Treatment of Pharmaceutical Wastewater Containing Tylosin in an Anaerobic – Aerobic Reactor System

Shreeshivadasan Chelliapan[1], Thomas Wilby[2] and Paul Sallis[3]

[1]Senior Lecturer/Head of Department, Department of Civil Engineering, College of Science and Technology (CST), University Technology Malaysia, (International Campus), 54100, Kuala Lumpur, Malaysia. Tel: 006-03-26154404; Fax: 006-03-26934844; E-mail: shreeshivadasan@ic.utm.my
[2]Senior Engineer, Eli Lilly and Company Limited, Speke, Liverpool L24 9LN, UK.
[3]Senior Lecturer, Environmental Engineering Group, School of Civil Engineering and Geosciences, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK.

Abstract: Effluents from manufacturing operations in the pharmaceutical industry, such as antibiotic formulation, usually contain recalcitrant compounds. An approach towards appropriate technology for the treatment of pharmaceutical wastewaters has become imperative due to strict water quality legislation for environmental protection. In the present study, an Up-Flow Anaerobic Stage Reactor (UASR) and a Porous Membrane Activated Sludge Reactor (PMASR), operating in series, were used to treat pharmaceutical wastewater containing the macrolide antibiotic Tylosin. The performance of UASR treating real pharmaceutical wastewater at various organic loading rates (OLR) (0.43 to 3.73 kg COD.m^{-3}.d^{-1}) was investigated. Effluent from the UASR was passed directly into a PMASR system in a continuous process. At a reactor OLR of 1.86 kg COD.m^{-3}.d^{-1} (hydraulic retention time (HRT), 4 d), the soluble COD reduction was around 70 - 75% (average specific degradation rate (SDR), 1.29 kg COD.m^{-3}.d^{-1}) an average of 95% Tylosin reduction was achieved in the UASR. During this period, the soluble COD removal efficiency of the PMASR was 63–69% (average SDR, 0.37 kg COD.m^{-3}.d^{-1}). The combined UASR – PMASR treatment system was slightly more effective with 87–90% COD and average 97% Tylosin removal. The results indicate successful treatment of the pharmaceutical wastewater, and confirm Tylosin degradation, providing further evidence that Tylosin can be degraded efficiently in anaerobic-aerobic environments.

Keywords: antibiotic, anaerobic-aerobic treatment, pharmaceutical wastewater, biodegradation, Tylosin

1. Introduction

Wastewaters produced from pharmaceutical industries, such as antibiotic manufacture and formulation, generally contain high levels of soluble organics, many of which are recalcitrant (Schroder, 1999). If these compounds are not removed by one-site treatment they will be discharged to sewage treatment plants (STPs) where they may disturb the biological process and the microbial ecology, and potentially affect receiving surface waters. Typically, pharmaceutical wastewater is characterized by high COD concentration, some pharmaceutical wastewaters having a COD as high as 80,000 mg.L^{-1} (Nandy and Kaul, 2001). Most published studies have investigated the removal of COD but ignored antibiotic removal during treatment. Although high COD removal efficiencies have been achieved, biological treatment is sometimes ineffective in the removal of antibiotics under some circumstances (Adams et al. 2002; Saravanane et al. 2001b). It is therefore important to investigate antibiotic degradation associated with COD removal in wastewater treatment processes.

For industrial wastewater treatment, an anaerobic process is often applied to reduce of the major part of the COD load, which is then followed by an aerobic treatment to oxidize the residual COD in the wastewater. This is because effluent from the anaerobic bioreactor usually has substantial amounts of residual COD even if its removal efficiency is above 90% (Zhou et al. 2006). Therefore, direct discharge to the environment immediately after

anaerobic treatment is rarely permitted, and post-treatment by an aerobic process is usually necessary. According to Field et al. (1995), an aerobic polishing step was required for complete mineralisation of aromatic amines and chlorinated aromatics treated by anaerobic digestion. In addition, process optimisation can be achieved by the use of a sequential anaerobic-aerobic treatment system, particularly when treating highly recalcitrant wastewaters (van Lier et al. 2001). Despite these considerations, there is a limited number of experimental studies investigating the treatment of pharmaceutical wastewaters in an anaerobic-aerobic reactor systems (Fox and Venkatasubbiah (1996); Buitrón et al. (2003); Zhou et al. (2006); Sponza and Demirden (2007).

Tylosin is a macrolide antibiotic produced by a strain of *Streptomyces fradiae*. It has good anti-bacterial activity against most pathogenic gram-positive bacteria, and some gram-negative bacteria, vibrio, spirochete, coccidian, etc. It is one of the first-choice drugs against infections caused by mycoplasma. The chemical structure of Tylosin is given in Figure 1.

The aim of this study was to investigate treatment of pharmaceutical wastewater that contains Tylosin in a sequential Up-Flow Anaerobic Stage Reactor (UASR) and Porous Membrane Activated Sludge Reactor (PMASR). The more specific objectives of this research were to assess the stability of reactor for measured parameters (e.g. COD and TOC removal) and to investigate the efficiency of Tylosin reduction in the UASR-PMASR system. To date, there is no reported study on the treatment of pharmaceutical wastewater containing macrolide Tylosin by a sequential anaerobic–aerobic reactor system.

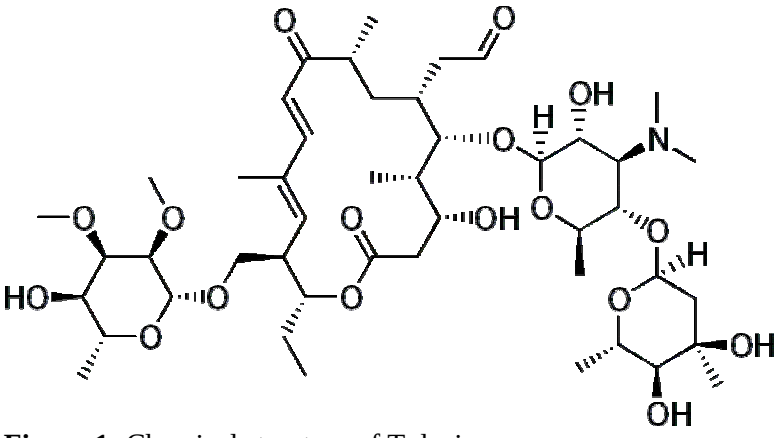

Figure 1: Chemical structure of Tylosin.

2. Materials and methods

2.1 Reactor configurations

A continuously fed laboratory scale UASR and a PMASR, operating in series, were used in this study. The operational set-up, flow diagram and reactor design are presented in Figure 2a. The UASR system comprise four identical cylindrical Plexiglas compartments (stages), 80 mm internal diameter by 640 mm height, linked in series and was developed according to the concept of Anaerobic Baffled Reactor (ABR) (Barber and Stuckey, 1999). The active volume of the UASR system was 11 L (4 stages of 2.75 L). 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 (Figure 2b). Each

stage was equipped with sampling ports at 100 mm intervals (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 12 mm internal diameter downcomer tube in the headplate that extended to within 15 mm 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, as each stage was placed on stepped platform having a 150 mm step height. The walls of the reactors were wrapped with a tubular polyvinyl chloride (PVC) water-jacket with 15 mm 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. Effluent from the UASR flowed to a PMASR for aerobic treatment. The aerobic reactor was cylindrical, 400 mm high and 155 mm diameter with an operational volume of 4.1 litres. It included a porous membrane 316 mm high and 145 mm diameter made from a polymer material (high density polyethylene, HDPE) with a wall thickness of 3.5 mm and nominal pore size of 5–8 μm which acted as a secondary clarifier. Watson Marlow 500 series peristaltic pump was used to supply the feed to the PMASR. Aeration was provided approximately 3 $L.min^{-1}$ from the laboratory compressed air supply through a standard aquarium diffuser stone located at the base of the reactor. The dissolved oxygen (DO) concentration was monitored and air flow regulated in order to maintain a minimum DO concentration of 2 $mg.L^{-1}$ at all times.

2.2 Pharmaceutical wastewater

The pharmaceutical wastewater was supplied by Eli Lilly & Company Ltd, Liverpool, UK and had the following characteristics; soluble COD, 7000 ± 800 $mg.L^{-1}$; soluble BOD_5, 3500 ± 500 $mg.L^{-1}$; sulphate, 2500 ± 500 $mg.L^{-1}$; Total Kjeldahl Nitrogen (TKN), 364 ± 50 $mg.L^{-1}$; pH, 5.2–6.8; and Tylosin concentration, 20 to 200 $mg.L^{-1}$.

2.3 Reactor operation

In general, the operation of UASR was carried out in five major steps (Table 1): start-up of UASR, acclimatisation to pharmaceutical wastewater, step increase in OLR (0.43–1.86 kg $COD.m^{-3}.d^{-1}$) by altering feed COD (1700 – 7450 $mg.L^{-1}$) at constant HRT (4 d), and then further step increments in OLR to 3.73 kg $COD.m^{-3}.d^{-1}$ by reducing the HRT from 4 to 2 d at constant feed COD (7450 $mg.L^{-1}$). Finally, the OLR was reduced again to 1.86 kg $COD.m^{-3}.d^{-1}$ (HRT 4 d) to determine the ability of the reactor to recover treatment efficiency (Table 1). The UASR was seeded with anaerobic digested sewage sludge from an anaerobic sludge digester at Hexham Municipal sewage treatment plant, Northumberland, UK. Effluent from the UASR was passed through a PMASR reactor after the UASR performance had approached steady-state at an OLR of 1.86 kg $COD.m^{-3}.d^{-1}$. Cramlington municipal wastewater treatment plant, Northumberland, UK, giving an initial mixed liquor suspended solids (MLSS) concentration of 2500 $mg.L^{-1}$. The reactor was then conditioned by feeding brewery wastewater (selected for ease of degradation, high COD value, and having well established use in continuous anaerobic reactors, Sallis and Uyanik, 2003) as substrate, at flow rate of 1 $L.d^{-1}$ (HRT of 4 d), the feed COD concentration being maintained between 500 and 1500 $mg.L^{-1}$. MLSS concentration was monitored in particular during the start up phase to understand the aerobic biomass growth kinetics in the reactor.

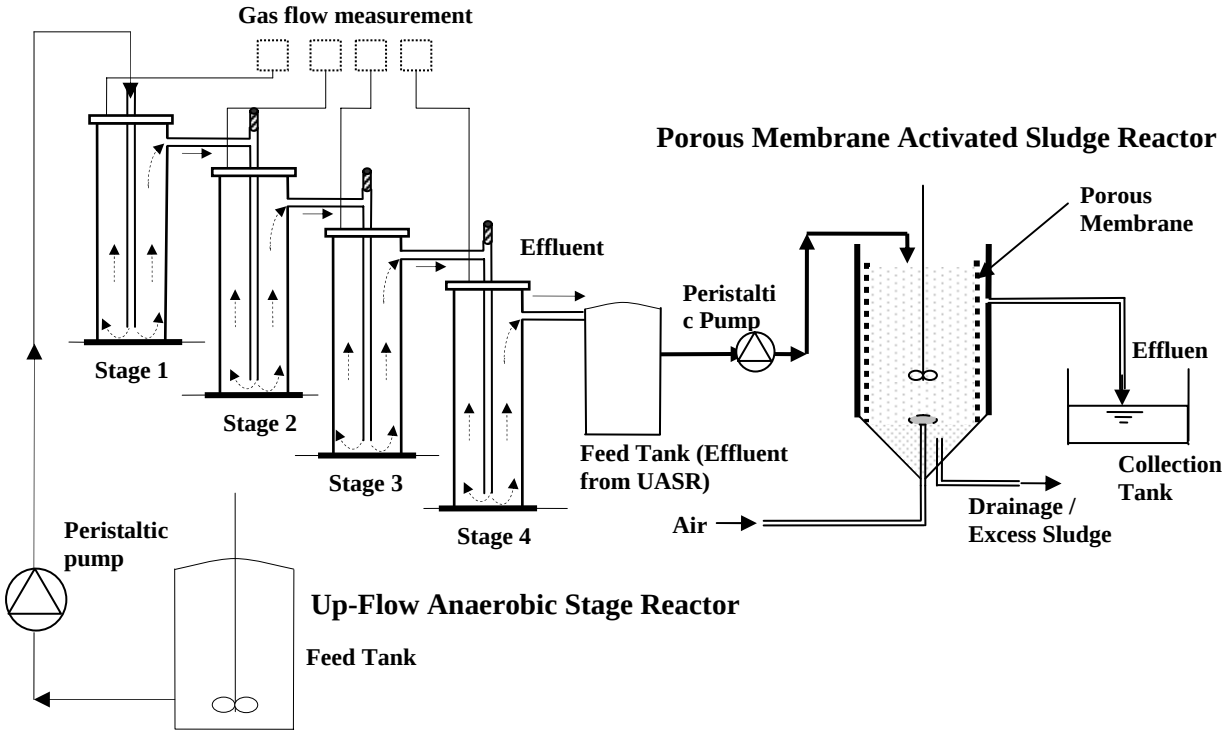

Figure 2a.

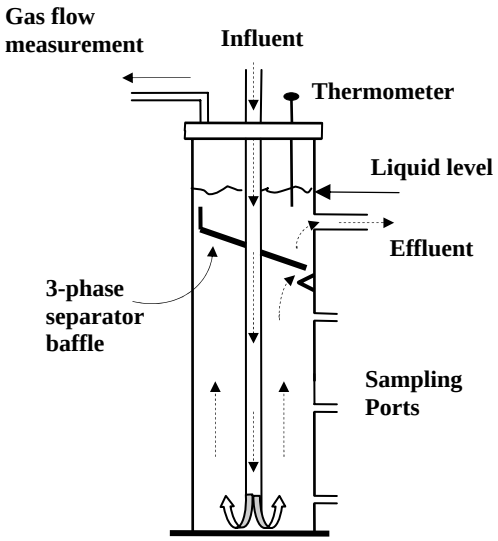

Figure 2b.

Figure 2: (a) Experimental set-up and flow chart of UASR-PMASR; **(b)** details of an individual UASR stage.

After PMASR start-up on brewery wastewater feed, sludge was acclimated to UASR effluent gradually (i.e. the UASR effluent was blended with brewery wastewater in increasing proportions, stepwise from 20 to 100% (as COD), with corresponding reductions in the brewery wastewater component). This achieved successful acclimatisation of biomass to the partially treated pharmaceutical wastewater components during the sensitive

start-up period by creating conditions of minimal organic load and hydraulic stresses. The reactor was aerated continuously, and pH was maintained in the range of 7.0–8.2 by adjustment of the feed pH with NaOH. Nutrient levels were maintained in the ratio of COD: N: P at 100:5:1 (Metcalf and Eddy, 2003). The biomass was considered to be acclimatised when MLSS concentration maintained constant levels (3000 ± 200 $mg.L^{-1}$). During the course of the investigation, a small problem developed from foaming in this reactor, probably due to surfactant chemicals in the pharmaceutical wastewater. This was controlled by addition of an antifoaming agent (Silicone Antifoaming Agent, BDH Laboratory Supplies, UK; Product Code 512K), which did not appear to inhibit the biomass activity.

Table 1: Summary of reactor operating conditions of UASR system

Step	Brewery (%)* wastewater	Antibiotic (%)* wastewater	Mean OLR ($kg\ COD.m^{-3}.d^{-1}$)	HRT (d)	Mean Feed COD ($mg.L^{-1}$)	Day
1	100 – 50	0 – 50	0.43	4.0	1700	–
2	50	50	0.43	4.0	1700	1
3	40 – 10	60 – 90	0.86	4.0	3450	41
	0	100	1.23	4.0	4900	82
	0	100	1.53	4.0	6100	109
	0	100	1.86	4.0	7450	166
4	0	100	2.48	3.0	7450	188
	0	100	2.98	2.5	7450	212
	0	100	3.73	2.0	7450	231
5	0	100	1.86	4.0	7450	250

*proportion based on COD.

2.4 Analytical methods

Sample analysis included chemical oxygen demand (COD), total organic carbon (TOC), pH, alkalinity, suspended solids (SS), volatile suspended solids (VSS), all according to Standard Methods (APHA, 1998). Available PO_4-P was determined by ion-chromatography (Dionex, DX-100 Ion Chromatograph), volatile fatty acids (VFA) by gas-liquid chromatography (Unicam 610 Series Gas Chromatograph with auto-injector and PU 4811 computing integrator) having operating conditions as follows: carrier gas: nitrogen at 20 $ml.min^{-1}$; column temperature 140 C; detector temperature: 180 C; injection port temperature: 180 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. TOC measurement was based on quantitative infrared analysis performed with a Total Organic Carbon Analyser (Shimadzu Model, TOC-5050A). Reactor gas composition (CO_2 and CH_4) was determined by gas chromatography (Becker model 403 Gas Chromatograph with Unicam 4815 computing integrator) under 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.

Average values of the measured parameters quoted for each OLR were based on the mean of four data points taken after three HRT periods for each OLR, i.e. when reactor approached near steady-state.

2.5 Tylosin Assay

Tylosin assay was performed by HPLC on a 20cm Nucleosil C18 analytical column eluted with 60 vols 2 mol.dm^{-3} sodium perchlorate ($NaClO_4$) and 40 vols of acetronitrile (CH_3CN). Tylosin factors were separated and detected at 280nm. The integrated chromatogram was normalised and the relative percentage of each Tylosin factor reported. Comparison of each Tylosin sample chromatogram with that of a Tylosin base reference standard chromatogram confirmed peak identity for quantification against a 3-point standard curve.

3. Results and discussion

3.1 UASR performance

During start-up, the UASR showed steady-state COD conversion efficiencies of 93%, and no substantial reductions in the COD removal efficiency when reactor feed was supplemented incrementally with pharmaceutical wastewater (Table 2). The preliminary phase (acclimatisation) confirmed that the methanogenic activity in the UASR could be maintained in the presence of pharmaceutical wastewater (total methane production was 1150 ml.d^{-1}, Table 2). Table 3 shows the pH levels in the UASR treating pharmaceutical wastewater when the OLR was increased gradually. The pH levels were generally stable (pH 6.3–7.8) in all stages of the UASR until the reactor OLR exceeded 2.98 kg COD.m^{-3}.d^{-1}. Consequently, at a reactor OLR of 3.73 kg COD.m^{-3}.d^{-1} (when the reactor HRT was reduced to 2 d) the pH in Stage 1 dropped to 5.7 due to the rapid production of VFAs resulting from increased acidogenic activity. At the same time, pH reduction was also observed in all subsequent reactor stages; the degree of pH reduction following the order Stage 1>2>3>4 which reflected the actual OLR of each stage (stage OLR followed the order 1>2>3>4 on account of the sequential degradation of the influent COD load as it passed through the reactor system; e.g. at a reactor OLR of 3.73 kg COD.m^{-3}.d^{-1} the actual OLR in Stage 1 was 14.92 kg COD.m^{-3}.d^{-1}, while actual OLR in Stage 4 was 1.75 kg COD.m^{-3}.d^{-1}). However, when the reactor OLR was reduced back to 1.86 kg COD.m^{-3}.d^{-1}, the pH in Stage 1 stabilised at 7.2 indicating that acidogenesis and methanogenesis had recovered balanced levels. From the pH data, it can be assumed that the metabolic processes differed between Stages 1 to 4 of the UASR system (particularly when OLR exceeded 2.48 kg COD.m^{-3}.d^{-1}) and this would cause each stage to favour a unique population of microorganisms.

The total VFA concentration in each stage of the reactor is shown in Table 3 and indicates a low concentration of total VFA (average 222 mg.L^{-1}) was present in the reactor effluent (Stage 4) when operated at OLR in the range 0.43 to 1.53 kg COD.m^{-3}.d^{-1} (4 d HRT). However, the effluent VFA concentration increased to 949 mg.L^{-1} when the reactor OLR was increased to 2.48 kg COD.m^{-3}.d^{-1}. Further increases in reactor OLR, by reducing the HRT, resulted in higher VFA concentrations being produced in the effluent. The highest of these was found when OLR was 3.73 kg COD.m^{-3}.d^{-1} with an average value of 3,310 mg.L^{-1} in Stage 1, 2,311 mg.L^{-1} in Stage 2, 1,522 mg.L^{-1} in Stage 3 and 1,468 in Stage 4. At high OLRs and low HRTs the relatively complex pharmaceutical wastewater

caused pre-acidification resulting in accumulation of COD (as VFA), which did not subsequently convert to methane, resulting in an accumulation of VFA. However, when the reactor OLR was reduced to 1.86 kg $COD.m^{-3}.d^{-1}$, recovery was almost immediate with the effluent VFA concentration being 280 $mg.L^{-1}$. The ability of the reactor to recover rapidly when OLR was returned to 1.86 kg $COD.m^{-3}.d^{-1}$ follows a similar pattern to that found in the COD (Figure 3) and pH levels, however, complete recovery of COD removal efficiency required more time than pH and VFA recovery.

Table 2: Summary of UASR performance during start-up and acclimatisation to pharmaceutical wastewater (average values when reactor approached steady-state).

Source	**Parameter**	**Start-up** (OLR 0.43 kg $COD.m^{-3}.d^{-1}$ and HRT 4d)	**Acclimatisation** (OLR 0.43 kg $COD.m^{-3}.d^{-1}$ and HRT 4 d)
Influent	COD ($mg.L^{-1}$)	1700 ($mg.L^{-1}$)	1700 ($mg.L^{-1}$)
	pH	7.2	7.2
Effluent	COD ($mg.L^{-1}$)	119 ($mg.L^{-1}$)	221 ($mg.L^{-1}$)
	COD removal (%)	93%	87%
	pH	7.6	7.7
	VFA	100 ($mg.L^{-1}$)	130 ($mg.L^{-1}$)
	Methane production	1350 ($ml.d^{-1}$)	1150 ($ml.d^{-1}$)

Biogas production was monitored in all stages throughout the operation of the reactor, particularly for the assessment of methanogenic activity. Table 3 shows the methane volume produced in each stage of the reactor system. It is evident that Stage 1 produced the greatest volume of methane when the reactor OLR was operating between 0.43–1.86 kg $COD.m^{-3}.d^{-1}$. However, at higher OLR (2.48 $COD.m^{-3}.d^{-1}$), the methane production in Stage 1 dropped dramatically from an average value of 3432 $ml.d^{-1}$ (at OLR 1.86 $COD.m^{-3}.d^{-1}$) to 1066 $ml.d^{-1}$, and this reduced further to 671 and 269 $ml.d^{-1}$ when the reactor was operated at OLR 2.98 and 3.73 $COD.m^{-3}.d^{-1}$, respectively. Considering the changes in pH, and VFA concentration, that occurred with these step increases in OLR (Table 3) it is likely that a large part of the methanogenic population was adversely affected by physico-chemical conditions created by the acidogens at higher OLR. However, this inhibitory effect was not permanent, and methane production in Stage 1 of the reactor system recovered to 3127 $ml.d^{-1}$ when the reactor OLR was reduced back to 1.86 kg $COD.m^{-3}.d^{-1}$. During the period of reduced methane productivity in Stage 1 (i.e at OLR 2.48 to 3.73 kg $COD.m^{-3}.d^{-1}$), Stages 3 and 4 each showed relatively higher levels of methane production compared to that at lower OLR, but this returned to previous levels when the OLR was reduced to 1.86 kg $COD.m^{-3}.d^{-1}$. In contrast, Stage 2 showed relatively constant levels of methane production over a wide range of OLR (1.86 to 3.73 kg $COD.m^{-3}.d^{-1}$).

Figure 3 shows temporal changes in the COD removal of the UASR treating pharmaceutical wastewater when the OLR was increased gradually. Initial fluctuations were attributed to technical problems with the peristaltic feed pump. At a reactor OLR of 1.86 kg $COD.m^{-3}.d^{-1}$ (HRT 4 d), the soluble COD reduction was around 70–75% (average SDR being 1.29 kg $COD.m^{-3}.d^{-1}$, Figure 4). However, when the OLR was increased to 2.48 kg $COD.m^{-3}.d^{-1}$ (by lowering the HRT, since the strength of the wastewater was limited) the

Table 3: Typical pH, VFA and methane production in UASR stages at different OLR (average values when reactor approached steady-state).

		OLR (kg COD.m^{-3}.d^{-1})								
UASR Stages	Parameter	0.43	0.86	1.23	1.53	1.86	2.48	2.98	3.73	1.86
Stage 1	pH	7.07	7.03	6.97	7.17	7.11	6.81	6.26	5.66	7.20
	VFA (mg.L^{-1})	586	287	783	477	702	2410	3313	3310	823
	Methane (ml.d^{-1})	791	1497	2170	2697	3432	1066	671	269	3127
Stage 2	pH	7.20	7.15	7.17	7.36	7.38	7.20	6.98	6.36	7.18
	VFA (mg.L^{-1})	356	154	645	451	593	1589	2276	2311	634
	Methane (ml.d^{-1})	414	437	469	796	1384	1532	1504	1359	1492
Stage 3	pH	7.31	7.26	7.32	7.55	7.58	7.55	7.15	6.78	7.24
	VFA (mg.L^{-1})	260	127	390	425	340	708	945	1522	400
	Methane (ml.d^{-1})	313	264	363	343	195	762	1063	1675	525
Stage 4	pH	7.83	7.57	7.78	7.84	7.84	7.77	7.41	6.94	7.80
	VFA (mg.L^{-1})	199	65	250	374	250	949	1127	1468	280
	Methane (ml.d^{-1})	184	128	320	291	228	606	925	775	358

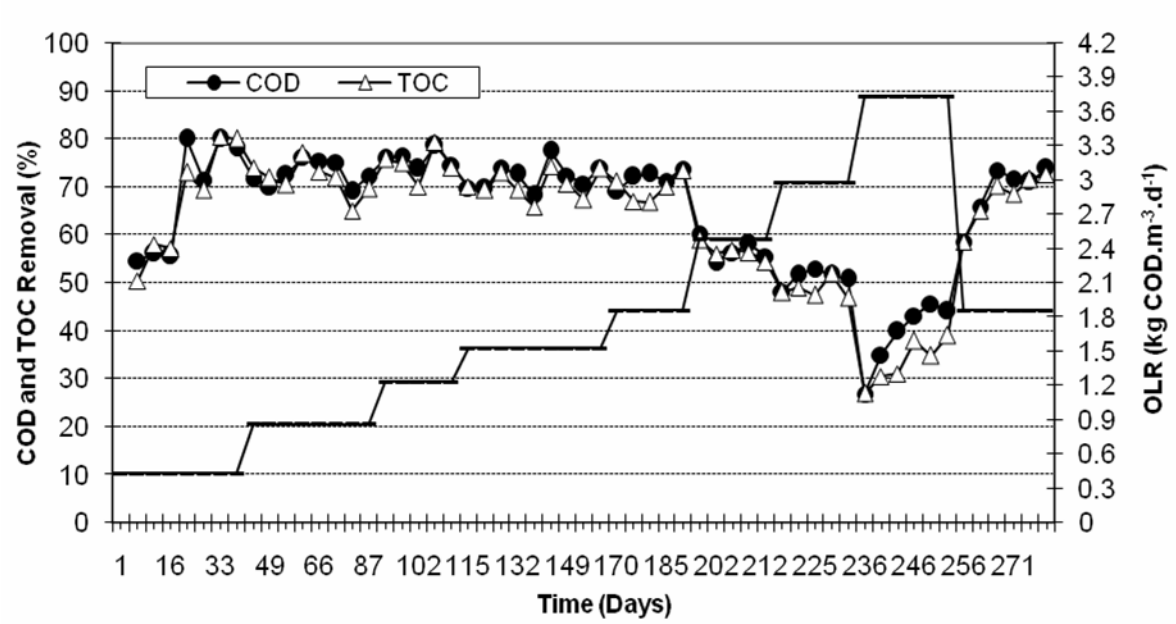

Figure 3: COD and TOC reduction profile of UASR at different OLR.

COD removal efficiency decreased gradually until only around 45% soluble COD removal (average removal when reactor approached steady-state) was observed at an OLR of 3.73 kg COD.m^{-3}.d^{-1} (SDR being 1.48 kg COD.m^{-3}.d^{-1}, Figure 4). The above results are consistent with observations made by Rodríguez-Martinez et al. (2005) in an UASB treating pharmaceutical wastewater containing Penicillin G macrolide antibiotics, who found that the COD removal efficiency was 90% at an OLR of 1.5 kg COD.m^{-3}.d^{-1} and HRT 11 d. However, when the OLR was increased to 2.09 kg COD.m^{-3}.d^{-1} by reducing the

HRT to 7 d, the COD removal efficiency dropped substantially to 70%. They also found that an increase in the OLR resulted in the accumulation of hydrogen sulphide in the biogas (sulphate in the feed was 3200 $mg.L^{-1}$) which affected the efficiency of the reactor; the presence of sulphide is known to inhibit the activity of methanogens (McCartney and Oleszkiewicz, 1991). It is generally known the application of anaerobic treatment processes for industrial wastewaters containing high amounts of sulphate has been problematic due to the production of hydrogen sulphide. The presence of H_2S in anaerobic digesters results from the action of sulphate-reducing bacteria (SRB) which utilise sulphate as terminal electron acceptor and compete with acetogens and methanogens for several key substrates in anaerobic digestion such as propionate, butyrate, ethanol and acetate (Oude-Elferink *et al.* 1994). Moreover, SRB are generally expected to out-compete other anaerobes in the presence of excess sulphate (O'Flaherty et al. 1998). The pharmaceutical wastewater used in this study also contained high concentration of sulphate (2500 ± 500 $mg.L^{-1}$) and sulphide production from this sulphate was thought to be one of the reasons for the poor performance of UASR during the period of high OLR (2.48–3.73 kg $COD.m^{-3}.d^{-1}$). Speece, 1996 has stated that at higher OLR, SRB have a competitive advantage over methanogens for substrates, and it is possible for hydrogen sulphide production to predominate over methane gas production. Fox and Venkatasubbiah (1996) reported that as influent pharmaceutical wastewater containing high sulphate was increased to 20% in an ABR, the reactor performance deteriorated (COD removal efficiency reduced from 50 to 20%) as the effluent sulphide concentration increased to inhibitory levels (more than 200 $mg.L^{-1}$). Kuscu and Sponza (2006) have demonstrated that hydrogen sulphide concentrations in the biogas increased from 160 ppm to 195 ppm when OLR was increased from 2.1 to 3.16 kg $COD.m^{-3}.d^{-1}$ in an ABR treating sulphate-containing wastewater (*p*-Nitrophenol). Consequently, it was thought that as OLR was increased (2.48–3.73 kg $COD.m^{-3}.d^{-1}$) in the UASR, the increasing load of Tylosin and sulphide production affected the methanogens, therefore, contributing to the lower process efficiency of the system. Another important point is that changes in HRT may have affected the operation of the UASR (increasing the OLRs to 3.73 kg $COD.m^{-3}.d^{-1}$ by reducing the HRT (4–2 d) reduced the COD removal efficiency to 45%). When the OLR was increased, the increasing acidogenic activity usually results in lower pH values; reduced methanogenic activity; increased COD and VFA in the effluent of the UASR. Even though it was expected that the UASR would be stable at high OLRs, it was not able to withstand the short HRT, probably due to the complexity of pharmaceutical wastewater which contained Tylosin and sulphate. In general, longer HRT can help the kinetics of degradation, i.e. more complex organics like polymers and recalcitrant simply have longer to be degraded. Nandy and Kaul (2001) have demonstrated that substrate removal efficiency increases with increase in HRT in anaerobic treatment of herbal-based pharmaceutical wastewater using fixed-bed reactor. Zhou et al. (2006) reported that when HRT of an ABR treating pharmaceutical wastewater containing antibiotics (Ampicillin and Aureomycin) was extended from 1.25 to 2.5 d, the COD removal efficiency increased from 77 to 85%. They also observed that the antibiotic removal efficiencies increased from 16 to 42% for Ampicillin and 26 to 31% for Aureomycin.

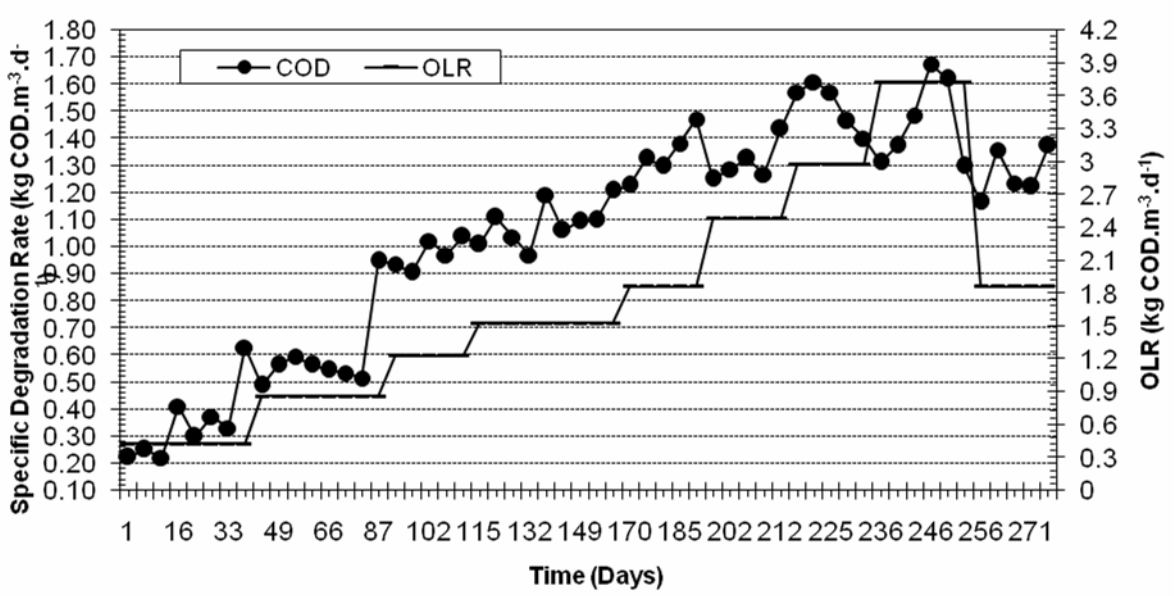

Figure 4: Specific degradation rate (SDR) profile of UASR at different OLR.

The organic content of the reactor feed and effluent was determined by measurement of the total organic carbon (TOC). The profile of TOC removal throughout the operation period was consistent with that of COD removal (Figure 3). Another factor associated with sulphate reduction (data not presented) is the fact that leads to the underestimation of organic matter degradation when this based on COD values because sulphide is oxidised during the COD test. Consequently, if there is large quantity of sulphide ions in the effluent, there will be a difference in the TOC and COD removal pattern. In the present study, the difference in COD and TOC removal profile was small (Figure 3), confirming that most sulphide partitions into the biogas (exchange between soluble sulphide and gaseous hydrogen sulphide being pH dependent). This is further supported by low levels of sulphide ion detected in the effluent of UASR (spot data of sulphide ion in UASR effluent showed 10–40 $mg.L^{-1}$ for all OLR investigated).

3.2 UASR-PMASR performances

Generally, an anaerobic process is applied to remove high concentrations of organic matter followed by an aerobic treatment to oxidise the residual organic matter. Given that influent COD is very high, effluent from anaerobic reactor can still have residual COD. Consequently, direct discharge of effluent from anaerobic reactor is not permitted, and post-treatment of anaerobic process effluent with an aerobic reactor is necessary. The effluent from UASR was further subjected to aerobic treatment (PMASR) to remove the residual COD. Figure 5 shows the results of COD and TOC removal from the UASR effluent by the PMASR during periods when the UASR was had an OLR above 1.86 kg $COD.m^{-3}.d^{-1}$. As a result, the feed to the PMASR contained variable influent COD values, caused by the different OLRs applied to the UASR. During the reactor start-up period with brewery wastewater (total duration was 21 days), the COD removal efficiency of the PMASR was around 89% (Figure 5), after which point the COD removal efficiency dropped to 76% when the reactor was being acclimated gradually to effluent from UASR.

From day 52, the PMASR was operated with 100% effluent from the UASR, and an HRT of 4 d. This led to a soluble COD removal efficiency for the PMASR of 63–69% (SDR being 0.37 kg $COD.m^{-3}.d^{-1}$, Figure 6). During this period, effluent from UASR contained 1800-2200 $mg.L^{-1}$ of COD (UASR operating at OLR of 1.86 kg $COD.m^{-3}.d^{-1}$). However, the COD removal efficiency dropped noticeably to an average 51% (SDR being 0.42 kg $COD.m^{-3}.d^{-1}$, Figure 6) during the period of high OLR in the UASR (2.48–3.73 kg $COD.m^{-3}.d^{-1}$), and recovered to around 66% (SDR being 0.38 kg $COD.m^{-3}.d^{-1}$, Figure 6) when the UASR was returned to an OLR of 1.86 kg $COD.m^{-3}.d^{-1}$. Changes in HRT of the UASR may have affected the aerobic biodegradation rate in the PMASR. When the PMASR was fed with anaerobic effluent from the UASR that operated with HRT of 4 d, the residual COD from the PMASR was stabilised at700 $mg.L^{-1}$, indicating substantial amount of COD that remained in the UASR effluent could be removed aerobically. However, when the HRT of the UASR was reduced from 4 to 2 d, significant changes occurred in the PMASR effluent (residual COD was 1000 $mg.L^{-1}$ when the OLR was 2.48–3.73 kg $COD.m^{-3}.d^{-1}$). Moreover, due to the nature of the pharmaceutical wastewater used in this study, a fraction of the wastewater is nonbiodegradabale under aerobic condition and complete removal of substrate cannot be expected due to the presence of the refractory material that could not be destroyed aerobically (Zhou et al. 2006). It is generally known that longer anaerobic treatment time could result in a decrease in the fraction of refractory organic materials since some refractory fraction under aerobic conditions could be decomposed at the acidogenic stage and some intermediates could be generated. These intermediates were more readily degraded under aerobic conditions.

The TOC removal efficiency was also investigated, the results showing a similar pattern to the COD removal profile with an average TOC removal in the PMASR of 69% observed during an OLR of 1.86 kg $COD.m^{-3}.d^{-1}$ in the UASR (Figure 5). The results indicate that a substantial amount of COD that remained in the UASR effluent could be removed aerobically by the PMASR. Furthermore, the results also signify that the pharmaceutical wastewater can be treated aerobically, and the combined UASR-PMASR system was successful in treating the pharmaceutical wastewater with typical total COD removal (Figure 8) in the range of 87-90% (when the UASR was operated at OLR 1.86 kg $COD.m^{-3}.d^{-1}$) and 70–80% (when the UASR was operated at OLR 2.48–3.73 kg $COD.m^{-3}.d^{-1}$).

The above results are comparable with Zhou et al. (2006) who reported around 73–90% COD removal in the aerobic and up to 97.8% removal in the combined anaerobic-aerobic treatment of pharmaceutical wastewater containing Ampicillin and Aureomycin antibiotics. Arslan-Alaton et al. (2004) reported 71% COD removal in the activated sludge treatment of Penicillin formulation effluent.

3.3 Tylosin reduction

Incomplete degradation of pharmaceuticals, especially antibiotics in wastewater treatment plants could be a contributing factor to the presence of antibiotics in receiving surface waters (Al-Ahmad et al. 1999). Because of their bacterial toxicity, antibiotics may play a key role in decreasing COD removal rates and their removal from pharmaceutical wastewater before discharge to sewer is at least as important as the overall removal of the COD fraction. In this study, Tylosin concentration in the pharmaceutical wastewater feed varied from 10 to 220 $mg.L^{-1}$ and Figure 7 shows the Tylosin degradation profile throughout the experimental study in the UASR. Tylosin removal efficiency fluctuated

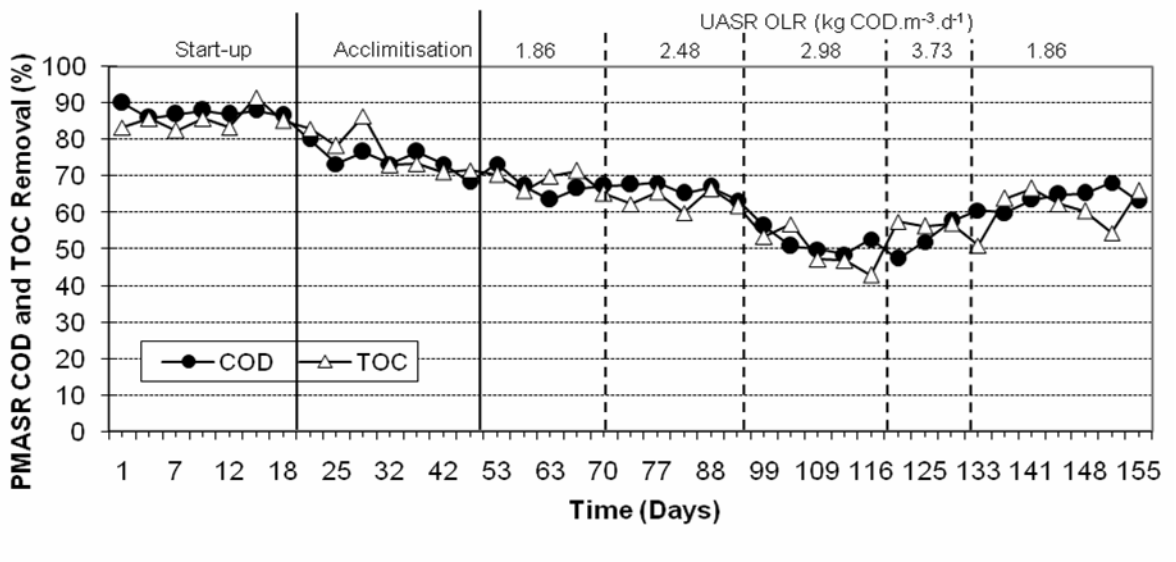

Figure 5: COD and TOC reduction profile of PMASR at different UASR organic loadings.

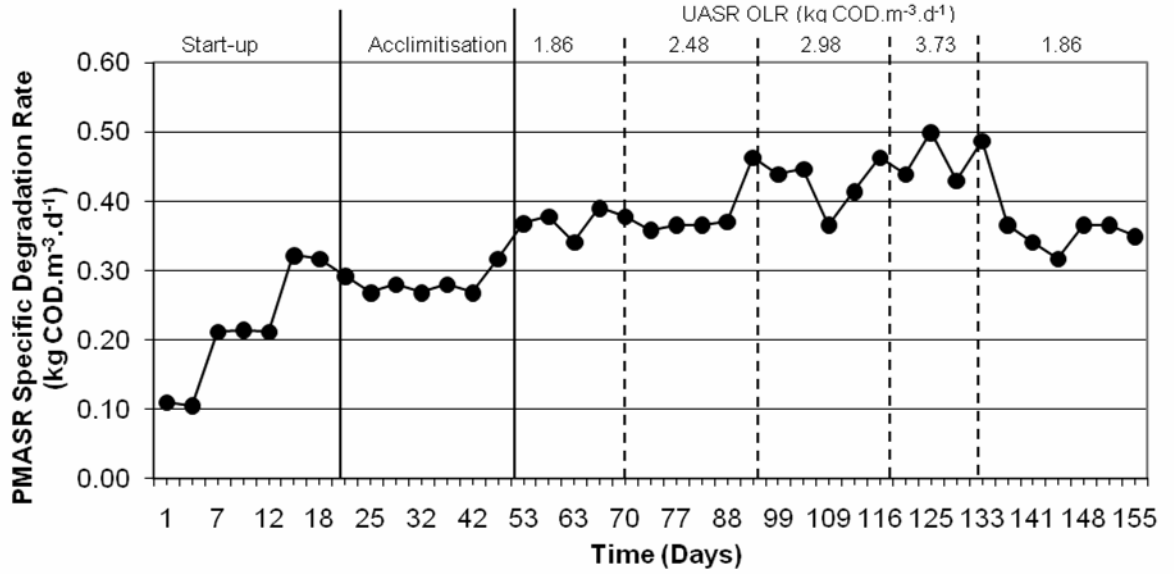

Figure 6: Specific degradation rate (SDR) profile of PMASR at different UASR organic loadings.

from 70–88% at OLR 1.86 kg $COD.m^{-3}.d^{-1}$, however, the removal efficiency remained relatively constant (93–99%) at OLR 2.48 - 3.73 kg $COD.m^{-3}.d^{-1}$. Similar removal trend was also observed when the reactor OLR was reduced to 1.86 kg COD m^{-1} d^{-1} (Figure 7), with an average Tylosin concentration in the treated wastewater of 3 $mg.L^{-1}$ for the all OLR investigated (Table 4). This confirms that Tylosin was readily degraded in the reactor under anaerobic conditions. In contrast to the COD removal profile, which showed reducing COD removal efficiency with increasing OLR, Tylosin concentration remained relatively constant in the reactor effluent throughout the experiment. These results are consistent with the view that typical wastewater concentrations of Tylosin have a relatively minor influence on the overall COD removal efficiency of UASR and do not inhibit substantially the activity of methanogenic populations. This result agrees with the study by Poels et al. (1984)

on effect of Tylosin on anaerobic digestion, which concluded that at concentration of 50 to 100 mg.L^{-1}, Tylosin had no inhibitory effect on methane production. Masse et al. (2000) reported that presence of Tylosin (110 mg.kg^{-1} in feed diets) did not effect the treatment of swine manure slurry in sequencing batch reactor (SBR). Kolz et al. (2005) showed that Tylosin can be reduced up to 90% in an anaerobic batch degradation of swine manure slurries with initial concentration of 195 mg.L^{-1}. However, they found that some residual Tylosin remained in the slurry after eight months of incubation, indicating incomplete degradation. More recently, Angenent et al. (2008) demonstrated that Tylosin with an average concentration of 1.6 mg/L in a swine waste can be degraded in a high-rate anaerobic digester (ASBR).

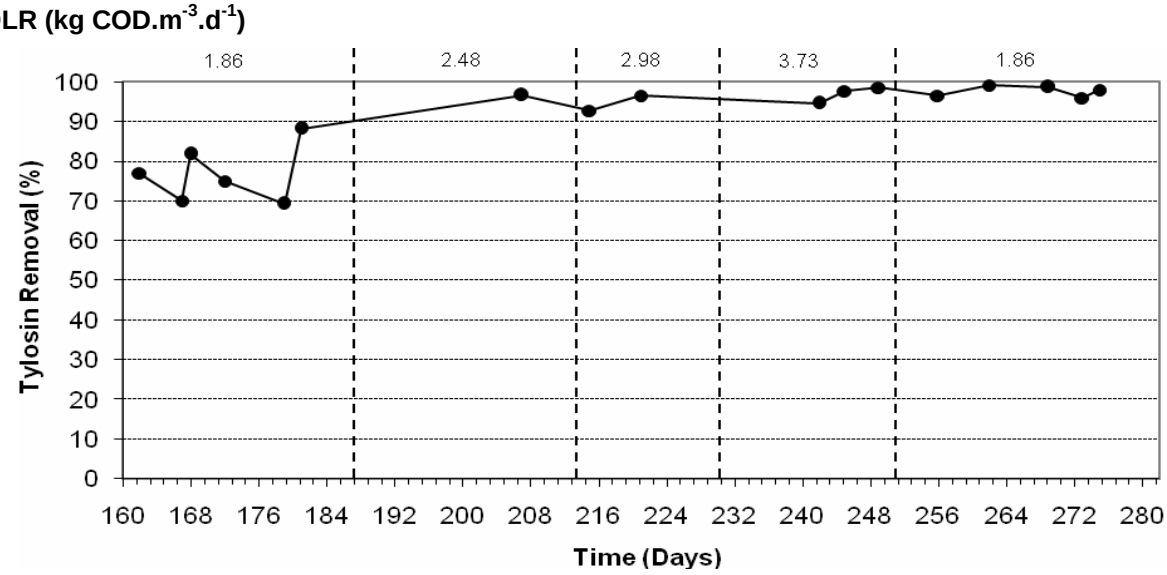

Figure 7: Tylosin reduction profile of UASR at different OLR.

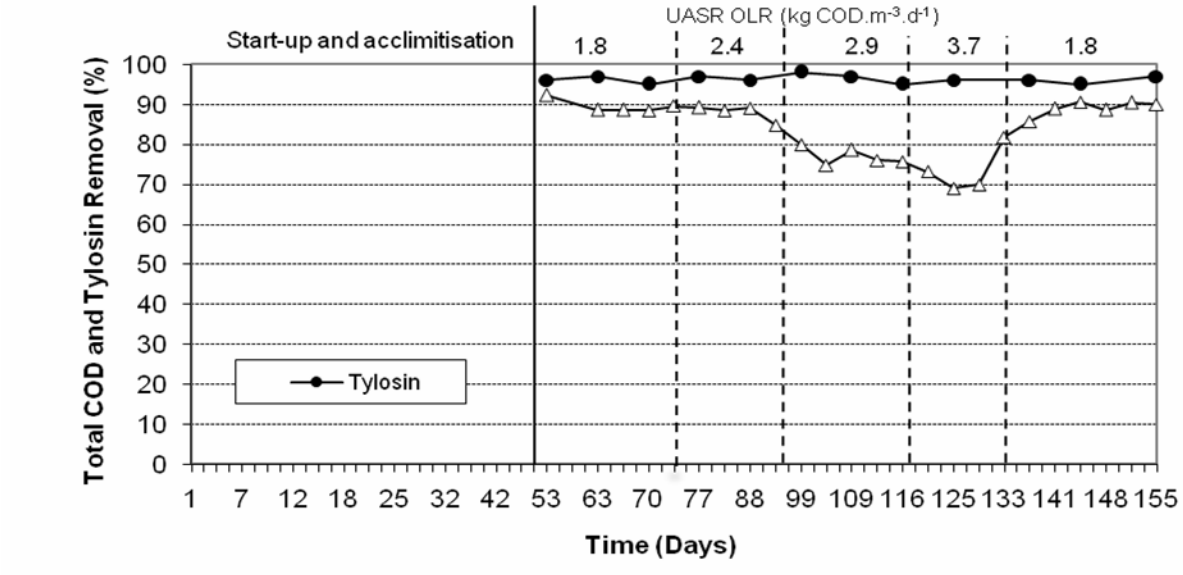

Figure 8: Total COD and Tylosin reduction profile of the combined treatment of UASR-PMASR.

Table 4: Summary of treatment performance of the pharmaceutical wastewater by UASR-PMASR (average values when reactor approached steady-state).

Parameter	Raw	UASR	PMASR	UASR-PMASR
COD ($mg.L^{-1}$)	7450	2000	700	–
COD removal (%)	–	73*	66*	89*
Tylosin ($mg.L^{-1}$)	< 200	3	2.7	–
Tylosin reduction (%)	–	95	10	97

*at UASR OLR 1.86 kg $COD.m^{-3}.d^{-1}$.

One essential point to consider is whether Tylosin removal from the UASR system was caused by sorption (adsorption to reactor sludge solids) or anaerobic degradation. Previous work on anaerobic degradation of Tylosin did not clearly state the reason for Tylosin disappearance. Loke et al. (2000) reported that the loss of Tylosin in batch anaerobic degradation experiments of pig manure was caused by a combination of sorption, abiotic transformation (e.g. hydrolysis) and biotic transformation (biodegradation); however, no further details were examined. Kolz et al. (2005) also showed that Tylosin disappearance in batch anaerobic degradation experiments of swine manure slurries was caused by abiotic and biotic degradation, but suggested strong sorption to slurry solids to be the main mechanism of Tylosin loss. Consequently, the loss of Tylosin in UASR system could be combination of sorption to sludge solids, abiotic and biotic degradation.

Feed to the PMASR contains an average Tylosin of 3 $mg.L^{-1}$ (effluent from UASR) and Figure 8 shows the Tylosin reduction in the combined UASR-PMASR system. The results showed that, an average Tylosin reduction for the combined system was 97% (Table 4) for the entire duration of the experiment, with the PMASR effluent having an average Tylosin value of 2.7 $mg.L^{-1}$ (plot of Tylosin removal in PMASR not presented). This result indicates that a small quantity of Tylosin was reduced (around 10% or 0.3 $mg.L^{-1}$) in the PMASR, since the majority of it was reduced in UASR (Table 4). Therefore, these results indicate that the aerobic reactor was not particularly effective in degrading Tylosin at the low starting concentrations that were applied experimentally. A similar observation was also reported by Zhou et al. (2006) when treating pharmaceutical wastewater containing Ampicillin and Aureomycin antibiotics in a combined anaerobic-aerobic reactor systems. Although the COD removal was high in the aerobic system (up to 90%), the removal efficiencies of the two antibiotics were less than 10%.

4. Conclusions

This study has demonstrated that the combination UASR-PMASR treatment system was effective in removing organic matter in pharmaceutical wastewater containing Tylosin. An average COD reduction of 73% at a HRT of 4 d and OLR of 1.86 kg $COD.m^{-3}.d^{-1}$ was achieved in the UASR, confirmed the biomass in the anaerobic reactor had acclimated to the antibiotic. Under these conditions, an average of 95% Tylosin reduction was achieved in the, indicated that this antibiotic could be degraded efficiently in the anaerobic reactor system. It was anticipated that as OLR was increased (2.48–3.73 kg $COD.m^{-3}.d^{-1}$) the increasing levels of Tylosin in the reactor feed would influence the microbial populations of the sludge, and therefore cause a detrimental effect on UASR treatment efficiency. However, this was not observed at levels of Tylosin typically present in the pharmaceutical wastewater (10–220 $mg.L^{-1}$). Further polishing of UASR effluent by a PMASR showed

successful treatment of residual COD in the effluent. Around 1300 $mg.L^{-1}$ COD was removed in PMASR, giving a typical effluent COD of 700 $mg.L^{-1}$ when the UASR OLR was 1.86 kg $COD.m^{-3}.d^{-1}$. In contrast, a small quantity of Tylosin was reduced (around 10% or 0.3 $mg.L^{-1}$) in the PMASR, showed that the aerobic reactor was not particularly effective in degrading Tylosin at the concentrations applied.

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