Psychrophilic one- and two-step systems for pre-treatment of winery waste water

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Abstract
The operation performance of a single and two (in series) laboratory UASB reactors (working volume of 2.7 l, 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 (OLR) applied were 4.7, 3.7 and 1.7 g COD/l/d (hydraulic retention times (HRTs) were around 1 d) at 9–11, 6–7 and 4–5°C, respectively. The average total COD removal for preacidified vinasse wastewater was around 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 COD/l/d under HRTs of 2 days at 10, 7 and 4°C, respectively). In situ determinations of kinetic sludge characteristics (Vₘ and Kₘ) revealed the existence of substantial mass-transfer limitations for the soluble substrates inside the reactor sludge bed. Therefore an application of higher recycle rations is essential for enhancement of UASB pre-treatment under psychrophilic conditions. The produced anaerobic effluents were shown to be efficiently post-treated aerobically - final effluent COD concentrations were around 0.1 g/l.

Keywords Biogas; COD removal; psychrophilic conditions; vinasse; UASB reactor

Introduction
A typical plant with primary wine production in Russia has facilities to process about 10,000 tons of grapes per production season (Kalyuzhnyi et al., 2000). Wastewater flows from various production steps are usually mixed together with cooling, washing and sewage flows. The resulting wastewater has a moderate temperature (around or below 20°C) and variable strength (> 10 g COD/l during the production season and < 1 g COD/l in the inter-seasonal period). Though some medium-scale wineries were equipped with aerobic treatment plants, the latter, however, work unsatisfactorily due to their frequent seasonal overloadings with the high-strength vinasse accompanied by a deficit of N- and P-sources. A possible solution of the problem can be an application of anaerobic pre-treatment at ambient temperatures for elimination of a major part of COD followed by aerobic post-treatment (Kalyuzhnyi et al., 2000). Thus, the primary objective of this paper was to examine the suitability of the upflow anaerobic sludge blanket (UASB) reactor for the pretreatment of winery wastewater at low temperatures (4–10°C) in terms of COD removal. The second objective of the work was to get more insights into the evolution of sludge kinetic characteristics under these conditions. Finally, the generated anaerobic effluents were assessed with regard to aerobic post-treatment.

Materials and methods
Reactors. Anaerobic pre-treatment studies were carried out in two laboratory UASB reactors (R1 and R2: diameter – 6.8 cm, height – 85 cm, working volume – 2.7 l). Operating temperatures of 10±1, 7±1 and 4±1 °C were maintained by placing the reactors in a refrig-
erator. Aerobic post-treatment of anaerobic effluents was performed at ambient temperatures (20–22°C) using an airlift reactor (diameter – 4.6 cm, height – 50 cm, working volume of 0.7 l) packed by stones. Air was continuously pumped through an external loop of the airlift reactor with a flow rate of 3 ml/min.

**Wastewater.** The UASB reactor influent was based on vinasse because this is the source of the majority of the COD present in winery wastewater (Kalyuzhnyi *et al.*, 2000). Raw vinasse was obtained in the laboratory by distillation of low quality red or white wines delivered by the Center of Certification of Food Products (Moscow). The chemical content of various raw vinasses used is presented in Table 1. Feeds were prepared by dilution of raw vinasse with tap water followed by addition of 1–5 g of Na₂CO₃ (for increase of alkalinity) as well as 1–2 g of NH₄Cl and K₂HPO₄ (both mainly for stabilization of nutrient content). Partial anaerobic preacidification of wastewater was achieved when necessary by leaving it in a closed vessel inoculated with a small quantity of washed-out anaerobic sludge for 1–2 days at ambient temperature (17–22°C). The effluent from the UASB reactor (runs 1–2, Table 1) was used as an influent for the aerobic post-treatment step.

**Seed sludges and schedule of runs.** UASB reactor R1 was seeded with 1 l (41 g volatile suspended solids (VSS)) of the mainly granular sludge, originating from an UASB reactor treating winery wastewater at 18–20°C (Kalyuzhnyi *et al.*, 2000). It was continuously operated at 9–11°C (run 1, duration – 5 months), 6–7°C (run 2, duration – 3 months) and 4–5°C (run 3, duration – 1.5 months), 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 – 2.5 months), 7°C (run 5, duration – 1.5 months) and 4°C (run 6, duration – 1.5 months), respectively. The secondary sludge from Moscow municipal aeration station was used to seed an airlift reactor (run 7, duration – 2 months).

**Analysis.** Biogas production was recorded by a gas meter “GSB-400” (Gasprador, USSR). All gas measurements are expressed at 0°C and standard pressure (760 mm Hg). Gas composition, ethanol and volatile fatty acids (VFA) were analyzed by gas chromatography; sugars, ammonia, phosphates and polyphenols were measured spectrophotometrically as described elsewhere (Kalyuzhnyi *et al.*, 2000). Determinations of specific sludge activities (small batch tests), in situ determination of sludge kinetic characteristics, treatment of sludge samples for microscopy were performed as described previously (Kalyuzhnyi *et al.*, 2000). All other analyses were performed using Standard Methods (APHA, 1985).

### Table 1  Some characteristics of raw vinasses used

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total COD, g/l</strong></td>
<td>129.0</td>
<td>115.0</td>
<td>196.0</td>
<td>90.0</td>
<td>92.0</td>
<td>123.0</td>
<td>160.0</td>
<td>155.0</td>
</tr>
<tr>
<td><strong>Sugars, g COD/l</strong></td>
<td>50.3</td>
<td>72.0</td>
<td>159.0</td>
<td>34.1</td>
<td>34.1</td>
<td>15.0</td>
<td>74.9</td>
<td>32.0</td>
</tr>
<tr>
<td><strong>Ethanol, g COD/l</strong></td>
<td>41.2</td>
<td>3.3</td>
<td>2.6</td>
<td>3.9</td>
<td>7.6</td>
<td>60.0</td>
<td>11.6</td>
<td>18.9</td>
</tr>
<tr>
<td><strong>VFA, g COD/l</strong></td>
<td>13.3</td>
<td>1.4</td>
<td>1.0</td>
<td>0.9</td>
<td>2.0</td>
<td>21.0</td>
<td>9.6</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>2.7</td>
<td>2.9</td>
<td>3.1</td>
<td>3.1</td>
<td>3.0</td>
<td>3.5</td>
<td>3.0</td>
<td>2.7</td>
</tr>
<tr>
<td><strong>Polyphenols, g/l</strong></td>
<td>1.0</td>
<td>1.2</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
<td>0.9</td>
<td>1.2</td>
<td>1.8</td>
</tr>
<tr>
<td><strong>Usage (run)</strong></td>
<td>1a</td>
<td>1a-1b</td>
<td>1b</td>
<td>2a</td>
<td>2b-3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
Results and discussion

One stage UASB pre-treatment

The performance data of a single UASB reactor under psychrophilic conditions are generalized in Table 2. When the reactor was fed with non-preacidified vinasse at 10°C (run 1a), an organic loading rate (OLR) was increased stepwise to 4–5 g COD/l/d with a total COD removal of around 70% (Table 2). A significant presence of propionate (predominant component) and acetate was observed in the effluents. However, only traces of sugars, ethanol and butyrate were detected in the reactor liquor, while 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 (Rebac, 1998; Kato et al., 1999). It should be also noted that a substantial increase (~20%) of sludge bed height had occurred at the end of run 1a. This was primarily due to a substantial growth of acidogens in the reactor, because a fluffy outer layer covering the granules was seen under microscopic observations of the sludge aggregates. Since such types of aggregates can provoke sludge flotation and create mass transfer limitations for substrates of propionate-degrading and aceticlastic bacteria which are usually located in the central part of aggregates (Rebac, 1998), it was decided to apply preacidification of wastewater in order to achieve a better COD removal. But feeding with preacidified vinasse (run 1b, Table 2) did not result in an enhancement of COD removal, however, the imposed OLRs were somewhat higher (6–7 g COD/l/d) than those applied during run 1a. Moreover, the effluent propionate concentrations often exceeded 1.5 g COD/l during days 95–119. Meantime, the substantial decolouration of effluents was also observed during run 1 – the average reduction of polyphenol content was around 40% (Table 2).

In order to have a deeper insight into the processes occurring in the psychrophilic UASB reactor, the sludge kinetic characteristics were assessed in situ, i.e., under reactor conditions (days 121–137, Table 3) as well as in small batch tests (day 113, Table 4). Satisfactory coincidence was observed for specific sludge activities determined by two different methods. Lower values of activities found in small batch tests (Table 4, day 113) in comparison with in situ determinations (Table 3, days 121–137) can be explained by better mixing conditions 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 above-mentioned supposition about the existence of mass transfer limitations inside the sludge bed of psychrophilic UASB reactor, because the significantly lower values of apparent half-

### Table 2  Operation performance of single UASB reactor (average values are given in brackets)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Run 1a</th>
<th>Run 1b</th>
<th>Run 2a</th>
<th>Run 2b</th>
<th>Run 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of experiment</td>
<td>0–67</td>
<td>68–158</td>
<td>159–185</td>
<td>197–222</td>
<td>251–273</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>9–11</td>
<td>9–10</td>
<td>6.5–7.5</td>
<td>6.5–7.5</td>
<td>3.5–5.0</td>
</tr>
<tr>
<td>Recycle ratio</td>
<td>1.26</td>
<td>1.26</td>
<td>1.26</td>
<td>1.116</td>
<td>1.116</td>
</tr>
<tr>
<td>$V_{up}$, m/h</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>OLR, g COD/l/d</td>
<td>0.3–5.1</td>
<td>1.4–7.3</td>
<td>3.2–4.6</td>
<td>2.8–3.5</td>
<td>1.1–2.7</td>
</tr>
<tr>
<td>HRT, days</td>
<td>0.8–5.1</td>
<td>0.5–1.6</td>
<td>0.85–0.87</td>
<td>0.9–1.3</td>
<td>1.14–1.17</td>
</tr>
<tr>
<td>Influent COD$_{boc}$, g/l</td>
<td>3.6–5.2</td>
<td>1.2–3.9</td>
<td>2.7–4.0</td>
<td>3.0–3.6</td>
<td>1.3–3.12</td>
</tr>
<tr>
<td>Effluent COD$_{boc}$, g/l</td>
<td>0.3–2.7</td>
<td>0.5–3.6</td>
<td>0.8–1.9</td>
<td>0.9–1.5</td>
<td>0.6–1.5</td>
</tr>
<tr>
<td>COD$_{rem}$ removal, %</td>
<td>48–92(72)</td>
<td>48–92(60)</td>
<td>52–79(68)</td>
<td>51–71(61)</td>
<td>15–72(57)</td>
</tr>
<tr>
<td>Gas production, l/l/day</td>
<td>0.9–1.1</td>
<td>0.8–0.9</td>
<td>0.4–0.8</td>
<td>0.1–1.3</td>
<td>0.1–0.3</td>
</tr>
<tr>
<td>$C_{H_2}$ in biogas, %</td>
<td>78–84(81)</td>
<td>78–82(80)</td>
<td>78–88(84)</td>
<td>72–88(82)</td>
<td>67–89(81)</td>
</tr>
<tr>
<td>Effluent pH</td>
<td>6.9–7.8</td>
<td>6.9–7.5</td>
<td>6.7–7.1</td>
<td>6.7–7.5</td>
<td>7.2–7.9</td>
</tr>
<tr>
<td>Effluent VFA, g COD/l</td>
<td>0.1–2.5</td>
<td>0.4–3.5</td>
<td>0.2–1.8</td>
<td>0.4–0.9</td>
<td>0.3–1.0</td>
</tr>
</tbody>
</table>
saturation constants were found in the EGSB reactor at $V_{up}$ of 10 m/h and similar temperature (Rebac, 1998). 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, though the overall quantity of the sludge in the reactor increased by 32% (from 41 to 54 g 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 COD/d/g VSS (Table 4, days 0 and 113) indicating about a substantial enrichment of the sludge by methanogenic bacteria. The fact that sludge enrichment (and not only sludge adaptation to low temperatures) was a prevailing reason of sludge activity increase is confirmed by a substantial increase of its specific aceticlastic activities assessed at 30°C (Table 4, days 0 and 113).

A decrease of temperature during run 2a to 7°C keeping a recycle ratio the same (1:2.6) did not result in deterioration of total COD removal (days 159–185), however, the OLRs imposed were somewhat lower (around 4 g COD/l/d) than those applied during run 1b (Table 2). Polyphenol removal also remained the same as during run 1b (around 40%, Table 2) but the effluent VFA concentrations decreased almost twice (days 159–185). Though the overall quantity of the sludge in the reactor further increased to 59 g VSS throughout run 2a, its specific activities (for all substrates tested) assessed at 7°C hardly changed in comparison with those assessed at 10°C during the previous run (Table 4, days 0 and 113).

A decrease of temperature during run 2a to 7°C keeping a recycle ratio the same (1:2.6) did not result in deterioration of total COD removal (days 159–185), however, the OLRs imposed were somewhat lower (around 4 g COD/l/d) than those applied during run 1b (Table 2). Polyphenol removal also remained the same as during run 1b (around 40%, Table 2) but the effluent VFA concentrations decreased almost twice (days 159–185). Though the overall quantity of the sludge in the reactor further increased to 59 g VSS throughout run 2a, its specific activities (for all substrates tested) assessed at 7°C hardly changed in comparison with those assessed at 10°C during the previous run (Table 4, days 0 and 113). However, a possible enrichment of the sludge by acetogenic and methanogenic microflora

**Table 3** Sludge kinetic characteristics assessed directly in a single psychrophilic UASB reactor (results are expressed as means±standard error)

<table>
<thead>
<tr>
<th>Parameter/Substrate</th>
<th>Ethanol</th>
<th>Butyrate</th>
<th>Propionate</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_m$, g COD/l/day</td>
<td>15.30±1.51</td>
<td>6.52±0.52</td>
<td>3.94±0.33</td>
<td>7.78±0.31</td>
</tr>
<tr>
<td>$K_m$, g COD/l</td>
<td>0.571±0.062</td>
<td>1.413±0.110</td>
<td>1.333±0.101</td>
<td>0.589±0.029</td>
</tr>
<tr>
<td>$A^*$, g COD/day/g VSS</td>
<td>0.612±0.061</td>
<td>0.217±0.017</td>
<td>0.121±0.011</td>
<td>0.370±0.013</td>
</tr>
</tbody>
</table>

**Table 4** Sludge activities (g COD/d/g VSS) determined in small batch tests, single UASB reactor, 3rd sampling port (results are expressed as means±standard error)

<table>
<thead>
<tr>
<th>Day</th>
<th>T, °C</th>
<th>Glucose</th>
<th>Ethanol</th>
<th>Butyrate</th>
<th>Propionate</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>not determined</td>
<td>not determined</td>
<td>not determined</td>
<td>not determined</td>
<td>0.103±0.003</td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td>not determined</td>
<td>not determined</td>
<td>not determined</td>
<td>not determined</td>
<td>0.629±0.008</td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>not determined</td>
<td>0.566±0.010</td>
<td>not determined</td>
<td>0.083±0.011</td>
<td>0.134±0.015</td>
</tr>
<tr>
<td>50</td>
<td>30</td>
<td>not determined</td>
<td>2.191±0.394</td>
<td>not determined</td>
<td>0.132±0.004</td>
<td>0.700±0.071</td>
</tr>
<tr>
<td>113</td>
<td>10</td>
<td>2.677±0.288</td>
<td>0.569±0.009</td>
<td>not determined</td>
<td>0.080±0.007</td>
<td>0.205±0.001</td>
</tr>
<tr>
<td>113</td>
<td>30</td>
<td>3.363±0.099</td>
<td>2.399±0.134</td>
<td>not determined</td>
<td>0.123±0.062</td>
<td>0.947±0.051</td>
</tr>
<tr>
<td>184</td>
<td>7</td>
<td>not determined</td>
<td>0.521±0.136</td>
<td>0.123±0.003</td>
<td>0.081±0.011</td>
<td>0.209±0.006</td>
</tr>
<tr>
<td>184</td>
<td>30</td>
<td>not determined</td>
<td>2.254±0.047</td>
<td>0.613±0.032</td>
<td>0.103±0.017</td>
<td>0.866±0.009</td>
</tr>
</tbody>
</table>
could be masked by a 3°C temperature drop, but the determination of specific sludge activities at 30°C did not confirm this supposition. From Table 4 (days 113 and 184), it is seen that all the sludge VFA degrading activities assessed at 30°C slightly dropped throughout run 2a. A further accumulation of large aggregates with irregular forms in the reactor sludge was observed at the end of this run compared to the previous run. In order 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 (Table 3, days 187–195). It is seen that a substantial drop in the apparent \( K_m \) values was observed. Again, the calculated specific sludge activities \( A^* \) (Table 3, days 187–195) were comparable to those assessed in small batch tests (Table 4, day 184).

Since an 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 (days 197–222, Table 2). As expected, an almost four-fold increase of \( V_{up} \) resulted in a better VFA removal though a total COD removal efficiency slightly decreased compared to run 2a. This was mainly due to an increased sludge washout because small sludge aggregates were continuously accumulated in the effluent recipient vessel during this run. Also a 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, nevertheless there was low gas production (Table 2). In spite of washout losses which were unfortunately not quantified, the overall quantity of the reactor sludge reached a value of 62.7 g 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. It should be noted that polyphenol removal efficiency substantially dropped during this run (Table 2). It is likely that relatively big (compared to VFA) molecules of these substances had substantial mass transfer difficulties to be degraded by large sludge aggregates accumulated in the reactor. In situ determinations of sludge kinetic characteristics after run 2b (Table 3, days 223–246) revealed a tendency of deterioration of apparent half-saturation constants \( K_m \) for all the substrate tested. This is logical because larger sludge aggregates create bigger mass transfer limitations for soluble substrates that kinetically result in increase of \( K_m \) values (Rebac, 1998). It should be noted that a noticeable increase of \( V_m \) value for propionate was found, manifesting a substantial enrichment of the sludge by propionate-degrading bacteria. It resulted in reduced effluent concentrations of propionate, which became comparable with acetate concentrations during days 197–222 (data not shown). Thus, contrary to the first 120 days, propionate was not a predominant VFA component in the effluent anymore.

A further decrease of working temperature to 4°C was accompanied by a decrease of OLR imposed during run 3 (Table 2). In general, the overall reactor performance was similar to that during run 2b though polyphenol removal efficiency further dropped to 20%. A sludge washout was also observed but it tended to decrease during this run. It was because a majority of fine sludge aggregates were already eliminated from the reactor during the precedent assessment of sludge kinetic characteristics accompanied by high \( V_{up} \) (days 223–246). Microscopic observation of the sludge showed an overwhelming predominance of fluffy large aggregates (4–5 mm) with irregular forms and it looked like flocculent ones. Such evolution of the sludge can be attributed to the fact that the reactor influent was not completely preacidified by the preacidification procedure applied, e.g., sometimes quite noticeable concentrations of ethanol (up to 2 g COD/l) and sugars (up to 0.6 g COD/l) entered to the reactor stimulating a development of fluffy acidogenic biomass which deteriorated the sludge quality. Thus, a control of preacidification efficiency seems to be essential for a stable pretreatment process of winery wastewater at low temperatures. In situ determinations of sludge kinetic characteristics after run 3 (Table 3, days 274–279) confirmed the above-mentioned observation that large sludge aggregates accumulated in the
reactor had increased values of apparent half-saturation constants $K_m$. It should be also noted that in the temperature range below 7°C, even a small (3°C from run 2 to run 3) temperature drop led to an 8–30% decrease of $V_m$ values and, correspondingly, specific sludge activities.

Two-stage UASB pretreatment

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

It is seen that at 10°C (run 4), reactor R1 was operated at an average OLR of 4.4 g COD/l/d (Table 5) providing an average COD$_{tot}$ removal of 46%. Acetate was a predominant product of preacidification while butyrate was hardly generated at all (data not shown). Meantime, ethanol (0.1–0.2 g COD/l) was sometimes detected in the effluents of this reactor. In turn, reactor R2 was maintained at an average OLR of 2.5 g COD/l/d (Table 5) removing 58% (on the average) of total COD. The latter characteristics could be higher but some sludge washout was observed in reactor R2 contrary to reactor R1 where sludge washout was negligible. Thus, an overall treatment efficiency of the combined system was 78% (on the average) on total COD (Table 5), i.e., higher than in a single UASB reactor (Table 2, run 1). Even more significant enhancement was obtained for polyphenol removal (compare Tables 2 and 5).

However, a substantial disintegration of the sludge occurred in reactor R2 approximately in the middle of this run, probably due to the lysis of abundant acidogenic bacteria presented in the seed sludge, because feeding influent to this reactor practically consists of acetate and propionate alone. 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. Despite its overall quantity decreased from 32.5 to 29.5 g VSS due to partial lysis and

### Table 5: Operation performance of two UASB reactors in series (average values are given in brackets)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Run 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of exp.</td>
<td>0–63</td>
<td>82–107</td>
<td>122–147</td>
</tr>
<tr>
<td>Temp., °C</td>
<td>9.5–10.5 (10.2)</td>
<td>6.5–7.5 (7.1)</td>
<td>3.5–4.5 (3.9)</td>
</tr>
<tr>
<td>Reactor R1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recycle ratio</td>
<td>1:1</td>
<td>1:18</td>
<td>1:1</td>
</tr>
<tr>
<td>OLR, g COD/l/d</td>
<td>3.2–5.5 (4.4)</td>
<td>0.8–4.0 (2.5)</td>
<td>2.3–4.2 (3.5)</td>
</tr>
<tr>
<td>HRT, days</td>
<td>0.8–1.3 (1.0)</td>
<td>0.8–1.2 (1.0)</td>
<td>1.0–1.1 (1.0)</td>
</tr>
<tr>
<td>Inf. COD$_{tot}$, g/l</td>
<td>3.1–5.4 (4.3)</td>
<td>1.6–3.9 (2.5)</td>
<td>2.5–4.2 (3.5)</td>
</tr>
<tr>
<td>Eff. COD$_{tot}$, g/l</td>
<td>1.0–3.9 (2.4)</td>
<td>0.4–2.8 (1.2)</td>
<td>1.3–3.1 (2.3)</td>
</tr>
<tr>
<td>COD$_{rem}$, %</td>
<td>16–76 (46)</td>
<td>24–80 (58)</td>
<td>19–52 (37)</td>
</tr>
<tr>
<td>Gas prod., l/l/d</td>
<td>0.1–0.6 (0.4)</td>
<td>0.1–0.4 (0.3)</td>
<td>0.1–0.4 (0.2)</td>
</tr>
<tr>
<td>CH$_4$ in biogas, %</td>
<td>68–90 (80)</td>
<td>45–92 (80)</td>
<td>70–89 (82)</td>
</tr>
<tr>
<td>Effluent pH</td>
<td>6.3–7.1 (6.6)</td>
<td>6.9–7.8 (7.4)</td>
<td>6.0–7.2 (6.7)</td>
</tr>
<tr>
<td>VFA$_{tot}$, g COD/l</td>
<td>0.7–3.0 (2.4)</td>
<td>0.2–2.0 (0.8)</td>
<td>1.3–2.6 (1.9)</td>
</tr>
<tr>
<td>P-phenol$_{rem}$, %</td>
<td>38–52 (45)</td>
<td>9–38 (22)</td>
<td>28–37 (31)</td>
</tr>
</tbody>
</table>

Combined system (R1+R2)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Run 4</th>
<th>Run 5</th>
<th>Run 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLR, g COD/l/d</td>
<td>1.6–2.8 (2.2)</td>
<td>1.2–2.1 (1.8)</td>
<td>1.0–1.4 (1.3)</td>
</tr>
<tr>
<td>HRT, days</td>
<td>1.6–2.5 (2.0)</td>
<td>2.0–2.2 (2.0)</td>
<td>1.6–2.0 (1.8)</td>
</tr>
<tr>
<td>COD$_{rem}$, %</td>
<td>36–91 (78)</td>
<td>42–89 (76)</td>
<td>60–86 (71)</td>
</tr>
<tr>
<td>P-phenol$_{rem}$, %</td>
<td>44–70 (57)</td>
<td>41–57 (50)</td>
<td>20–56 (44)</td>
</tr>
</tbody>
</table>
washout, aceticlastic activity of this sludge determined in small batch tests increased substantially at both 10 and 30°C (Table 6, days 0 and 63). It showed a significant enrichment of the R2 sludge by methanogenic bacteria. 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, days 65–73 and compare them with those from Table 3). However, apparent half-saturation constants K_m for all the substrates tested were found to be higher (Table 7) under 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 VSS, however, its aceticlastic activity determined in small batch tests decreased both at 10 and 30°C (Table 6, days 0 and 63). This is logical because this reactor mainly performed the acidogenic conversions. The sludge from reactor R1 at the end of run 4 consists of large flocculent aggregates (3–4 mm) with a significant presence of fine particles also witnessing to a partial disintegration of the seed sludge. A cause of this phenomenon is not quite clear. Probably too intensive development of acidogenic bacteria had a detrimental effect on the stability of the large aggregates.

After a shift of a combined system to 7°C (run 5), an average OLR in reactor R1 was maintained as 3.5 g COD/l/d (Table 5). Under these conditions, reactor R1 removed 37% of total COD (on the average) and an average polyphenol removal was 31%. The effluent of this reactor, predominantly consisting of acetate and propionate (days 82–107), was pumped into reactor R2. Its average OLR was thus 2.2 g COD/l/d (Table 5). Under the conditions imposed, reactor R2 removed 61% of total COD (on the average) and an average polyphenol removal was 27%. Thus, an overall treatment efficiency of combined system at 7°C was 76 and 50% (on the average) with regard to total COD and polyphenols, respectively (Table 5), i.e., 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 (1–2 mm) and fine particles at the end of this run (possible cause of this

### Table 6

Aceticlastic sludge activities (g COD/d/g VSS) determined in small batch tests (two UASB reactors in series, results are expressed as means ± standard error)

<table>
<thead>
<tr>
<th>Day</th>
<th>T, °C</th>
<th>R1</th>
<th>R2</th>
<th>Day</th>
<th>T, °C</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>0.128±0.003</td>
<td>107</td>
<td>7</td>
<td>0.068±0.001</td>
<td>0.145±0.005</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>30</td>
<td>0.780±0.028</td>
<td>107</td>
<td>30</td>
<td>0.670±0.013</td>
<td>1.146±0.013</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>10</td>
<td>0.097±0.002</td>
<td>117</td>
<td>30</td>
<td>0.055±0.010</td>
<td>0.140±0.013</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>30</td>
<td>0.695±0.047</td>
<td>1.337±0.008</td>
<td>147</td>
<td>4</td>
<td>0.658±0.014</td>
<td>1.083±0.023</td>
</tr>
</tbody>
</table>

### Table 7

Sludge kinetic characteristics assessed directly in reactor R2 (means ± standard error)

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

A* = sludge activity calculated as in Table 3.
phenomenon is mentioned above). A low but continuously observed washout of the sludge has resulted in that its overall quantity in reactor R1 hardly changing (31.7 g VSS at the end of run 5) but its aceticlastic activity determined in small batch tests slightly decreased (Table 6, day 107). Similarly, aceticlastic activity of the sludge from reactor R2 determined in small batch tests also slightly decreased (Table 6, day 107) accompanied by a decrease of the overall sludge quantity in the reactor (25.2 g VSS at the end of run 5) due to lysis and washout. However, the sludge quality seems to increase – a substantial presence of 1–2 mm granules was found under visual and microscopic observations of the sludge samples taken at day 107. In situ assessment of kinetic parameters confirmed the existence of moderate mass transfer limitations under reactor conditions with \( V_{up} \) of 0.55 m/h (see \( K_m \) values in Table 7, days 113–119). Meantime, a noticeable increase of propionate-degrading activity \( A^* \) of the sludge calculated from these data corresponds well with the low propionate concentrations observed during days 88–106 (data not shown).

After a decrease of working temperature to 4°C (run 6), an average OLR imposed on reactor R1 was 2.5 g COD/l/d while an average COD\(_{tot}\) removal achieved was 37% (Table 5). However, a preaacidification was complete because practically only acetate and propionate were detected in the effluent. In turn, at an average OLR imposed to reactor R2 of 1.7 g COD/l/d, an average total COD removal achieved was 53% (Table 5). Thus, an overall efficiency of a combined system was 71% with substantial decolouration of effluent (an average polyphenol removal was 44%, Table 5). Comparing these values to those obtained with a single UASB reactor (run 3, Table 2), one can again state that a combined system showed substantially higher treatment efficiencies, especially with regard to polyphenol removal. No significant changes occurred with the sludges in both reactors throughout run 6 compared to the previous run. Both sludges mainly consisted of granule-like aggregates (~ 2 mm in diameter) though a moderate quantity of fluffy aggregates and fine particles were also present in reactor R1. Due to negligible washout, the overall quantity of the sludge slightly increased in both reactors at the end of run 6 (33.2 and 26 g VSS for reactors R1 and R2, respectively). The specific activities of the sludges determined in situ (Table 7, days 148–156) and in small tests (Table 6, day 147) also changed significantly compared to run 5.

### Aerobic post-treatment of anaerobic effluents

Since the effluents after anaerobic treatment of vinasse are sometimes quite persistent to the aerobic post-treatment (de Bazua et al., 1991), anaerobic effluents produced in our case were processed via airlift reactor taken as an example of such a post-treatment. From generalized results of Table 8, it is seen that the average COD\(_{tot}\) removal was 82% giving an average effluent concentration of 0.11 g COD\(_{tot}\)/l (Table 8). A major part (0.07 g/l, on average) of effluent COD consisted of acetate and propionate indicating that the efficiency of aerobic post-treatment can be improved (if necessary) but it was not a primary goal of the research described in this paper. Thus, a psychrophilic UASB pretreatment of winery wastewater followed by a proper aerobic post-treatment step is expected to be an adequate solution to meet the requirements for a final effluent discharge.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of experiment</td>
<td>0–55</td>
<td>Effluent COD(_{tot}), g/l</td>
<td>0.04–0.17 (0.11)</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>20–25 (22)</td>
<td>COD(_{tot}) removal, %</td>
<td>72–90 (82)</td>
</tr>
<tr>
<td>OLR, g COD/l/d</td>
<td>0.32–2.06 (0.84)</td>
<td>Effluent pH</td>
<td>8.4–8.9 (8.6)</td>
</tr>
<tr>
<td>HRT, days</td>
<td>0.25–1.27 (0.86)</td>
<td>Effluent VFA, g COD/l</td>
<td>0.03–0.11 (0.07)</td>
</tr>
<tr>
<td>Influent COD(_{tot}), g/l</td>
<td>0.40–1.15 (0.61)</td>
<td>Polyphenol removal, %</td>
<td>13–44 (31)</td>
</tr>
</tbody>
</table>
Conclusions

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 total COD removal was around 60% at OLRs of 4.7, 3.7 and 1.7 g COD/l/d (HRTs were around 1 d) for 9–11, 6–7 and 4–5°C, respectively. For two UASB reactors in series treating non-preacidified vinasse, the average total COD removal was higher (> 70%) at the OLRs for a whole system of 2.2, 1.8 and 1.3 g COD/l/d and HRTs of around 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 an application of higher recycle ratios is essential for enhancement of UASB pre-treatment under psychrophilic conditions. The produced anaerobic effluents were shown to be efficiently post-treated aerobically – final effluent COD concentrations were around 0.1 g/l. A successful operation of the UASB reactors at quite low temperatures (4–10°C) opens some perspectives for application of high-rate anaerobic pre-treatment at ambient temperatures, e.g. in southern regions of Russia where the main national wineries are located.

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References
