Influence of Lower Substrate pH and Retention Time on the Efficiency of a UASB Bioreactor Treating Winery Waste Water

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Submitted for publication: February 1999
Accepted for publication: June 1999
Key words: UASB bioreactor, anaerobic digestion, winery waste water, retention time, pH

A mesophilic laboratory-scale upflow anaerobic sludge bed (UASB) bioreactor design was evaluated for the treatment of winery waste water. In the first experimental study, the hydraulic retention time (HRT) was shortened from 24 h to 13 h which led to an increase in the organic loading rate (OLR) from 6,34 to 10,12 kgCOD.m^{-3}.d^{-1}. The recovery rate of the bioreactor, in terms of pH stabilisation was much slower for HRT's less then 14 h, suggesting that the optimum operational HRT had been reached. At this HRT the average COD removals were between 93 and 97% and the removal rate was 10,94 kgCOD.m^{-3}.d^{-1}. The second experimental study was the reduction in substrate pH from 7,5 to 5,0. This study was performed to investigate the lowest substrate pH that the active microbial population in the bioreactor could handle so as to reduce neutralisation costs and acclimatise the microbes to lower pH's. The lowest operational pH reached was 5,1 with a COD removal of over 90%. This has a considerable impact on the economic aspects of the winery waste water treatment process as neutralisation of the waste water will not be necessary before introducing it to the UASB bioreactor.

Wineries and Distilleries use large water volumes and produce large amounts of waste water which makes them potential candidates for effluent reuse. Due to the winery effluents characteristic acidic nature and peak in organic content over the harvesting season, it has the potential to cause considerable environmental problems (Borja *et al.*, 1994). These effluents contain organic acids and their salts, soluble proteins and carbohydrates as well as various inorganic compounds which are normal constituents of wine (Moosbrugger *et al.*, 1993a). It is therefore the responsibility of the winery to treat and dispose their waste water in the most environmentally friendly method possible.

Industrial effluent disposal in South Africa usually results in high treatment levies from the local authorities (Strydom *et al.*, 1995). These wastes present a series of problems to biological purification plants because of the need for prior treatment to establish conditions suitable for the development of the microorganisms responsible for the process and because of the long biomass retention time if an acceptable treatment system is to be obtained (Sales *et al.*, 1987). Many local authorities are now insisting that industries undertake some form of effluent treatment so as to protect the environment (Trnovec & Britz, 1998). Another problem which could cause surface or groundwater contamination is further pollution when untreated effluents are either discharged into the environment or used directly as irrigation water (Strydom *et al.*, 1995; Strydom *et al.*, 1997).

To enable the winery and distillery industry to contribute to water conservation, an efficient and cost-effective effluent treatment technology has to be developed. Considerable interest has been shown in the application of anaerobic digestion to waste waters from the food industry since the nature and strength of the waste water often provides ideal conditions for the digester operation (Trnovec & Britz, 1998). Anaerobic digestion is one of the most feasible methods of treating winery waste water as it achieves substantial COD reductions, while producing a low biomass (Lettinga & Hulshoff-Pol, 1991; Toffelmire, 1972; Water Research Commission, 1987). Another advantage is the fact that no excess sludge is formed that will also eventually have to be disposed (Heunis, 1986). The major advantage is energy recovery in the form of biogas, specifically methane as up to 95% of the organic matter in a waste stream can be converted into biogas (Weber *et al.*, 1984).

The aim of this study was to evaluate a mesophilic laboratory-scale UASB as an option for the treatment of winery waste water. The impact of reducing the substrate pH and hydraulic retention times, on the overall operational efficiency, will also be determined.

MATERIALS AND METHODS

Bioreactor: In this study laboratory-scale upflow anaerobic sludge blanket bioreactors (UASB) with an operational volume of 2,3 L, were used. The design combined a UASB system with an open gas/solids separator at the top of the bioreactor (Fig. 1). The gas exited via the top, while substrate was introduced at the bottom of the bioreactor. The overflow of the bioreactor emptied through a U-shaped tube to prevent any atmospheric oxygen from entering the system. The upflow velocity within the reactor was set at 2 m.h^{-1} using effluent recycling as shown in Fig. 1. The bioreactor temperature was maintained at 35°C by insulating it with heating tape and an electronic control unit (Meyer *et al.*, 1983). The volume of the biogas was determined using a manometric unit equipped with an electronically controlled counter and a gas-tight valve. The biogas volumes were corrected to standard temperature and pressure. The substrate was fed semi-continuously to the bioreactor by means of a peristaltic pump (Watson-Marlow 101) controlled by an electronic timer.

Acknowledgements: The authors sincerely thank Winetech and THRIP for financial assistance. The awarding of an FRD and Harry Crossley bursary to L.C. Ronquest is gratefully acknowledged.

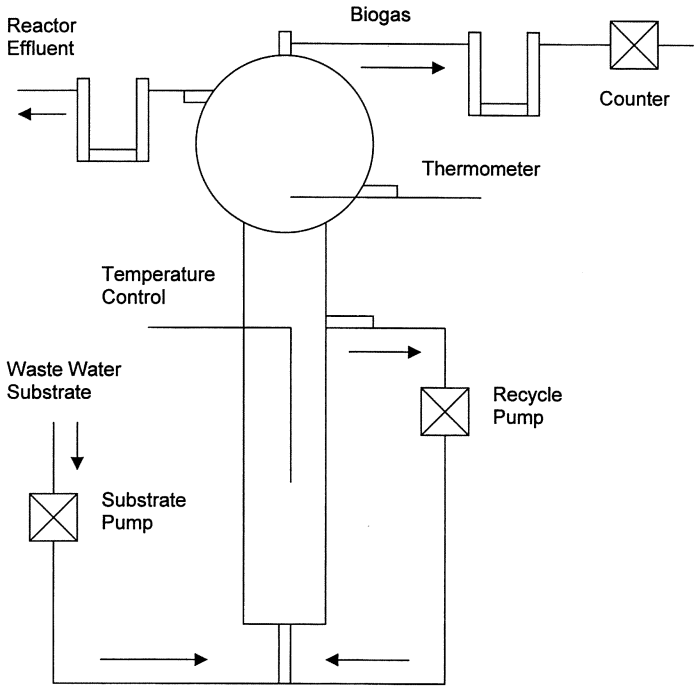

FIGURE 1
Laboratory-scale upflow anaerobic sludge blanket bioreactor.

Sludge inoculated bioreactor: A UASB bioreactor was seeded just with 1 000 g raw anaerobic sludge obtained from the Kraaifontein Sewage works and was used to represent a conventional UASB bioreactor that has no selected seeding inoculum specific for the treatment of winery waste water. The reactor was then fed with winery waste water supplemented with 5 g/L sodium lactate, 500 mg/L K_2HPO_4, 500 mg/L urea and 1 mL trace elements for 5 d during start-up. The pH was adjusted to 8,5 and the HRT was set at 2,2 d. The final winery substrate COD throughout the experiment varied between 1 750 and 3 400 mg/L.

Experimental bioreactor: This UASB bioreactor was seeded with a 700 g mixture of water drained anaerobic granules from an anaerobic batch reactor treating cannery effluent (Roos, 1998). This gave a settled sludge bed height of 50 cm. The bioreactor was allowed to stabilise for 24 h in order to allow the bacterial community to acclimatise and was then fed with a substrate containing a $^1/_3$ volume winery effluent, $^1/_3$ volume cannery effluent (Roos, 1998) and $^1/_3$ volume UASB effluent (Trnovec & Britz, 1998) for 7 d at a HRT of 2,5 d. The percentage winery effluent was gradually increased, accompanied by a decrease in the percentage UASB effluent, as the reactor reached a stable-state. Stable-state is defined as a state which can be maintained indefinitely, without system failure, and during which the variation in bioreactor performance parameters is less than 10% (Cobb & Hill, 1990). Within 20 d a 100% winery effluent as substrate was used at a HRT of 24 h. During the study the substrate COD varied between 1 800 and 2 800 mg/L. The variation in COD was due to the seasonal differences in effluent composition.

Substrate: Winery waste water contains residual organic acids, soluble proteins and carbohydrates which are normal constituents of wine (Table 1) (Moosbrugger *et al.*, 1993a; Mr Herman du Preez, Bottelary Winery, personal communication, 1998). The winery effluent was supplemented with 500 mg/L urea and 500 mg/L K_2HPO_4 to prevent any nitrogen or phosphorus limitation during the start-up period. The pH was set at 7,5 and the COD of the raw effluent that was used as bioreactor substrate averaged 2 595 mg/L (1 480 - 4 655 mg/L).

Analytical methods: The following parameters were monitored according to Standard Methods (APHA, 1992: pH; alkalinity; total solids (TS); total volatile solids (TVS); total non-volatile solids (TNVS); chemical oxygen demand (COD); orthophosphate phosphorus; and total Kjeldahl nitrogen (TKN) were determined colorimetrically using a DR2000 spectrophotometer (Hach Co. Loveland, CO) and standardised procedures (APHA, 1992). The general mineral analyses were done colorimetrically according to standard Hach procedures using a DR2000 spectrophotometer (Hach Co. Loveland, CO).

The total volatile fatty acids (TVFA) were determined using a Varian (Model 3700) gas chromatograph, equipped with a flame ionisation detector and a 30 m Fused Silica capillary column with 007 bonded FFAP stationary phase (Quadrex Co., New Haven). The column temperature was initially held at 105°C for 2 min, then increased at a rate of 8°C min^{-1} to 190°C. The detector and inlet temperatures were set at 300°C and 130°C respectively and nitrogen gas was used as carrier gas at a flow of 6,1 $mL.min^{-1}$.

The biogas composition was determined on a Fisons Gas Chromatograph, equipped with a thermal conductivity detector and 2,0 m x 2,0 mm i.d. column packed with Hayesep Q (Supelco, Bellefonte, PA), 80/100 mesh. The oven temperature was set at 45°C and helium was used as carrier gas at a flow rate of 40 $mL.min^{-1}$.

RESULTS AND DISCUSSION

Effluent composition: The average composition of winery waste water from five local wineries sampled over the period April 1997 to April 1998 is given in Table 1. The data clearly indicates a waste water with a high organic content and varying pH, but it was generally acidic, although occasionally it was more alkaline which corresponds to cleaning operations using sodium hydroxide (Torrijos & Moletta, 1997). The COD concentrations vary according to the specific winery operation, which is dictated by the grape harvesting seasons. The winery waste water had insufficient phosphate (19,0 ml/L) and nitrogen (14,0 mg/L) and as a result these nutrients had to be supplemented to ensure no limitations for microbial growth. Ideally, the C:N:P ratio for anaerobic digestion should be in the range of 100 : 1-10 : 1-5 (Iza *et al.*, 1991). In contrast the winery waste waters C:N:P ratio was 81:1:1.35 and therefore needs some nitrogen and phosphate supplementation.

Sludge inoculated bioreactor: An UASB bioreactor, seeded just with sewage sludge was used to represent a conventional UASB bioreactor that has no selected seeding inoculum specific for the treatment of winery waste water. The UASB bioreactor's pH varied between 5,5 and 7,5 throughout the start-up which clearly indicates an unstable state. The COD removal, even after 90 d, never reached 70% (Fig. 2). This clearly shows a long and poor start-up period as the bioreactor continually needed re-seeding due to wash-out and this is characteristic of UASB's seeded with just sewage sludge. This implies the need for a more easily settlable and well-defined seeding inoculum so the re-seeding and continuous wash-out does not occur.

TABLE 1
Composition of winery effluent (15 batches of approximately 25 1-5 plants) randomly obtained during the period of April 1997 to April 1998 (mg/L).

Parameter	Concentration	
	Average	Range
COD	2 595	1 480 – 4 655
Alkalinity	248,0	0 – 650
pH	6,9	3,9 – 8,2
Phosphate	19,0	6,8 – 39,0
Total Solids	25,0	21,0 – 30,0
Volatile Solids	10,0	6,0 – 14,0
Non-volatile Solids	15,0	15,0 – 16,0
Total Kjeldahl Nitrogen	14,0	6,3 – 26,3
Carbohydrates	0,0	–
Proteins	0,0	–
Na	97,3	97,2 – 97,4
K	77,0	76,0 – 78,0
Ca	28,0	27,8 – 28,2
Mg	17,0	16,9 – 17,1
Fe	1,3	1,2 – 1,4
Cl	189,0	–
CO_3	0,0	–
HCO_3	159,9	154,0 – 165,9
SO_4	24,5	24,0 – 25,0

Experimental bioreactor: The implementation of a gradual conditioning step for the granule inoculum to adpat to winery waste water was performed to investigate whether this would influence the start-up and operational efficiency of the bioreactor. The adddition of a $^1/_3$ volume cannery effluent and $^1/_3$ volume UASB effluent to the $^1/_3$ volume winery effluent substrate for 7 d was done in order to provide additional viable and active acidogenic organisms, and so that the acidogens in the granules accustomed to cannery effluent had a chance to adapt to the new substrate.

During this start-up phase it took the bioreactor only 17 d to reach an optimum operational condition in terms of COD removal (90%), bioreactor pH (average 7,3) and biogas production (2,3 $l.d^{-1}$) (Fig. 2). The addition of a $^1/_3$ volume UASB effluent to the substrate, which is rich in acidogenic bacterial organisms, was probably a major influence in reaching the enhanced start-up period. These acidogenic bacterial cultures present in the additionally added UASB effluent are viable and active organisms conditioned to the UASB environment. Within 20 d the bioreactor with an HRT of 24 h reached a COD removal efficiency of above 90% and an OLR of 6,3 kg $COD.m^{-3}.d^{-1}$. This stable state was further maintained for nearly a 100 d. The bioreactor was now ready for further studies to investigate the limits of operational efficiency in terms of HRT and substrate pH. These start-up results compare very favourably to studies performed by Austermann-Haun *et al.* (1997) on treating distillery waste water with a fixed-film methane reactor and achieving a COD removal efficiency of about 80% at a COD loading rate of 4 - 6 $kgCOD.m^{-3}.d^{-1}$.

Reduction in HRT: Hydraulic retention time (HRT) plays a major role during bioreactor start-up and performance behaviour. This can be attributed to the quantity, quality and activity of the biomass immobilized at different HRT's (Zhang & Noike, 1994; Rubindamayugi *et al.*, 1992). It is thus extremely important, specifically with respect to the economic implications accociated with start-up and treatment times, to be able to treat waste water as quickly and as efficiently as possible. It is therefore necessary to investigate the efficiency of the UASB while treating winery effluent at shortened HRT's.

During this study the HRT was shortened from 24 h to 13 h over a series of eight steps as summarised in Table 2. This reduction resulted in an increase in OLR from 6,34 to 10,12 $kgCOD.m^{-3}.d^{-1}$. While reducing the HRT it was found that the bioreactor pH remained fairly constant (7,3 to 6,95), but the alkalinity of the bioreactor gradually decreased from 1 625 to 750 mg/L. This large decrease in alkalinity as the HRT reached 13 h can probably be attributed to the accumulation of volatile fatty acids (VFA) and a subsequent pH drop, which occurs when a bioreactor enters a more unstable state (Moosbrugger *et al.*, 1993b). According to Duff & Kennedy (1982) and Lane (1984),

TABLE 2
UASB bioreactor operating conditions and average efficiency after stable state had been reached while reducing the HRT.

Parameter	Steps							
	1	2	3	4	5	6	7	8
HRT (h)	24	22	20	19	18	17	14	13
Substrate COD (mg/L)	2755	2414	2540	2454	2560	2099	2730	2384
Substrate pH	6,8	6,8	6,8	6,8	6,8	6,8	6,8	6,8
COD Removal (%)	89	96	94	93	92	88	97	98
OLR ($kgCOD.m^{-3}.d^{-1}$)	6,34	6,06	7,01	7,13	7,85	6,82	10,76	10,12
Bioreactor effluent pH	7,31	7,27	7,16	7,14	7,08	7,12	6,95	7,04
Alkalinity (mg/L $CaCO_3$)	1625	nd	nd	nd	1460	nd	825	750
Biogas ($l.d^{-1}$)	3,68	4,21	4,06	4,34	4,26	3,79	3,22	3,52
Y_{biogas} ($m^3.kg^{-1}.COD_{removed}$)	0,284	0,315	0,268	0,285	0,257	0,275	0,134	0,154

nd = not determined.

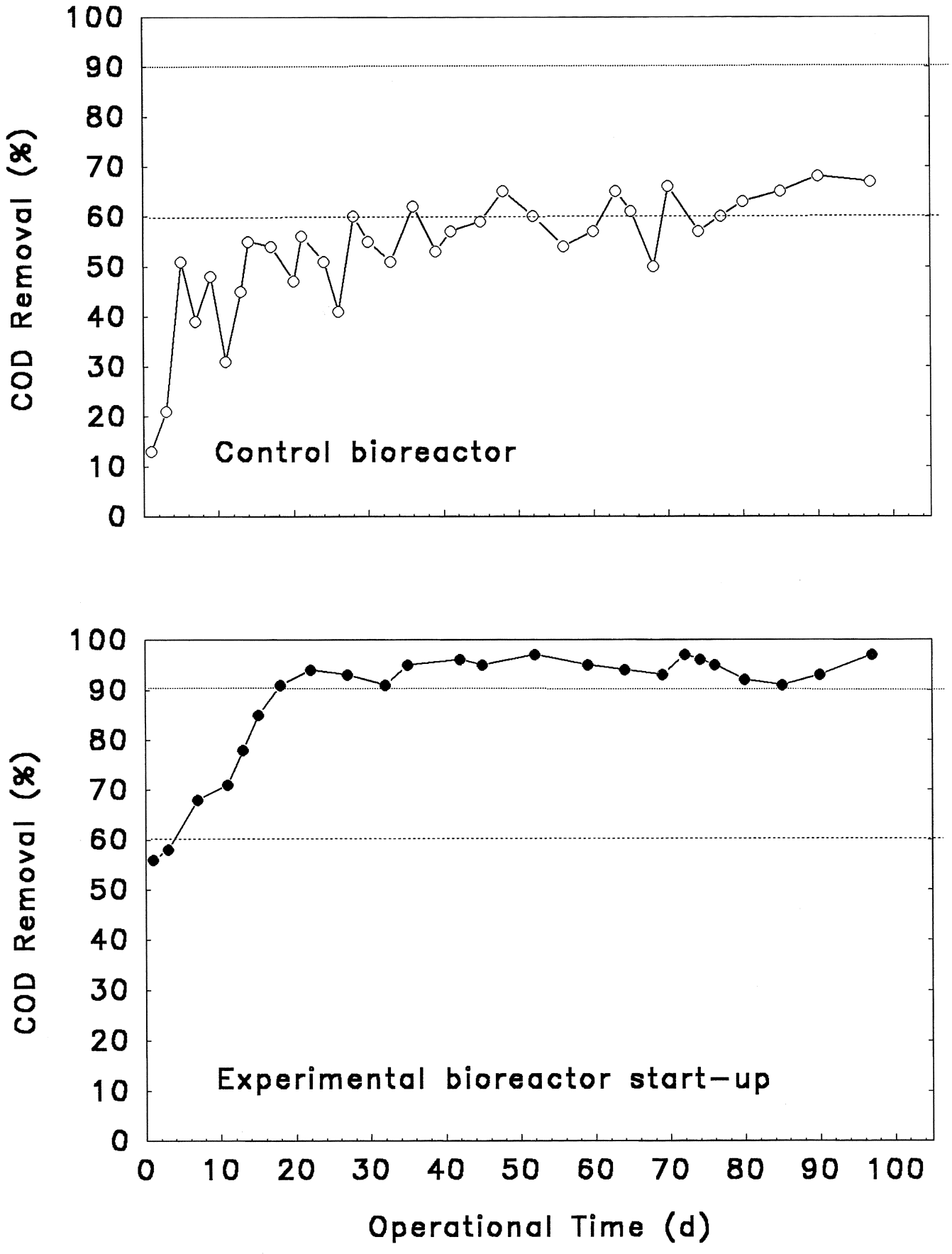

FIGURE 2

Comparison between the control and experimental bioreactor's operational efficiencies during start-up (O = control bioreactor; ● = experimental bioreactor).

alkalinity plays an important role in minimising overloading effects and is a good indicator of instability (Borja & Banks, 1995).

It was also found that at HRT's of less than 17 h the bioreactor's recovery rate, in terms of pH stabilisation just after the HRT was changed, was slower (up to 5 d) than that found at the longer HRTs of above 17 h. The pH recovery after increasing the OLR has been shown to be a good indicator of impending bioreactor system failure (Hill & Bolte, 1989). Moosbrugger *et al.* (1993b) reported that under unbalanced operational conditions, such as sudden increased organic loading, VFA's can accumulate in the system resulting in a reduction in the alkalinity level, leading to a decline in pH. Once the microbial biomass has recovered and stabilised the excess VFA's can normally be metabolised within a short time and thus lead to a pH and alkalinity stabilisation of above 7,0 and 1 300 mg/L respectively (Myburg & Britz, 1993).

At an HRT of 14 h, when the bioreactor effluent pH dropped to lower than 7,0, it was furthermore observed that granules started to wash-out from the reactor. These washed-out granules, as opposed to well-settled, round and smooth granules, had hairlike protrusions on them (Fig. 3). A possible explanation for this occurrence is that at low pH values, particularly below 6,0, certain filamentous organisms can begin to grow (Schwartz *et al.*, 1980). These organisms are known to be the main reason for bulking (Sezgin *et al.*, 1978). According to Cetin & Sürücü (1990) at these low pH values bioreactors have the lowest settling velocity and highest turbidity. These hair-like protrusions could also, according to Riedel & Britz (1993), be the result of extracellular polysaccharides which could develop during environmentally stressed conditions such as organic overloading.

The bioreactor needed an extended operational period (up to 8 d) in order to recover in terms of pH and alkalinity at HRT's below 14 h and it was therefore decided that even though the bioreactor was achieving good COD removals of above 90%, the bioreactor had in fact reached its shortest stable operational HRT. It was thus concluded that any sudden changes in normal operating parameters would impact negatively on the bioreactor effluent pH, alkalinity and COD removal efficiency. Optimum operational conditions were taken as an average COD removal of between 93 to 97% and a removal rate of 10,44 $kgCOD.m^{-3}.d^{-1}$ when the HRT was reset at 14 h. The HRT achieved when compared to the literature is not as short as reached by Borja & Banks (1995) or Trnovec & Britz (1998) where HRT's of 8 and 10 h were achieved while treating ice-cream and cannery waste water respectively, but the data was significantly better than efficiency achieved by Strydom *et al.* (1995) of 1,7 d when treating a dairy effluent using a hybrid digester. However, it must be remembered that ice-cream and cannery waste waters have a high carbohydrate content compared to winery waste water which automatically makes them easily degradable and therefore more easily treatable using anaerobic digestion.

Reduction in substrate pH: The bioreactor pH affects the enzymatic activity as well as the growth rate of the bacterial community involved in anaerobic digestion. The limits of pH for bacterial growth and thus for waste water treatment processes is generally given between 4 and 9 (Cetin & Sürücü, 1990). The bioreactor pH is maintained by the buffering capacity of the system, which is dictated by the alkalinity (Borja & Banks, 1995). Thus, it is clear that pH has a direct influence on the growth of the microbial populations present in the bioreactor and therefore directly influences the treatment efficiency as well.

During the sampling period (Table 1) it was found that winery waste water can be discharged from the winery at a pH of as low as 3,9 normally must then be neutralised to at least pH 7,0 before being introduced to the bioreactor in order to prevent stressing the active microbial population. It was therefore decided to determine the lowest substrate pH at which an UASB bioreactor could still operate efficiently. This would help reduce the neutralisation costs which influence the economic aspects of this treatment process and would condition the microbes present in the bioreactor to lower pH's.

In this section, the pH of the winery substrate was gradually reduced in 13 steps from 6,7 to 5,0 and the bioreactor response was closely monitored. The HRT of the bioreactor was maintained at 14 h throughout the study and changes were implemented only once a COD removal of at least 90% was attained and the

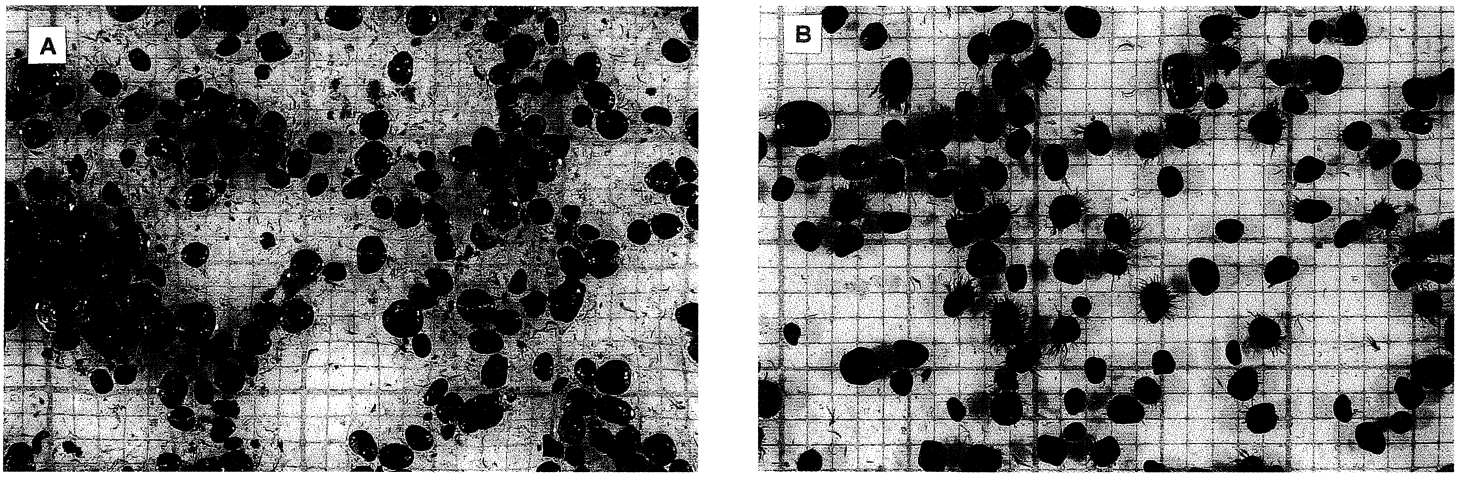

FIGURE 3
Granuals from the UASB bioreactor during stable conditions (A) and granules with hair-like protrusions obtained during unstable conditions (B) while treating winery waste water.

TABLE 3
UASB bioreactor operating conditions and average efficiency after stable state had been reached while reducing substrate pH.

Parameter	Steps												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Substrate COD (mg/L)	2384	2562	2468	2141	2366	2306	2454	2759	2803	2704	2608	2601	2595
COD Removal (%)	98	96	95	96	87	94	91	89	95	91	92	93	88
HRT (h)	14	14	14	14	14	14	14	14	14	14	14	14	14
OLR ($kgCOD.m^{-3}.d^{-1}$)	9,39	10,10	9,73	8,44	9,33	9,09	9,68	10,88	11,05	10,66	10,28	10,26	10,05
Substrate pH	6,7	6,6	6,4	6,2	6,1	6,0	5,8	5,6	5,5	5,4	5,2	5,1	5,0
Bioreactor effluent pH	7,16	7,08	6,95	7,10	7,31	7,41	7,31	7,25	7,29	7,37	7,03	7,18	7,29
Alkalinity (mg/L $CaCO_3$)	750	750	850	950	1550	1775	1875	1625	1700	1575	1325	1450	1500
Biogas ($l.d^{-1}$)	3,78	5,20	5,38	5,06	4,47	4,24	3,45	3,35	3,90	3,33	2,72	2,70	2,90

system showed stable state conditions. The bioreactor response to the changes in substrate pH could be divided into four clear phases. The first phase ended when a substrate pH of 6,4 (step 3) was implemented. The bioreactor effluent pH dropped to 6,95 and the alkalinity was 850 mg/L (Table 3). Due to the drop in pH and rather low alkalinity, which is used as an indicator of bioreactor instability (Borja & Banks, 1995) it was expected that the bioreactor would not handle any furhter substrate pH changes. However, this was not the case. When the winery substrate pH was reduced further (phase 2), to 6,2 (step 4), the bioreactors pH was found to increase to 7,10 and the alkalinity began to improve until a level of 1 875 mg/L was reached (substrate pH 5, 8, step 7). In the next phase (3), substrate pH changes after step 7 lead to very little change in alkalinity with the effluent pH remaining above 7,25.

The fourth phase occurred at step 10 whereafter a slow drop in alkalinity and changes in bioreactor effluent pH indicated that the bioreactor was beginning to reach the minimum substrate pH in terms of operational efficiency. The bioreactor recovery period, in terms of pH stabilisation after the substrate pH was set at 5,4 was longer than found with the higher substrate pH's. However, the bioreactor could still maintain an alkalinity and effluent pH of above 1 325 mg/L and 7,0 respectively.

Once the substrate pH reached 5,0 the bioreactor was allowed to recover for 5 d. However, recovery was very slow. The result was a drop in the bioreactor pH to 6,5 and a dramatic reduction in biogas yield to 1,2 $l.d^{-1}$. Even though, the COD removal was above 88%, the slow recovery and drop in biogas production indicated that the lowest operational substrate pH had been reached and any further lowering of the substrate pH would result in system failure.

CONCLUSIONS

Torrijos & Moletta (1997) suggested that the best solution for treating winery waste water is an aerobic process, specifically a sequencing batch reactor. The results from this study clearly show that using an UASB design is also an effective treatment option (HRT = 14 h; COD removal > 90%; substrate pH 5,1), which does not involve producing and subsequent removal of excess sludge which greatly contributes to the total operating costs. It was possible to operate the bioreactor at HRT's of as short as 14 h, but values below this resulted in extended recovery periods in terms of pH stabilisation. The UASB bioreactor used in the study was operated at a substrate pH of as low as 5,0, but the slow recovery and drop in biogas production indicated that this was the lowest operational substrate pH. This implies that an UASB could treat fresh winery waste water with little or no neutralisation (depending on the winery effluent pH) at a substrate pH of 5,1 in 14 h with an average COD removal of 93% at organic loadings of between 8,44 and 11,05 $kgCOD.m^{-3}.d^{-1}$. These operational procedures and parameters could lead to a significant reduction in operational start-up and efficiency. The results in terms of HRTs, OLRs and substrate pH are highly efficient when compared to results reported by Cheng *et al.* (1990) and Torrijos & Moletta (1997) using a modified UASB bioreactor and a sequencing batch reactor to treat winery waste water respectively. However, a pilot-scale and later a full-scale operational UASB which could be implemented in a winery, would need to be investigated in order to verify these results on industrial scale.

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