Environmental Technology, Vol. 21. pp 919-925

THE UASB TREATMENT OF WINERY WASTEWATER UNDER SUBMESOPHILIC AND PSYCHROPHILIC CONDITIONS

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(Received 22 November 1999; Accepted 10 March 2000)

ABSTRACT

The start-up and operational performance of two laboratory UASB reactors (working volume of 2.6 l) treating diluted vinasse (1-17 g COD l^{-1}) were investigated at 35 °C (run 1, without recycle), 19-21°C (run 2, without recycle), 18-20 °C (run 3, with recycle 1:1) and 9-10 °C (run 4, with recycle 1:2.6). The reactors for runs 1 and 2 were seeded with flocculant mesophilic sludge. For runs 3 and 4, the reactor seed sludge was upgraded by addition of 30% of psychrotrophically adapted granular sludge. A successful start-up of the reactors for all runs was achieved in 2-3 months. The maximum applied organic loading rates (OLR) were 15.9, 6.5, 12.5 and 7.2 g COD l^{-1}d^{-1} for runs 1-4, respectively. Hydraulic retention times at these loadings were around or less than 1 day. The total COD removals achieved under these OLR were higher than 85% for the first 3 runs and higher than 60% for run 4 with substantial decoloration of effluents (reduction of polyphenol content varied between 45-67%).

Keywords: COD removal, psychrophilic, submesophilic, UASB reactor, vinasse.

INTRODUCTION

Due to an unfavourable climate for grape cultivation in the overwhelming majority of territory, Russia is not one of the significant wine producers of the world, although at least 70 medium to small-scale wineries currently operate in the southern regions of the country. A typical plant with primary wine production has facilities to process about 10 000 tons of grape per production season, which usually lasts for 20 to 60 days in autumn [1]. Wastewater flows from various production steps are usually mixed together with cooling, washing and sewage flows (Table 1).

Table 1. Characteristics of wastewater produced by typical winery processing 10 000 tons of grape per year (adapted from [1]).

Wastewater characteristics	Source of wastewater					Average concentration, g l^{-1}
	Grape processing	Vinasse	Cooling + washing	Sewage and other	Total per year	
Volume:						
per day, m^3	150^{*}	295^{*}	220^{*}	30^{*}-20^{**}	695^{*}-20^{**}	-
per year, m^3	3,000	5,900	4,400	600^{*}-$5,100^{**}$	19,000	-
Temperature, °C	20^{*}	80-85^{*}	18-25^{*}	20^{*}-18^{**}	-	-
pH	6.4^{*}	3.8-4.1^{*}	10.8^{*}	7.1^{*}-7.2^{**}	-	5.4^{*}-7.2^{**}
COD, tons	30^{*}	198^{*}	4.12^{*}	0.13^{*}-0.74^{**}	234	16.8^{*}-0.2^{**}
BOD, tons	16.8^{*}	122^{*}	3.98^{*}	0.1^{*}-0.51^{**}	143	10.3^{*}-0.1^{**}
VSS, tons	6.6^{*}	111^{*}	2.16^{*}	0.09^{*}-0.56^{**}	120	8.6^{*}-0.11^{**}
N-NH$_3$, kg	22.8^{*}	155^{*}	14.4^{*}	5.2^{*}-30.0^{**}	227	0.014^{*}-0.006^{**}
Phosphate, kg	20.1^{*}	72^{*}	2.8^{*}	2.2^{*}-6.6^{**}	104	0.007^{*}-0.001^{**}
Polyphenols, tons	1.215^{*}	1.905^{*}	0.04^{*}	-	3.2	0.23^{*}
Tartaric acids, tons	6.42^{*}	35.2^{*}	0.48^{*}	-	42.1	3.03^{*}

*during the production season (20 days)
**in inter-seasonal period

The resulting wastewater has a moderate temperature (around or below 20 °C) and variable strength (> 10 g COD l^{-1} during the production season and < 1 g COD l^{-1} in the inter-seasonal period). Though some medium-scale wineries are equipped with aerobic treatment plants, these work unsatisfactorily, if at all, because of their frequent seasonal overloadings with the high-strength vinasse accompanied by a deficit of N- and P-sources (Table 1). Such an exploitation practice as well as an absence of treatment facilities on small-scale wineries has led to the severe pollution of soils, ground waters, rivers and lakes in the surrounding areas.

A possible solution of the problem can be an application of anaerobic treatment. This has several well known advantages in comparison with aerobic treatment, especially for treatment of high-strength wastewater : no energy needs for aeration (on the contrary, generation of energy in the form of biogas), substantially reduced nutrient requirements, high organic loading rates (OLR) etc. [2]. From the other side, an implementation of conventional anaerobic treatment (especially in the countries with a cold climate such as Russia) is often hindered by the necessity of maintaining an operation temperature (mesophilic or thermophilic) which is significantly higher than ambient temperatures. However, the recent developments in anaerobic high-rate reactor technology substantially extend its potential with regard to low temperature regimes [2,3]. Thus, the primary objective of this study is to examine the suitability of the upflow anaerobic sludge blanket (UASB) reactor for the treatment of winery wastewater under submesophilic (18-20 °C) and psychrophilic (9-10 °C) conditions in terms of COD removal and methane production. A classical mesophilic regime (35 °C) was also assessed as a reference control. The second objective of the work was to obtain more insight into the sludge kinetic characteristics at low temperatures.

MATERIALS AND METHODS

UASB reactors

Investigations were carried out in two laboratory UASB reactors (A and B, diameter, 6.8 cm, height, 85 cm, total working volume, 2.6 l) made from transparent plastic and equipped with six sampling ports along the reactor height. Operating temperatures of 35±1 and 10±1 °C were maintained by placing the corresponding reactor into thermostat 'TS-80' (Mashzavod, Odessa, USSR) or refrigerator 'Snaige' (Alitus, Lithuania), respectively. The submesophilic conditions were imposed by keeping the reactors under ambient temperature in the laboratory (18-21 °C). Feeding of the reactors as well as recycle of effluent (when necessary) was achieved using peristaltic pumps NP-1M (Kievpribor, Kiev, USSR), P-1 (Pharmacia, Sweden) and Masterflex L/S (Cole Parmer, USA).

Wastewater

The reactor influent was based on vinasse because this is the source of the majority of the COD present in winery wastewater (Table 1). Raw vinasse was obtained in the laboratory by distillation of low quality red or white wines delivered by the Centre of Certification of Food Products (Moscow). The chemical content of various raw vinasses used is presented in Table 2. Feeds were prepared by dilution of raw vinasse with tap water or treated effluent followed by addition of sodium carbonate (for increase of alkalinity) as well as ammonium chloride and di-potassium hydrogen phosphate (both mainly for stabilisation 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 sludge for 1-2 days at ambient temperature (18-20 °C). Feed characteristics are summarised in Table 3.

Seed sludge and schedule of runs

Reactors A and B were seeded with 800 ml (approximately 30 g volatile suspended solids VSS) of mesophilic sludge originating from an UASB reactor treating pig manure wastewater [4]. Initially they were operated at 35 °C (run 1, duration - 3 months) and 19-21 °C (run 2, duration - 4 months), respectively. No recycle of effluent was applied for these runs. After termination of run 1, reactor A

Table 2. Chemical content of raw vinasses used.

	Vinasse sample						
Characteristics	1	2	3	4	5	6	7
COD, g l^{-1}	99	86	210	104	129	115	196
Sugars, g COD l^{-1}	33.0	28.8	67.0	35.4	50.3	72.0	159
Ethanol, g COD l^{-1}	64.2	19.8	98.8	57.6	41.2	3.3	2.6
VFA, g COD l^{-1}	1.5	1.8	2.4	1.1	13.3	1.4	1.0
pH	2.5	3.0	3.0	3.0	2.7	2.9	3.1
Polyphenols, g l^{-1}	0.95	1.2	0.48	0.81	0.96	1.2	0.9
N-NH_3, mg l^{-1}	8.4	5.6	75.6	58.8	ND	ND	ND
Phosphate, g l^{-1}	0.265	0.325	0.161	0.267	0.22	0.19	0.25
Usage (run)	1	1	2-3	2-3	4	4	4

ND - not determined

Table 3. Main feed characteristics.

Influent	COD_{tot}, ($g\,l^{-1}$)	VFA, ($g\,COD\,l^{-1}$)	Na_2CO_3, ($g\,l^{-1}$)	NH_4Cl, ($g\,l^{-1}$)	K_2HPO_4, ($g\,l^{-1}$)	pH
Non-preacidified	1-17	1-2	1-5	0-2	0-2	7.0-8.0
Preacidified	2-12	2-9	1-5	0-2	0-2	6.5-6.9

with the sludge inside was stored without feeding under 4-8 °C for 7 months before it was used for operation at 18-20 °C (run 3, duration - 3 months). The reactor sludge was upgraded (30%) with psychrotrophically adapted sludge originating from an EGSB reactor treating synthetic wastewater [3]. When this run was finished, reactor A was again kept in a cool room without feeding or sludge removal for 4 months and then used for the experiments at 9-10 °C (run 4, duration -5 months). Recycle of effluent was applied for runs 3 and 4 to decrease mass transfer limitations between the sludge and the feed inside the reactor [5] (recycle ratios were 1:1 and 1:2.6, respectively).

Analysis

Biogas production was recorded by a GSB-400 gas meter (Gaspribor, USSR). All gas measurements are expressed at 0°C and standard pressure (760 mm Hg). Feed input to the UASB reactors was monitored by measuring the accumulated outflow on a daily basis. Gas composition, ethanol and volatile fatty acids (VFA) were analysed by gas chromatography as described by Kalyuzhnyi *et al.* [6]. Sugars, ammonia, phosphates and polyphenols were measured spectrophotometrically as described elsewhere [7]. Determinations of specific sludge activities (small batch tests without stirring) and treatment of sludge samples for microscopy were performed as described previously [6]. All other analyses were performed using Standard Methods [8].

Assessment of sludge kinetic parameters in reactor conditions

For *in situ* determination of sludge kinetic characteristics, the reactor was operated temporarily in batch-mode. Before starting the experiments, the reactor was kept unfed (but with effluent recycle) for 1-2 days in order to deplete all remaining biodegradable COD. At time zero, the concentration of assessed substrate (propionate, butyrate, acetate or ethanol) was set at 0.5-2 g COD l^{-1} and its depletion was monitored. The substrate depletion data were fitted to the integrated Michaelis-Menten equation using non-linear least-squares analysis [9].

RESULTS AND DISCUSSION

Mesophilic (35 °C) and submesophilic (18-21 °C) regimes

Although the seed sludge was mainly flocculent [5] and had been stored unfed for a period of 5 months before these experiments, it quickly adapted to the new feeding substrate – diluted vinasse. After 45 days - which can be considered as a start-up period - the mesophilic reactor A (run 1) had reached an organic loading rate (OLR) of 8 g COD $l^{-1}d^{-1}$ and a total COD reduction of around 90% (data not shown). Thereafter, OLR was increased in stages to a maximum of 16 g COD·$l^{-1}d^{-1}$ as hydraulic retention time (HRT) was reduced to around 1 day. Results of reactor steady-state operation (duration 5 HRT) under these conditions (Table 4) demonstrate that high treatment efficiency (COD removal > 90%) as well as substantial decoloration of effluents (reduction of polyphenol content was greater than 60%) can be achieved readily. An essential condition for such stable operation is maintenance of sufficient feed alkalinity (e.g., via addition of carbonate or dilution of raw vinasse by already carbonised effluent). Otherwise, a sudden acidification of the reactor medium may occur followed by reactor failure.

At the end of this run, the distribution of the most important process variables along the reactor height was assessed (Figure 1). It is seen that a clear division of reactor volume between acidogenic (first 10-15 cm from the bottom with pH values less than 6) and methanogenic zones can be observed (Figure 1b). Accordingly, the lowest specific aceticlastic methanogenic activity of the sludge (Figure 1a) was found in the first sampling port due to unfavourable conditions for methanogenic bacteria. In contrast, the sludge from the middle and upper parts of the sludge bed had very high methanogenic activity (Figure 1a) ensuring low levels of VFA in the effluent (Figure 1b and Table 4). The microscopic examination of sludge samples taken at the end of the run 1 showed a prevailing presence of well-formed granules in comparison with the other sludge aggregates observed (data not shown).

The running of reactor B under submesophilic conditions (19-21 °C, run 2) was initiated under an OLR of 1.5 g COD $l^{-1}d^{-1}$. A gradual increase of the OLR (over 3 months) resulted in the development of a stable treatment process with an OLR of about 6 g COD $l^{-1}d^{-1}$ and a total COD removal efficiency of more than 90% (Table 4). However, a sudden lifting of the sludge was observed upon further increase of OLR. In order to recover operational stability, the reactor was stopped for 1 day, allowing the sludge to settle down. The feeding of the reactor was then renewed at an OLR of 3 g COD $l^{-1}d^{-1}$ with a subsequent increase (over 10 days) to 6.5 g COD $l^{-1}d^{-1}$. Although 90% treatment efficiency

Table 4. Steady-state operation performance of UASB reactors treating diluted vinasse[*]

Parameters	Run 1	Run 2	Run 3	Run 4
Temperature, °C	35	19-21	18-20	9-10
Seed sludge	MF[#]	MF[#]	From run 1[$]	From run 3
Recycle ratio	No recycle	No recycle	1:1	1:2.6
Maximum OLR applied, g COD $l^{-1}d^{-1}$	15.9	6.5	12.5	7.2
HRT, days	1.1	0.68	1.1	0.85
Influent COD_{tot}, g l^{-1}	17.5	4.4	13.7	6.1
Effluent COD_{tot}, g l^{-1}	1.2±0.1	0.4±0.2	2.0±0.9	2.3±0.3
COD_{tot} removal, %	93.1±0.6	90.9±4.5	85.4±6.6	62.3±4.9
Gas production, l $l^{-1}d^{-1}$	5.5±0.3	2.5±0.3	5.3±0.6	0.87±0.06
CH_4 in biogas, %	63±2	75±3	77±2	80±2
Effluent pH	7.3±0.1	7.6±0.1	7.1±0.2	7.0±0.1
Total effluent VFA, g COD l^{-1}	0.20±0.08	0.20±0.07	0.44±0.31	2.1±0.4
Polyphenol removal, %	67.0±5.5	ND	ND	45.1±9.5

[*] Results expressed as means ± standard errors (n=3);
[#] MF – mesophilic flocculent;
[$] Upgraded with 30% of psychrotrophically adapted sludge;
ND – not determined.

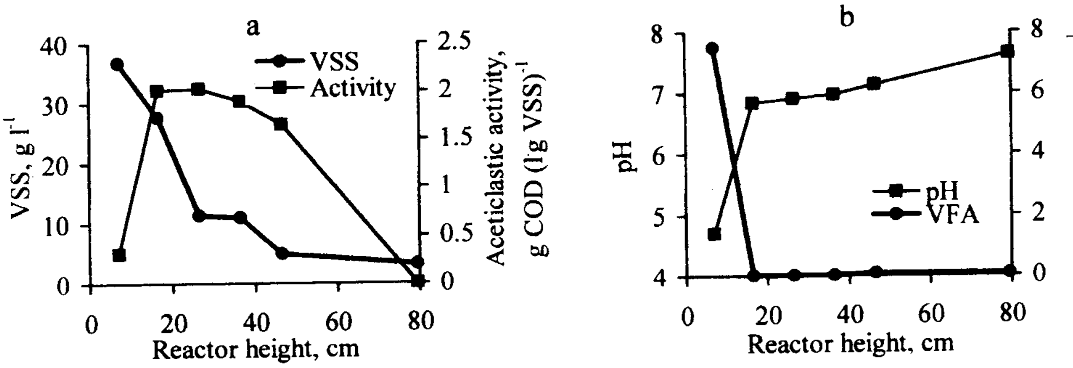

Figure 1. Profile characteristics of the mesophilic UASB reactor under OLR of 15.9 g COD $l^{-1}d^{-1}$ (35 °C). (a) - VSS and aceticlastic activity; (b) pH and VFA.

was achieved at this OLR, sludge flotation occurred again. The procedure for recovery of reactor stability was then repeated twice but the result was the same. It is likely that the OLR of around 6 g COD $l^{-1}d^{-1}$ was the maximum, consistent with stable operation for reactor B, which contained mainly non-adapted flocculent mesophilic sludge and was operated under submesophilic conditions (19-21 °C) without effluent recycle. The main reason for the phenomenon of sludge lifting was the poor sludge quality which was clearly revealed by electron microscopy. The sludge samples taken at the end of run 2 contained mainly irregular form aggregates, appearing to be flocs (data not shown). This indicated that practically no granulation occurred under submesophilic conditions compared with good granule growth under mesophilic conditions with the same seed sludge (run 1). Thus, the operational temperature has a crucial impact on formation of well-settled granules from the flocculent seed sludge.

The importance of seed sludge quality was demonstrated during run 3, when reactor A, having granular (mixed mesophilic/psychrophilic) sludge, was switched to submesophilic conditions (18-20 °C). The 3-months adaptation of the sludge to this temperature regime allowed an OLR of about 12 g COD $l^{-1}d^{-1}$ to be achieved with a reasonable treatment efficiency of around 85% total COD (Table 4). Any difficulties including sludge lifting were not observed at all. The sludge remained mainly in a granular form according to visual and microscopic observations with concentration amounting to 50 g VSS l^{-1} in the sludge bed zone (end of run 3). Moreover, the overall quantity of the sludge in the reactor increased from 29.1 to 41.4 g VSS throughout the run, and its specific aceticlastic activity assessed at 20 °C also increased from 0.31 to 0.41 g COD (g VSS d)$^{-1}$. The latter fact indicates that there was a substantial enrichment of the sludge by methanogenic bacteria. Thus, the application of granular seed sludge led to the development of a stable treatment process with an OLR twice that possible with flocculent seed sludge under similar submesophilic conditions. It should be noted that the application of effluent recycle (1:1) during run 3 (Table 4) also could have some additional positive influence on the UASB reactor performance due to decreasing mass transfer limitations [3,5].

Psychrophilic regime (9-10 °C)

The performance data of reactor A switched to psychrophilic conditions are shown in Tables 4-5 and Figure 2. The first 6 weeks (period I) can be considered as a reactor start-up, when an OLR was increased stepwise to 2 g COD $l^{-1}d^{-1}$ (Figure 2a) with a total COD removal of around 80% (Figure 2c). Further increase of OLR to 4-5 g COD $l^{-1}d^{-1}$ (days 44-67, period II) led to a drop in total COD removal to 60-70% (Figure 2c) with a significant presence of propionate (predominant component) and acetate in the effluents (Figure. 2b and d). 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 [3]. It should be also noted that a substantial increase (~20%) of sludge bed height had occurred after 2 months of reactor operation. This was due primarily to a substantial growth of

Table 5. Performance data of the psychrophilic UASB reactor (9-10 °C). Average values are given in parentheses.

Period	Days	Substrate	OLR, g COD $l^{-1}d^{-1}$	COD_{rem}, %	VFA_{eff}, g COD l^{-1}
I	0-43	NPV*	0.3-2.5 (1.8)	72-92 (85)	0.03-0.51 (0,24)
II	44-67	NPV*	3.1-5.1 (4.2)	48-80 (63)	0.40-2.53 (1.28)
III	68-94	PV#	3.7-5.2 (4.4)	42-75 (58)	0.39-2.67 (1.41)
IV	95-119	PV#	5.9-7.5 (6.8)	21-81 (53)	0.25-6.48 (2.80)
V	120-138	Batch operation for determination of kinetic parameters			
VI	139-148	Propionate	1.4-2.5 (2.1)	52-92 (65)	0.04-1.00 (0.43)
VII	149-158	PV#	5.0-5.3 (5.3)	58-88 (68)	0.12-1.10 (0.83)

*NPV – non-preacidified vinasse;
#PV – preacidified vinasse.

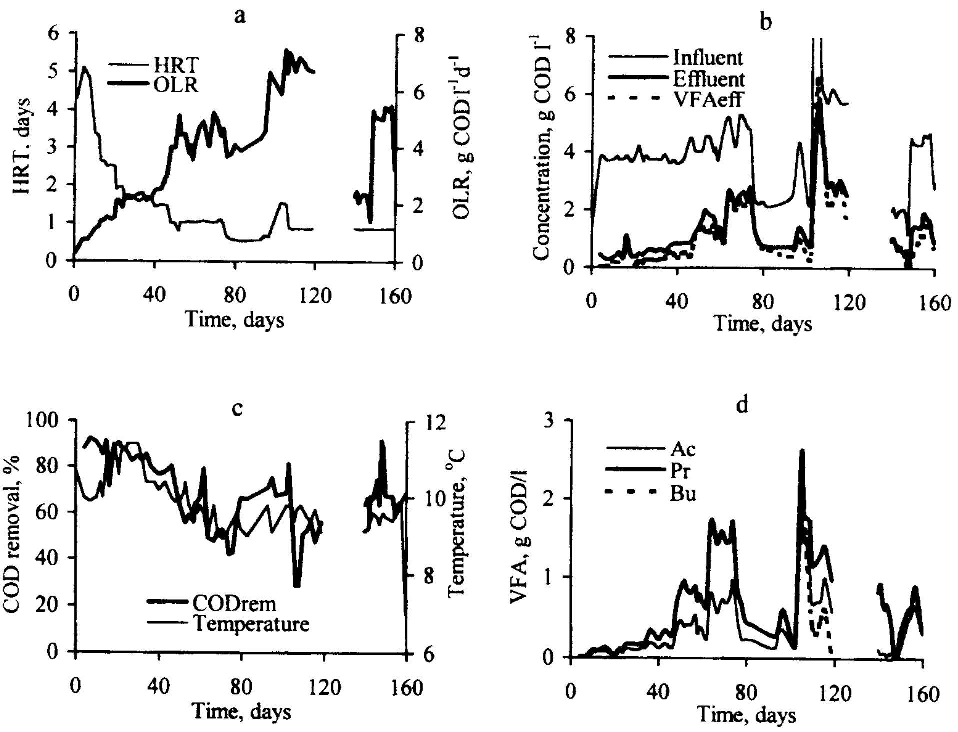

Figure 2. Operation parameters and efficiency of the psychrophilic UASB reactor: (a) HRT and OLR; (b) Total COD influent, total COD effluent and effluent VFA-COD concentrations; (c) COD removal and operation temperature; (d) Effluent acetate, propionate and butyrate concentrations.

acidogens in the reactor, because a fluffy outer layer covering the granules was seen under microscopic observations of the sludge aggregates. Similar granule changes were found on the anaerobic treatment of sugar-containing influents [3]. 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 [3,5], it was decided to apply preacidification of wastewater in order to achieve better COD removal. However, feeding with preacidified vinasse (days 68-94, period III) did not result in any enhancement of COD removal (Figure 2c) though the OLRs were similar to those applied during period II (Table 5). Further increase of OLR (days 95-119, period IV) led to a further drop in COD removal to around 50% (Figure 2c) while the effluent propionate concentrations often exceeded 2 g COD·l^{-1} (Figure 2d).

In order to have 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 120-138, period V) as well as in small batch tests. Satisfactory coincidence was observed for specific sludge activities determined by two different methods (Table 6). Lower values of activities found in small batch tests in comparison with *in situ* determinations 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 greater than 1.0 g COD·l^{-1} under reactor conditions (Table 6), which supports the above-mentioned supposition about the existence of mass transfer limitations inside the psychrophilic sludge bed, because the significantly lower values of apparent half saturation constants were found in the EGSB reactor under superficial velocity V_{up} of 10 m h^{-1} and similar temperature [9]. This fact together with the relatively low V_m assessed for propionate (Table 6) explains why the latter was prevalent in the reactor effluent. Simple estimations using the kinetic parameters from Table 6 clearly demonstrate that the imposed propionate loading exceeded the propionate assimilative capacity of the sludge at least twice during periods III and IV. This was further confirmed by direct experiment when propionate alone was fed to the reactor (days 139-148, period VI). It is seen that the propionate removal amounted to only 65% (Table 5, Figure 2) even at a relatively low OLR of 2 g COD l^{-1}d^{-1}. Meanwhile, it is likely that some enrichment of the sludge by propionate-degrading bacteria occurred during periods of V and VI, because a restoration of reactor feeding with preacidified vinasse (days 149-158, period VII) resulted in a moderate increase of both total COD and propionate removal at an OLR as high as 5 g COD l^{-1}d^{-1} compared with periods III and IV when similar OLRs were applied (Table 5, Figure 2). The better COD removal at psychrophilic temperatures can be obtained under improved mixing conditions inside the UASB reactor (e.g., by increasing superficial velocity) [3].

A relatively successful operation of the UASB reactor during runs 3 and 4 at low temperatures opens some perspectives for application of high-rate anaerobic treatment at ambient temperatures, e.g., in southern regions of Russia where the main national wineries are located. Anaerobic effluents thus produced could comply with the restrictions for a discharge into municipal sewers or be easily post-treated on existing aerobic tanks.

CONCLUSIONS

The application of granular seed sludge has a crucial impact on development of a stable treatment process under submesophilic (18-20 °C) and psychrophilic (9-10 °C) conditions. Formation of granules with good settling properties from flocculent seed sludge occurs more readily under mesophilic conditions than under submesophilic conditions.

On the basis of kinetic determinations, substantial mass-transfer limitations for soluble substrates have been found in a classical (low V_{up} values) UASB reactor operating under psychrophilic conditions. This fact together with decrease of specific sludge activities under low temperatures resulted in a retarded assimilation of VFA (mainly propionate) in psychrophilic regime. To enhance a propionate removal, mixing conditions in the reactor should be improved (e.g., by transformation of the classical UASB reactor into the EGSB reactor).

Table 6. Sludge kinetic characteristics assessed directly in the psychrophilic UASB reactor (9-10 °C; V_{up} = 0.1 m h^{-1}; results expressed as means ± standard errors, n=2).

Substrate	V_m (g COD l^{-1}d^{-1})	K_m (g COD l^{-1})	Activity 1 g COD (g VSS d)$^{-1}$	Activity 2 g COD·(g VSS d)$^{-1}$
Ethanol	27.5±0.5	1.43±0.09	0.82±0.12	0.57±0.09
Butyrate	6.5±0.1	1.41±0.05	0.20±0.01	ND
Propionate	3.9±0.9	1.33±0.10	0.12±0.01	0.08±0.01
Acetate	16.6±2.1	1.73±0.11	0.51±0.05	0.21±0.02

Activity 1 – sludge activity calculated from the Michaelis-Menten equation on the basis of assessed V_m and K_m and substrate concentration of 2 g COD l^{-1} (total VSS in the reactor was 53.2 g);
Activity 2 – sludge activity determined from small batch tests (non-stirred);
ND – not determined.

ACKNOWLEDGEMENTS

We thank Dr Salih Rebac for delivering psychrophilic sludge and Dr Alla Nozhevnikova for providing microscopic examination of the sludge samples. The financial supports of INTAS (grant N96-2045 'Methane production in low temperature environments') and the Netherlands Organisation for Scientific Research (NWO grant 'Use of anaerobic and microaerobic systems for wastewater treatment') are gratefully acknowledged.

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