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EFFECT OF HYDRAULIC RETENTION TIME ON UP-FLOW ANAEROBIC STAGE REACTOR PERFORMANCE AT CONSTANT LOADING IN THE PRESENCE OF ANTIBIOTIC TYLOSIN

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Abstract - The present investigation was aimed at determining the impact of the macrolide antibiotic Tylosin in reduced HRT at constant organic loading rate (OLR) by varying feed substrate concentration in an up-flow anaerobic stage reactor (UASR). The antibiotic concentration was maintained at 200 $mg.L^{-1}$, at constant OLR of 1.88 kg $COD.m^{-3}.d^{-1}$, by varying feed substrate concentration to the UASR and the HRT was decreased gradually from 4 to 1 d. Throughout the operation period, brewery wastewater was used as simple feed substrate to elevate the concentration of easily biodegradable carbon in comparison with the concentrations of more recalcitrant Tylosin substrate. The reactor alkalinity was controlled in all the stages of UASR by adding 1000 – 2000 $mg.L^{-1}$ $CaCO_3$. Results showed the total COD removal efficiency at 4 d HRT was around 92%, after which point there was a slight decrease at 3 and 2 d HRT (average 82%), and this was reduced further (average 77%) at a HRT of 1 d. The UASR showed stable operation with effluent volatile fatty acid (VFA) less than 300 $mg.L^{-1}$ throughout the experimental period (HRT 4 – 1 d). Moreover, the average methane yield ($CH_4.kg\ COD_r^{-1}$) showed a relatively constant profile and was largely unaffected by HRT in all the stages of UASR. These results show that bacteria were readily adapted to wastewater containing Tylosin at lower HRTs and did not affect the reactor performance substantially.
Keywords: Antibiotic; Anaerobic digestion; Hydraulic retention time; Tylosin; UASR.

INTRODUCTION

A number of studies have been carried out in the literature on the effect of hydraulic retention time (HRT) on anaerobic digestion. In the start-up of a hybrid anaerobic digester treating soluble synthetic sugar wastes, Guiot et al. (1989) found that the soluble COD content of the effluent increased with decreasing HRT. An increase in influent flow rate of 100 and 150% for 5 and 10 h was carried out in an anaerobic downflow filter treating slaughterhouse wastewater (Borja et al. 1994). Effluent suspended solids increased, which was probably due to the increase in gas production brought about by the increase in the OLR and hydraulic shear. Zhang and Noike (1994) evaluated the influence of HRT on the performance and bacterial trophic populations in an anaerobic continuous stirred tank reactor (CSTR) treating starch at 35°C. The results demonstrated that methanogenesis occurred in the acidogenic phases, even with HRTs as short as 1.5 h, and that retention time was a significant factor in selecting for the predominant microbial

*To whom correspondence should be addressed species. Chua et al. (1997) evaluated the responses of an anaerobic fixed-film reactor (AFFR) to hydraulic shock loading by varying HRT at constant organic loading and showed the AFFR was tolerant of hydraulic shock loadings and the COD removal efficiency was only slightly affected. The influence of HRT (136 – 6 h) at a constant organic loading rate of 2 kg COD.m^{-3}.d^{-1} was studied by Cadi et al. (1994) in an anaerobic membrane bio-reactor treating synthetic wastewater containing starch and showed the COD removal efficiency was not greatly affected even at HRT of 6 h. More recently, Climenhaga and Banks (2008) reported stable reactor performance when a single stage anaerobic digester treating food waste was operated at a constant OLR with different HRT. Other workers (Nandy and Kaul, 2001; Sanchez et al. 2002; Kuscu and Sponza, 2007; Nain and Jawed, 2006; Feng et al. 2008; Rincon et al. 2008; Araujo et al. 2008) have also looked at the influence of HRT on reactor performance.

There are very few published studies investigating the effect of HRT in staged reactors or anaerobic baffled reactors (ABR). Xing and Tilche (1992) assessed the effect of HRT on the hybrid ABR at a constant loading of 10 kg COD.m^{-3}.d^{-1} and found that COD reduction dropped from 75% to 40% when the HRT was changed from 1 d to 6 d. Nachaiyasit and Stuckey (1997) evaluated the performance of an ABR at HRT 20, 10 and 5 h at a constant feed COD of 4000 mg.L^{-1} (using synthetic carbohydrate-protein substrate) and showed a decrease in COD removal rate to 98, 90 and 52%, respectively. Moreover, they also investigated the effect of shock loads at constant OLR (4.8 kg COD.m^{-3}.d^{-1}) with decreasing HRT and concluded that COD removal efficiency was affected only slightly (98%, 97% and 96% at HRT 20, 10 and 6.6 h, respectively). Baloch and Akunna (2003) studied the effect of rapid hydraulic shock loads on the performance of a granular bed baffled reactor (GBBR) using synthetic wastewater containing glucose as the main organic compound and showed high COD removal (94% to 97%) for all shock loadings (2.5 to 20 kg COD.m^{-3}.d^{-1}) upon decreasing HRT (48 – 6 h). Recently, Kuscu and Sponza (2009) demonstrated that, as the HRT decreased from 10.38 d to 2.5 d, the COD removal efficiencies decreased slightly from 94% to 92 % in an ABR treating nitrobenzene. In summary, the literature above indicates that it is impossible to predict the effect of HRT on anaerobic treatment systems, since it depends on reactor configuration, type of feed and characteristics, organic loading rate, type of biomass and method used to evaluate performance. Accordingly, each new system therefore requires specific investigation.

The novel up-flow anaerobic stage reactor (UASR) was developed according to the concept of the anaerobic baffled reactor (ABR) (Barber and Stuckey, 2000), where each stage of the reactor represents a separate compartment. A stage reactor can provide high treatment efficiency since recalcitrant substrates will be in an environment more conducive to degradation (Ghaniyari-Benis et al. 2009). The innovative design of the UASR results in the separation of acidogenesis and methanogenesis, which has potential benefits for reactor performance. With no moving parts or mechanical mixing, no requirement for biomass with unusual settling properties, and a high degree of stability to hydraulic and organic shock loads, the stage reactor has the potential to be applied economically as a pre-treatment system for many trade effluents (Barber and Stuckey, 2000; van Lier et al. 2001; Zhu et al. 2008; Krishna et al. 2008; Liu et al. 2009). Furthermore, the reactor configuration provides protection to the methanogenic biomass from toxic compounds in the influent (Barber and Stuckey, 2000).

The macrolide antibiotic Tylosin, which is produced by a strain of *Streptomyces fradiae*, has been widely used for the treatment of pneumonia, arthritis and dysentery caused by Mycoplasma, Bacillus Pastorianus, etc (Prats et al. 2002). It has good anti-bacterial activity against most pathogenic organisms such as gram-positive bacteria, some gram-negative bacteria, vibrio, spirochetes, coccidia, etc. (Sarmah et al. 2006). In addition, Tylosin is also incorporated into animal feed to improve growth rate and feed efficiency (Sarmah et al. 2006). There are contradictory reports on the inhibitory effects of Tylosin on anaerobic treatment performance. Partial inhibition of propionate and butyrate uptake was observed in anaerobic batch reactors with Tylosin concentrations ranging from 25 to 250 mg.L^{-1} (Sanz et al. 1996). Masse et al. (2000) reported that the presence of Tylosin (110 mg.kg^{-1} in feed diets) did not effect the treatment of swine manure slurry in a sequencing batch reactor (SBR). However, a decrease in methane production was observed by Loftin et al. (2005) after addition of Tylosin at a concentration of 1 – 25 mg.L^{-1}. More recently, Angenent et al. (2008) reported that Tylosin, at an average concentration of 1.6 mg.L^{-1} in swine waste, was degraded rapidly when fed to an anaerobic sequencing batch reactor (ASBR) and does not affect the methane yield. On the other hand, Shimada et al. (2008) observed a decrease in the rates of methane production and propionate uptake when Tylosin was added to an ASBR at a concentration of 1.67 mg.L^{-1}, while the total methane production did not change in their study. The literature above indicates apparent contradictions and may be attributed to the type of biomass, Tylosin concentration, reactor configurations, start-up conditions and the methods used to evaluate performance. Furthermore, long-term exposure to macrolide antimicrobials may result in the acclimatization of the anaerobic biomass.

In our previous study (Chelliapan et al. 2006); we demonstrated the treatment of pharmaceutical wastewater containing the macrolide antibiotic Tylosin in an up-flow anaerobic stage reactor (UASR). Because that experiment investigated higher OLR (2.48 – 3.73 kg COD.m^{-3}.d^{-1}) by a combination of increasing the feed COD concentration and reducing the HRT (4 - 2d) at constant feed concentration (7450 mg.L^{-1}), it is not clear whether the OLR or HRT influenced the UASR performance. Additionally, the influence of elevated Tylosin concentrations on the UASR process performance was also evaluated using additions of Tylosin phosphate concentrate (100 – 800 mg.L^{-1} at HRT 4 d). Therefore, this further investigation was conducted to examine the effect of reducing HRT on the performance of UASR at constant organic loading in the presence of the macrolide antibiotic Tylosin.

MATERIALS AND METHODS

Up-Flow Anaerobic Stage Reactor (UASR)

The UASR system (Figure 1) consists of four identical cylindrical Plexiglas compartments (stages), 80 mm in internal diameter by 640 mm in height, linked in series, and was constructed for the present study. The active volume of the UASR system was 11 L (4 stages of 2.75 L). The operational set-up, flow diagram and the reactor design are presented in Fig. 1a. Each stage of the reactor had a 3-phase separator baffle, angled at 45^{o} and placed 50 mm below the effluent ports, to prevent floating granules from washing out with the effluent (Fig. 1b). Each stage was equipped with sampling ports at 100 mm intervals (the lowest being 30 mm from the base) that allowed biological solids and liquid samples to be withdrawn from the sludge bed. The influent wastewater entered through a 12mm internal diameter downcomer tube in the headplate that extended to within 15mm of the reactor base and allowed feed to flow upward through the sludge bed. Effluent from each stage of the reactor flowed by gravity to the next, since each stage was placed on stepped platform having a 150 mm step height. The walls of the reactors were wrapped with a tubular PVC water-jacket, 15mm in internal diameter, to maintain the reactor temperature at 37°C. Peristaltic pumps (Watson Marlow 100 series) were used to control the influent feed rate to the first stage of the UASR. Gas production was monitored separately for each stage using an optical gas-bubble counter (Newcastle University) having a measurement range of 0 – 1.5 L.hr^{-1} and precision within ±1%.

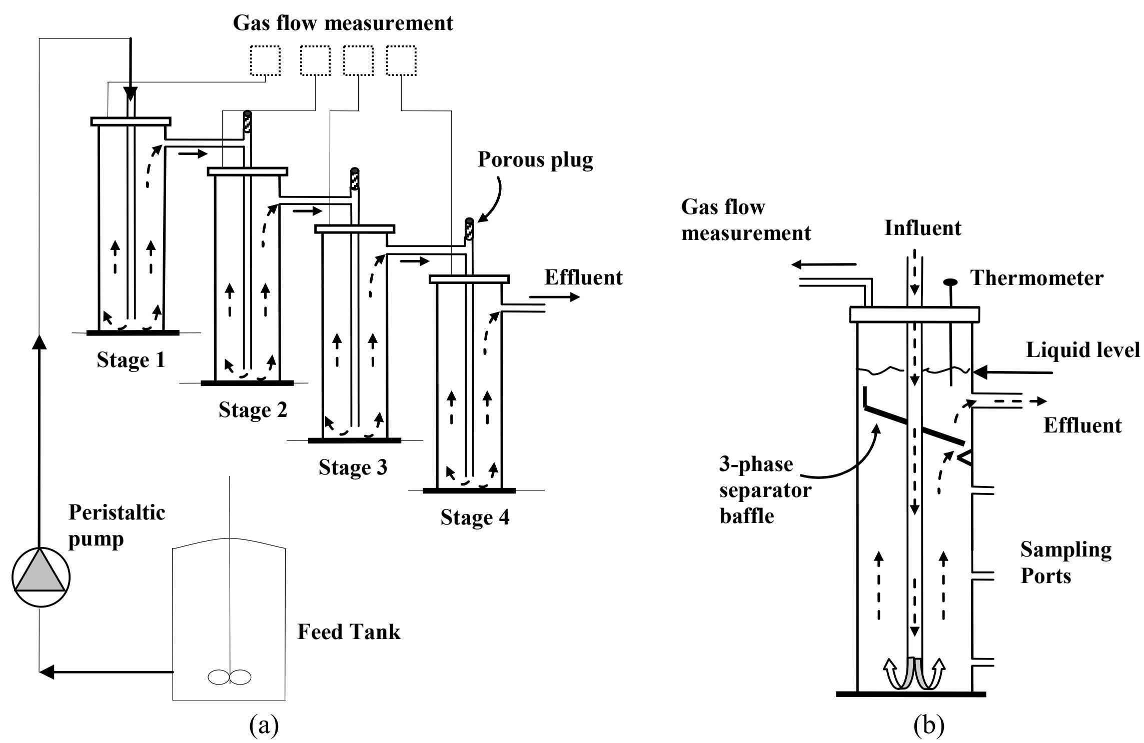

Figure 1: (a) UASR system and flow regime; (b) details of an individual UASR stage

Reactor Operation

The UASR was seeded with anaerobic digested sewage sludge (Hexham Municipal sewage treatment plant, Northumberland, UK). This was sieved to pass 2mm mesh, giving a solid content of 21,500 mg $TSS.L^{-1}$ (13,400 mg $VSS.L^{-1}$). 1.2 L of sieved sludge was added to each reactor stage, the remaining volume being filled with tap water, to give a final sludge concentration of 5850 mg $VSS.L^{-1}$. After seeding, the headplates were attached and the headspace above each reactor stage was flushed with nitrogen gas to displace residual air from the system before introducing the feed. The reactors were allowed to stabilize at 37 ^{0}C for 24 h without further modification.

The UASR used in this experimental study was operated as a continuation of the study of the effect of elevated Tylosin concentrations (Chelliapan et al. 2006). During that study (first phase), a series of experiments investigating the influence of Tylosin concentrations on UASR was assessed using additions of Tylosin phosphate concentrate for a period of 290 days (reactor biomass had been adapted to Tylosin gradually). In the first phase, the start-up of the reactor was established with a brewery wastewater feed (Sallis and Uyanik, 2003) and the reactor was operated with an OLR of 0.43 – 1.88 kg $COD.m^{-3}.d^{-1}$ and a HRT of 4 d, which achieved the necessary acclimatisation of the sludge and stable operation required for the second phase of the investigation.

Once the reactor had reached the steady state (93% COD removal from brewery wastewater), the feed to the reactor was supplemented with Tylosin in the form of Tylosin phosphate concentrate (supplied by Eli Lilly & Company Ltd, Liverpool, UK). The concentration of Tylosin was maintained at 200 $mg.L^{-1}$, at a constant OLR of 1.88 kg $COD.m^{-3}.d^{-1}$ and the HRT was decreased gradually from 4 to 1 d by varying feed substrate concentration to the UASR (7500 – 1850 $mg.L^{-1}$, Table 1). During the experiment, COD, pH, VFA, biogas production and solids washout were measured according to standard methods. The brewery wastewater was used as a feed throughout the UASR operations due to its ease of degradation, high COD values, and well established use in continuous anaerobic reactors (Uyanik et al. 2002; Sallis and Uyanik, 2003). The COD contributed by Tylosin was taken into consideration while preparing the feed (adjusted with brewery wastewater to the desired final COD). Nutrients and trace elements were added in order to provide a balanced feed to the reactor (COD: N: P, 250:7:1). The trace elements deficiency of brewery wastewater was corrected by adding a trace elements solution (Sallis and Uyanik, 2003). The brewery wastewater had alkalinity of around 90 $mg.L^{-1}$ as $CaCO_3$; the mixed liquor in all reactor stages was 1000 to 2000 $mg.L^{-1}$ as $CaCO_3$.

Sampling and Analysis

Supernatant liquor, gas and sludge samples were taken separately from each stage for analysis. In addition, the gas production rate was determined separately for each stage. Sample analysis included chemical oxygen demand (COD), pH, alkalinity, total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_3-N), suspended solids (SS), and volatile suspended solids (VSS), all according to Standard Methods (APHA, 1985). Available PO_4-P was determined by ion-chromatography (Dionex, DX-100 Ion Chromatograph) and volatile fatty acids (VFA) by gas-liquid chromatography (Unicam 610 Series Gas Chromatograph with auto-injector and PU 4811 computing integrator) with operating conditions as follows: carrier gas: nitrogen at 20 $mL.min^{-1}$; column temperature: 140^{o}C (isothermal); detector temperature: 180^{o}C; injection port temperature: 180^{o}C; column dimensions: 2000 mm long x 2 mm I.D. glass packed with 10% AT-1000 on 80/100 Chromosorb W-AW; detector type: flame ionisation detector. Biogas composition (CO_2 and CH_4) was determined by gas chromatography (Becker model 403 Gas Chromatograph with Unicam 4815 computing integrator) with the following operating conditions; carrier gas: helium at 50$mL.min^{-1}$, column temperature 55°C, metal column dimensions: 2000 mm long x 4mm I.D. packed with Porapak Q, detector: thermal conductivity.

RESULTS AND DISCUSSION

pH

The pH is an essential factor to control during anaerobic digestion. The methane-producing microorganisms have optimum growth in the pH range between 6.6 and 7.6 (Rittmann and McCarty, 2001), although stability may be achieved in the formation of methane over a wider pH range (6.0 – 8.0). pH values below 6.0 and above 8.3 should be avoided, as they can inhibit the methane-forming microorganism (Chernicharo, 2007). The pH levels were generally stable (pH 7.1 – 7.9) in all stages of the UASR when the reactor was operated at 4 d HRT (Figure 2). However, when the reactor HRT was reduced to 3 d, the pH in Stage 1 dropped to 6.7 due to the increased acidogenic activity. During this period, there were no major changes in the pH of Stages 2, 3 and 4. Further reduction in the HRT (2 and 1 d), did not cause substantial change in the pH profile of the reactor system with average pH in Stage 1 being 6.5; Stage 2, 7.0, Stage 3, 7.4 and Stage 4, 7.8. In theory, the pH in Stage 1 should be lower than in Stages 2, 3, and 4 due to horizontal separation of acidogenesis and methanogenesis (Nachaiyasit and Stuckey, 1997). When the reactor HRT was changed to 1 day (actual HRT in Stage 1 was 6 h), the accumulation of VFAs, particularly in Stage 1, overcame the buffering capacity of the wastewater and, as a result, the pH steadily decreased to 6.5. According to Rittmann and McCarty (2001), a pH in the range of 6.5 to 8.2 is not detrimental to anaerobic processes. Consequently, in this study, it can be assumed that the bacteria adapted well to the HRT change at the levels of Tylosin present (Table 1) and were not adversely affected by the pH reduction resulting from the reduced HRT.

COD Removal

Figure 3 depicts the total soluble COD removal efficiency in the UASR and the fractional contribution to the total COD removal by each stage over time. The total COD removal efficiency at 4 d HRT was around 92%, after which point there was a slight decrease at 3 and 2 d HRT (average 82%), and this was reduced further (average 77%) at a HRT of 1 d. This indicates that COD removal efficiency became less efficient and more variable with the HRT reduction. Importantly, the results show that a dramatic effect on the reactor performance did not occur; however, partial inhibition by the macrolide antibiotic may have caused the COD to decrease more at HRT 1 d than would have been the case if Tylosin had not been present. It is notable that the COD removal efficiency in Stage 1 dropped dramatically (from an average of 82% to 40%) when the HRT was reduced from 4 d to 1 d, indicating increasing acidogenic activity in Stage 1. A low HRT caused pre-acidification, resulting in accumulation of COD (as VFA), which did not subsequently convert to methane, resulting in an accumulation of VFA. This result agrees with the trend observed by Nachaiyasit and Stuckey (1997), who showed that little change occurred in COD removal efficiency when an ABR was operated at constant OLR (4.8 kg $COD.m^{-3}.d^{-1}$) with decreasing HRT (20 – 6.6 h). They concluded that, at low OLRs, there is enough biomass to metabolise the feed, and, even when the substrate concentration decreases with decreasing HRT, the mass transfer into the flocs is sufficient to remove most of the substrate. Another possible reason for the drop in treatment efficiency (to 77%) in this study at HRT 1 d might be due to the high applied Tylosin load (2200 $mg.d^{-1}$). Although Tylosin degradation rate was not determined in the present study, it is possible that partial inhibition of bacterial biomass by Tylosin may have resulted in lower methanogenic activity to such an extent that the VFAs were not well metabolised, resulting in the increased effluent COD. Moreover, the difference in COD removal efficiency of 15% (difference between HRT 4 and 1 d) may be due to more recalcitrant molecules needing longer time for bacterial degradation in the anaerobic biomass. However, the minimal effect on reactor performance confirms that the UASR reactor was efficient at low HRTs and, therefore, a short HRT was not responsible for the large drop in treatment efficiency (to less than 30%) that was seen when high OLR had been achieved with real pharmaceutical wastewater by decreasing the HRT to 2 d rather than increasing the COD of the feed (Chelliapan et al. 2006).

Table 1: Operational characteristics of the UASR during the study of the effect of Tylosin on reactor performance at reduced HRT

OLR* (kg $COD.m^{-3}.d^{-1}$)	Feed Flow rate ($L.d^{-1}$)	HRT (days)	Tylosin ($mg.d^{-1}$)	Influent COD ($mg.L^{-1}$)	Day
1.88	2.75	4.0	550	7500	1
1.88	3.67	3.0	734	5650	19
1.88	5.50	2.0	1100	3750	44
1.88	11.00	1.0	2200	1850	65

*provided by brewery wastewater

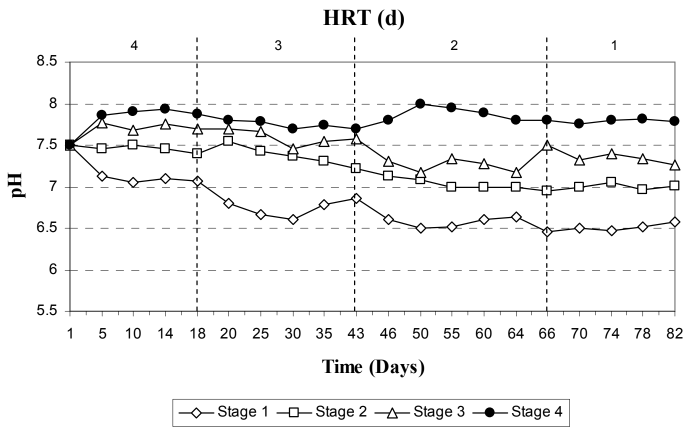

Figure 2: pH profile in each stage of the UASR at reduced HRT.

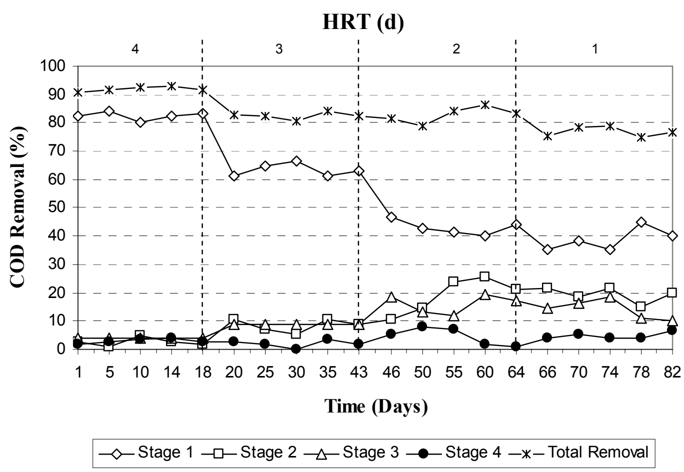

Figure 3: Total COD removal (%) of the UASR and fractional contribution (%) to the total COD removal by each stage at various HRT.

VFAs

It is well documented that high VFA concentrations in anaerobic processes cause the inhibition of methanogenesis (Anderson et al. 2003). Under conditions of overloading and in the presence of inhibitors, methanogenic activity cannot remove hydrogen and volatile organic acids as quickly as they are produced. The result is the accumulation of acids and the depression of pH to levels that also inhibit the hydrolysis or acidogenesis phase. It has also been shown that, even when process pH is optimal, the accumulation of VFAs may contribute to a reduced rate of hydrolysis of the solid organic substrate (Banks and Wang, 1999). Organic acids such as acetic, propionic, butyric and isobutyric acids are central to evaluating the performance of anaerobic digestion (Rittmann and McCarty, 2001). Figure 4 shows the total VFA concentration in each stage of the UASR at different HRT. The total VFA concentration in Stage 1 fluctuated from around 500 to 800 mg.L^{-1} (HRT 4 – 3 d), but stabilised at 700 – 800 mg.L^{-1} when the HRT was reduced to 2 – 1 d. The results support the pH profile in Figure 2. Stages 2, 3 and 4 showed minor fluctuations at HRT 4 – 2 d, but stabilised at around 100 – 300 mg.L^{-1} at HRT 1 d. Since stable reactor performance in an ABR (treating highly concentrated industrial dye effluent with COD up to 4500 mg.L^{-1}) is indicated by an effluent total VFA concentration below 500 mg.L^{-1} (Bell et al. 2000), it can be concluded that UASR operation (inlet COD of 7500 mg.L^{-1}) was stable since the effluent VFA was less than 300 mg.L^{-1} (Stage 4) throughout the experimental period (HRT 4 – 1 d).

Typical VFA intermediates in the UASR stages taken on day 18, 43, 64 and 82 (based on the end of each operational cycle when the reactor approached the steady-state) at each investigated HRT are given in Table 2. It is apparent that high concentrations of propionic acid were detected in all the UASR stages (propionic was higher than acetic) and contributed substantially to the total VFA concentration. The accumulation of propionate was probably due to the activity of the acidogenic bacteria that produce propionate, being greater than that of the microorganisms that convert substrate directly to acetate and more active than those bacteria responsible for propionate degradation (Aquino and Stuckey, 2004). The acetic acid concentration in the reactor effluent (Stage 4) was below 55 mg.L^{-1} for all the HRT investigated, indicating efficient conversion to methane and carbon dioxide. Other intermediates such as isobutyric, n-valeric, iosvaleric and n-valeric acids were also detected in all the UASR stages and showed reduced levels in later stages, which is consistent with the sequential degradation of fatty acids in a staged reactor.

It is interesting to note that the lower COD removal efficiency of the UASR at 1 d HRT was not the result of high VFA concentrations in the reactor effluent as these were present at a lower concentration than at 4 d HRT, as indicated by the Stage 4 concentration (i.e., the UASR effluent) in Figure 4. Consequently, the lower COD removal efficiency that resulted from the short (1 d) HRT was probably due to the incomplete degradation of the complex, more recalcitrant, organic fraction of the feed at the shorter contact time.

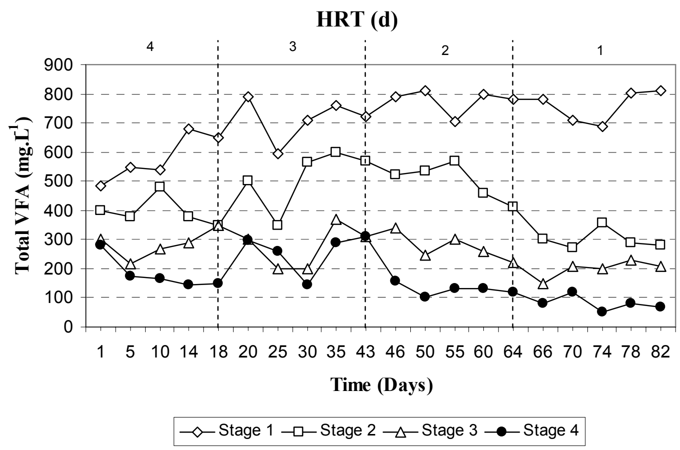

Figure 4: Total VFA profile in each stage of the UASR at different HRT.

Table 2: Typical VFA intermediates in the UASR stages taken on day 18, 43, 64 and 82 at each HRT investigated when the reactor approached the steady-state

UASR Stages	VFAs (mg.L^{-1})	HRT (d)			
		4	3	2	1
Stage 1	Acetic	98	127	160	187
	Propionic	451	499	466	443
	Isobutyric	10	9	1	2
	n-Butyric	27	15	59	43
	Isovaleric	16	14	31	31
	n-Valeric	48	56	73	96
Stage 2	Acetic	88	112	164	57
	Propionic	197	431	110	60
	Isobutyric	12	6	13	10
	n-Butyric	8	14	38	66
	Isovaleric	15	9	34	20
	n-Valeric	30	8	58	67
Stage 3	Acetic	50	33	121	121
	Propionic	265	254	51	69
	Isobutyric	3	1	11	2
	n-Butyric	1	8	9	7
	Isovaleric	19	7	21	8
	n-Valeric	10	2	8	1
Stage 4	Acetic	32	55	20	19
	Propionic	89	234	76	32
	Isobutyric	1	1	1	1
	n-Butyric	8	14	9	5
	Isovaleric	11	5	14	12
	n-Valeric	8	1	1	1

Biogas Production

Figure 5 shows that the total methane production in the UASR remained relatively constant (average 7600 $mL.d^{-1}$) when the reactor was operated at 4 to 2 d HRT. Nevertheless, a 12% reduction was observed (average 6700 $mL.d^{-1}$) when HRT was reduced to 1 d. Methane production in each stage differed, with Stage 1 generally producing more methane than the other stages. The proportion of CH_4 in the biogas was typically 68 – 82% (CO_2 being 6 – 28%) (data not presented) in all the stages of the reactor, regardless of the HRT, confirming high methanogenic activity in the reactor system. Furthermore, the average methane yield showed a relatively constant profile and was largely unaffected by HRT in all the stages throughout the experimental period (Figure 6), with Stage 1 having 0.28; Stage 2, 0.31; Stage 3, 0.32 and Stage 4, 0.35 m^3 CH_4.kg COD_r^{-1}. These values compare with the theoretical methane yield of 0.395 m^3 CH_4.kg COD_r^{-1}. The lower values of methane yield, particularly in Stage 1, were most likely caused by solids accumulation in the early stages, which were higher than in the later stages of the UASR (data not presented). In general, these results show that bacteria were readily adapted to wastewater containing Tylosin at lower HRTs (except Stage 1 when operated at 1 d HRT) and did not affect the reactor performance to a large extent.

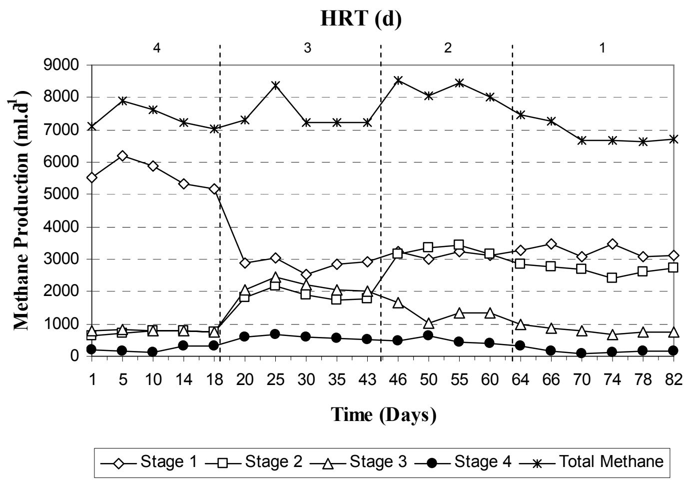

Figure 5: Methane production in each stage of the UASR at reduced HRT.

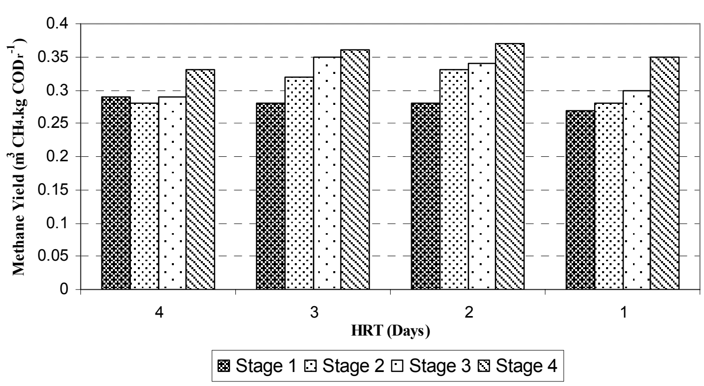

Figure 6: Mean methane yield in each stage of the UASR at reduced HRT (each mean taken from data during the final three HRT of each HRT investigated when the reactor approached the steady-state).

CONCLUSION

From the experimental results it can be concluded that only a minor reduction in COD removal efficiency was observed when the reactor was operated at high feed flow rate (1 d HRT). The UASR performance depends on a number of factors: HRT, OLR and the concentration of Tylosin. Although Tylosin degradation rate was not monitored in this study, results showed stable performance in terms of pH, methane production and methane yield. Lower COD removal efficiency, especially in Stage 1 at shorter HRT was probably due to incomplete degradation of the more recalcitrant feed. In general, the minimal effect of the antibiotic on overall reactor performance confirms that the bacteria were adapted to Tylosin at low HRTs.

ABBREVIATIONS

ABR	anaerobic baffled reactor
COD	chemical oxygen demand
CSTR	continuous stirred tank reactor
GBBR	granular bed baffled reactor
HRT	hydraulic retention time
OLR	organic loading rate
TKN	total Kjeldahl nitrogen
UASR	up-flow anaerobic stage reactor
VFA	volatile fatty acids
VSS	volatile suspended solid

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