Tolerance of the antibiotic Tylosin on treatment performance of an Up-flow Anaerobic Stage Reactor (UASR)

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**ABSTRACT**

Tylosin has been considered inhibiting COD removal in anaerobic digestion. In this study it is proven that this is not always the case. Accordingly, elevated concentrations of Tylosin (100–800 mg L\(^{-1}\)) could be tolerated by the anaerobic system. The influence of Tylosin concentrations on an up-flow anaerobic stage reactor (UASR) was assessed using additions of Tylosin phosphate concentrate. Results showed high efficiency for COD removal (average 93\%) when Tylosin was present at concentrations ranging from 0 to 400 mg L\(^{-1}\). However, at Tylosin concentrations of 600 and 800 mg L\(^{-1}\) treatment efficiency declined to 85\% and 75\% removal respectively. The impact of Tylosin concentrations on archaeal activity were investigated and the analysis revealed that archaeal cells dominated the reactor, confirming that there was no detectable inhibition of the methanogens at Tylosin levels between 100 and 400 mg L\(^{-1}\). Nevertheless, the investigation showed a slight reduction in the number of methanogens at Tylosin levels of 600 and 800 mg L\(^{-1}\). These results demonstrated that the methanogens were well adapted to Tylosin. It would not be expected that the process performance of the UASR would be affected, not even at a level well in excess of those appearing in real wastewater from a Tylosin production site.

**Key words** | antibiotic, anaerobic digestion, methanogens, tylosin, UASR

**INTRODUCTION**

It is important to understand the impact of antibiotic on the treatment plant operation and to know what effect this may have on the mixed populations of anaerobic bacteria. In particular, critical methanogenic populations could suffer reduced growth rate, which could influence substrate removal efficiency and methane production. Unfortunately, there are not many experimental studies investigating the effects of antibiotics on the performance of anaerobic digestion. A study of the effect of antibiotic Chloramphenicol on the performance of an up-flow anaerobic sludge bed (UASB) reactor was carried out by Sanz & Rodrı́guez (1996) and showed COD removal efficiency dropped from 80\% to zero when 40 mg L\(^{-1}\) of Chloramphenicol was added to the feed. In studying the effects of certain antibiotics on biogas production in anaerobic digestion of pig waste slurry, Lallai et al. (2002), found that Thiampenicilol and Amoxicillin had an inhibitory effect on methane production, while Oxytetracycline did not. The influence of Erythromycin macrolide antibiotic on anaerobic treatment of pharmaceutical wastewater was investigated by Amin et al. (2004) and showed reduction in biogas production and COD removal efficiency at concentration of 200 mg L\(^{-1}\). Zhou et al. (2006) showed that the antibiotics Ampicillin and Aureomycin did not affect the performance of an anaerobic baffled reactor (ABR) treating pharmaceutical wastewater. In summary, the literature above indicates that it is impossible to predict the effect of antibiotics on anaerobic treatment systems, and each new system therefore requires specific investigation.
The current study focused on the macrolide antibiotic Tylosin which is produced by a strain of *Streptomyces fradiae*. The main component of the mixture is Tylosin A, Tylosin B, Tylosin C and Tylosin D (*Kolz et al. 2005*). Tylosin has been widely used for the treatment of pneumonia, arthritis and dysentery caused by Mycoplasma, Bacillus Pastorianus, etc (*Prats et al. 2002*). 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 & Rodríguez 1996*). 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). 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. In contrast, 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, while the total methane production did not change in their study. The literature above indicates clear contradictions and may possibly be attributed to the type of biomass, Tylosin concentration, reactor configurations, start-up conditions and the methods used to evaluate performance of anaerobic treatment system. Furthermore, long term exposure (long HRT) to macrolide antimicrobials may result in the acclimation of the anaerobic biomass.

The up-flow anaerobic stage reactor (UASR) is similar in design and application to the anaerobic baffled reactor (ABR) (*Barber & Stuckey 1999*), where each stage of the reactor represents a separate compartment. The ABR can be described as a series of up-flow sludge blankets (UASBs), which does not require granulation for its operation. A series of vertical baffles (regarded as UASBs) forces the wastewater to flow under and over them as it passes from inlet to outlet. Bacteria within the reactor gently rise and settle due to flow characteristics and gas production in each compartment of the ABR. Similarly, the UASR has a modular design, where each stage of the reactor was an UASB and had a three phase separator baffle to retain biomass. A stage reactor can provide high treatment efficiency since recalcitrant substrates will be in an environment more conducive to degradation (*Speece 1996*). The 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 be applied economically as a pre-treatment system for many trade effluents. Furthermore, the reactor configuration provides protection to the methanogenic biomass from toxic compounds in the influent (*Barber & Stuckey 1999*).

The aim of this research was to investigate the effects of synthetic Tylosin phosphate concentrations on an UASR system, which was previously studied for treating real pharmaceutical wastewater containing Tylosin (*Chelliapan et al. 2006*). The influence of Tylosin shock load on process performance and the potential impact on archeal activity in the UASR was also investigated. Although the wastewater concentration of Tylosin from antibiotic manufacturing sites usually falls within a typical range (e.g. 10–220 mg L\(^{-1}\)), considerable variation outside the normal range is possible as a result of discarded batches and equipment failure. Consequently, it is important to understand the impact of such variability on the treatment plant operation and to know what effect this may have on the mixed populations of anaerobic bacteria.

**MATERIALS AND METHODS**

**Up-flow anaerobic stage reactor (UASR)**

The UASR system comprise four identical cylindrical Plexiglas compartments (stages), 80 mm internal diameter by 640 mm height, linked in series, 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 Figure 1a. Each stage of the reactor had a 3-phase separator baffle, angled at 45\(^\circ\) and placed 50 mm below the effluent ports, to prevent floating granules from washing out with the effluent (*Figure 1b*). 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 PVC water-jacket, 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. 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%.

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 (Kasapgil 1994).

**Reactor operation**

The UASR was seeded with anaerobic digested sewage sludge (Hexham Municipal sewage treatment plant, Northumberland, UK). This was sieved to pass 2 mm mesh, giving solids 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 head-plates 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°C for 24 h without further modification.

The reactor was fed with brewery wastewater (used for reactor start-up) (described by Uyanik 2001) supplemented with Tylosin in the form of Tylosin phosphate concentrate (supplied by Eli Lilly & Company Ltd, Liverpool, UK).

The concentration of Tylosin was increased stepwise from 100 mg L\(^{-1}\) to 800 mg L\(^{-1}\) and then reduced to 200 mg L\(^{-1}\). To assess the rate at which the reactor returned to stable operation, a Tylosin shock load was also investigated by increasing the Tylosin concentration in a single step from 200 mg L\(^{-1}\) to 800 mg L\(^{-1}\), before being reduced again to 200 mg L\(^{-1}\). Throughout the experiment, UASR was supplied with constant COD loading of 1.88 kg COD m\(^{-3}\) d\(^{-1}\) from brewery wastewater at an HRT of 4 d. It should be noted that the actual OLR in Stage 1 was 7.5 kg COD m\(^{-3}\) d\(^{-1}\) (HRT in Stage 1 was 1 d). The brewery wastewater was used as a feed due to its ease of degradation, high COD values, and well established use in continuous anaerobic reactors (Sallis & Uyanik 2003). The COD contributed by Tylosin was taken into consideration while preparing the feed (adjusted with brewery wastewater for desired final COD).

**Sampling and analysis**

Supernatant liquor, gas and sludge samples were taken separately from each stage for analysis. In addition, gas production rate was determined separately for each stage. Sample analysis included COD, pH, alkalinity, total Kjeldahl nitrogen (TKN), ammonium nitrogen (\(\text{NH}_3\)-N), suspended solids (SS), 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), volatile fatty acids (VFA) by gas-liquid chromatography (Unicam 610 Series Gas Chromatograph with auto-injector and...
PU 4811 computing integrator). Reactor gas composition (CO₂ and CH₄) was determined by gas chromatography (Becker model 403 Gas Chromatograph with Unicam 4815 computing integrator).

**Microbial community analysis**

Samples of sludge from each stage of the UASR were collected through reactor sampling ports located nearest to the vertical mid-point of the sludge bed. An aliquot of these samples was fixed for in situ hybridisation using 4% paraformaldehyde and ethanol (as described by Chelliapan 2006). Total cell counts using 4',6-diamidino-2-phenylindole (DAPI) were performed and means were calculated from 20 randomly chosen fields of view (FOV) for each sample. DAPI-staining was used to quantify the relative proportion of the bacterial (EUB338, Amann et al. 1990; Daims et al. 1999) and archaeal (ARC915, Amann et al. 1999) cells. Further insight into the archaeal community structure was also gained by using two family-genus probes, MX825 (Rocheleau et al. 1999) probe for Methanoseta and MS821 (Rocheleau et al. 1999) probe for Methanosaetace. The number of cells for each group specific probe was determined and means were calculated from 5 to 10 randomly chosen FOV for each sample. Cells were visualised using a Zeiss standard microscope 14 (Carl Zeiss) or confocal laser scanning microscope (CLSM). Statistical analysis for valid cell counting was determined according to Davenport & Curtis (2004). For DAPI-stained counting, the FOV was determined for twenty random observations. The variance within these levels was determined using nested analysis of variance with the MINITAB V14 program ((Minitab Inc., Philadelphia, USA).

**RESULTS AND DISCUSSION**

**UASR performance**

Total soluble COD removal efficiency in the UASR system operating on synthetic wastewater with various Tylosin concentrations is illustrated in Figure 2. Generally, Tylosin concentrations at or below 400 mg L⁻¹ had a minimal effect on reactor performance, and COD removal efficiency had a mean value of 93% under these conditions. When Tylosin concentration was increased to 600 mg L⁻¹, the reactor showed a small decrease in the effluent COD removal efficiency (to 85%), and this was reduced further (average 75%) at a Tylosin concentration of 800 mg L⁻¹. These results show that even at high concentrations (600–800 mg L⁻¹) Tylosin caused only a minor inhibitory effect on the microorganisms within the UASR. The higher residual COD that accounted for the reduction in COD removal efficiency at the 600–800 mg L⁻¹ dose level may have been the result of partial degradation of Tylosin to a more recalcitrant degradation product; however, the GC analysis method for Tylosin quantification could not confirm whether such breakdown products were formed.

When the Tylosin concentration was reduced to 200 mg L⁻¹, the average COD removal efficiency improved rapidly back to 93%, confirming the biomass had not suffered permanent inhibition. The antibiotic shock load (800 mg L⁻¹) imposed on day 247 resulted in a sharp decrease in the COD removal efficiency (to 75%), but this was similar to that observed in the preceding period when the 800 mg L⁻¹ concentration had been achieved gradually over a period of 145 days. The recovery of COD performance was very rapid when Tylosin was returned to 200 mg L⁻¹. This result shows that once acclimatised to Tylosin, the biomass could tolerate rapid changes in Tylosin concentration without substantial or persistent loss in performance.

It was found that most of the influent COD was removed in Stage 1 (around 80–86%) at Tylosin concentrations of 0–400 mg L⁻¹, with smaller degree of removal occurring in Stages 2, 3 and 4 (each less than 10%). When Tylosin concentration was increased to 600 and 800 mg L⁻¹, COD removal in Stage 1 was reduced to around 72 and 65% respectively, with unchanged removal in the remaining stages. This shows that most of the bacterial inhibition by Tylosin occurred in Stage 1 where the actual concentration was presumably highest (Tylosin analysis was not conducted for individual stages).

Figure 3 shows total VFAs found in the reactor effluent over time. Generally, during reactor start-up the VFA concentration in the reactor effluent was constant at less than 100 mg L⁻¹. When the reactor was exposed to a Tylosin concentration of 100 mg L⁻¹, the VFAs in all the stages
showed lower values than without Tylosin (except for Stage 3); 550 mg L\(^{-1}\) in Stage 1; 350 mg L\(^{-1}\) in Stage 2; 200 mg L\(^{-1}\) in Stage 3 and 40 mg L\(^{-1}\) in Stage 4. Further increments in Tylosin concentration to 200 and 400 mg L\(^{-1}\) showed slight reduction in total VFAs in stages 1, 2 and 3. However, when the Tylosin concentration was increased to 600 mg L\(^{-1}\) the total VFA in Stage 1 of UASR increased to an average value of 1050 mg L\(^{-1}\) with an average 90 mg L\(^{-1}\) in Stage 4. The total VFAs in Stages 2 and 3 during this period were not affected much and show similar profiles to those at Tylosin concentration at 400 mg L\(^{-1}\). When the reactor was exposed to Tylosin concentration of 800 mg L\(^{-1}\) the VFA in all the stages increased (except for Stage 1), with levels of 1050 mg L\(^{-1}\) in Stage 1; 750 mg L\(^{-1}\) in Stage 2; 570 mg L\(^{-1}\) in Stage 3 and 400 mg L\(^{-1}\) in Stage 4. The VFAs in all the stages returned to their previous lower levels when the Tylosin concentration was reduced again to 200 mg L\(^{-1}\) on day 215. These results could indicate that Tylosin stimulated the acidogenic populations or was itself easily degraded by such populations; however, a more likely explanation is the possibility that Tylosin was at least partially inhibitory to some of the methanogenic populations at these elevated concentrations, leading to an imbalance between acidogenesis and methanogenesis which gave rise to subsequent increases in VFA concentrations.

When the reactor was exposed to Tylosin shock load from 200 to 800 mg L\(^{-1}\), the VFA concentration in Stage 1 increased again to high levels (average 1200 mg L\(^{-1}\)) with Stage 4 having an average value of 370 mg L\(^{-1}\). The VFA data also corresponds to the measured pH data (not provided) as a consequence of the physico-chemical relationship between these parameters. Bell (2002) stated that a stable reactor performance is indicated by an effluent total VFA concentration below 500 mg L\(^{-1}\), therefore, it was concluded that the UASR operation was stable throughout the test period.

The methane yield (m\(^{3}\) methane produced per kg COD removed) can be useful parameter to assess the performance of an anaerobic reactor and Table 1 shows that mean steady state methane yield was relatively constant in Stages 2, 3 and 4 of the reactor system (0.291–0.377 m\(^{3}\) CH\(_{4}\) kg COD\(_{r}^{-1}\) for all investigated Tylosin levels). However, the methane yield dropped gradually in Stage 1 when the Tylosin concentration was increased from 400 to 800 mg L\(^{-1}\) and then recovered when the Tylosin level was reduced to 200 mg L\(^{-1}\). The theoretical methane yield at 35°C is 0.395 m\(^{3}\) CH\(_{4}\) kg COD\(_{r}^{-1}\) (Speece 1996). The lower values of methane yield in all the stages of the UASR, but particularly in Stage 1, were most likely caused by methanogenic populations were partially inhibited by Tylosin. However, methane yield in Stage 1 of the reactor system recovered to 0.327 m\(^{3}\) CH\(_{4}\) kg COD\(_{r}^{-1}\) when the Tylosin concentration was reduced back to 200 mg L\(^{-1}\). Stages 2, 3 and 4 showed a relatively constant level of methane yield for all Tylosin concentrations studied.

The antibiotic shock load (800 mg L\(^{-1}\)) imposed on day 247 resulted in a decrease in methane yield (0.261 m\(^{3}\) CH\(_{4}\) kg COD\(_{r}^{-1}\)), but this was similar to that observed in the preceding period when the 800 mg L\(^{-1}\) concentration was achieved gradually over a period of 145 days (Table 1). The recovery of methane yield was rapid when Tylosin was returned to 200 mg L\(^{-1}\). Furthermore, methanogens were active in all the stages of the reactor system, even in Stage 1 at the highest Tylosin feed concentration (OLR in Stage 1 was 7.5 kg COD m\(^{-3}\) d\(^{-1}\)) which clearly demonstrates the resistance of methanogens to Tylosin. Angenent et al. (2008) reported similar findings where Tylosin at an average concentration of 1.6 mg L\(^{-1}\) did not inhibit the methane production when it was fed to an anaerobic sequencing batch reactor (ASBR).

### Table 1

<table>
<thead>
<tr>
<th>Tylosin (mg L(^{-1}))</th>
<th>Methane yield (m(^{3}) CH(<em>{4}) kg COD(</em>{r}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (start-up)</td>
<td>0.322</td>
</tr>
<tr>
<td>100</td>
<td>0.294</td>
</tr>
<tr>
<td>200</td>
<td>0.281</td>
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<tr>
<td>400</td>
<td>0.301</td>
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<tr>
<td>600</td>
<td>0.271</td>
</tr>
<tr>
<td>800</td>
<td>0.253</td>
</tr>
<tr>
<td>200 (shock load)</td>
<td>0.317</td>
</tr>
<tr>
<td>800 (shock load)</td>
<td>0.261</td>
</tr>
<tr>
<td>200</td>
<td>0.327</td>
</tr>
</tbody>
</table>

Table 1 shows methane yield in each stage of UASR at various Tylosin levels (each mean values taken when reactor approached steady-state at each investigated Tylosin levels).
More lately, Stone et al. (2009) reported Tylosin (1.1 mg L\(^{-1}\)) did not affect methane and carbon dioxide generation during batch anaerobic swine manure digestion. Thus, the methane yield results show that methanogens tolerate Tylosin at a level well in excess of those expected in real wastewater from a Tylosin production site and confirms UASR is a process that could be applied in practice. Some may argue Tylosin is hardly biodegradable and could contribute to high COD in the effluent; however, we believe, the anaerobic treatment system (UASR) operated to efficiently remove most of the general COD associated with fermentation waste residues in the real pharmaceutical wastewater containing Tylosin. Further polishing by aerobic degradation would be viable if tight discharge consent applied (i.e. aerobic polishing after anaerobic digestion process is better than using aerobic to degrade all COD).

**Bacterial populations**

The presence of antibiotics in anaerobic treatment processes may inhibit the microbial populations in the reactor sludge. The antibiotic Monensin was found to inhibit methanogenic activity at a concentration of 0.1 mg L\(^{-1}\) in a laboratory scale UASB reactor treating synthetic wastewater (Thavesiri et al. 1994). Sanz & Rodriguez (1996) reported that Erythromycin (250 mg L\(^{-1}\)) did not inhibit methanogenic activity in batch anaerobic experiments, however, partial inhibitory (25–45\%) effects was observed for Doxycycline, Tylosin and Streptomycin on the propionic and butyric acid-utilising bacteria. Amin et al. (2004) also reported that Erythromycin did not affect methanogenic activity in the treatment of synthetic pharmaceutical wastewater in an anaerobic sequencing batch reactor (ASBR) at concentration of 200 mg L\(^{-1}\). More recently, Loftin et al. (2005) discovered partial inhibition (20–45\%) of Sulfonamides, Tetracyclines, Lincomycin and Tylosin at concentration 1–25 mg L\(^{-1}\) in batch experiments containing anaerobic lagoon slurries. Consequently, it is important to examine the potential impact of Tylosin concentration on archaeal activity in UASR.

Percentages of DAPI-stained cells detected by FISH with probes for archaea (ARC915) and bacteria (EUB338) in each stage of the UASR, for each investigated Tylosin level are presented in Figure 4, and show an abundance of archaeal cells hybridized with ARC915 compared to bacterial cells (acidogenic bacteria) hybridized with EUB338 in all stages of UASR at each sampling date. The biomass samples taken from the reactor on day 109 represented sludge which had been exposed to a Tylosin dosage of 100 mg L\(^{-1}\), and showed that 74.3\% (SD = 14.8\%) of the microbial population in Stage 1 was made up of archaeal cells. It had been concluded in earlier experiments that these organisms were responsible for the majority of the COD reduction, indicated by high amount of methane volume produced during this period (Table 1). The archaeal cells also dominated the microbial population in Stages 2, 3 and 4 of the reactor. The hybridisation in all UASR stages at this period showed bright fluorescence, indicating high metabolic activity, which supports the evidence from the COD and methane results (Figure 2 and Table 1). The biomass samples taken from the reactor on day 164 represented sludge which had been exposed to Tylosin at a concentration of 600 mg L\(^{-1}\), and Figure 4 shows that the archaeal population reduced slightly in all the UASR stages compared to lower doses, although statistical comparison (t-test with 95\% confidence level) showed no significant difference in the means of archaeal numbers compared to day 144 (400 mg L\(^{-1}\)) in Stage 1 (P = 0.560). It is notable that the COD and methane results showed a minor decrease under these conditions, indicating not significant effect to the methanogens. On day 213, when the reactor was fed with 800 mg L\(^{-1}\) Tylosin, the archaeal cells decreased further to 60.5\% (SD = 17.2\%) in Stage 1 and increased slightly to 79.0\% (SD = 16.8\%) in Stage 4 (Figure 9). These results are substantiated by the reduction of methane yield (Table 1) observed during this period. Statistical comparison (t-test with 95\% confidence level) showed a significant difference in the means of archaeal numbers between the stages (e.g. in Stage 1, P = 0.012) confirming partial inhibition of the methanogens at higher Tylosin concentrations.

In summary, these results show that Tylosin did not affect the methanogenic activity up to 400 mg L\(^{-1}\); however, partial inhibition was observed when the concentration was increased to 600–800 mg L\(^{-1}\).

**Composition of archaeal population in UASR sludge**

The most important methanogenic transformations in anaerobic digestion are the acetoclastic reaction and the reduction of carbon dioxide. It has been estimated from stoichiometric relations that about 70\% of the methane is produced via the acetate pathway (Hobson & Wheatley 1993). Very few known species can perform this acetoclastic methane production, whereas nearly all known methanogenic species are able to produce methane from hydrogen and carbon dioxide. Among the species that are able to utilise acetate (acetoclastic) are Methanosaeta and Methanosarcina. In addition to this acetoclastic activity, Methanosarcina sp. is also capable of using methanol, methylamines and sometimes hydrogen and carbon dioxide as growth substrates, while Methanosaeta sp. is
restricted to growth only on acetate (Schmidt & Ahring 1996). Accordingly, to gain further insight into the acetoclastic methanogens making up the archaeal population, the samples from the UASR stages were hybridised with probe MX825 (Methanosaeta) and MS821 (Methanosarcina). Figure 5 shows the percentage of archaeal cells in each stage of UASR at each sampling point, and indicates that Methanosaeta cells (high affinity for acetate) dominated the reactor stages at all the Tylosin level studied, with exception in Stage 1 on day 213 (Tylosin level 800 mg L\(^{-1}\)), where Methanosarcina cells (poor affinity for acetate) dominated Stage 1. This is probably due to high volatile fatty acids being found in Stage 1 at this time (Figure 3). Methanosarcina cells were also detected in all the stages with the number increasing when the reactor was operated at a higher Tylosin concentrations; e.g. in Stage 1 at 100 mg L\(^{-1}\), 19.4% (SD = 13.2%) and at 800 mg L\(^{-1}\), 43.6% (SD = 18.9%). This observation is consistent with recent results obtained by Shimada et al. 2008 and Stone et al. 2009, where abundance of Methanosaeta and Methanosarcina cells were found in batch anaerobic reactors when Tylosin was added. Furthermore, it was reported that Methanosaeta is not sensitive to Tylosin due to differences in 23S rRNA binding sites (Shimada et al. 2008).

CONCLUSIONS

It can be concluded that extremely high levels of Tylosin in the feed of the UASR were still compatible with stable performance. Despite Tylosin being antibiotic methanogens appear to function well with archaeal cells dominated UASR stages, confirming that there was no detectable inhibition at the Tylosin levels studied. Accordingly, Tylosin would not be
expected to influence the process performance of the reactor at the levels typically present in the real pharmaceutical wastewater. The experiment also confirmed the stability of the UASR to potential shock loads that may occur from time to time on such production sites.

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