

# Two-Step Upflow Anaerobic Sludge Bed System for Sewage Treatment Under Subtropical Conditions with Posttreatment in Waste Stabilization Ponds

LUCAS SEGHEZZO,<sup>\*,1</sup> ANÍBAL P. TRUPIANO,<sup>1</sup> VIVIANA LIBERAL,<sup>1</sup>  
PATRICK G. TODD,<sup>2</sup> MARÍA E. FIGUEROA,<sup>1</sup>  
MARCELO A. GUTIÉRREZ,<sup>1</sup> ANA C. DA SILVA WILCHES,<sup>1</sup>  
MARTÍN IRIBARNEGARAY,<sup>1</sup> RAQUEL G. GUERRA,<sup>1</sup>  
ANGÉLICA ARENA,<sup>1</sup> CARLOS M. CUEVAS,<sup>1</sup> GRIETJE ZEEMAN,<sup>2</sup>  
AND GATZE LETTINGA<sup>2</sup>

<sup>1</sup>Universidad Nacional de Salta, Laboratorio de Estudios Ambientales (CIUNSa-INENCO), Buenos Aires 177, A4402FDC Salta, Argentina, E-mail: lucas@unsa.edu.ar; and <sup>2</sup>Department of Agrotechnology and Food Sciences, Sub-Department of Environmental Technology, Wageningen University, Bomenweg 2, 6703 EV Wageningen, The Netherlands

## Abstract

A pilot-scale sewage treatment system consisting of two upflow anaerobic sludge bed (UASB) reactors followed by five waste stabilization ponds (WSPs) in series was studied under subtropical conditions. The first UASB reactor started up in only 1 mo (stable operation, high chemical oxygen demand [COD] removal efficiency, low volatile fatty acids concentration in the effluent, alkalinity ratio above 0.7, biogas production above 0.1 Nm<sup>3</sup>/kg of COD<sub>removed</sub>). Removal efficiencies up to 90% were obtained in the anaerobic steps at a hydraulic retention time of 6 + 4 h (80% removal in the first step). Fecal coliform removal in the whole system was 99.9999% (99.94% in anaerobic steps and 99.98% in WSPs). COD balances over UASB reactors are provided. A minimum set of data necessary to build COD balances is proposed. Intermittent sludge washout was detected in the reactors with the COD balances. Sludge washout from single-step UASB reactors should be monitored and minimized in order to ensure constant compliance with discharge standards, especially when no posttreatment is provided. The system combined

\*Author to whom all correspondence and reprint requests should be addressed.

high COD and fecal coliform removal efficiency with an extremely low effluent concentration, complying with discharge standards, and making it an attractive option for sewage treatment in subtropical regions.

**Index Entries:** Anaerobic treatment; sewage; subtropical regions; upflow anaerobic sludge bed reactors; waste stabilization ponds.

## Introduction

The full-scale application of upflow anaerobic sludge bed (UASB) reactors for sewage treatment at moderate temperatures seems within reach (1–3). In temperate and subtropical climates, low hydrolysis of suspended solids (SS), bad sludge quality, and lower removal efficiencies are expected, particularly at temperatures below 20°C (2). Presettling of sewage, two-stage systems, and hybrid reactors have been proposed to improve the retention and degradation of SS under these conditions (2,4,5). In tropical countries, UASB reactors treating sewage showed chemical oxygen demand (COD) removal efficiencies of about 65%, with some reports of up to 80% in underloaded reactors (6). Hydraulic retention time (HRT) applied fluctuates around 6 h, aiming at an upflow velocity ( $V_{up}$ ) of about 0.75 m/h, according to reactor height. At lower temperatures, reported results differ widely, depending on factors such as environmental conditions, sewage concentration, operational parameters, type and dimensions of the reactor, amount and quality of the inoculum (1). COD removals of up to 79% were reported at 21°C and 9-h HRT (7). A similar efficiency was achieved at 20°C and 8-h HRT, using anaerobic granular sludge as inoculum (8). A COD removal efficiency of 67% was reported in a laboratory UASB reactor inoculated with highly active granular sludge, when treating raw sewage at 13°C (2). Low-strength presettled sewage was also efficiently treated in a UASB reactor at a mean sewage temperature of 21.6°C, with short periods at about 13°C (9). Similar results were reported in refs. 2 and 5. In a two-stage anaerobic system treating sewage at 17°C, overall COD removal efficiency achieved was 69% (5). Specific two-step configurations yielded COD removal efficiencies similar to values found in tropical areas (2). Analysis of data from several works reviewed by Seghezzo et al. (1) indicates that average COD removal efficiencies of 41.7, 52.8, and 69.1% have been observed at temperatures below 15°C, between 15 and 22°C, and above 22°C, respectively. It is accepted that removal efficiency decreases at lower temperatures (4). Based on data in the literature, an interesting relationship among temperature, COD removal efficiency, and HRT was obtained (10).

COD balances over UASB reactors might be a useful tool to gain insight into the flow of organic matter through the reactor, assess the process performance, validate methods and assumptions, and predict outputs. A COD balance is based on the fact that if a constant flow and load is applied, and organic matter does not accumulate in the treatment system (steady-state conditions), the daily mass of influent COD is equal to the sum of the daily mass of COD leaving the system in one of several possible forms (methane,

excess sludge, effluent COD, among others). van Haandel and Lettinga (4) proposed a basis for the construction of COD balances. Reports of COD balances in UASB reactors treating sewage have been scarce (11). However, some investigators have provided information about their systems that could lead to the formulation of COD balances (2,5). No references have been found for coupled COD balances in two-step UASB systems. A standardized methodology to build COD balances over UASB reactors is still lacking.

The effluent of anaerobic reactors has to be further treated in order to remove nitrogen and phosphorus (when reuse is not possible) and fecal coliforms, the most commonly used indicator of pathogenic microorganisms. Waste stabilization ponds (WSPs) are being studied as a posttreatment method for anaerobically treated sewage because they are among the most efficient and cost-effective methods (4,12). Sludge washed out from a UASB reactor was effectively trapped in a polishing pond, but the sludge growth in the bottom of the pond was so low (including dead, settled algae) that desludging of the pond was deemed not necessary during its useful life-span (13). Therefore, an additional advantage of WSPs is that sludge discharges from UASB reactors (a major factor in operational costs) can be reduced or even eliminated. Local kinetic constants are necessary to accurately design WSPs, although most of the WSPs in northern Argentina have been designed using extrapolations, adaptations, or regional constants (14).

The objectives of the present study were to assess the startup and subsequent operation of a two-step UASB system for sewage treatment under subtropical conditions, followed by five WSPs in series for posttreatment, and to build and contribute to the standardization of COD balances over UASB reactors.

## Materials and Methods

### *Reactor Operation*

Experiments were performed in a pilot plant located in Salta, Argentina (24° 51' latitude south, 65° 29' longitude west, 1187 m above sea level). Mean ambient temperature, measured over a 22-yr period (1971–1992) at a meteorological station 12 km away from the site, was  $16.5 \pm 0.2^\circ\text{C}$  (15). Statistical comparisons and confidence intervals (CIs) were built at a level of significance ( $\alpha$ ) of 0.05 (5%). The flow sheet and dimensions of the pilot plant are presented in Fig. 1. Thirteen sampling ports (diameter = 3/4 in.) along the reactors allowed liquid and sludge sampling. Up to 2 m high, ports were separated 0.20 m (10 ports); from 2 to 3.50 m, the distance between ports was 0.50 m (three additional ports). The last port was 0.45 m below the effluent exit.

Polyvinyl chloride (PVC) tubes and hoses (id = 1 in.) were used in the reactors for influent and effluent distribution. A transparent PVC tube (id = 3 mm) was used for the influent of WSPs. Retention valves were installed in the reactors at the influent entrance to prevent sludge from

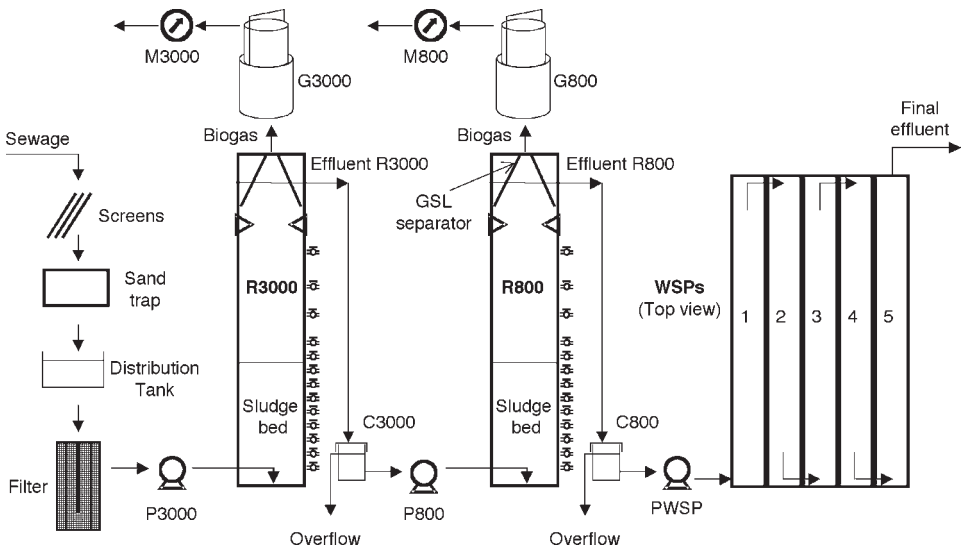


Fig. 1. Schematic diagram of pilot plant (not to scale). R3000: first UASB reactor (height = 3.95 m; diameter = 1 m; volume = 3.10 m<sup>3</sup>); R800: second UASB reactor (height = 3.95 m; diameter = 0.5 m; volume = 0.766 m<sup>3</sup>); WSPs (1–5) (length = 3 m; width = 0.5 m; mean depth = 0.94 m; mean volume = 1.39 m<sup>3</sup>; total volume = 6.97 m<sup>3</sup>). P3000, P800, and PWSP: peristaltic pumps; G3000 and G800: gas collectors (collection volume = 0.3 m<sup>3</sup>); M3000 and M800: gas meters; C3000 and C800: intermediate pumping containers (effective volume = 10 L); GSL = gas-solid-liquid.

flowing out during pump maintenance operations and power cuts. The influent of R3000 was screened through a double 5-mm filter to prevent clogging, as suggested in ref. 16. Watson Marlow 701 I/R, 621 I/R, and 313 S peristaltic pumps equipped with Marprene<sup>®</sup> tubing were used to feed R3000, R800, and WSPs. Flow rates could be freely changed between a wide range in all units. Liquid was pumped in R800 and WSPs from two intermediate 10-L containers (C3000 and C800) in which the effluents from R3000 and R800 were discharged. Surplus was eliminated through overflow pipes. The containers were not tightly sealed, but they remained always closed to minimize stripping of dissolved methane. Biogas was accumulated in 0.3-m<sup>3</sup> gas collectors and automatically measured in domestic gas meters (ABB ELSTER and Schlumberger Gallus 2000), operating electric valves (Jefferson), and switches (Neumann CB 130). Reactors, ponds, and gas collectors were constructed of polyester reinforced with glass fiber at a local company (JJS Industrias Plásticas y Mecánicas).

## Sewage

Raw sewage was submitted to preliminary treatment (screens and sand trap) before being fed into the system. In the reactors, influent was distributed through one inverted inlet pipe located 5 cm from the bottom. Partially digested sewage sludge from conventional anaerobic digesters and anaerobic granular sludge from a UASB reactor treating presettled

sewage were used as inoculum for R3000 and R800, respectively (15% [v/v]). Specific methanogenic activity (SMA) of the inocula was determined according to ref. 17. Analyses were performed according to ref. 18 or using HACH® micromethods.

### *Sample Analyses*

Two times a week, composite samples were taken before and after each unit (0.5 L every 3 h for 24 h). Samples were kept at 4°C until analyzed. Total COD ( $\text{COD}_{\text{tot}}$ ), paper-filtered COD ( $\text{COD}_{\text{filt}}$ ) (Schleicher & Schuell 595 ½ 4.4-µm paper filters), and membrane-filtered (dissolved) COD ( $\text{COD}_{\text{dis}}$ ) (Schleicher & Schuell ME 250.45-µm membrane) were determined in the samples. Suspended and colloidal COD ( $\text{COD}_{\text{sus}}$  and  $\text{COD}_{\text{col}}$ , respectively) were calculated as ( $\text{COD}_{\text{tot}} - \text{COD}_{\text{filt}}$ ) and ( $\text{COD}_{\text{filt}} - \text{COD}_{\text{dis}}$ ), respectively. The effluent of WSPs was filtered through Whatman GF/C 1.2-µm glass microfiber filters to retain algae, and COD of the filtered sample was measured ( $\text{COD}_{\text{fl.2}}$ ).

### *Sludge and Scum Layer*

The increase in sludge bed was visually followed using the sampling ports. No excess sludge was discharged during the study. Sludge composite samples (an equal volume from all ports in which sludge could be extracted) were taken and analyzed for COD, solids, and sludge volume index (SVI). Scum layer was regularly withdrawn and analyzed as sludge. Sludge and scum layer COD was measured in crushed and diluted samples. Total suspended solids (TSS) and volatile suspended solids (VSS) of sludge and scum layer were determined on centrifuged samples; the supernatant was filtered through Schleicher & Schuell 589 4.4-µm ashless paper filter, and the retained solids were added to the measured TSS/VSS.

### *Determination of Methane Content*

Methane content in the biogas was determined by stripping  $\text{CO}_2$  in a closed, upside down serum bottle with a 5% NaOH solution, and collecting the displaced liquid in a graduated cylinder. The content of other gases in the biogas, such as hydrogen sulfide, was neglected. Dissolved methane in the effluent of anaerobic reactors was calculated according to Henry's law, assuming that equilibrium concentrations were reached. Atmospheric pressure at the site was 0.866 atm.

### *Dimensioning of Ponds*

Dimensioning of the ponds was based on the assumption that the death rate of pathogenic microorganisms follows first-order kinetics (19). Considered values for  $K_{b,20}$  (die-off constant at 20°C) and  $\theta$  (temperature coefficient) were  $1.5 \text{ d}^{-1}$  and 1.17, respectively (14,20). With these kinetic constants, expected fecal coliform removal efficiency (calculated for five completely mixed tanks in series) was 99.99%.

Table 1  
Basic Sewage Composition Since First Day of Operation

Parameter	Mean values $\pm$ CI
Temperature ( $^{\circ}\text{C}$ )	$23.6 \pm 0.10$
pH	$7.77 \pm 0.18$
COD <sub>tot</sub> (mg/L)	$370.8 \pm 34.2$
TS (g/L)	$0.75 \pm 0.08$
VS (g/L)	$0.34 \pm 0.01$
Settleable solids (mL/L)	$4.85 \pm 1.8$
Alkalinity (mg $\text{CaCO}_3$ /L at pH 4.3)	$176.0 \pm 6.5$
VFA (mg/L)	$19.0 \pm 3.9$

Results and Discussion

During the period under consideration (summer), air temperature was  $24.9 \pm 1.37^{\circ}\text{C}$ . Basic sewage composition is shown in Table 1. SMA of the inocula was 0.078 and 0.033 g of COD- $\text{CH}_4$ /(g of VSS $\cdot$ d) for R3000 and R800, respectively. Mean sludge characteristics during the experiences are shown in Table 2. Scum layer composition was similar to that of the sludge.

Startup

HRTs applied during startup were 8 h, 4 h, and 15 d in R3000, R800, and WSPs, respectively. The inoculum of R3000 was completely washed out during the first week of operation. The amount of sludge then increased 9.3% of reactor volume per month. The reactor was not inoculated again. The inoculum of R800 was not washed out, although the applied  $V_{up}$  was higher, probably because settleability of the inoculum (determined through the SVI) was better, and biogas production was lower. Sludge increased in R800 2.3% of reactor volume per month. In R3000, considered to be the most critical step of the system, all conditions proposed in ref. 21 to indicate stable operation and/or to decide on an increase in organic load, were met within 1 mo of operation: COD removal efficiency reached 80% at the end of the month; the ratio between alkalinity measured at pH 5.75 and 4.3 was higher than 0.7 in the effluent, while volatile fatty acids (VFA) remained low; and biogas production was  $>0.1 \text{ Nm}^3/\text{kg}$  of COD<sub>removed</sub>, with a stable  $\text{CH}_4$  content of 90%.

Operation

Table 3 shows the HRT applied and removal efficiencies in the system for three different periods: period I (startup), period II (after startup, same hydraulic conditions), and period III (HRT in R3000 decreased to 6 h). COD<sub>tot</sub> removal in R3000 reached 80% at the end of the first month (period I), and stayed constant during period II. In period III, at a lower HRT, COD<sub>tot</sub> removal was not significantly lower in R3000. COD<sub>tot</sub> removal

Table 2  
Sludge Characteristics<sup>a</sup>

Reactor	Parameter (mean values ± CI)					
	TS (g/L)	VS (g/L)	TSS (g/L)	VSS (g/L)	SVI (mL/g TS)	COD (g/L)
R3000	66.3 ± 16.9	31.8 ± 10.5	32.3 ± 9.3	14.6 ± 4.8	16.7 ± 6.2	55.2 ± 3.8
R800	91.0 ± 48.6	43.4 ± 23.7	87.6 ± 44.6	40.8 ± 22.1	10.7 ± 5.0	97.4 ± 48.6

<sup>a</sup>Values are referred to wet sludge volume.



Table 3  
HRT and Removal Efficiencies in System<sup>a</sup>

Period	Days	HRT	COD	Mean removal efficiency (%)			
				Total	R3000	R800	Anaerobic
I	0–28	R3000: 8 h	COD <sub>tot</sub>	78.5	62.2	27.2	72.5
		R800: 4 h	COD <sub>sus</sub>	88.1	85.0	5.9	85.9
		WSPs: 15 d	COD <sub>col</sub>	42.1	-1.6	18.8	17.5
			COD <sub>dis</sub>	50.6	13.0	29.0	38.2
II	28–91	R3000: 8 h	COD <sub>tot</sub>	77.7 (86.1)	80.4	8.1	82.0
		R800: 4 h	COD <sub>sus</sub>	85.4	94.5	-18.2	93.5
		WSPs: 15 d	COD <sub>col</sub>	37.7	16.8	32.6	43.9
			COD <sub>dis</sub>	35.1	26.9	3.5	29.5
III	91–126	R3000: 6 h	COD <sub>tot</sub>	74.6 (89.8)	78.3	52.6	89.7
		R800: 4 h	COD <sub>sus</sub>	82.5	76.5	72.8	93.6
		WSPs: 15 d	COD <sub>col</sub>	73.3	94.5	-334.8	76.0
			COD <sub>dis</sub>	13.7	44.7	38.9	66.2

<sup>a</sup>Removal efficiencies were calculated from average concentrations in each period. Values in parentheses were calculated based on algae-free final effluent.



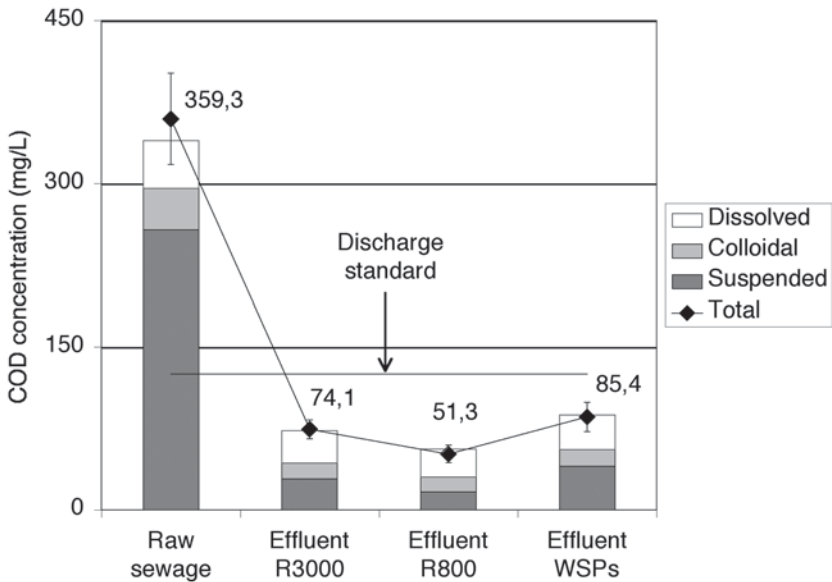


Fig. 2. COD fractions through the system. The startup period was not included. CIs are shown as error bars. The sum of suspended, colloidal, and dissolved COD is slightly different from total COD values because the latter come from a larger set of data.

increased during this period in R800, owing to a higher  $COD_{tot}$  concentration in the influent.  $COD_{col}$  and  $COD_{dis}$  removal efficiency increased steadily in R3000 since the beginning of the experience. A clear trend was not observed in R800 for these fractions. In period III,  $COD_{sol}$  removal efficiency decreased in R3000 but increased sharply in R800. Negative removal of  $COD_{sol}$  in R800 during period II is attributed to washout of seed sludge.  $COD_{tot}$  removal achieved in R3000 at 6-h HRT ( $V_{up} = 0.66$  m/h) was similar, or even higher, to that reported in tropical countries. The performance of the system in winter needs to be assessed before drawing final conclusions. However, data from the last 7 yr indicate that annual mean sewage temperature is 21.6°C, and a drastic drop in removal efficiency is not likely to occur.

In Fig. 2, COD fractions through the system are shown (startup period not included). The effluent of R3000 already complied with local  $COD_{tot}$  discharge standards (22) and could also comply with Council Directive 91/271/EEC on Urban Waste Water Treatment, dictated by the European Union Council of Ministers (23) (125 mg of  $COD_{tot/L}$  in both cases). Extremely low concentrations were always observed in the effluent of R800. Algae grown in the ponds were responsible for the increase in  $COD_{sol}$  in the final effluent, but it still complied with discharge standards, making a final clarification step unnecessary. After the first anaerobic step,  $COD_{col}$  and  $COD_{dis}$  remained rather constant. Methane content in the biogas was 90% in R3000 and 95% in R800. In terms of COD removal and effluent concentration, a second anaerobic step seems unnecessary, especially when the

first step is operated at high HRT (6–8 h), and a relatively high sewage temperature (typical for summertime). A second step could be important in wintertime (when slower conversion of entrapped SS is expected in the first step), or when the first step operates at low HRT (4 to 5 h). WSPs, designed to remove fecal coliform, are subutilized with respect to COD removal, and could easily treat remnant COD in the effluent of R3000 (24). Therefore, a one-step UASB reactor followed by WSPs could be enough to treat sewage under subtropical conditions.

### *Removal of Fecal Coliform*

Removal of fecal coliform in the entire system was 99.9999% (99.94% in the anaerobic steps and 99.98% in the WSPs). Final effluent concentration remained below 300 most probable number (MPN)/100 mL, complying with the World Health Organization's recommended guideline for unrestricted irrigation (1000 MPN/100 mL) (25), and local discharge standards (2000 MPN/100 mL) (22). Removal in WSPs approached that expected from design calculations, validating the hydraulic model of completely mixed tanks in series. It was reported that plug-flow regime is difficult to achieve in practice (12). Contrary to what is reported in literature (2,4), very high fecal coliform removal was observed in the UASB reactors. WSPs will provide new data to determine accurate kinetic constants for the design of full-scale systems.

### *COD Balances*

COD balances were based on the following equation:

$$I - E - G - D - B - W - S = 0$$

in which *I* is influent, *E* is effluent, *G* is gaseous methane, *D* is dissolved methane, *B* is sludge bed, *W* is sludge washout, and *S* is sulfate reduction. The terms of the balance are indicated for both reactors in Fig. 3. In Fig. 4, COD balances for the entire experimental period are presented. Sulfate was never detected in the effluent of R3000, and, therefore, there was no sulfate reduction in R800. In R800, terms *D* and *W* were calculated as net values, since dissolved methane and sludge from R3000 enter into R800. The exact amount of sludge from R3000 that actually enters into R800 is subject to discussion.

The concentration of dissolved methane in the influent of R800 was assumed to be in equilibrium with the methane concentration of biogas in R3000. Diffusive stripping of dissolved methane between R3000 and R800 was neglected, assuming that the methane concentration gradient in the gas phase was very small. This assumption was based on the following: (1) The effluent of R3000 was always exposed to an atmosphere of biogas, either in the space around the GSL separator device on top of the reactor, in the pipes, and in C3000; (2) temperature and pressure were constant; and (3) retention time in C3000 was very short (about 1 min). Convective stripping from the top of the reactor and from C3000 was also neglected, as long

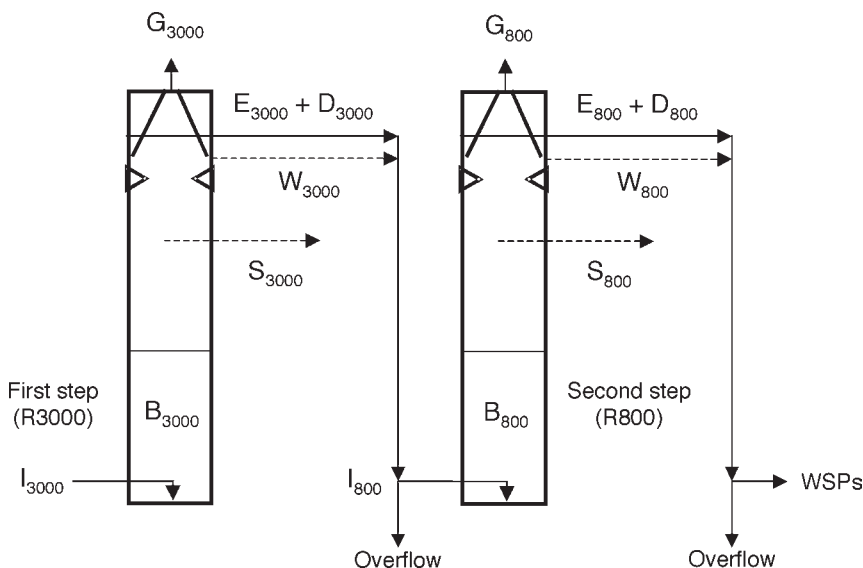


Fig. 3. COD balances over two-step UASB reactors.  $I$  = influent,  $E$  = effluent,  $G$  = gaseous methane,  $D$  = dissolved methane (net value in R800),  $B$  = sludge bed,  $W$  = sludge washout (net value in R800),  $S$  = sulfate reduction, WSPs = water stabilization ponds.

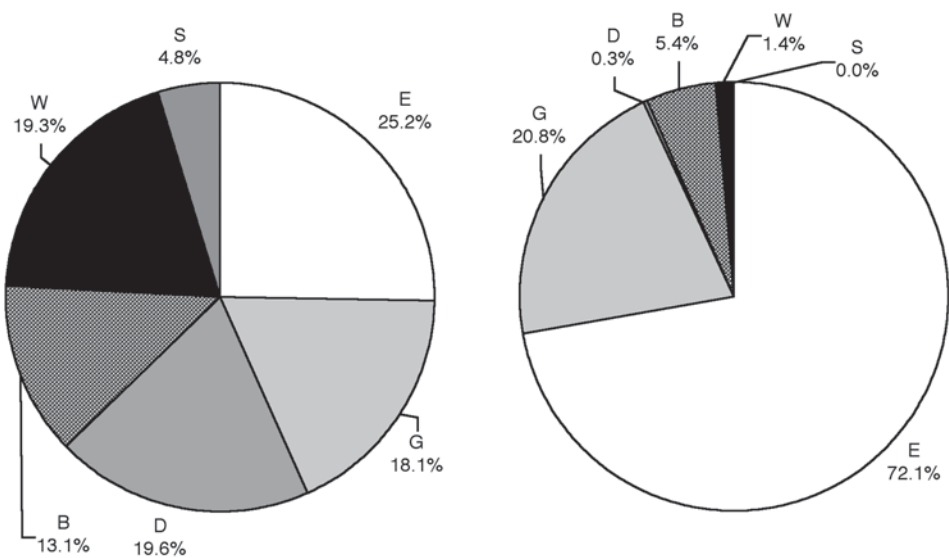


Fig. 4. COD balances for entire experimental period in R3000 (left) and R800 (right). Slices represent the terms of the balance as percentage of influent COD. In R800, term  $W$  represents sludge washin (negative washout), which was proportionally subtracted from all other terms. There was no sulfate reduction in R800.

as these units were always closed. Additional dissolution of methane took place in R800 (as a whole) only when temperature decreased in this reactor. When temperature in R800 was higher than in R3000, some methane left the

liquid phase and was collected as gas in G800, until a new equilibrium was reached. In Fig. 4 (right), term  $D$  is positive, and indicates newly dissolved methane. It was also assumed that dissolved methane was not found as COD in laboratory analyses, because it left the liquid phase during sampling, storage, and measuring (more than 24 h exposed to the air).

In periods II and III, some sludge accumulated in the bottom of intermediate pumping containers and was incidentally observed in a couple of grab samples. Sludge was not clearly detected in 24-h composite samples, probably because washout was not constant, while effective sampling time was very short (a few seconds every 3 h, only on sampling days). COD balances seem to confirm that some washout really occurred. In fact, missing COD accounted for a significant proportion of influent COD<sub>tot</sub> in R3000 (19.3%), without explanation other than intermittent sludge washout. On the other hand, excess COD was detected in R800 (1.4%). In Fig. 4 (right), term  $W$  is negative, and represents sludge “washin” (negative washout). The fate of this fraction is uncertain, and it was proportionally subtracted from all other terms. Sludge washin in R800 was far lower than expected from the calculated amount of sludge washed out from R3000. Because of the higher upflow velocity, most of this sludge flowed through R800, and only a minor fraction could be intercepted and retained.

Variability in sludge COD measurements could also introduce errors in the balances. Potential errors in the determination of sludge bed increase and scum layer formation during the first months of operation will be reduced in long-term balances, when a better steady state is expected. Intermittent sludge washout should be experimentally detected (and measured). Improved design of the GSL separator device may be needed to minimize washout. Solids retention time calculated according to ref. 4 and 26 was 233 d in R3000, and 771 d in R800, more than enough to achieve sufficient hydrolysis and methanogenesis at working temperatures (27). A distinction between solids from the sludge bed (secondary sludge) and particles from the influent that were not retained in the reactor (primary sludge) is difficult to make. Intermittent washout (if confirmed) would be an indication that secondary sludge is being lost, because short circuits over the sludge bed (main cause of primary solids washout) are assumed to be a constant phenomenon (28). At the end of period II, sludge SMA in R3000 was 0.050 g of COD-CH<sub>4</sub>/(g of VSS·d). Sludge from R800, subjected to very low organic loading rate and high  $V_{up}$ , showed almost no activity at the end of period II. Basic variables needed to build the COD balances were flow rate, temperature, influent COD, effluent COD, biogas production, biogas composition, sludge increase, and sulfate concentration. Sludge withdrawn for analysis and days without operation (power cuts, maintenance, and so on) have also been accounted for in the balances.

## Conclusion

The startup of a two-step UASB system for sewage treatment under subtropical conditions, with posttreatment in five WSPs in series, was suc-

cessfully achieved in only 1 mo. HRT in the anaerobic steps during startup was 8 + 4 h. Removal efficiencies up to 90% were observed at an HRT of 6 + 4 h (about 80% in the first step). Efficiency in the second step might improve in wintertime, when solids degradation in the first step is expected to be lower. The use of coupled COD balances allowed a better understanding of the process and was important in detecting intermittent sludge washout, which was not observed in routine sampling. A minimum set of data needed to build COD balances over UASB reactors is proposed. Fecal coliform removal in the whole system was 99.9999% (99.94% in the anaerobic steps and 99.98% in the WSPs). The system complied with discharge standards for COD and fecal coliform.

Washout from single-step UASB reactors should be monitored and minimized in order to avoid losses of valuable biomass, and to ensure constant compliance with discharge standards, especially when no post-treatment is provided. Attention should be directed toward refining sludge and scum layer assessment (sampling, volume estimation, COD measurements). The system combined high COD and fecal coliform removal efficiency with extremely low effluent concentration, making it an attractive option for sewage treatment in subtropical regions.

## Acknowledgments

We acknowledge valuable comments from I. Upton and W. Tejerina. Analyses were performed at the Laboratorio de Estudios Ambientales from CIUNSA and INENCO (Research Institute on Non Conventional Energy Sources). Gasnor S.A. provided the gas meters. Wageningen University (The Netherlands), the Netherlands Foundation for the Advancement of Tropical Research (WOTRO), the International Foundation for Science, Aguas de Salta S.A., and the Research Council of the National University of Salta (CIUNSA) funded this work.

## References

1. Seghezzo, L., Zeeman, G., van Lier, J. B., Hamelers, H. V. M., and Lettinga, G. (1998), *Bioresour. Technol.* **65**, 175–190.
2. Elmitwalli, T. A. (2000), PhD thesis, Wageningen University, The Netherlands.
3. Singh, K. S. and Viraraghavan, T. (2001), in *Proceedings of the 9th World Congress on Anaerobic Digestion 2001*, Pt. 1, van Velsen, A. F. M. and Verstraete, W. H., eds., Technologisch Instituut, Antwerp, Belgium, pp. 493–498.
4. van Haandel, A. C. and Lettinga, G. (1994), *Anaerobic Sewage Treatment: A Practical Guide for Regions with a Hot Climate*, John Wiley & Sons, Chichester, England.
5. Wang, K. (1994), PhD thesis, Wageningen Agricultural University, The Netherlands.
6. Wiegant, W. M. (2001), in *Anaerobic Digestion for Sustainable Development, Papers of the Farewell Seminar of Prof. Dr. Ir. Gatzke Lettinga*, van Lier, J. and Lexmond, M., eds., Environmental Protection and Resource Conservation Foundation (EPZRC), Wageningen, The Netherlands.
7. Lettinga, G., Roersma, R., and Grin, P. (1983), *Biotechnol. Bioeng.* **25**, 1701–1723.
8. de Man, A. W. A., van der Last, A. R. M., and Lettinga, G. (1988), in *Proceedings of the 5th International Symposium on Anaerobic Digestion*, Hall, E. R. and Hobson, P. N., eds., IAWPRC, Bologna, Italy, pp. 197–208.

9. Seghezzo, L., Guerra, R. G., González, S. M., Trupiano, A., Figueroa, M. E., Cuevas, C. M., Zeeman, G., and Lettinga, G. (2000), *Water Sci. Technol.*, submitted.
10. Zakkour, P. D., Gaterell, M. R., Griffin, P., Gochin, R. J., and Lester, J. N. (2001), *Water Res.* **35**, 4137–4149.
11. Kalogo, Y. (2000), PhD thesis, Gent University, Belgium.
12. Cavalcanti, P. F. F., van Haandel, A., and Lettinga, G. (2001), *Water Sci. Technol.* **44**(4), 237–245.
13. Cavalcanti, P. F. F., van Haandel, A., and Lettinga, G. (2002), *Water Sci. Technol.* **45**(1), 75–81.
14. Liberal, V., Cuevas, C. M., Trupiano, A. P., and Bohuid, E. (1998), in *Proceedings of the 26th Interamerican Congress on Sanitary and Environmental Engineering*, Lima, Peru (in Spanish).
15. Arias, M. and Bianchi, A. R. (1996), *Estadísticas Climatológicas de la Provincia de Salta*, Dirección de Medio Ambiente y Recursos Naturales de la Provincia de Salta, INTA EEA Salta and Gobierno de la Provincia de Salta, eds., Salta, Argentina (in Spanish).
16. Haskoning and WAU. (1994), *Anaerobic Treatment of Domestic Sewage Under Tropical Conditions*, DECOM Manual (Design, Construction, Operation and Maintenance of UASB reactors for domestic wastewater), Wageningen Agricultural University, the Netherlands.
17. DET (Department of Environmental Technology). (1994), *Manual of Laboratory Methods and Procedures for Anaerobic Wastewater Treatment*, Wageningen Agricultural University, The Netherlands.
18. APHA, AWWA, and WEF. (1995), *Standard Methods for the Examination of Water and Wastewater*, 19th ed., Eaton, A. D., Clesceri, L. S., and Greenberg, A. E., eds., American Public Health Association, American Water Works Association, and Water Environment Federation, Washington DC.
19. Marais, G. V. R. (1974), *J. Environ. Eng. Div.* 100, EE1, 119.
20. von Sperling, M. (1996), *Princípios do tratamento biológico de águas residuárias. Volume 3, Lagoas de estabilização*, Sanitary and Environmental Engineering Department-DESA, Federal University of Minas Gerais, Brazil (in Portuguese).
21. Noyola Robles, A. (1994), in *Proceedings of the 3rd Latin American Workshop and Seminar Anaerobic Wastewater Treatment*, Viñas, M., Soubes, M., Borzacconi, L., and Muxi, L., eds., Universidad de la República, Montevideo, Uruguay (in Spanish).
22. SeMADeS (Secretary of the Environment and Sustainable Development). (2001), Resolution no. 011, Salta, Argentina (in Spanish).
23. European Union Council of Ministers. (1991), *Off. J. Eur. Comm.* 135/40.
24. Chernicharo, C. A. L., van Haandel, A. C., Cybis, L. F., and Foresti, E. (2001), in *Proceedings of the 9th World Congress Anaerobic Digestion 2001*, Pt. 1, van Velsen, A. F. M. and Verstraete, W. H., eds., Technologisch Instituut, Antwerp, Belgium, pp. 747–752.
25. WHO (World Health Organisation). (1989), Technical Report Series 778, WHO, Geneva, Switzerland.
26. Zeeman, G. and Lettinga, G. (1999), *Water Sci. Technol.* **39**(5), 187–194.
27. Zeeman, G., Sanders, W., and Lettinga, G. (2000), *Water Sci. Technol.* **41**(1), 9–16.
28. van der Meer, R. R. (1979), PhD thesis, Wageningen Agricultural University, The Netherlands.