RESEARCH ARTICLE

Operation of a slow rate anaerobic digester treating municipal secondary sludge

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This study was designed to evaluate the performance of a slow rate anaerobic digester in treating secondary sewage sludge received from one local municipal wastewater treatment plant. The digester was fed by secondary sewage sludge without any previous thickening. A series of three independent batch experiments was investigated at an operation time of 60 days. The total solids (TS) in the influent sludge contained a percentage of organic matter of 59, 63 and 54%, a concentration of volatile suspended solids (VSS) of 23.7, 29.2 and 27.8 g L^{-1} and a chemical oxygen demand (COD) of 51.8, 32.9 and 65.7 g L^{-1} for the three experiments, respectively. The operation of anaerobic digestion was stable, with no noticeable scum or foaming problems. The COD reduction in each experiment reached 29, 21 and 45% in the sludge and 95, 85 and 82% in the supernatant. The microbial indicators were surveyed by sampling the sludge throughout the digester operation and counting the number of bacteria in the sampled sludge. Counted bacteria included the total culturable, the total and fecal coliform groups, *Pseudomonas aeruginosa* and fecal streptococci. The percentage removal of the indicator bacteria was higher for fecal streptococci (99.9%) than for coliform bacteria (96.3%), which in turn was higher than for *P. aeruginosa* (95.6%). Parasitological analysis was also performed on multiple sludge samples by determination of protozoa and helminth eggs. Protozoa (*Eimeria* and *Entamoeba*), helminth eggs (*Ascaris*, *Trichuris*, *Toxocara*, *Hymenolepis*) and mites were detected in the influent sludge, and particularly among the helminth eggs , only *Trichuris* was detected in the

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effluent sludge.

The treatment of municipal and industrial wastewater produces sludge, which either must be used for beneficial purposes or requires disposal (Malina, 1993). Sludge can be considered as an aqueous suspension of particles together with some compound salts (Gillium and Lecomte, 1999). Secondary sludge consists predominantly of excess biomass produced during the biological process (Ramalho, 1983). About half of the incoming organic pollution load is converted into secondary sludge, which contains 0.5 to 2% solids (Malina, 1993; Winkler, 1993). Since a large number of the enteric bacteria and viral pathogens presented in untreated sewage become associated with wastewater solids, many are not completely removed during sewage treatment processes and are merely transferred to wastewater sludge (Farrah and Bitton, 1983). The latter is further treated to convert it into a small volume of stable material with high solid content and low level of heavy metals, pathogens and persistent organic substances (Winkler, 1993). Anaerobic digestion processes are widely recognised as particularly suitable for highly polluted wastewater treatment and for the stabilisation of primary and secondary sludges (Genovesi et al. 1999).

There are few reports on anaerobic digestion of secondary sewage sludge yielded by up flow anaerobic sludge blanket reactors (UASB), because it is considered that sludge has achieved stabilisation inside the UASB reactors. However, the optimisation of UASB reactors allows sewage treatment at a low hydraulic retention time; therefore, the produced sludge may not achieve stabilisation, and it may even be necessary to include a supplemental stabilisation process out of the reactor (Fernandes, 1998).

Sewage sludge disposal is a serious worldwide problem. Because of increased environmental awareness and stringent environmental standards governing the disposal of sewage sludge (set by different environmental protection agencies), its utilisation in agricultural production has been gaining increasing interest and attention in recent years. It offers economic and nutrient recycling advantages over the traditional disposal options, such as incineration for dry sewage and sea disposal (Stone et al. 1998). Nevertheless, potential risks derived from the accumulation of heavy metals and organic compounds, as well as pathogen contamination, must be taken into consideration.

A nine-month sampling program was carried out in a RALF (anaerobic reactor of fluidised sludge) reactor, a variation of UASB reactor, located in a local urban wastewater treatment plant. The purpose of this program was to investigate the seasonal fluctuations on the physicochemical characteristics and on the levels of the pollution indicator bacteria in excess sludge yielded by the RALF reactor, as well as to investigate the sanitary efficiency of low rate anaerobic digestion as a supplemental stabilisation process. Thus, a total of three independent batch experiments, carried out with an anaerobic digester at an operation time of 60 days, was evaluated through physicochemical, microbiologic and parasitologic analysis. The indicators chosen included total culturable, the total and fecal coliform groups, *Pseudomonas aeruginosa* and fecal streptococci. Parasitological analysis was also performed on multiple sludge samples by determination of protozoa and helminth eggs. Furthermore, the viability of utilisation of sewage sludge in agricultural lands was analysed considering the environmental risks.

Materials and Methods

Influent sludge

Experiments were carried out with sewage sludge, without any previous thickening, obtained from one of the local municipal wastewater treatment plants (Maringá-Paraná, Brazil). The characterisation of influent sludge included all the parameters described in Analytical Methods. The total solids (TS) in the influent sludge contained a percentage of organic matter of 59, 63 and 54%, concentration of volatile suspended solids (VSS) of 23.7, 29.2 and 27.8 g L^{-1} and a chemical oxygen demand (COD) of 51.8, 32.9 and 65.7 g L^{-1} for the three experiments, respectively. Total coliforms were enumerated on the order of 10^4-10^5 MPN mL^{-1} and fecal coliforms on the order of 10^4 MPN mL^{-1} in the influent sludge. The wastewater treatment system consists of a screening/grit device for the removal of coarse solids followed by a RALF reactor. In the treatment sequence, in the wastewater treatment plant, the sewage sludge produced is spread on drying beds. These beds contain gravel for rapid drainage and are exposed to outdoor conditions. In each experiment, the sewage sludge was collected approximately every three to four months during the discharge of excess sludge from the RALF reactor, before draining and drying in the drying beds.

Schematic of sludge treatment process

Laboratory experiments were carried out in a 70-liter cylindrical anaerobic digester with a fixed cover, manufactured in polyvinyl chloride and without mixing and temperature control (Figure 1). A series of three independent batch experiments was performed over a period of nine months (Table 1), for an operation time of 60 days at room temperature. The produced biogas was collected by water displacement in a biogas collection unit, periodically measured, and later corrected to standard pressure and temperature (1atm and 0ºC).

Microbial enumeration

Total and fecal coliform bacteria were determined by multiple tube fermentation and enumerated by most-probable-number (MPN) estimates by using standard techniques (APHA, 1998). Total coliform bacteria were also determined in all experiments using the 3M Petrifim plates (3M Brazil, São Paulo) according to manufacturer's instructions. Samples of 1 mL of homogenised sludge and dilutions of these in saline solution (0.85%) were spread on

3M Petrifim plates. Numbers reported are an average of three replicate plates.

Estimation of culturable bacteria in influent sludge, as well as measuring changes during sludge treatment, followed the pour plate method (APHA, 1998). Samples of 0.1 mL of homogenised sludge and their dilutions in saline solution (0.85%) were spread on agar plates. Media for bacterial analyses were obtained from Difco Laboratories (Detroit, MI) or from BBL Microbiology Systems (Cockeysville, MD). The solid media employed and the bacteria enumerated were as follows: Endo, coliform bacteria; KF, fecal streptococci; Cetrimide, Pseudomonas; Nutrient Agar, total culturable. All plates were incubated at 37ºC for 24-48 h. The plates of KF were incubated inside the anaerobe jar (BBL Microbiology Systems). All colonies on plates were counted soon after incubation period. Numbers reported are an average of two replica plates.

Colonies were classified according to their cellular morphologies by brightfield microscopy of Gram-stained preparations. BBL Crystal Identification Systems (Becton Dickinson) for the detection of enteric bacteria/non fermenters/gram-negative were used according to manufacturer's instructions. The isolated colonies from the Cetrimide medium were also investigated by using the antibiotic resistance test, according to method of Acar and Goldstein, 1996.

Parasites eggs detection

Parasites examinations in multiple sewage sludge samples were also made for determination of protozoa and helminth, and consisted in concentration and counting according to method of Faust et al., method of Ritchie and method of Hoffman et al. described by Rocha, 2000, and Brug's modified Baermann's method, described by Golvan, 1977.

Analytical methods

Measurements of total suspended solids (TSS), fixed suspended solids (FSS) and volatile suspended solids (VSS), chemical oxygen demand (COD), oils and greases and pH in sewage sludge and supernatant followed Standard Methods (APHA, 1998). Nitrogen (Kjeldahl) was measured according to Adolfo Lutz Institute Analytical Norms (Normas Analíticas do Instituto Adolfo Lutz, 1985). Protein was measured according to the method of Lowry, described by Tavares et al. 1995, using bovine serum albumin as protein standard. Volatile acids (VA) and alkalinity (AL) were measured according to the method of Silva, described by Paixão et al. 2000. Humidity, total organic matter and organic carbon were measured according to the ignition method described by Kiehl, 1985. For the measurement of metals (Cu, Zn, Fe and Cr) and nutrients (Mg, Ca, K, P and Mn), the samples were digested according to the nitric-percloric methodology (Malavolta et al. 1997). With the exception of K and P, the readings of the elements were accomplished by using an atomic absorption spectrometer GBC 932 AA (Scientific

Equipment PTY). Potassium was measured by emission flame photometry and total phosphorous was measured through the metavanadate colorimetric method (Malavolta et al. 1997). Biogas composition was monitored by gas chromatography on a Varian 1420 chromatograph (Porapak Q column, 2m, 1/8 in, 50ºC, carrier gas helium at a flow rate of 27 mL min^{-1}).

Results and Discussion

Monitoring of digester

Characteristics of sludges in the three experiments are reported in Table 2. Initial concentration of oils and greases in the sewage sludge was 196, 120 and 180 mg L^{-1} in each experiment, respectively. In general, the excess sludge discharged by the RALF presented seasonal characteristics because of variations in incoming wastewater and variations in the performance of the treatment process. The influent total solids concentration ranged from 5 to 8%, of which an average of 59% was organic matter. Influent pH, TSS, VSS, protein and COD concentration in the influent sludge varied, respectively, from 7.2 to 7.5, 37.9 to 56.3 g L^{-1}, 23.7 to 29.2 g L^{-1}, 9.8 to 18.7 g L^{-1} and 32.9 to 65.7 g L^{-1}. Metal concentrations of influent sludge also presented variations, especially for chromium and iron that ranged from 0.04 to 0.1 g Kg^{-1} and from 39.0 to 63.3 g Kg^{-1}, respectively. Characteristics of the supernatant formed in each experiment are reported in Table 3.

The batch experiments were operated at an average room temperature throughout the anaerobic digestion of 23.1, 24.6 and 25.2ºC (daily average temperature basis), respectively. Because of technical problems, the VSS content was not measured during the first experiment. Removal efficiency of VSS reached 43 and 20% in sludge and 98 and 81% in the supernatant of the second and third experiments, respectively. This different reduction in VSS achieved in the supernatant was probably because of the sedimentation of suspended solids. The efficiency of protein removal in sludge reached 25, 28 and 27% in each experiment. The protein reduction achieved in all experiments may have occurred due to the reduction of microbial cells in the sludge. COD reduction reached 29, 21, and 45% in the sludge and 95, 85, and 82% in the supernatant in each experiment, respectively. This low COD reduction was probably because of two main reasons: firstly, the organic matter is transformed into other compounds such as organic acids that increases soluble COD; secondly, the stabilised sludge is transformed into mineralised organic matter that also contributes to COD. The higher COD reduction achieved in the supernatant in all experiments was because of both the digestion of organic matter and the sedimentation of most suspended solids, thus obtaining a final liquid with lower COD concentration and, consequently, lower BOD. These results show that organic matter both in the sludge and in the supernatant was digested, sensibly decreasing the pollutant load.

The ratio of volatile acids to alkalinity (VA/AL) represents a useful and sensitive monitoring tool of digestion, which increases rapidly after a process upset and then decreases with recovery (Ripley et al. 1986). By maintaining a constant VA/AL below 0.25, the buffering capacity of the system can be maintained (WPCF, 1985). VA/AL average ratios in each experiment were 0.16, 0.20 and 0.13, respectively, showing that digestion developed regularly (data not shown).

Daily biogas production measured at standard temperature and pressure and room temperature during sample collection is shown in Figure 2, Figure 3 and Figure 4 for each experiment. In the first and second experiments, biogas had an average composition of 87% methane and 13% carbon dioxide. These results may indicate the dissolution of carbon dioxide in the water of the biogas collection unit. During the third experiment, biogas was collected by water displacement in acidified water (pH<2) to minimize dissolution of carbon dioxide. In this experiment, biogas presented an average composition of 74% methane and 26% carbon dioxide. Such results are in agreement with Chernicharo, 1997 who reported that the typical proportions of methane and carbon dioxide in biogas from anaerobic digestion of domestic wastewaters are 70 to 80% and 20 to 30% respectively. It was observed that a drop in biogas production occurred in those days in which temperature decreased. The ratio of biogas production to influent volume was 1.1, 0.5 and 0.3 (L L^{-1}) for each experiment, respectively. Results also showed that 1 g of influent VSS produced an average of 0.05, 0.02 and 0.01 L digestion gas for the three experiments, respectively.

Fecal contamination indicators

Wastewater sludge may harbor a wide range of microbial pathogens and parasites, which present potential health hazards to humans. Since anaerobic digestion is widely used, most researchers have studied survival of enteric pathogenic bacteria under anaerobic conditions (Carrington, 1991; Gantzer et al. 2001). In general, anaerobic treatment can reduce the number of pathogens, but usually it does not completely eliminate them.

Figure 5 shows the total and fecal coliform groups enumerated in each batch experiment, throughout the digester operation, based on MPN procedure. Total coliforms in the digester ranged from 10^4 to 10^5 MPN mL^{-1} in the influent sludge and from 10^4 to 10^3 MPN mL^{-1} in the effluent sludge, with an average reduction of 90.2%. Fecal coliforms on the order of 10^4 MPN mL^{-1} were enumerated in the influent sludge and on the order of 10^0 MPN mL^{-1} were enumerated in the effluent sludge, with an average reduction of 99.9%. Fecal coliform bacteria levels in the digester effluent sludge was small and no bacterial growth was observed at the minor dilution (10^{-1}), which may indicate numbers < 10 MPN/100 mL. The reduced number of fecal coliform bacteria in the digester effluent may indicate that the system was working efficiently, with a

significant reduction of pathogens in the sludge. Similar results were obtained by using colony counts on solid media for total and fecal coliforms and Petrifim plates for total coliforms (data not shown).

Many investigations have shown that in biological wastewater treatment process there are seasonal fluctuations in the levels of pollution indicator bacteria (Monfort and Baleux, 1991; Bahlaoui et al. 1997). However, as indicative of stability, the number of organisms obtained in sewage sludge discharged by RALF reactor varied less than 2 orders of magnitude during the period of study. Furthermore, the percentage of removal of pollution-indicator bacteria was always higher for fecal coliform than for total coliform. This agrees with results obtained by Farrah and Bitton, 1983, who observed, under anaerobic digestion of sludge at 28ºC, that the values for daily change in *S. typhimurium*, *S. faecalis* and *E. coli* did not exhibit large temporal variability (0.6 to 0.9 $\log_{10}$ L^{-1}). Thus, total culturable, coliform bacteria, P. *aeruginosa* and fecal streptococci were determined on the third experiment (Figure 6) as an additional indicator of fecal contamination. The numbers are based on colony counts on solid media (CFU g^{-1}).

Total culturable in the digester was enumerated at the order of 10^7 MPN mL^{-1} in the influent sludge and 10^5 MPN mL^{-1} in the effluent sludge, reaching a reduction of 95.2%. Coliform bacteria in the digester were on the order of 10^6 MPN mL^{-1} in the influent sludge and 10^5 MPN mL^{-1} in the effluent sludge, reaching a reduction of 96.3%. Isolated colonies from Cetrimide plates were Gram stained, and the oxidase test was carried out. All colonies tested were gram-negative, oxidase-negative bacilli, identified as *Pseudomonas aeruginosa* by the BBL Crystal Identification Systems (Becton Dickinson). *P. aeruginosa* in the digester was enumerated at the order of 10^5 MPN mL^{-1} in the influent sludge and 10^4 MPN mL^{-1} in the effluent sludge, reaching a reduction of 95.6%. Fecal streptococci in the digester were on the order of 10^4 MPN mL^{-1} in the influent sludge and 10^0MPN mL^{-1} in the effluent sludge, reaching a reduction of 99.9%. The average bacterial concentration in the effluent sludge was always lower than in the influent sludge. The indicator bacteria removal percentage was higher for fecal streptococci than for coliform bacteria, which in turn was higher than for *Pseudomonas*.

Antibiotic resistance of enteric bacteria

Previous studies have shown that waste effluents from hospitals contain higher levels of antibiotic-resistant enteric bacteria than waste effluents derived from other sources (Guardabassi et al. 1998). It is generally agreed that the selection and dissemination of resistant bacteria in nature should be avoided, in order to ensure effective treatment against infectious disease in human and to maintain an ecological balance which favours predominance of a susceptible bacterial biota in nature.

In this study, *Pseudomonas aeruginosa* was used as a pathogenic bacteria indicator of antibiotic resistance in the sewage sludge. *P. aeruginosa* was chosen as the target bacterium, due to their extreme nutritional versatility. In comparison to levels of antibiotic resistance reported in literature for clinical isolates (Murray, 1996), *P. aeruginosa* isolated from sludge was generally more susceptible to antibiotic agents (data not shown).

Parasites eggs

Considering the option of using sludge for agricultural purposes, it is of special importance to take into account the presence of parasite eggs because they are extremely resistant to most of the treatments used to stabilise effluents, and many epidemiological studies for humans reveal a significant health risk with nematode eggs (Gaspard et al. 1997). Parasitological analysis was performed on multiple sewage sludge samples for determination of protozoa and helminth eggs (Table 4). Two genera of enteric protozoa, *Eimeria,* and *Entamoeba* were observed in the influent sludge samples. Of these genera, only *Eimeria* was present in the digester effluent sludge.

Coccidiosis, caused by eimerian parasites, produces enteritis of varying severity depending on the species of parasite and on the infectious dose. The disease detrimentally affects meat and milk production by disrupting nutrient absorption from the intestinal tract of domestic agricultural animals (Schito and Barta, 1997).

Regarding to human health risks, the four most important genera found in sludge were *Ascaris, Trichuris, Hymenolepis,* and *Toxocara* (Table 4). Soil-transmitted helminth infections (*i.e. Ascaris lumbricoides, Trichuris trichiura,* and hookworm), estimated to affect approximately one thousand million persons, represents a major public health problem in poor and developing countries (Scolari et al. 2000). Except for *Trichuris*, the parasitological examinations in effluent sludge were all negative. In addition, microscopic examination of sewage sludge, carried out throughout anaerobic digestion, demonstrated the presence of mites' eggs. In the indoor environment, mites and fungi are two of the most important causes of asthma and rhinitis in people.

Agronomic parameters

From an agricultural point of view, the use of sewage sludge as fertilizer has restricted applications based on heavy metal content. Such limitations were published in the European Directive 86/278/EEC (Council of the European Communities, 1986). Table 2 shows that none of the heavy metals measured was over the maximum established limits.

The agricultural value of sludge mainly derives from its nutrient content. Sludge, like other organic fertilizers, has long-term beneficial effects on the soil: organic matter contained in sewage sludge improves the physical properties of soil such as aggregate stability, water retention and infiltration, and reduce soil compactibility (Stone et al. 1998). In addition to nutritious content, the organic matter and the C/N ratio are important parameters of the sludge fertilizing potential. Table 2 shows that this sludge had a high content of organic matter and nitrogen, with a neutral pH, and moderate levels of phosphorus and potassium. The sludge C/N ratio determined in this work, which reflects the value of sludge as a nitrogen supplier, is lower in sludge (9 to 10) than in low-urine cow manure (12 to 17) and plant residues (30 to 40), as presented by Strauss, 1985. Therefore, this sludge has fertilizing potential despite the need for complementation with mineral sources due to their unbalanced nutritious content.

In Paraná, Brazil, the state sanitation company classifies the sludge according to the American CFR Part 503 regulation and specifies class A sludge (10^3/g dry wt basis) for urban sludge spreading on agricultural land (Andreoli et al. 1999). The presented data show that the fecal coliforms in influent sludge must be reduced to below detectable levels to reach the land-use criteria. Therefore, the effluent sludge can fulfil the criteria for category A sludge, especially if the entire process, such as the anaerobically digested sludge analyses after being air-dried on drying beds, has been considered.

Concluding Remarks

Municipal sewage sludges are routinely utilised on agricultural lands in various parts of the world. Land application is likely to remain as a major option for the future, particularly for smaller plants that are closer to disposal sites. In the state of Paraná-Brazil, whose economy is essentially based on agriculture, it is vital to consider the sludge application to agricultural land. For this option, the presence of pathogens, heavy metals and organic contaminants is important. Applying the anaerobic technology properly, the risk from pathogens is reduced. The results show that the number of indicator bacteria substantially decreases when the sludge is submitted to anaerobic digestion. Parasitological analysis performed on multiple sewage sludge samples for determination of protozoa and helminth eggs also showed that among numerous microorganisms detected in the influent sludge, only *Trichuris* was detected in the effluent sludge. In addition, *P. aeruginosa* isolated from sludge presented a level of antibiotic resistance lower than the ones reported in literature for clinical isolates, thus representing no risk to bacterial biota in nature. Regarding to the heavy metal content, it is not a limiting factor in the use of this sludge as a soil conditioner. However, the phosphorus and potassium content of sludges may be too low to satisfy specific plant uptake requirements in some land application systems.

Analysis of anaerobically digested sludge after being air-dried on drying beds would be helpful in the viability of land application of treated sludge. Based on this data,

criteria for estimating pollution mobilisation capacity and behaviour in the environment could be developed. However, considerable progress has been achieved in this direction by providing information concerning the efficiency of anaerobic digestion of secondary sludge in decreasing the bacteria population and the determination of possible pollution levels for land application of the treated sludge.

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APPENDIX

Tables

Table 1. Period of work for the three independent experiments performed at operation time 60 days.

Experiment	Period of work
I	July-September/2000
II	October-December/2000
III	February-April/2001

Table 2. Characteristics of sludges in each experiment.

Experiment	I		II		III	
Characteristic	Initial	Final	Initial	Final	Initial	Final
pH	7.2	7.3	7.5	7.1	7.4	7.0
TSS (g L^{-1})	37.9	--	46.4	39.2	56.3	36.7
FSS (g L^{-1})	14.2	--	17.2	22.6	28.5	14.5
VSS (g L^{-1})	23.7	--	29.2	16.6	27.8	22.2
COD (g L^{-1})	51.8	36.6	32.9	25.9	65.7	36.3
Protein (g L^{-1})	18.7	14.0	9.8	7.1	13.5	9.9
Alkalinity (mg CaCO$_3$ L^{-1})	508	1,076	442	762	781	1,171
Volatile Acid (mg CH$_3$COOH L^{-1})	126	210	109	118	130	188
Humidity (% w/w)	93	91	95	91	92	90
Total Organic Carbon (% w/w)	33	19	35	30	30	32
Total Organic Matter (% w/w)	59	59	63	54	54	57
Cu (g Kg^{-1})	0.4	0.4	0.3	0.3	0.3	0.3
Zn (g Kg^{-1})	1.8	1.7	2.0	2.2	0.4	0.6
Fe (g Kg^{-1})	39.0	34.7	48.7	54.4	63.3	61.6
Cr (g Kg^{-1})	0.1	0.1	0.1	0.1	0.04	0.03
Nitrogen (Kjeldahl) (% w/w)	3.5	3.2	3.6	3.4	3.3	3.1
C/N	9.3	10.1	9.6	8.9	9.2	10.1
P (g Kg^{-1})	8.1	7.6	6.7	7.0	7.1	7.0
K (g Kg^{-1})	1.0	1.0	1.3	1.0	1.0	1.2
Ca (g Kg^{-1})	20.6	22.4	15.5	14.7	21.0	17.2
Mg (g Kg^{-1})	4.1	4.6	3.0	2.9	3.7	3.1
Mn (g Kg^{-1})	0.2	0.2	0.6	0.6	0.3	0.3

Table 3. Characteristics of supernatant in each experiment.

Experiment	I		II		III	
Supernatant	Initial*	Final	Initial*	Final	Initial*	Final
PH	7.3	7.3	7.5	7.0	7.6	7.2
TSS (mg L^{-1})	1,370	–	2,925	30	2,030	880
FSS (mg L^{-1})	410	–	1,215	0	830	270
VSS (mg L^{-1})	960	–	1,710	30	1,200	610
COD (mg L^{-1})	9,720	434	1,203	180	4,259	746

*supernatant formed at the 5th day

Table 4. Microorganisms groups observed in sewage sludge samples throughout the anaerobic digestion.

Microorganisms groups	Operation time (d)			
	0	20	40	60
Protozoa				
Eimeria spp.	+	+	+	+
Entamoeba spp.	+	-	-	-
Helminth				
Ascaris spp.	+	+	-	-
Trichuris spp.	+	-	-	+
Toxocara spp.	+	+	+	-
Hymenolepis spp.	-	+	-	-
Mites	++	++	+	+

(-) absence of microorganisms.
Protozoa: (+) 1 to 5 cysts or oocysts per slide.
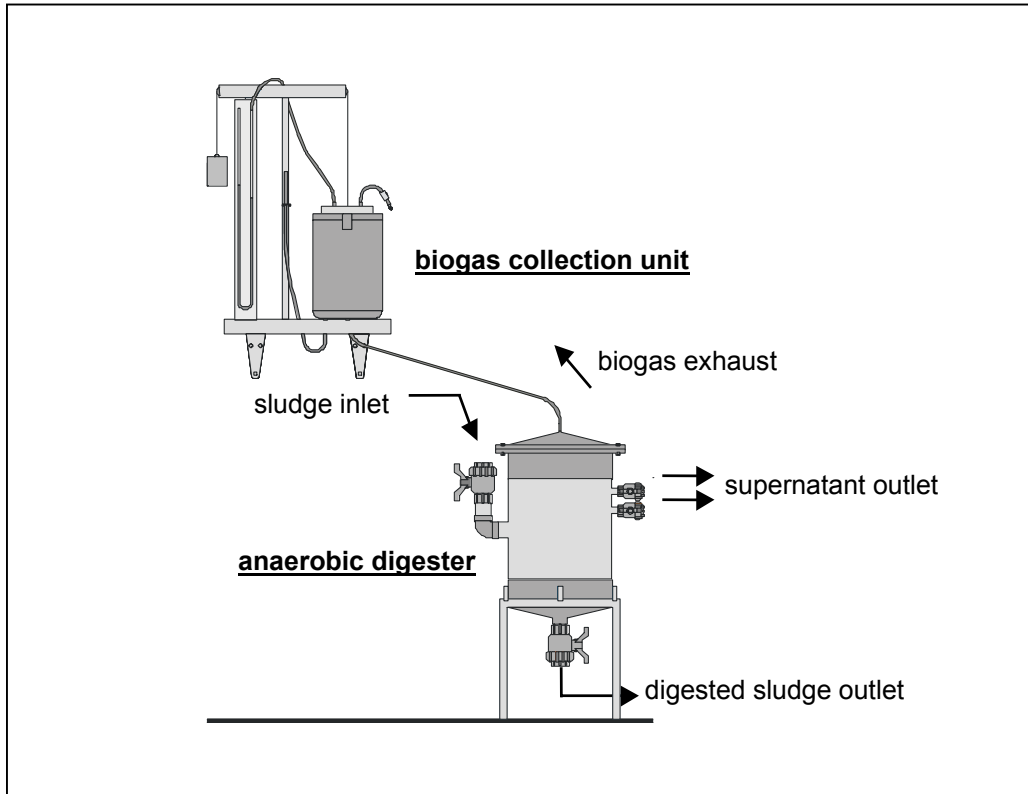Helminth: (+) 1 to 2 eggs per slide, (++) 3 to 10 eggs per slide.

Figures

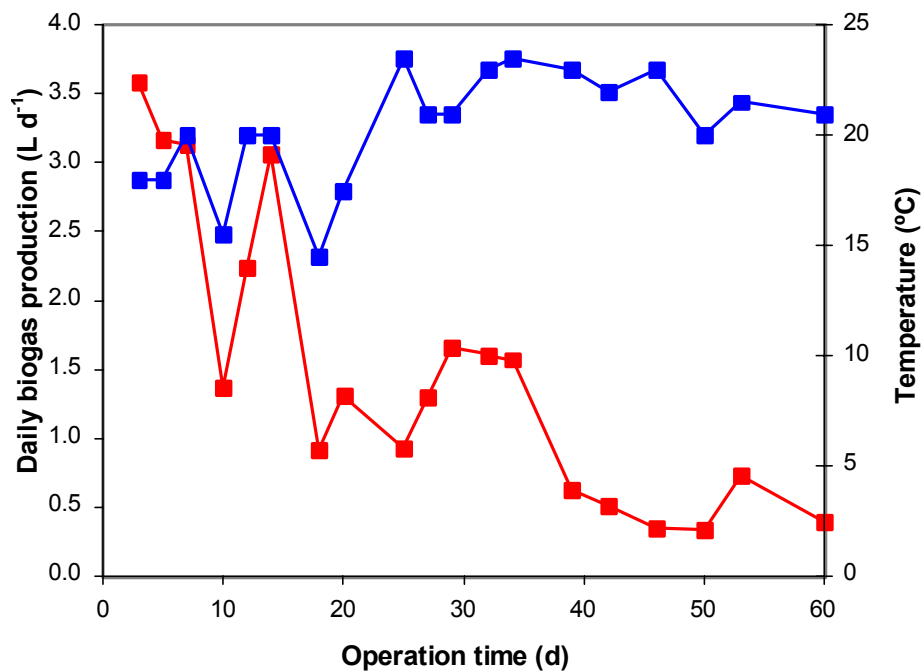**Figure 1. Schematic diagram of the experimental system.**

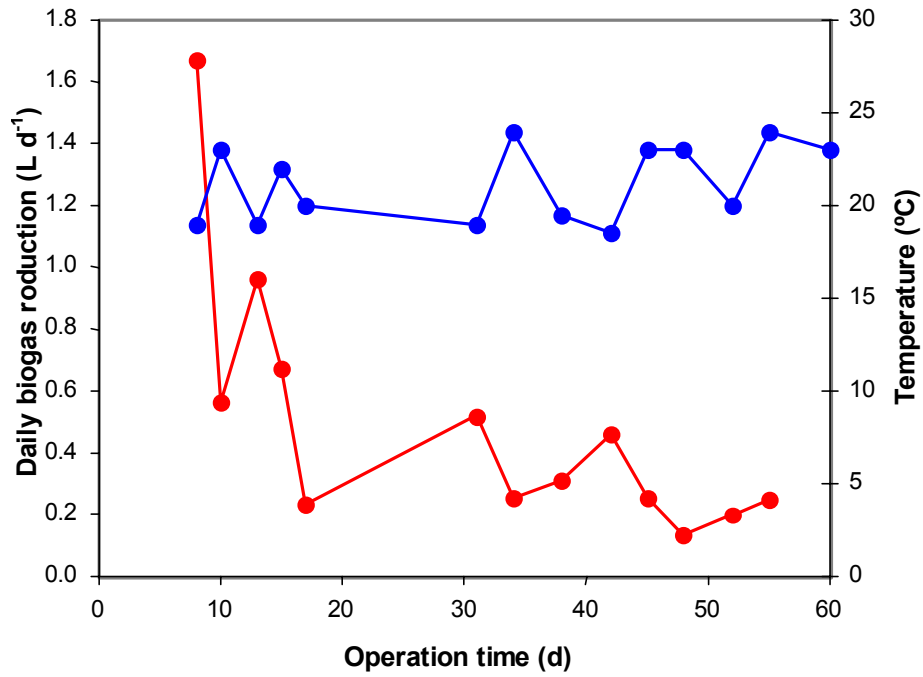**Figure 2. Biogas production (■) and room temperature at the moment of sampling (■) in experiment 1.**

Figure 3. Biogas production (●) and room temperature at the moment of sampling (●) in experiment 2.
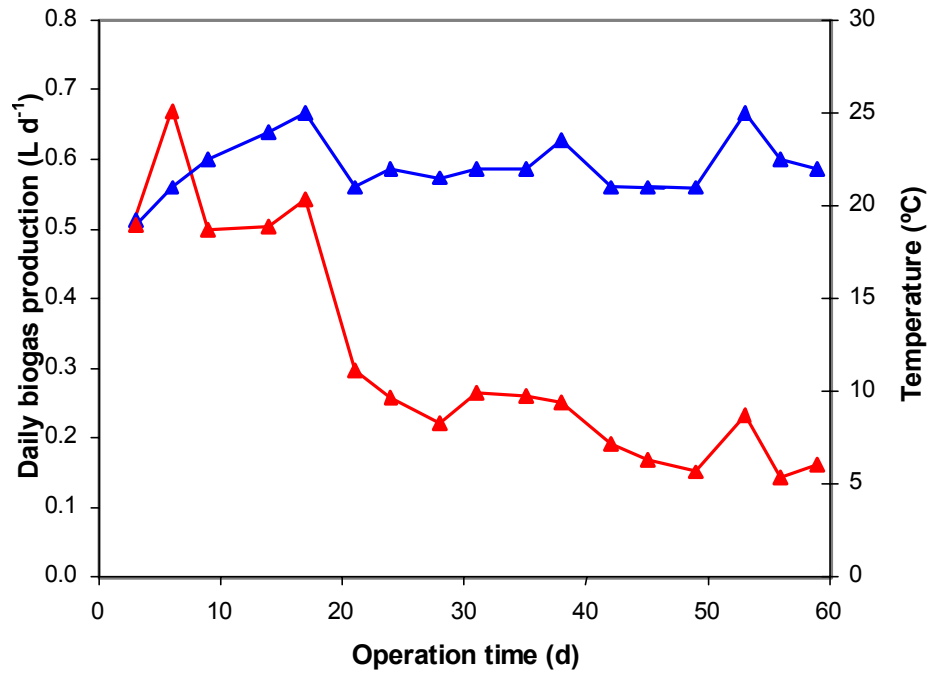

Figure 4. Biogas production (▲) and room temperature at the moment of sampling (▲) in experiment 3.
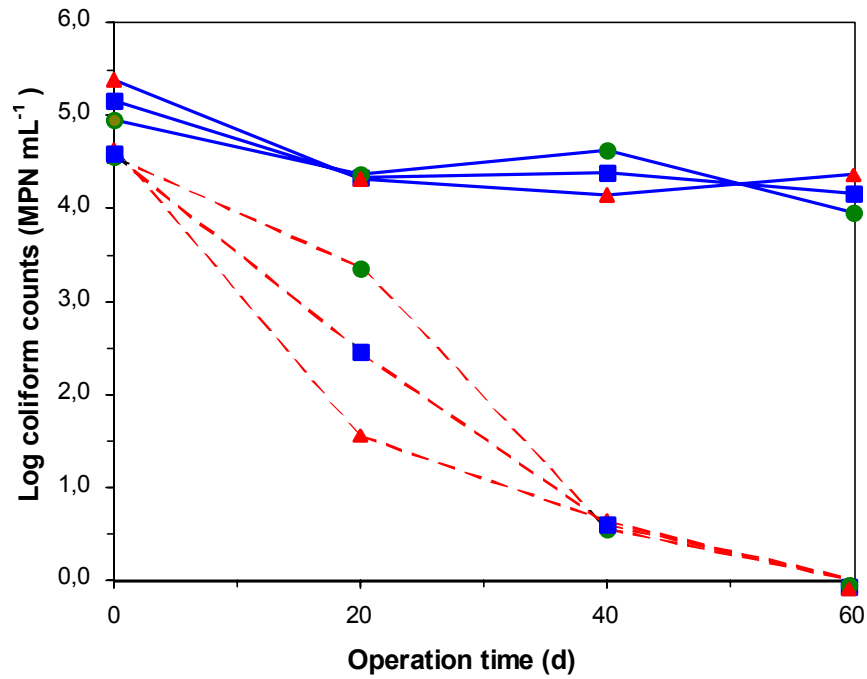
Figure 5. Total (——) and fecal (------) coliform groups in sewage sludge based on most-probable number: experiment 1 (■); experiment 2 (●); experiment 3 (▲).
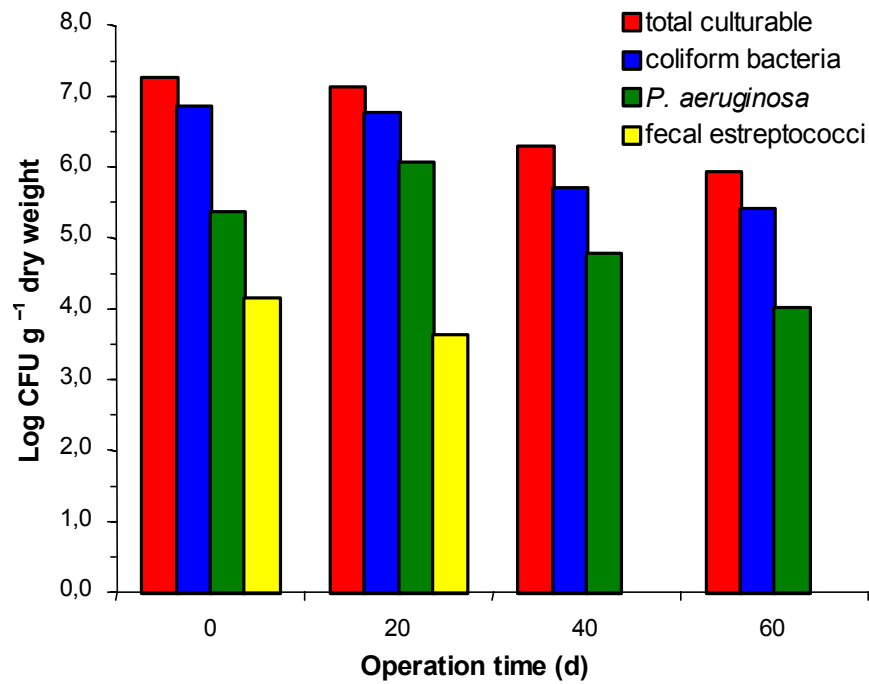

Figure 6. Different bacterial types counting of the experimental system along 60 d operating.