importantremoval efficiency during the UASB systems operation, with values of 63.3% and 27.5%

Table 4. Removal of macro and micronutrients by two serial UASB reactors.

	Experiment 1				Experiment 2			
	Influent	Effluent UASB1	UASB2	Removal UASB1+ UASB2	Influent	Effluent UASB1	UASB2	Removal UASB1+ UASB2
	mg L^{-1}			(%)	mg L^{-1}			(%)
Macronutrients								
TKN	642.1 ±248.4^{a}(8)	754.7 ±234.2^{A}(9)	474.2 ±248.4$^{\underline{a}}$(8)	23.9 ±18.2$^{\Delta}$(8)	1004.0 ±314.2^{a}(5)	1238.5 ±314.2^{A}(5)	521.4 ±314.2$^{\underline{a}}$(5)	49.1 ±23.0$^{\Delta}$(5)
N-am.	153.5 ±45.9^{b}(16)	304.3 ±45.9^{B}(15)	356.5 ±45.9$^{\underline{a}}$(14)	N.R	306.5 ±53.6^{a}(10)	522.0 ±56.2^{A}(10)	402.8 ±53.6$^{\underline{a}}$(10)	N.R
TP	82.4 ±16.2^{a}(8)	85.9 ±15.3^{A}(9)	29.9 ±16.2$^{\underline{a}}$(8)	63.3 ±29.1$^{\Delta}$(8)	91.8 ±20.5^{a}(5)	77.8 ±20.5^{A}(5)	57.4 ±22.9$^{\underline{a}}$(4)	27.5 ±41.2$^{\Delta}$(4)
K	10.4 ±6.3^{a}(10)	11.3 ±6.7^{A}(9)	12.1 ±7.06$^{\underline{a}}$(8)	N.R	18.3 ±8.9^{a}(5)	23.1 ±8.9^{A}(5)	23.4 ±8.9$^{\underline{a}}$(5)	N.R
Ca	10.6 ±4.2^{a}(10)	12.4 ±4.4^{A}(9)	5.9 ±4.6$^{\underline{a}}$(8)	43.4 ±35.2$^{\Delta}$(8)	18.2 ±5.8^{a}(5)	16.5 ±5.8^{A}(5)	8.5 ±5.8$^{\underline{a}}$(5)	20.4 ±44.5$^{\Delta}$(5)
Mg	5.0 ±1.2^{a}(10)	5.6 ±1.2^{A}(9)	3.6 ±1.9$^{\underline{a}}$(8)	27.2 ±17.2$^{\Delta}$(8)	8.1 ±1.7^{a}(5)	7.0 ±1.7^{A}(5)	4.2 ±1.7$^{\underline{a}}$(5)	41.0 ±12.6$^{\Delta}$(5)
Na	186.6 ±58.8^{a}(10)	198.3 ±62.0^{A}(9)	174.2 ±65.8$^{\underline{a}}$(8)	N.R	164.0 ±83.2^{a}(5)	154.7 ±83.2^{A}(5)	202.5 ±83.2$^{\underline{a}}$(5)	N.R
Micronutrients								
Fe	77.0 ±50.1^{a}(10)	96.2 ±52.8^{A}(9)	21.7 ±56.0$^{\underline{a}}$(8)	69.2 ±62.6$^{\Delta}$(8)	160.2 ±70.8^{a}(5)	166.0 ±70.8^{A}(5)	68.5 ±70.8$^{\underline{a}}$(5)	N.R
Zn	8.3 ±17.1^{a}(10)	42.4 ±18.1^{A}(9)	2.6 ±19.2$^{\underline{a}}$(8)	46.8 ±180.4$^{\Delta}$(8)	15.4 ±24.2^{a}(5)	23.7 ±24.2^{A}(5)	13.0 ±24.2$^{\underline{a}}$(5)	N.R
Mn	11.2 ±3.2^{a}(10)	12.5 ±3.4^{A}(9)	6.2 ±3.6$^{\underline{a}}$(8)	43.9 ±31.9$^{\Delta}$(8)	15.2 ±4.5^{a}(5)	17.7 ±4.55^{A}(5)	10.0 ±4.5$^{\underline{a}}$(5)	N.R
Cu	1.6 ±2.2^{a}(10)	4.3 ±10.9^{A}(9)	0.6 ±2.3$^{\underline{a}}$(8)	58.9 ±135.9$^{\Delta}$(8)	2.2 ±2.9^{a}(5)	6.22 ±2.9^{A}(5)	3.3 ±3.3$^{\underline{a}}$(5)	N.R

Different letters (Tukey test, $p<0.05$) mean statistical difference.Comparisons between influents (lowercase), UASB1 (uppercase) and UASB2 (underline lowercase). (X) Number of samples for the calculation of the average.N.R: Not removal.

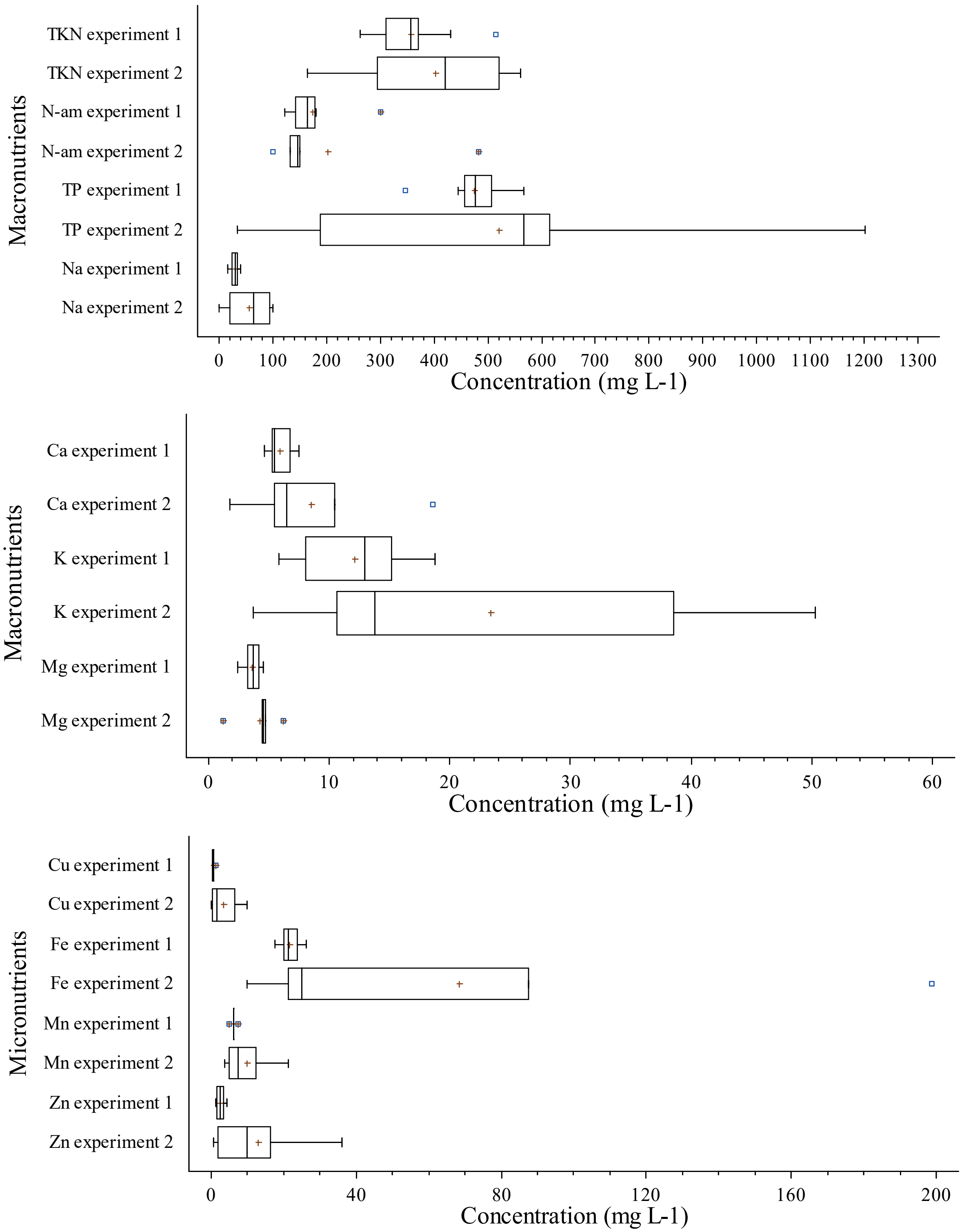

Figure 5. Macro- (TKN, N-am, TP, K, Ca, Mg and Na) and micronutrients (Fe, Zn, Mn and Cu) in the effluent of two serial UASB reactors.

in experiments 1 and 2, respectively, and could be used in microbial activities in reactors as a substrate for the syntheses of adenylates, nucleic acids and phospholipids (Möller and Müller, 2012).

Nitrogen, phosphorous and K are important nutrients that can improve soil productivity, therefore, the liquid and sludge effluent from the anaerobic reactors are a valuable source for the agricultural sector (Sakar et al., 2009).

K, Ca and Mg are also keycompounds for the anaerobic digestion of microorganisms. Their concentrations in the influent ranged between 3.6 and 23.4mg L^{-1} and their maximal removal was 43% in the serial UASB reactors (Figure 5). The nutrients removal may have been caused by the cellular synthesis and/or physical-chemical processes, such as precipitation and sludge adsorption (Möller and Müller, 2012). Consequently, their concentrations in the effluent did not exceed 23.4 mg L^{-1} (Table 4). Ca and Mg are required by crops in substantial amounts (Sakar *et al.,* 2009); therefore, the effluent could be reused as a source of such a nutrient.

The Na concentrations in the influent may have originated from animal food in the cattle production. Low or no removal of Na was obtained by the serial UASB reactors due to their low ability for nutrient removal from DMW in anaerobic processes. The high solubility of Na in the liquid prevents its sedimentation in the sludge and increases its concentrations in the effluent (Duda, 2010). Therefore, lower Na concentrations could be applied to animal food, because of the difficulty of removal from biological systems and potential negative impacts on the soil salinization during re-use in fertigation. As a consequence, a further treatment of the effluent is necessary for the removal of excess Na.

Fe, Zn, Mn and Cu were also present in the influent (Table 4), probably due to the diet, dietary supplements, and water consumed by animals, and eliminated as urine and feces (Sakar et al., 2009). For example, Cu and Zn are supplemented as trace metals in transition dairy cows, as promoters of the antioxidant and immunity system (Spears and Weiss, 2008). Some products such as Cu proteinate, Cu sulfate, Zn methionine, and Zn proteinate (Spears and Weiss, 2008) used for animal growth could be a source of metals in the manure. Their presence during the anaerobic digestion is important for the cellular growth, because they act as coenzymes or cofactors (Manyi-Loh *et al.,* 2013).

Fe was the nutrient of highest removal by the anaerobic system, probably due to the formation of phosphate ($Fe_3(PO_4)_2$), hydroxides ($Fe(OH)_2$) or carbonate ($FeCO_3$) retained in the sludge (Möller and Muller, 2012; Page *et al.,* 2015). However, no metal characterization in the sludge was performed for the confirmation of such a hypothesis.

Zn, Mn and Cu exert a positive effect on the anaerobic digestion, as they increase the methane production, substrate utilization, and reactor stability (Manyi-Loh *et al.,* 2013). However, the presence of nutrients may not be correlated with the methane biosynthesis, since both influents showed similar quantities of the aforementioned elements (Table 2 and 3). Low concentrations were detected in the effluent, which is a positive result, since metals such as Fe and Cu can accumulate in agricultural soils (Sheets *et al.,* 2015).

At least 30.8% of the nutrients quantified in the substrate of the system were found in the effluent and could be recycled (Figure 5). No significant difference was observed between the experiments, which proved HRT exerted no effect on the removal of the macro- and micronutrients. Similar results were reported by Castrillón *et al.* (2002) during the treatment of cattle manure in UASB reactors for HRT between 22.5 and 7.3 days. The authors concluded the metals removal was not related to the HRT. Fe was the micronutrient of higher concentration in cattle manure and the anaerobic system removed a minimal of 50.4% of this metal.

Experiment 2 showed no removal of K, Na, Fe, Zn and Cu, probably as a consequence of the sludge washing evidenced by the reduction of solids in some sludge sampling points of the reactor (data not shown). Sludge can accumulate nutrients and cations and be used as a potential substrate source for microbial activities and a stimulator in the sludge granulation process (Annachhatre and Bhamidimarri, 1992). Thereby, when the sludge is washed out from the reactors, the concentration of the nutrients in the liquid effluent increases.

Similar performances were reported by Ogejo and Li (2010), who studied the treatment of DMW in co-digestion with turkey processing wastewater in an attached growth digester. They observed small changes in the K, Ca, Mg, Zn, Cu, Mn and Na concentrations; however, such elements exerted no direct effect on the biogas production.

The Brazilian Regulation (Normative Instruction No.25 of July 23rd, 2009) allows the controlled application of effluents from animal wastes in soil and crops. The minimal concentrations in the fluid product of Ca, Mg, Cu, Mn and Zn for soil application are 5000, 5000, 500, 500, and 500 mg L^{-1}, and 3000, 3000, 500, 200 and 500 mg L^{-1} for foliar application or fertigation, respectively. A minimal soluble concentration in water for nitrogen is 10000 mg L^{-1}. All elements referenced by the Brazilian Regulation were observed in the effluent from serial UASB reactors, however, in inferior concentrations. Therefore, DMW can potentially be reused in fertigation and a higher OLR could be applied in the anaerobic systems for the achievement of the minimal nutrients concentrations required in fluid products.

The effluent of the two serial UASB systems and a commercial fertilizer, *NPK 04-14-08* (applied over forage *B. brizantha* used for cattle dairy nutrition) were compared. Only 70 L ha^{-1} of the effluent of experiment 2 are required for the same amount of nitrogen, phosphorous and potassium available for fertilizer *NPK 04-14-08*, with 571.4 kg ha^{-1} (Lima *et al.,* 2010). The UASB system tested could irrigate up to 3.4 hectares monthly and 41.4 hectares annually, which mean significant savings for farmers could be achieved by the setting-up of full scale reactors.

The two serial UASB system produces energy and an effluent from DMW containing macro and micronutrients and low concentrations of organic matter. The effluent could be a source of nutrients for fertigation. However, a post-treatment is required for the removal of sodium and microorganisms.

Sludge from serial UASB reactors during anaerobic treatment

The sludge from UASB reactors was evaluated by a VS/TS relation at different sampling points (Table 5), with values between 0.65 and 0.73 for experiments 1 and 2. TS and VS increased in experiment 2, probably due to the accumulation of solids in the reactor by the cell growth and precipitation of organic and inorganic compounds, as explained in previous sections.

UASB1 showed sludge washout during the operational period. In contrast, UASB2 was effective in retaining the excess sludge and providing superior effluent quality. The sludge retention in the second reactor sustains the importance of serial UASB reactors in the overall performance.

The reuse of the anaerobic sludge from the two serial UASB reactors was evaluated according to the Brazilian Regulation (CONAMA 375), which has established that sludge can be reused for agricultural purposes when the VS/TS relation is lower than 0.7. This value indicates organic matter stabilization and avoids high microbial activities when sludge is applied to soils (CONAMA, 2006). Both reactors produced organic fertilizers due to the active biomass present in the sludge adapted for the loading of organic shocks (Latif *et al.,* 2011).

Table 5. Solids in the anaerobic sludge of the serial UASB reactors.

	VS/TS			
	UASB1		UASB2	
Experiment **Sample points**	**1**	**2**	**1**	**2**
1	0.73±0.05^{a}(4)	0.69±0.03^{a}(2)	0.65±0.06^{a}(4)	0.71±0.07^{a}(2)
2	0.73±0.05^{A}(4)	0.69±0.08^{A}(2)	0.67±0.06^{A}(4)	0.71±0.07^{A}(2)
3	0.73±0.05$^{\underline{a}}$(4)	0.73±0.07$^{\underline{a}}$(2)	0.69±0.06$^{\underline{a}}$(4)	0.71±0.07$^{\underline{a}}$(2)
4	0.72±0.05$^{\underline{A}}$(4)	0.73±0.07$^{\underline{A}}$(2)	0.71±0.05$^{\underline{A}}$(4)	0.71±0.07$^{\underline{A}}$(2)

Different letters (Tukey test, p<0.05) mean statistical difference. Comparisons between reactor points 1 (uppercase) and 2 (lowercase) for UASB1 and UASB2 separately. (X) Number of samples for the calculation of the average.

CONCLUSIONS

Dairy manure wastewater treated in two serial UASB reactors showed a maximum bioenergy productivity of 0.73 L CH_4 L^{-1} d^{-1} and maximal methane yield of 0.19 L CH_4 g $COD_{rem.}^{-1}$ with superior methane production in UASB1. The methane yields were low in both reactors; therefore, superior performance could be achieved through the separation of the metabolic phases.

High removals of COD, SST and SSV between 71.2 and 78.6% produced at a 7.5 day HRT showed the capacity of the system to replace conventional systems, such as anaerobic biodigesters.

Nutrients, such as nitrogen, phosphorus, K, Ca, Mg, Na, Fe, Zn, Mn and Cu were found in the effluent of the serial UASB system. The content of nitrogen, phosphorous and K in the effluent showed potential for agricultural fertigation; however, the high Na concentration present in the DMW represents a risk for soil application.

Sludge from the UASB reactors showed appropriate characteristics for the soil fertilization, according to the Brazilian Regulation, and a potential by-product for application in forage plants.

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NOMENCLATURE

BA - Bicarbonate alkalinity, mg L^{-1}.
COD - Chemical oxygen demand, mg L^{-1}.
CSTR - Continuous Stirred Tank Reactors.
DMW - Dairy manure wastewater.
HRT - Hydraulic retention time, d.
IA - Intermediate alkalinity, mg L^{-1}.
N-am - Ammonia nitrogen, mg L^{-1}.
OLR - Organic load rate, g $COD_{total}L^{-1}$ d^{-1}.
PA - Partial alkalinity, mg L^{-1}.
TA -Total alkalinity, mg L^{-1}.
TKN - Total Kjeldahl nitrogen, mg L^{-1}.
TP - Total phosphorus, mg L^{-1}.
TS - Total solids, mg L^{-1}.
TSS - Total suspended solids, mg L^{-1}.
UASB - Upflow Anaerobic Sludge Blanket.
VFA - Volatile fatty acids, mg L^{-1}.

VS - Volatile solids, mg L^{-1}.

VSS - Volatile suspended solids, mg L^{-1}.

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