

One- and Two-Stage Upflow Anaerobic Sludge-Bed Reactor Pretreatment of Winery Wastewater at 4–10°C

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Abstract

The operating performance of a single and two (in series) laboratory upflow anaerobic sludge-bed (UASB) reactors (2.7-L working volume, recycle ratio varied from 1:1 to 1:18) treating diluted wine vinasse was investigated under psychrophilic conditions (4–10°C). For a single UASB reactor seeded with granular sludge, the average organic loading rates (OLRs) applied were 4.7, 3.7, and 1.7 g of chemical oxygen demand (COD)/(L·d) (hydraulic retention times [HRTs] were about 1 d) at 9–11, 6 to 7, and 4 to 5°C, respectively. The average total COD removal for preacidified vinasse wastewater was about 60% for all the temperature regimes tested. For two UASB reactors in series, the average total COD removal for treatment of non-preacidified wastewater exceeded 70% (the average OLRs for a whole system were 2.2, 1.8, and 1.3 g of COD/[L·d] under HRTs of 2 d at 10, 7, and 4°C, respectively). *In situ* determinations of kinetic sludge characteristics (apparent V_m and K_m) revealed the existence of substantial mass transfer limitations for the soluble substrates inside the reactor sludge bed. Therefore, application of higher recycle ratios is essential for enhancement of UASB pretreatment under psychrophilic conditions. The produced anaerobic effluents were shown to be efficiently posttreated aerobically: final effluent COD concentrations were about 0.1 g/L. Successful operation of the UASB reactors at quite

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low temperatures (4–10°C) opens some perspectives for application of high-rate anaerobic pretreatment at ambient temperatures.

Index Entries: Biogas; chemical oxygen demand removal; psychrophilic conditions; vinasse; upflow anaerobic sludge-bed reactor.

Introduction

A typical plant with primary wine production in Russia has facilities to process about 10,000 t of grapes per production season, which lasts from 20 to 60 d in autumn (1). Wastewater flows from various production steps are usually mixed together with cooling, washing, and sewage flows. The resulting wastewater has a moderate temperature (about or below 20°C) and variable concentration (>10 g of chemical oxygen demand [COD]/L during the production season and <1 g of COD/L in the interseasonal period). Although some medium-scale wineries are equipped with aerobic treatment plants, the latter, however, work unsatisfactorily owing to their frequent seasonal overloadings with the high concentrated vinasse accompanied by a deficit of N- and P-sources. A possible solution to this problem could be to apply anaerobic pretreatment at ambient temperatures to eliminate a major part of COD followed by aerobic posttreatment (1).

Thus, the primary objective of the present study was to examine the suitability of the upflow anaerobic sludge-bed (UASB) reactor concept for the pretreatment of winery wastewater at low temperatures (4–10°C) in terms of COD removal. The second objective was to obtain more insight into the evolution of sludge kinetic characteristics under these conditions.

Materials and Methods

Reactors

Anaerobic pretreatment studies were carried out in two laboratory UASB reactors (R1 and R2: 6.8 cm diameter, 85 cm height, 2.7-L working volume) made from transparent plastics and equipped with six sampling ports along the reactor height. Operating temperatures of 10 ± 1 , 7 ± 1 , and $4 \pm 1^\circ\text{C}$ were maintained by placing the reactors in a refrigerator (Snaige, Lithuania). Aerobic posttreatment of anaerobic effluents was performed at ambient temperatures (20–25°C) using an airlift reactor (4.6 cm diameter, 50 cm height, 0.7-L working volume) made from glass and packed by stones. Air was continuously pumped through an external loop of the airlift reactor at a flow rate of 3 L/min. Feeding of the reactors as well as recycling of effluent was achieved using the peristaltic pumps NP-1M (Kievpribor, USSR), P-1 (Pharmacia, Sweden), Masterflex L/S, and Masterflex C/L (both from Cole Parmer).

Wastewater

The UASB reactor influent was based on vinasse because this is the source of the majority of the COD present in winery wastewater (1). Raw

Table 1
Characteristics of Raw Vinasses Used

Characteristic	Vinasse							
	1	2	3	4	5	6	7	8
COD _{tot} (g/L)	129	115	196	90	92	123	160	155
Sugars (g COD/L)	50.3	72.0	159	34.1	34.1	15.0	74.9	32
Ethanol (g COD/L)	41.2	3.3	2.6	3.9	7.6	60.0	11.6	18.9
VFA (g COD/L)	13.3	1.4	1.0	0.9	2.0	21.0	9.6	1.7
pH	2.7	2.9	3.1	3.1	3.0	3.5	3.0	2.7
Polyphenols (g/L)	0.96	1.2	0.9	0.85	1.0	0.9	1.2	1.82
Usage (run)	1a	1a–1b	1b	2a	2b–3	4	5	6

vinasse was obtained in the laboratory by distilling low-quality red or white wines delivered by the Center of Certification of Food Products (Moscow). Table 1 presents the chemical content of the various raw vinasses used. Feeds were prepared by dilution of raw vinasse (Table 1) with tap water followed by the addition of 1–5 g of Na₂CO₃ (to increase alkalinity) as well as 1 to 2 g of NH₄Cl and K₂HPO₄ (both mainly to balance nutrient content). Partial anaerobic preacidification of wastewater was achieved when necessary by leaving it in a closed vessel for 1 to 2 d at ambient temperature (17–22°C). The effluent from the UASB reactor (runs 1 and 2, Table 1) was used as an influent for the aerobic posttreatment step.

Seed Sludges and Schedule of Runs

UASB reactor R1 was seeded with 1 L (41 g of volatile suspended solids [VSS]) of the mainly granular sludge, originating from a UASB reactor treating winery wastewater at 18–20°C (1). It was continuously operated at 9–11°C (run 1, duration of 5 mo), 6 to 7°C (run 2, duration of 3 mo), and 4 to 5°C (run 3, duration of 1.5 mo), respectively. After termination of run 3, approximately a half quantity of the sludge was withdrawn from reactor R1 to seed reactor R2. Both the reactors then operated in series at 10°C (run 4, duration of 2.5 mo), 7°C (run 5, duration of 1.5 mo), and 4°C (run 6, duration of 1.5 mo), respectively. The secondary sludge from Kur'yanovskaya municipal aeration station (Moscow) was used to seed an airlift reactor (run 7, duration of 2 mo).

Analysis

Biogas production was recorded by a gas meter, GSB-400 (Gaspribor, USSR). All gas measurements are expressed at 0°C and standard pressure (760 mmHg). Feed input to the reactors was monitored by measuring the accumulated outflow on a daily basis. Gas composition, ethanol, and volatile fatty acids (VFA) were analyzed by gas chromatography (2). Sugars, ammonia, phosphates, and polyphenols were measured spectrophotometrically as described elsewhere (3). Determinations of specific sludge

activities (small batch tests) and treatment of sludge samples for microscopy were performed as described previously (2). All other analyses were performed using standard methods (4). Data were statistically analyzed using the program Descriptive Statistics (Microsoft Excel).

Assessment of Sludge Kinetic Parameters in Reactor Conditions

For *in situ* determination of sludge kinetic characteristics, the UASB reactors were temporarily operated in batch mode. Before starting the experiments, the reactor was kept unfed (but with effluent recycle) for 1 to 2 d to deplete all remaining biodegradable COD. At time zero, the concentration of assessed substrate (propionate, butyrate, acetate, or ethanol) was set at 1 to 2 g of COD/L and its depletion was monitored. The substrate depletion data were fitted to the integrated Michaelis-Menten equation using nonlinear least-squares analysis (1,5).

Results and Discussion

One-Stage UASB Reactor Pretreatment

The performance data of a single UASB reactor under psychrophilic conditions are presented in Fig. 1 and generalized in Table 2. When the reactor was fed with nonpreacidified vinasse at 10°C (run 1a), an organic loading rate (OLR) was increased stepwise to 4 to 5 g of COD/(L·d) with a total COD (COD_{tot}) removal of about 70% (Fig. 1A–C, Table 2). A significant presence of propionate (predominant component) and acetate was observed in the effluents (Fig. 1D). However, only traces of sugars, ethanol, and butyrate were detected in the reactor liquor, and the headspace gas hydrogen concentration was negligible. These facts clearly demonstrate that low temperatures affect the various stages of anaerobic digestion differently, with propionate conversion becoming the rate-limiting step (6,7). Note also that a substantial increase (~20%) of sludge bed height had occurred at the end of run 1a. This was likely owing to substantial growth of acidogens in the reactor, because a fluffy outer layer covering the granules was seen under microscopic observations of the sludge aggregates. Similar granular changes were found in the low-temperature anaerobic treatment of sugar containing influents (6). Because such types of aggregates can provoke sludge instability and flotation and create mass transfer limitations for substrates of propionate-degrading and aceticlastic bacteria that are usually located in the central part of aggregates (6,8), we decided to apply preacidification of wastewater in order to achieve better COD removal. However, feeding with preacidified vinasse (run 1b, Table 2) did not result in enhanced COD removal (Fig. 1C), but the imposed OLRs were somewhat higher (6 to 7 g of COD/[L·d]) than those applied during run 1a (Fig. 1A). Moreover, the effluent propionate concentrations often exceeded 1.5 g of COD/L during d 103–119 (Fig. 1D). Also note that the substantial decoloration of effluents was observed during run 1; the average reduction in polyphenol content was about 40% (Table 2, Fig. 1E).

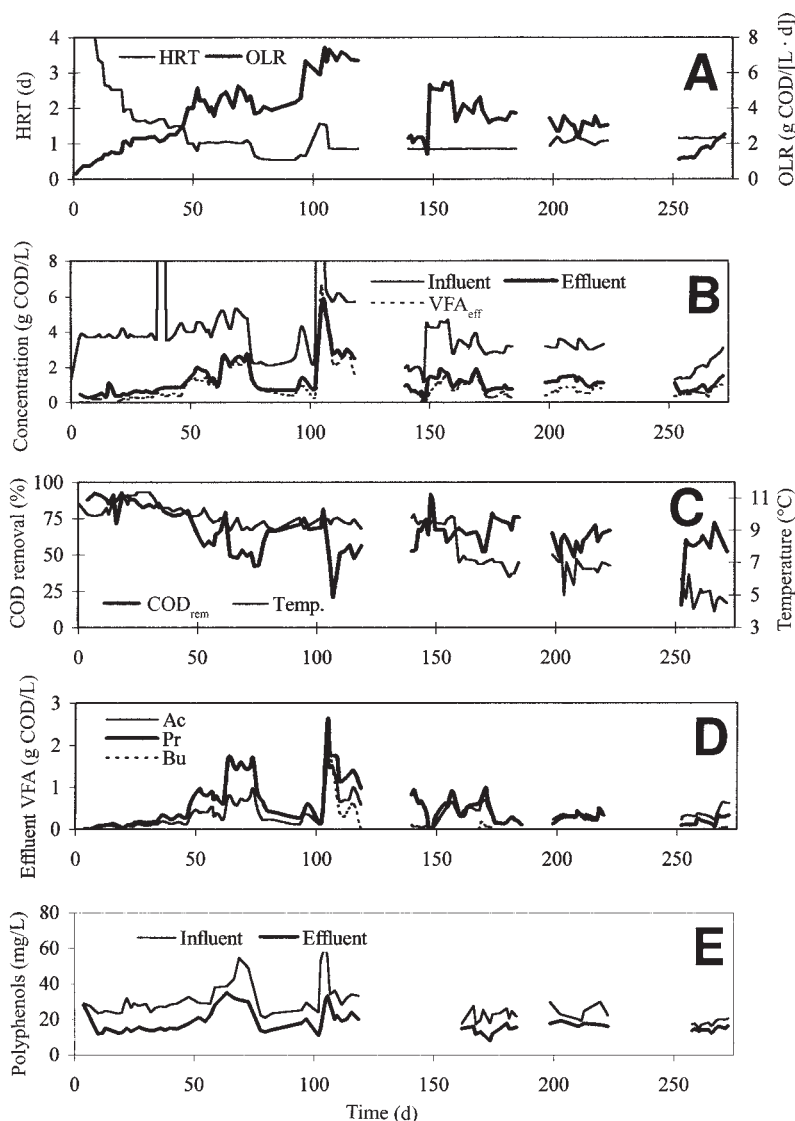


Fig. 1. Operation parameters and efficiency of a single UASB reactor treating diluted vinasse wastewater. (A) HRT and OLR. (B) Influent and effluent COD_{tot} concentrations and VFA effluent concentration. (C) COD_{tot} removal and temperature. (D) Effluent acetate, propionate, and butyrate concentrations. (E) Influent and effluent polyphenol concentrations.

To gain deeper insight into the processes occurring in the psychrophilic UASB reactor, the sludge kinetic characteristics were assessed *in situ*, i.e., under reactor conditions (d 121–137, Table 3) as well as in small batch tests (d 113, Table 4). A satisfactory coincidence was observed for specific sludge activities determined by two different methods. Lower values of activities found in small batch tests (d 113, Table 4) in comparison to *in situ* determinations (d 121–137, Table 3) can be explained by better mixing con-

Table 2
Operation Performance of Single UASB Reactor Treating Diluted Vinasse^a

Parameter	Run 1a ^b	Run 1b ^c	Run 2a ^c	Run 2b ^c	Run 3 ^c
Days of experiment	0–67	68–158	159–185	197–222	251–273
Temperature (°C)	9–11 (10.3)	9–10 (9.5)	6.5–7.5 (6.8)	6.5–7.5 (6.6)	3.5–5.0 (4.8)
Recycle ratio	1:2.6	1:2.6	1:2.6	1:11.6	1:11.6
V_{up} (m/h)	0.1	0.1	0.1	0.36	0.36
OLR (g COD/[L·d])	0.3–5.1 (2.7)	1.4–7.3 (4.7)	3.2–4.6 (3.7)	2.8–3.5 (3.1)	1.1–2.7 (1.7)
HRT (d)	0.8–5.1 (1.9)	0.5–1.6 (0.9)	0.85–0.87 (0.86)	0.9–1.3 (1.1)	1.14–1.17 (1.15)
Influent COD _{tot} (g/L)	3.6–5.2 (4.0)	1.2–9.9 (4.2)	2.7–4.0 (3.2)	3.0–3.6 (3.2)	1.3–3.1 (2.0)
Effluent COD _{tot} (g/L)	0.3–2.7 (1.0)	0.5–3.6 (1.8)	0.8–1.9 (1.0)	0.9–1.5 (1.2)	0.6–1.5 (0.8)
COD _{tot} removal (%)	48–92 (72)	48–92 (60)	52–79 (68)	51–71 (61)	15–72 (57)
Gas production (L/[L·d])	0.9–1.1 (1.0)	0.8–0.9 (0.9)	0.4–0.8 (0.6)	0.1–1.3 (0.4)	0.1–0.3 (0.2)
CH ₄ in biogas (%)	78–84 (81)	78–82 (80)	78–88 (84)	72–88 (82)	67–89 (81)
Effluent pH	6.9–7.8 (7.3)	6.9–7.5 (7.1)	6.7–7.1 (6.9)	6.7–7.5 (7.1)	7.2–7.9 (7.5)
Effluent VFA (g COD/L)	0.1–2.5 (0.7)	0.4–3.5 (1.6)	0.2–1.8 (0.8)	0.4–0.9 (0.6)	0.3–1.0 (0.6)
Polyphenol removal (%)	25–52 (40)	25–72 (39)	32–55 (39)	11–43 (25)	11–25 (20)

^a Average values are given in parentheses.
^b Nonpreacidified influent.
^c Preacidified influent.

Table 3
Sludge Kinetic Characteristics Assessed Directly in a Single Psychrophilic UASB Reactor^a

Parameter / substrate	Ethanol	Butyrate	Propionate	Acetate
Days 121–137 (10°C, V_{up} = 0.1 m/h, total reactor VSS = 53.2 g)				
V (g COD/[L·d])	15.30 ± 1.51	6.52 ± 0.52	3.94 ± 0.33	7.78 ± 0.31
K_m (g COD/L)	0.571 ± 0.062	1.413 ± 0.110	1.333 ± 0.101	0.589 ± 0.029
A^* (g COD/[d·g VSS]) ^b	0.612 ± 0.061	0.217 ± 0.017	0.121 ± 0.011	0.370 ± 0.013
Days 187–195 (7°C, V_{up} = 6 m/h, total reactor VSS = 59.1 g)				
V (g COD/[L·d])	9.93 ± 0.48	5.26 ± 0.22	2.48 ± 0.40	4.69 ± 0.27
K_m (g COD/L)	0.032 ± 0.001	0.258 ± 0.052	0.092 ± 0.009	0.205 ± 0.009
A^* (g COD/[d·g VSS]) ^b	0.452 ± 0.023	0.215 ± 0.009	0.109 ± 0.018	0.197 ± 0.012
Days 223–246 (7°C, V_{up} = 6 m/h, total reactor VSS = 62.7 g)				
V (g COD/[L·d])	9.04 ± 0.41	4.55 ± 0.19	4.71 ± 0.33	4.87 ± 0.41
K_m (g COD/L)	0.137 ± 0.005	0.268 ± 0.014	0.190 ± 0.008	0.215 ± 0.027
A^* (g COD/[d·g VSS]) ^b	0.368 ± 0.018	0.181 ± 0.030	0.187 ± 0.014	0.191 ± 0.017
Days 274–279 (4°C, V_{up} = 6 m/h, total reactor VSS = 65 g)				
V (g COD/[L·d])	6.36 ± 0.70	4.17 ± 0.14	3.78 ± 0.25	4.03 ± 0.21
K_m (g COD/L)	0.097 ± 0.011	0.265 ± 0.040	0.352 ± 0.025	0.201 ± 0.005
A^* (g COD/[d·g VSS]) ^b	0.239 ± 0.010	0.155 ± 0.006	0.135 ± 0.009	0.154 ± 0.010

^aResults are expressed as means ± SE.
^b A^* , sludge activity calculated from the Michaelis-Menten equation on the basis of assessed V_m and K_m and substrate concentration of 2 g of COD/L (as in small batch tests).

Table 4
Sludge Activities (g COD/[d·g VSS]) Determined in Small Batch Tests, Single UASB Reactor, Third Sampling Port^a

Day	T (°C)	Glucose	Ethanol	Butyrate	Propionate	Acetate
0	10	ND	ND	ND	ND	0.103 ± 0.003
0	30	ND	ND	ND	ND	0.629 ± 0.008
50	10	ND	0.566 ± 0.010	ND	0.083 ± 0.011	0.134 ± 0.015
50	30	ND	2.191 ± 0.394	ND	0.132 ± 0.004	0.700 ± 0.071
113	10	2.677 ± 0.288	0.569 ± 0.009	ND	0.080 ± 0.007	0.205 ± 0.001
113	30	3.363 ± 0.099	2.399 ± 0.134	ND	0.123 ± 0.062	0.947 ± 0.051
184	7	ND	0.521 ± 0.136	0.123 ± 0.003	0.081 ± 0.011	0.209 ± 0.006
184	30	ND	2.254 ± 0.047	0.613 ± 0.032	0.103 ± 0.017	0.866 ± 0.009

^aResults are expressed as means ± SE. ND, not determined.

ditions in the working UASB reactor. However, apparent half saturation constants K_m for all the substrates tested were found to be quite high (Table 3) under reactor conditions (superficial velocity V_{up} of 0.1 m/h), which supports the aforementioned supposition of the existence of mass transfer limitations inside the sludge bed of the psychrophilic UASB reactor, because the significantly lower values of apparent half saturation constants were found in the expanded granular sludge-bed reactor at a V_{up} of 10 m/h and similar temperature (5). This fact, together with the relatively low V_m assessed for propionate (Table 3), explains why the latter was prevalent in the reactor effluent. Simple estimations using the kinetic parameters from Table 3 clearly demonstrate that the imposed propionate loading exceeded the propionate assimilative capacity of the sludge at least twice during d 95–119 (Fig. 1).

Despite the significant presence of fluffy voluminous (up to 4 mm in diameter) aggregates, the sludge remained mainly in a granular form at the end of run 1 according to visual and microscopic observations. Moreover, although the overall quantity of the sludge in the reactor increased by 32% (from 41 to 54 g of VSS) throughout run 1, its specific aceticlastic activities assessed at 10°C in small batch tests increased twice—from 0.103 to 0.205 g of COD/(d·g VSS) (d 0 and 113, Table 4), indicating about a substantial enrichment of the sludge by methanogenic bacteria. The fact that namely sludge enrichment (and not only sludge adaptation to low temperatures) was a prevailing reason for the increase in sludge activity was confirmed by a substantial increase in its specific aceticlastic activities assessed at 30°C (d 0 and 113, Table 4).

Decreasing the temperature to 7°C during run 2a and keeping a recycle ratio the same (1:2.6) did not result in deterioration of COD_{tot} removal (d 159–185, Fig. 1C); however, the OLRs imposed were somewhat lower (about 4 g of COD/[L·d]) than those applied during run 1b (Table 2, Fig. 1A). Polyphenol removal also remained the same as during run 1b (about 40%, Table 2), but the effluent VFA concentrations decreased almost twice as much (d 159–185, Fig. 1D). Although the overall quantity of the sludge in the reactor further increased to 59 g of VSS throughout run 2a, its specific activities (for all substrates tested) assessed at 7°C almost did not change in comparison with those assessed at 10°C during the previous run (d 113 and 184, Table 4). Further accumulation of large aggregates with irregular forms in the reactor sludge was observed at the end of this run compared with the previous run.

To illustrate that the mass transfer limitations inside the psychrophilic sludge bed can be substantially reduced, the sludge kinetic characteristics were assessed directly in the reactor at V_{up} as high as 6 m/h (d 187–195, Table 3). A substantial drop in the apparent K_m values was observed. Again, the calculated specific sludge activities A^* (d 187–195, Table 3) were comparable with those assessed in small batch tests (d 184, Table 4).

Since application of high V_{up} was accompanied by a heavy sludge washout, the recycle ratio was increased to only 1:11.6 (V_{up} of 0.36 m/h)

during run 2b (d 197–222, Fig. 1, Table 2). As expected, during run 2b an almost fourfold increase in V_{up} resulted in better VFA removal (Fig. 1D), although COD_{tot} removal efficiency slightly decreased (Table 2) compared with run 2a. This was mainly owing to increased sludge washout because small sludge aggregates were continuously accumulated in the effluent recipient vessel during this run. Also, further expansion of the sludge bed occurred (~10% from the beginning of run 2b). This process was sometimes accompanied by a sudden lifting of some parts of the sludge bed, although of low gas production (Table 2). In spite of washout losses that unfortunately were not quantified, the overall quantity of the reactor sludge reached a value of 62.7 g of VSS at the end of run 2b. Fluffy large (3–5 mm) aggregates with relatively random presence of more fine particles were predominant in the sludge composition. Note that polyphenol removal efficiency substantially dropped during this run (Fig. 1E, Table 2). It is likely that relatively big (compared to VFA) molecules of these substances had substantial mass transfer difficulties in order to be degraded by large sludge aggregates accumulated in the reactor.

In situ determinations of sludge kinetic characteristics after run 2b (d 223–246, Table 3) revealed a tendency toward deterioration of apparent half-saturation constants K_m for all the substrates tested. This is logical because larger sludge aggregates create bigger mass transfer limitations for soluble substrates that kinetically result in an increase in apparent K_m values (9). Note that a noticeable increase in V_m value for propionate was found manifesting about a substantial enrichment of the sludge by propionate-degrading bacteria. It resulted in reduced effluent concentrations of propionate, which became comparable with acetate concentrations (d 197–222, Fig. 1D). Thus, contrary to the first 120 d (Fig. 1D), propionate was no longer a predominant VFA component in the effluent.

A further decrease in working temperature to 4°C was accompanied by a decrease in OLR imposed during run 3 (Fig. 1, Table 2). In general, the overall performance of the reactor was similar to that during run 2b although polyphenol removal efficiency further dropped to 20%. Sludge washout was also observed but it tended to decrease during this run, because a majority of fine sludge aggregates was already eliminated from the reactor during the precedent assessment of sludge kinetic characteristics accompanied by high V_{up} (d 223–246). Microscopic observation of the sludge showed an overwhelming predominance of fluffy large aggregates (4 to 5 mm) with irregular forms that looked flocculent. Such evolution of the sludge can be attributed to the fact that the reactor influent was not completely preacidified by the preacidification procedure applied; for example, sometimes quite noticeable concentrations of ethanol (up to 2 g of COD/L) and sugars (up to 0.6 g of COD/L) entered the reactor stimulating development of fluffy acidogenic biomass that deteriorated sludge quality. Thus, control of preacidification efficiency seems to be essential for a stable pre-treatment process of winery wastewater at low temperatures.

In situ determinations of sludge kinetic characteristics after run 3 (d 274–279, Table 3) confirmed the aforementioned observation that large sludge aggregates accumulated in the reactor had increased values of apparent half-saturation constants K_m . Also note that in the temperature range below 7°C, even a small drop in (3°C from run 2 to run 3) temperature led to an 8–30% decrease in V_m values and, correspondingly, specific sludge activities.

Two-Stage UASB Reactor Pretreatment

To control preacidification of wastewater with the aim of enhancing COD removal, two UASB reactors were combined in series. Reactor R1 mainly served as the preacidifier to generate VFA for feeding reactor R2. A high recycle ratio (1:18) was applied in reactor R2 in order to decrease mass transfer limitations, whereas the recycle ratio in reactor R1 was kept at a low level (1:1) because diffusional limitations are not very important for the fast acidogenic step. As mentioned in Materials and Methods, the sludge from run 3 consisting predominantly of fluffy large aggregates was used as a seed for both reactors. The results of operational performance of this combined system are presented in Figs. 2 and 3 and are generalized in Table 5.

At 10°C (run 4), reactor R1 was operated at an average OLR of 4.4 g of COD/(L·d) (Fig. 2A, Table 5), removing on average 46% of COD_{tot} (Fig. 2C). Acetate was a predominant product of preacidification (Fig. 2D) whereas butyrate was practically not generated at all. Ethanol (0.1–0.2 g of COD/L) was sometimes detected in the effluents of this reactor. By contrast, reactor R2 was maintained at an average OLR of 2.5 g of COD/[L·d] (Fig. 3A, Table 5), removing 58% (on average) of COD_{tot} (Fig. 2C). The latter characteristics could be higher, but some sludge washout was observed in reactor R2, contrary to reactor R1 in which sludge washout was negligible. Thus, an overall treatment efficiency of the combined system was 78% (on average) on COD_{tot} (Table 5), i.e., higher than in a single UASB reactor (Table 2, run 1). Even more significant enhancement was obtained for polyphenol removal (Tables 2 and 5).

However, substantial disintegration of the sludge occurred in reactor R2 approximately in the middle of this run, probably owing to lysis of abundant acidogenic bacteria presented in the seed sludge because feeding influent to this reactor practically consists of only acetate and propionate. At the end of run 4, the sludge from reactor R2 contained mainly small granule-like aggregates (maximal diameter of 1.5 mm) and fine particles. In spite of the decrease in overall quantity from 32.5 to 29.5 g of VSS, owing to partial lysis and washout, aceticlastic activity of this sludge determined in small batch tests substantially increased both at 10 and 30°C (d 0 and 64, Table 6). A significant enrichment of the R2 sludge by methanogenic bacteria was observed. Assessment of kinetic parameters performed at the same V_{up} (0.55 m/h) as in a working reactor confirmed this observation not only for aceticlastic but also for propionate-degrading bacteria (see calculated sludge activities A^* in Table 7, d 65–73, and compare them with those from Table 3). However, apparent half-saturation constants K_m for all the

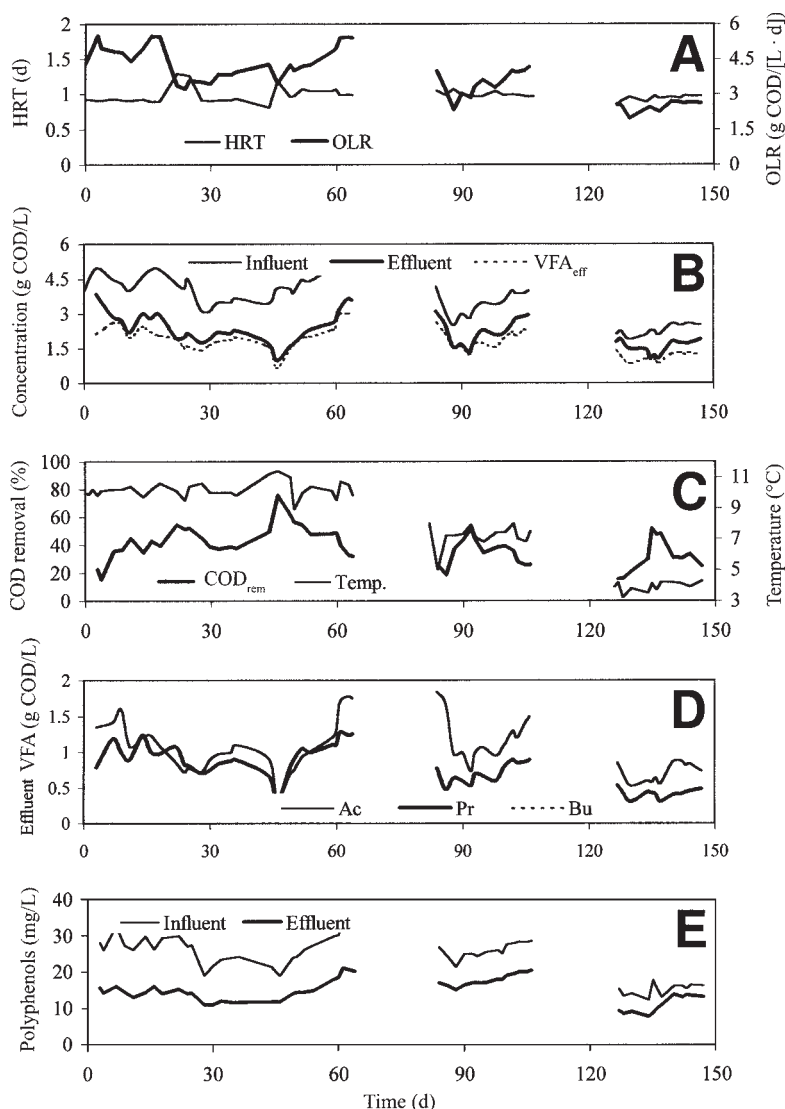


Fig. 2. Operation parameters and efficiency of reactor R1 (first in series) treating nonpreacidified vinasse wastewater. **(A)** HRT and OLR. **(B)** Influent and effluent COD_{tot} concentrations and VFA effluent concentration. **(C)** COD_{tot} removal and temperature. **(D)** Effluent acetate, propionate, and butyrate concentrations. **(E)** Influent and effluent polyphenol concentrations.

substrates tested were found to be higher (Table 7) under the conditions of reactor R2 than those previously determined at V_{up} as high as 6 m/h (Table 3), indicating moderate mass transfer limitations inside the sludge bed of reactor R2.

Contrary to reactor R2, the overall quantity of the sludge in reactor R1 slightly increased from 30 to 32 g of VSS; however, its aceticlastic activity determined in small batch tests decreased both at 10 and 30°C (d 0 and 64,

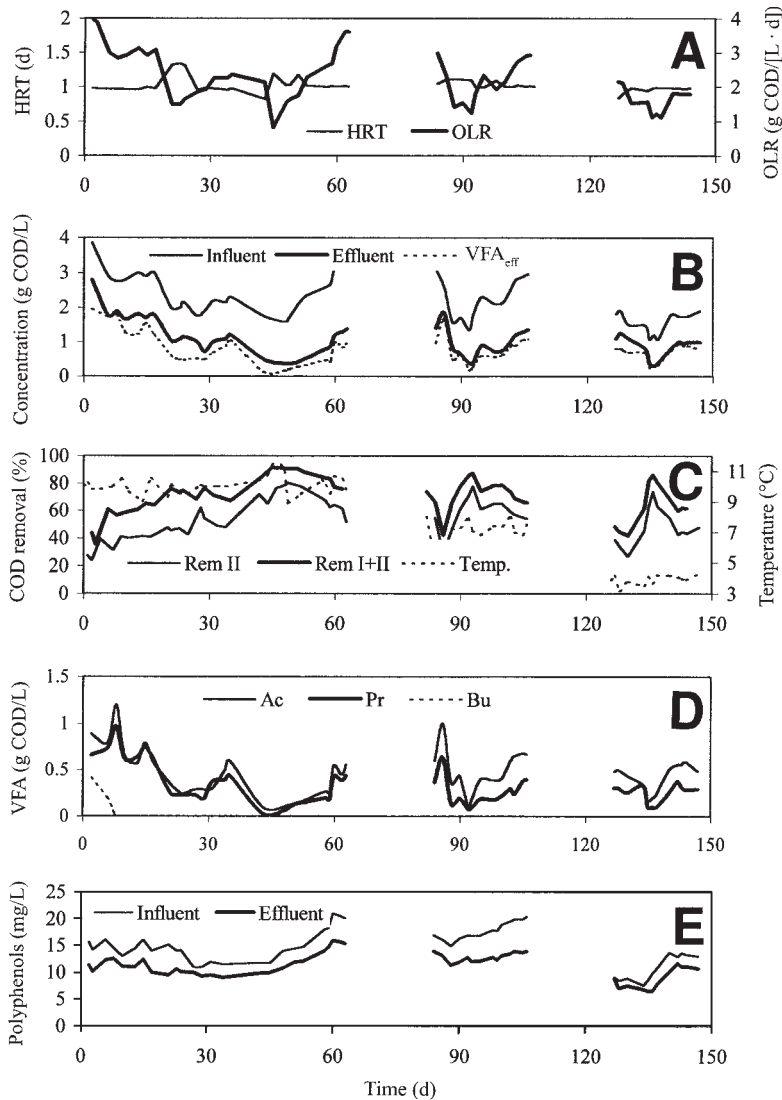


Fig. 3. Operation parameters and efficiency of reactor R2 (second in series) treating effluent from reactor R1. (A) HRT and OLR. (B) Influent and effluent COD_{tot} concentrations and VFA effluent concentration. (C) COD_{tot} removal and temperature. (D) Effluent acetate, propionate, and butyrate concentrations. (E) Influent and effluent polyphenol concentrations.

Table 6). This is logical because this reactor mainly performed acidogenic conversions. The sludge from reactor R1 at the end of run 4 consisted of large flocculent aggregates (3 to 4 mm) with significant presence of fine particles, indicating partial disintegration of the seed sludge too. The cause of this phenomenon is not clear. Probably too intensive development of acidogenic bacteria owing to a switch from preacidified to nonpreacidified influent had a detrimental effect on the stability of the large aggregates.

Table 5
Operational Performance of Two UASB Reactors in Series Treating Diluted Nonpreacidified Vinasse^a

Parameter	Run 4		Run 5		Run 6	
Days of experiment	0–63		82–107		122–147	
Temperature (°C)	9.5–10.5 (10.2)		6.5–7.5 (7.1)		3.5–4.5 (3.9)	
Reactor	R1	R2	R1	R2	R1	R2
Recycle ratio	1:1		1:1		1:1	
OLR (g COD/[L·d])	3.2–5.5 (4.4)	0.8–4.0 (2.5)	2.3–4.2 (3.5)	1.2–3.0 (2.2)	2.0–2.7 (2.5)	1.5–2.2 (1.7)
HRT (d)	0.8–1.3 (1.0)	0.8–1.2 (1.0)	1.0–1.1 (1.0)	1.0–1.1 (1.0)	0.8–1.0 (0.9)	0.8–1.0 (0.9)
Influent COD _{tot} (g/L)	3.1–5.4 (4.3)	1.6–3.9 (2.5)	2.5–4.2 (3.5)	1.4–3.1 (2.3)	1.9–2.6 (2.4)	1.1–1.9 (1.5)
Effluent COD _{tot} (g/L)	1.0–3.9 (2.4)	0.4–2.8 (1.2)	1.3–3.1 (2.3)	0.4–1.9 (1.0)	1.1–1.9 (1.5)	0.3–1.2 (0.7)
COD _{tot} removal (%)	16–76 (46)	24–80 (58)	19–52 (37)	29–78 (61)	25–52 (37)	43–74 (53)
Gas production (L/[L·d])	0.1–0.6 (0.4)	0.1–0.4 (0.3)	0.1–0.4 (0.2)	0.1–0.3 (0.2)	0.1–0.2 (0.15)	0.1–0.3 (0.2)
CH ₄ in biogas (%)	68–90 (80)	45–92 (80)	70–89 (82)	72–91 (86)	50–82 (76)	52–83 (78)
Effluent pH	6.3–7.1 (6.6)	6.9–7.8 (7.4)	6.0–7.2 (6.7)	7.0–8.2 (7.6)	6.4–6.9 (6.7)	7.2–7.7 (7.5)
VFA _{eff} (g COD/L)	0.7–3.0 (2.4)	0.2–2.0 (0.8)	1.3–2.6 (1.9)	0.3–1.6 (0.7)	0.8–1.4 (1.1)	0.3–0.9 (0.6)
P-phenol removal (%)	38–52 (45)	9–38 (22)	28–37 (31)	18–31 (27)	11–39 (28)	10–28 (22)
Combined system (R1 + R2)						
OLR (g COD/[L·d])	1.6–2.8 (2.2)		1.2–2.1 (1.8)		1.0–1.4 (1.3)	
HRT (d)	1.6–2.5 (2.0)		2.0–2.2 (2.0)		1.6–2.0 (1.8)	
COD _{tot} removal (%)	36–91 (78)		42–89 (76)		60–86 (71)	
P-phenol removal (%)	44–70 (57)		41–57 (50)		20–56 (44)	

^a Average values are given in parentheses.

Table 6
Aceticlastic Sludge Activities (g COD/[d·g VSS]) Determined
in Small Batch Tests, Two UASB Reactors in Series

Day	T (°C)	R1 ^a	R2 ^b
0	10		0.128 ± 0.003
0	30		0.780 ± 0.028
63	10	0.097 ± 0.002	0.177 ± 0.001
63	30	0.695 ± 0.047	1.337 ± 0.008
107	7	0.068 ± 0.001	0.145 ± 0.005
107	30	0.670 ± 0.013	1.146 ± 0.013
147	4	0.055 ± 0.010	0.140 ± 0.013
147	30	0.658 ± 0.014	1.083 ± 0.023

^aThird sampling port.

^bSecond sampling port.

Table 7
Sludge Kinetic Characteristics Assessed Directly
in Reactor R2, Two UASB Reactors in Series^a

Substrate	V_m (g COD/[L·d])	K_m (g COD/L)	A^* (g COD/[d·g VSS]) ^b
Days 65–73 (10°C, $V_{up} = 0.55$ m/h, total reactor VSS = 29.5 g)			
Ethanol	9.73 ± 0.17	0.211 ± 0.030	0.786 ± 0.014
Propionate	5.55 ± 0.34	0.482 ± 0.041	0.399 ± 0.025
Acetate	4.68 ± 0.08	0.450 ± 0.039	0.329 ± 0.005
Days 113–119 (7°C, $V_{up} = 0.55$ m/h, total reactor VSS = 25.2 g)			
Propionate	5.90 ± 0.08	0.528 ± 0.037	0.496 ± 0.007
Acetate	3.45 ± 0.31	0.402 ± 0.042	0.305 ± 0.028
Days 148–156 (4°C, $V_{up} = 0.55$ m/h, total reactor VSS = 62.7 g)			
Propionate	4.76 ± 0.11	0.485 ± 0.010	0.514 ± 0.012
Acetate	3.38 ± 0.01	0.488 ± 0.022	0.280 ± 0.001

^aResults are expressed as means ± SE.

^b A^* , sludge activity calculated from the Michaelis-Menten equation on the basis of assessed V_m and K_m and substrate concentration of 2 g of COD/L (as in small batch tests).

After a shift in the combined system to 7°C (run 5), an average OLR in reactor R1 was maintained as 3.5 g of COD/(L·d) (Fig. 2A, Table 5). Under these conditions, reactor R1 removed 37% of COD_{tot} (on average) and average polyphenol removal was 31% (Fig. 2C,E; Table 5). The effluent of this reactor, which predominantly consisted of acetate and propionate (d 82–107, Fig. 2D) as well as traces of ethanol and polyphenols, was pumped into reactor R2. Its average OLR was 2.2 g of COD/(L·d) (Fig. 3A, Table 5). Under the conditions imposed, reactor R2 removed 61% of COD_{tot} (on average) and average polyphenol removal was 27% (Fig. 3C,E; Table 5). Thus, an overall treatment efficiency of the combined system at 7°C was 76

and 50% (on average) for COD_{tot} and polyphenols, respectively (Table 5)—again higher than in a single UASB reactor (run 2, Table 2).

During run 5, the sludge in reactor R1 continued to disintegrate and was represented by small aggregates (1 to 2 mm) and fine particles at the end of this run (the possible cause of this phenomenon is mentioned in the discussion of run 4). Because of the low but continuous washout of the sludge, its overall quantity in reactor R1 almost did not change (31.7 g of VSS at the end of run 5), but its acetoclastic activity determined in small batch tests slightly decreased (d 107, Table 6).

Similarly, acetoclastic activity of the sludge from reactor R2 determined in small batch tests also slightly decreased (d 107, Table 6) and was accompanied by a decrease in the overall sludge quantity in the reactor (25.2 g of VSS at the end of run 5) owing to lysis and washout. However, the sludge quality seemed to increase—a substantial presence of 1- to 2-mm granules was found under visual and microscopic observations of the sludge samples taken at d 107. *In situ* assessment of kinetic parameters confirmed the existence of moderate mass transfer limitations under reactor conditions with a V_{up} of 0.55 m/h (see K_m values in Table 7, d 113–119). Furthermore, a noticeable increase in propionate-degrading activity A^* of the sludge calculated from these data corresponded well with the relatively low propionate concentrations observed during d 88–106 (Fig. 3D).

After a decrease in working temperature to 4°C (run 6), the average OLR imposed on reactor R1 was 2.5 g of COD/(L·d) (Fig. 2A), and the average COD_{tot} removal achieved was 37% (Fig. 2C, Table 5). However, preacidification was complete because practically only acetate and propionate were detected in the effluent (Fig. 2D). By contrast, at an average OLR imposed to reactor R2 of 1.7 g of COD/(L·d) (Fig. 3A), the average COD_{tot} removal achieved was 53% (Fig. 3C, Table 5). Thus, the overall efficiency of the combined system was 71% with substantial decoloration of effluent (average polyphenol removal was 44%) (Table 5). By comparing these values to those obtained with a single UASB reactor (run 3, Table 2), one can again state that the combined system showed substantially higher treatment efficiencies, especially regarding polyphenol removal.

No significant changes occurred with the sludges in both reactors throughout run 6 compared with the previous run. Both sludges mainly consisted of granule-like aggregates (~2 mm in diameter) although a moderate quantity of fluffy aggregates and fine particles was also present in reactor R1. Because of negligible washout, the overall quantity of the sludge slightly increased in both reactors at the end of run 6 (33.2 and 26 g of VSS for reactors R1 and R2, respectively). The specific activities of the sludges determined *in situ* (d 148–156, Table 7) and in small tests (d 147, Table 6) also did not change significantly compared with those of run 5.

The results obtained during two-stage UASB pretreatment indicate that the combined system with two reactors in series has higher removal efficiencies and significantly better operation stability compared with a single UASB pretreatment at temperatures as low as 4–10°C. Any difficul-

Table 8
Operation Performance
of Airlift for Posttreatment of Anaerobic Effluents^a

Parameter	Run 7
Days of experiment	0–55
Temperature (°C)	20–25 (22)
OLR (g COD/[L·d])	0.32–2.06 (0.84)
HRT (d)	0.25–1.27 (0.86)
Influent COD _{tot} (g/L)	0.40–1.15 (0.61)
Effluent COD _{tot} (g/L)	0.04–0.17 (0.11)
COD _{tot} removal (%)	72–90 (82)
Effluent pH	8.4–8.9 (8.6)
Effluent VFA (g COD/L)	0.03–0.11 (0.07)
Polyphenol removal (%)	13–44 (31)

^aAverage values are given in parentheses.

ties in a combined system performance including sludge lifting or heavy washout were not observed at all. Note, however, that if one takes into account the overall volume of both reactors, the single UASB reactor was operated at higher OLRs (but with preacidified wastewater) than the OLRs imposed on the combined system treating nonpreacidified wastewater. Thus, the application of the two-stage UASB reactor system implies higher capital and operational costs, which should be taken into account when considering possible implementation of low temperature anaerobic pretreatment. On the other hand, a single-stage UASB reactor operating at psychrophilic temperatures seems to need at least partial preacidification of wastewater in order to ensure its more or less stable operation.

Aerobic Posttreatment of Anaerobic Effluents

Since the effluents after anaerobic treatment of vinasse are sometimes quite persistent in the aerobic posttreatment (10), anaerobic effluents produced in our case were processed via airlift reactor taken just as an example of such a posttreatment. The corresponding results are generalized in Table 8. The average COD_{tot} removal was 82%, giving an average effluent concentration of 0.11 g of COD_{tot}/L (Table 8). A major part (0.07 g/L, on average) of effluent COD consisted of acetate and propionate (data not shown), indicating that the efficiency of aerobic posttreatment can be improved (if necessary), but it was not a primary goal of the research described herein. Thus, psychrophilic UASB pretreatment of winery wastewater followed by a proper aerobic posttreatment step is expected to be an adequate solution to meet the requirements for a final effluent discharge.

Conclusion

Winery wastewater can be satisfactorily pretreated in UASB reactors at temperatures as low as 4–10°C. For one-stage treatment of preacidified

vinasse, the average COD_{tot} removal was about 60% at OLRs of 4.7, 3.7, and 1.7 g of COD/(L·d) (HRTs were about 1 d) for 9–11, 6 to 7, and 4 to 5°C, respectively. For two UASB reactors in series treating nonpreacidified vinasse, the average COD_{tot} removal was higher (>70%) at the OLRs for a whole system of 2.2, 1.8, and 1.3 g of COD/(L·d) and HRTs of about 2 d at 10, 7, and 4°C, respectively. *In situ* determinations of kinetic sludge characteristics (V_m and K_m) revealed the existence of substantial mass transfer limitations for the soluble substrates inside the reactor sludge bed. Therefore, application of higher recycle ratios is essential for enhancement of UASB pretreatment under psychrophilic conditions. The produced anaerobic effluents were shown to be efficiently posttreated aerobically—final effluent COD concentrations were about 0.1 g/L. Successful operation of the UASB reactors at quite low temperatures (4–10°C) opens some perspectives for application of high-rate anaerobic pretreatment at ambient temperatures, e.g., in the southern regions of Russia where the main national wineries are located.

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