Case Study

Thermophilic Aerobic Treatment of Mycelia Sludge and Antibiotic Wastewater

C. Furlong[1]; P. J. Sallis[2]; and T. Wilby[3]

Abstract: A major waste stream from antibiotic production is mycelia sludge. This paper explores the use of an aerobic thermophilic bioreactor with solids separation and chemical treatment (acid hydrolysis and oxidation) of excess sludge to treat this waste. This system was evaluated for reduction of total chemical oxygen demand (TCOD) and volatile suspended solids (VSS). The investigation was split into three phases. Phase 1 was the treatment of 5% [weight/volume (w/v)] mycelia waste (with an average TCOD of 92,400 mg/L and VSS of 46,000 mg/L). Phase 2 was the treatment of 1% (w/v) mycelia waste in combination with effluent from the antibiotic recovery plant (average TCOD of 36,000 mg/L and VSS of 8,900 mg/L), and Phase 3 was as for Phase 2 with chemical treatment. The system was run for 92 days without accumulation of organic or inorganic solids, even though no sludge was wasted from the reactor. The mycelia sludge proved to be readily biodegradable in Phase 1 (average TCOD destruction was 82% and average VSS destruction was 89%). An acclimatization period of 37 days was required when the mixed antibiotic wastewater was introduced in Phase 2. After this period, the average TCOD destruction was 83%. Chemical treatment in Phase 3 improved and stabilized VSS destruction (from 76% in Phase 2 to 87% in Phase 3). This study demonstrates that this system can be used to effectively treat mycelia sludge, both alone and combined with a mixed antibiotic wastewater, with minimal excess sludge formation. **DOI: 10.1061/(ASCE)EE.1943-7870.0000643.**

CE Database subject headings: Waste treatment; Biological processes; Aerobic treatment; Waste digestion; Sludge; Wastewater management.

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Introduction

Antibiotic production is a multibillion dollar industry, and several hundred types of antibiotics are used in human and veterinary medicine. Data are limited on world antibiotic consumption rates, but it has been estimated that between 100,000 and 200,000 tons are consumed per annum (Wise 2002). No data are available on the amount of waste produced by this industry, but it is known that waste from this industry contains residual amounts of the products manufactured. The production of antibiotics produces two major sources of waste, wastewater and waste microbial cells, so-called mycelia sludge. Mycelia sludge poses several problems for biological treatment, having high levels of suspended solids and many recalcitrant organics (Schröder 1999). Many authors have researched biological treatment of wastewater from antibiotic production (Chelliapan et al. 2006; Lapara et al. 2001; Oktem et al. 2007), but until recently, the treatment of mycelia sludges has been ignored (Zupančič and Gotvajn 2009).

This paper investigates thermophilic aerobic treatment of mycelia sludge. The process has been used successfully to treat other high-strength wastewater streams and sludges such as pulp wastewater (Lapara and Alleman 1999), livestock waste (Juteau et al. 2004), food processing wastewater (Lasik et al. 2010), and sewage sludge (Lui et al. 2010). Furthermore, these systems have been shown to be robust when exposed to antibiotics, and to remove antibiotic-resistant organisms efficiently (Han et al. 2009).

Thermophilic systems have many advantages compared to mesophilic systems, such as faster degradation rates, inactivation of pathogens, low sludge yield, reduction in retention times, and improved stability (Stanton et al. 2001), and when combined with solids separation and chemical treatment of excess sludge, no waste sludge is produced (Rozich and Bordacs 2002). These systems have a reputation for instability attributable to foaming, which is intrinsic because of reduced surface tension at thermophilic temperatures, high cellular concentration, longer sludge ages that increase the extracellular polymeric substances, and high aeration rates (Lapara and Alleman 1999).

The aim of this research was to evaluate the use of thermophilic aerobic treatment with solids separation and chemical treatment as a potential zero sludge generation treatment for mycelia sludge alone and in combination with wastewater containing tylosin, avilamycin, and apramycin antibiotics, and to quantify the removal efficiency of the antibiotic tylosin by this system.

Methods and Materials

A bench-scale system was set up as shown in Fig. 1. The reactor was seeded with activated sludge from the Howdon municipal sewage treatment plant (Newcastle, UK) and fed with synthetic sewage (Pholchan et al. 2008) before this trial.

The reactor had a volume of 20 L and a working volume of 10 L, which was maintained by a twice-weekly batch procedure.

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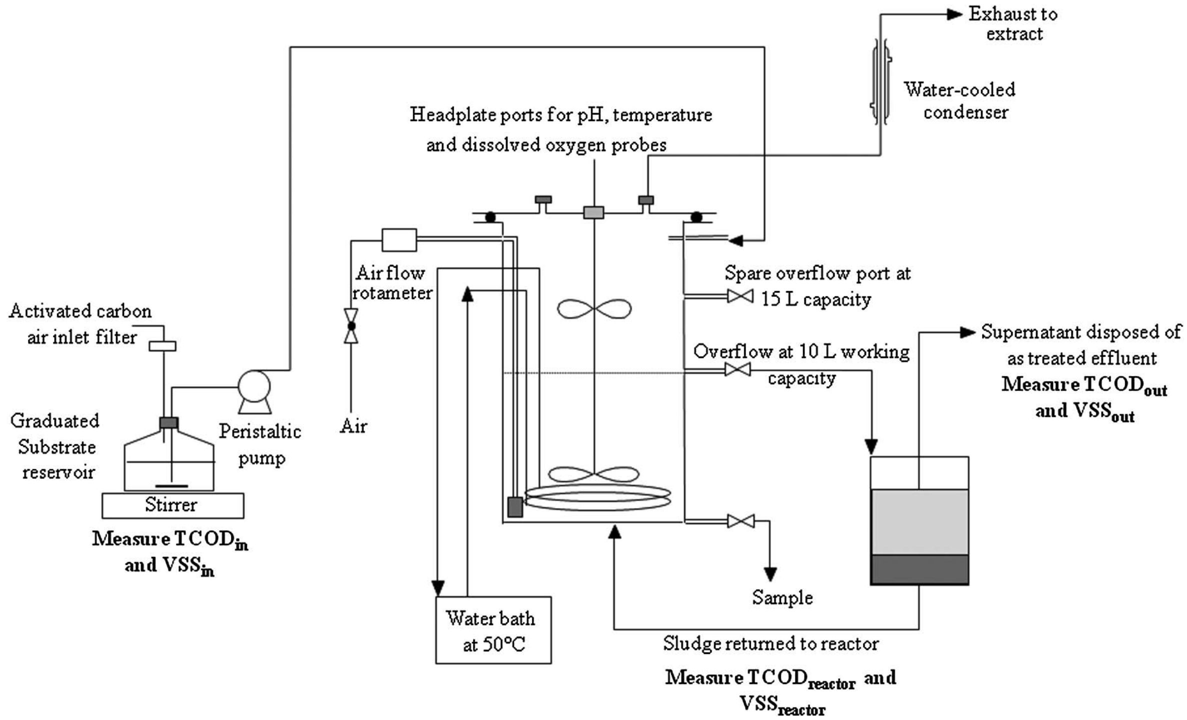

Fig. 1. Experimental setup for aerobic thermophilic experiments

The excess contents of the reactor, plus one liter of the reactor contents, were drawn from an outlet and centrifuged for 20 min at 2,000 revolutions per minute (rpm) (rotor diameter of 35 cm). A sample of the supernatant (the effluent) was kept for analysis. The biomass pellet was diluted to one liter using the supernatant and returned to the reactor. The excess supernatant was then disposed after analysis.

Autothermal operation could not be achieved because of thermal losses at bench-scale. To maintain the operating temperature of 50°C, an external heating jacket was used. A condenser on the air outlet was used to minimize the loss of water vapor.

The condenser also served as a release point for any transient foam production. All other outlets were sealed. Air was supplied by a compressed air line to a bubble diffuser at a rate of 360 L/h. The reactor was stirred by an impeller (diameter 6 cm, height 2 cm) at a rate of 220 rpm. The impeller shaft had a second foam breaker impeller (same dimensions) at the 15 L height. The reactor was fitted with four internal vertical baffles (projecting 2 cm from the wall of the reactor) to aid mixing and to minimize the formation of anaerobic pockets. A 5 L feed reservoir was stirred at approximately 100 rpm. The reactor was fed by a peristaltic pump every 15 min. The feed rate could be adjusted to achieve the required reactor loading rate (mg COD L^{-1} d^{-1}).

The experimental investigation was split into three phases, as shown in Table 1. The mixed antibiotic wastewater consisted of the effluents in the volume ratio of 60% tylosin effluent, 24% avilamycin effluent, and 16% apramycin effluent. The differing proportion of wastewater reflects their production on site. The blended feed was stored at 4°C prior to use. The characteristics of the feed from each phase can be seen in Table 2. The operating conditions for the reactor are given in Table 3. Results were collected over a 92 day period.

Table 1. Description of Experimental Phases

Phase	Day	Feed	Chemical treatment
1	0–23	5% mycelia waste	No
2	24–65	1% mycelia waste + mixed antibiotic effluent	No
3	66–92	1% mycelia waste + mixed antibiotic effluent	Yes

Chemical Treatment

During Phase 3, the reactor contents were chemically treated to control the accumulation of biological solids and recalcitrant substances once the system had reached steady state, and to solubilize the recalcitrant components of the sludge. The amount of material to be chemically treated was calculated by using Eq. (1) (Rozich, personal communication, 2002).

$$V_t = \frac{\mathrm{VSS}_{ac}}{\mathrm{VSS}_r} \tag{1}$$

where V_t = volume of reactor contents to be treated each day, (L/d); VSS_{ac} = excess VSS accumulation, (mg/d); VSS_r = concentration in reactor, (mg/L). The procedure was undertaken weekly; therefore, the volume calculated using Eq. (1) was multiplied by 7. The sludge was placed into a flask, the pH was adjusted to 3.0 using sulphuric acid [20% volume/volume (v/v)], and hydrogen peroxide was added according to the calculation based on the total chemical oxygen demand (TCOD) of the sample using Eq. (2) (Rozich, personal communication, 2002).

$$V_{hp} = \frac{\mathrm{TCOD}_r \times V_t}{4000} \tag{2}$$

where V_{hp} = volume of pure hydrogen peroxide to be added each day, (mL/d); TCOD_r = total COD concentration in reactor, (mg/L). Hydrogen peroxide was added to the flask over a 10 min period by mixing. The flask was capped with a rubber septum and pierced with two needles for pressure equalization. The flask was then

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Table 2. Characteristics of the Feed

Parameter	Units	Feed for Phase 1: 5% Mycelia solids, Batch 1	Feed for Phase 1: 5% Mycelia solids, Batch 2	Mixed antibiotic effluent (average)	Feed for Phases 2 and 3: Mixed antibiotic effluent and 1% mycelia solids (average)
Total COD (TCOD)	mg/L	84,000	95,333	20,658	38,385
Soluble COD (SCOD)	mg/L	5,050	3,053	16,037	16,687
TCOD:SCOD	ratio	16.63	31.23	1.29	0.43
Total suspended solids (TSS)	mg/L	54,000	42,587	7,021	16,609
VSS	mg/L	51,000	39,020	6,182	15,122
NVSS	mg/L	3,000	3,367	839	1,568
Total dissolved solids (TDS)	mg/L	15,833	2,999	29,576	31,164
Nonvolatile dissolved solids (NVDS)	mg/L	15,000	272	6,874	8,333
Volatile dissolved solids (VDS)	mg/L	833	2,727	22,702	22,831
Total Kjeldahl nitrogen (TKN-N)	mg/L	2,800	2,940	1,891	2,446
pH		6.00	5.89	6.51	7.64

Table 3. Reactor Operating Conditions

Parameter	Operating condition
Reactor loading rate (mg COD L^{-1} d^{-1})	152–155
Hydraulic retention time (days)	Phase 1: 18–26 Phases 2 and 3: 9–10
Temperature (°C)	50 ± 1
Solids separation	Centrifugation 2,000 rpm for 20 min
Chemical treatment	Acid hydrolysis and oxidation

incubated at 50°C for 24 h. The chemically treated sludge was blended with the reactor feed, and this mix of feed and chemically treated sludge was characterized before feeding to the reactor.

Analytical Methods

Samples from the feed (influent), reactor contents, and the effluent were analyzed for total suspended solids (Standard Methods 2540D), volatile and nonvolatile suspended solids (Standard Methods 2540E), and TCOD (Standard Methods 5220D) [American Public Health Association (APHA) 1998]. The pH of the reactor was monitored throughout the experiment (Standard Methods 4500-H^+B; APHA 1998). Spot samples of feed and effluent in Phases 2 and 3 were analyzed for tylosin (Chelliapan et al. 2006).

Results and Discussion

Reactor Stability

During Phase 1, the TCOD within the reactor increased by 29%, the nonvolatile suspended solids (NVSS) decreased slightly (−11%), and the VSS increased slightly (9%) (Fig. 2).

The decrease in NVSS can be explained by the visible formation of mineral deposits on the reactor surfaces, and precipitate that was thought to be struvite ($MgNH_4PO_4 \cdot 6\ H_2O$). This is because the conditions in this type of system are known to favor crystallization (Juteau et al. 2004). The components that form struvite are present because of the breakdown of cells and the retention of their byproducts [phosphate and magnesium, accelerated protein hydrolysis and inhibited nitrification (ammonia)] (Stanton et al. 2001). Analysis of the mineral deposits would need to be undertaken to prove this hypothesis. If they were found to be struvite, as suspected, the

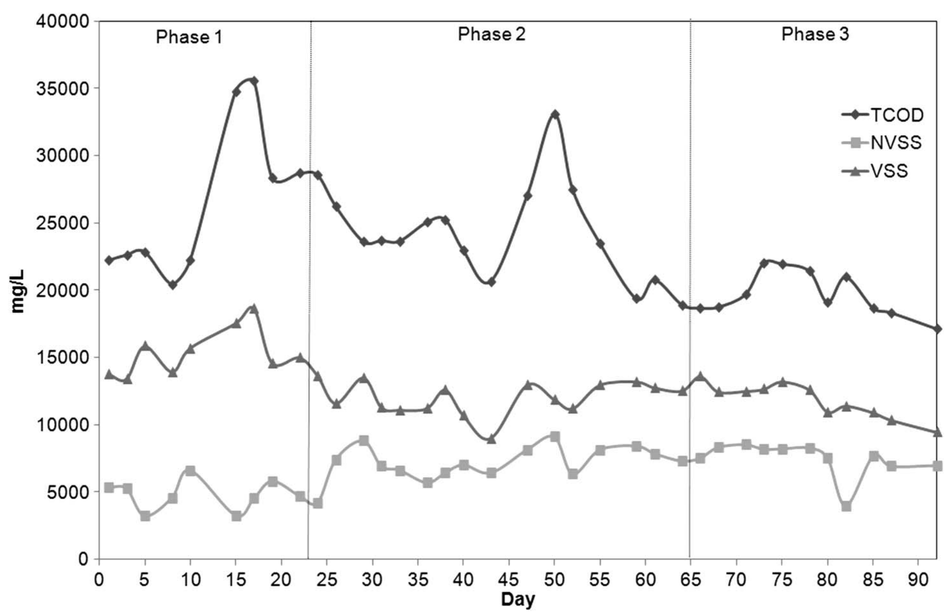

Fig. 2. TCOD, VSS, and NVSS levels in the reactor during the three phases

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treatment of mycelia waste using this process would produce a valuable and useful byproduct.

During Phase 2, the TCOD within the reactor decreased by 34% and the VSS decreased by 16%, but the NVSS increased by 55% (Fig. 2). The lower levels of NVSS in the feed during this phase (Table 2) was thought to be caused by the minerals remaining in solution in the reactor (no increase in visible mineral deposits were noted during this phase). The accumulation of NVSS was controlled by the introduction of chemical treatment in Phase 3, a 4% decrease was observed during this phase (Fig. 2).

Change of the feed at the start of Phase 2 did not affect the reactor biomass adversely. Biomass concentration decreased slightly from an average of 15,389 mg/L VSS (standard deviation 1,756 mg/L) in Phase 1 to 12,009 mg/L VSS (standard deviation 1,236 mg/L) in Phase 2 (Fig. 2). A short period of foaming (3 days) was experienced when the mixed feed was introduced at the start of Phase 2 because of the acclimatization of the biomass within the reactor. Importantly, there was no accumulation of TCOD within the reactor (Fig. 2). This indicated that the TCOD within two feeds was readily biodegradable. The introduction of chemical treatment in Phase 3 decreased the fluctuations in TCOD of the reactor contents (Fig. 2). The standard deviation of the reactor TCOD was 3,539 mg/L in Phase 2, compared to 1,641 mg/L in Phase 3. This was because of the conversion of recalcitrant chemical oxygen demand (COD) into nonrecalcitrant forms during chemical treatment. The NVSS and VSS remained stable during Phase 3 (Fig. 2). The pH of the reactor ranged from 8.01–8.36 over the entire experiment, which falls within the typical range for thermophilic systems (Lapara and Allenman 1999).

COD and VSS Reduction

The average degree of TCOD and VSS reduction (comparing the feed with the centrifuged effluent) were calculated for each phase of the experiment (Table 4). Mycelia sludge was found to be readily degradable with an average TCOD reduction of 82% and VSS reduction of 89% (Table 4). The TCOD reduction fell from 80% at the end of Phase 1 (day 22, Fig. 3) to 64% on day 24 at the start of Phase 2 (day 24, Fig. 3). However, by the end of Phase 2 (day 59, Fig. 3), the TCOD removal had increased to 82%, indicating a 37-day acclimatization period was required for the reactor to achieve efficient removal of the mixed antibiotic effluent. After acclimatization in Phase 2, the average TCOD reduction was 83%, but the average TCOD reduction during Phase 3 when chemical treatment was used was 82% (Table 4), showing the introduction of chemical treatment in Phase 3 did not improve the TCOD removal. Interestingly, a higher overall TCOD reduction was gained by this one-stage process compared to a four-stage up-flow anaerobic blanket that was treating a similar wastewater in which the estimated average COD removal rate was 67% (Chelliapan et al. 2006).

In Phase 1, the average VSS reduction was very stable at 89% (standard deviation 1.51%). The VSS of the feed during Phase 1 ranged from 39,020–51,000 mg/L. However, when the mixed antibiotic effluent was introduced into the feed (Phase 2), the average VSS reduction decreased to 76% and the reduction rate became more variable, with a standard deviation of 9.01% (Fig. 4). No acclimatization period was needed for the stable reduction of VSS because of the majority of the solids (60% of the VSS) in the feed coming from the mycelia waste that had been used in

Table 4. Summary of the Averaged TCOD and VSS Reduction Rates across Each Phase

Phase	Days	Average TCOD reduction (%)	Average VSS reduction (%)
1	0–23	82 (Standard deviation = 4.28)	89 (Standard deviation = 1.51)
2	24–58[a]	65 (Standard deviation = 5.65)	76 (Standard deviation = 9.01)
	59–65[b]	83 (Standard deviation = 0.60)	
3	66–92	82 (Standard deviation = 2.06)	87 (Standard deviation 3.31)

[a]During acclimatization.
[b]After acclimatization.

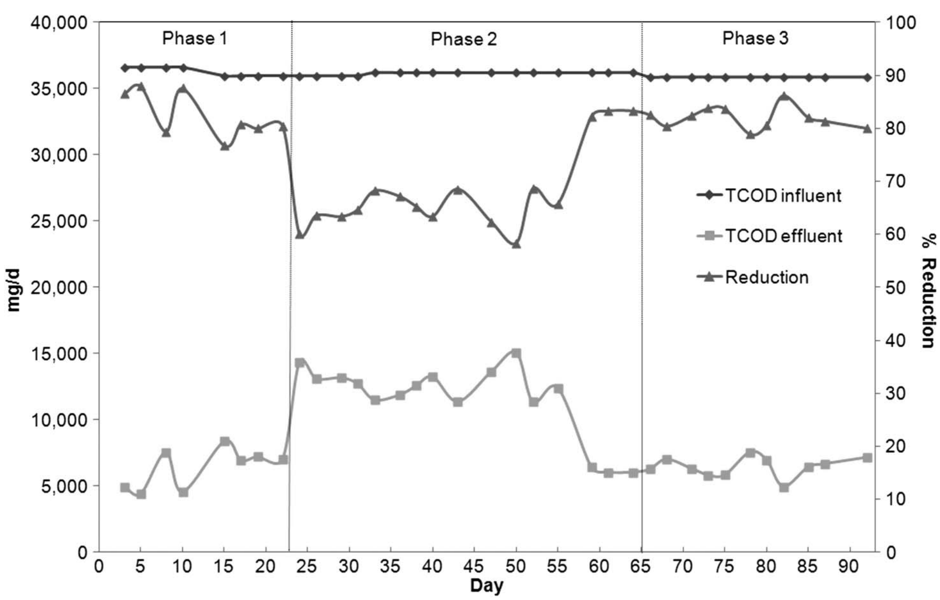

Fig. 3. TCOD of the influent, effluent, and percentage reduction

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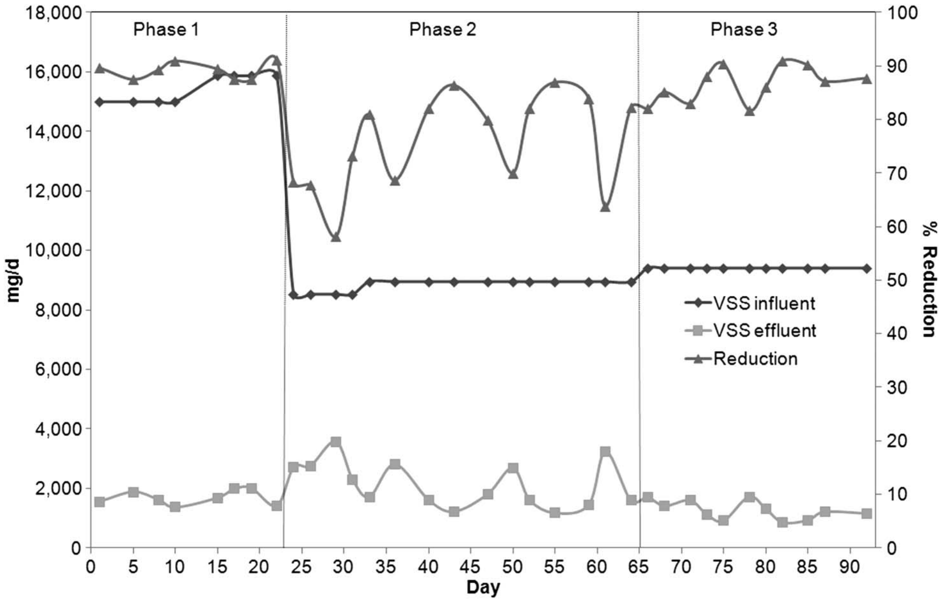

Fig. 4. VSS of the influent, effluent, and percentage reduction

the feed during Phase 1 (Table 2). The reduction in VSS reduction efficiency is thought to have resulted from the inhibitory effects of the mixed antibiotic effluent.

The introduction of chemical treatment in Phase 3 improved the VSS reduction further and stabilized the short-term fluctuations in reduction (Fig. 4), as shown by the relative standard deviations from Phase 2 (9.01%, Table 4) and Phase 3 (3.31%, Table 4). This was likely to be attributable to the chemical oxidation of recalcitrant solids in reactor biomass.

Tylosin Reduction

Tylosin was introduced in Phases 2 and 3 as a component part of the mixed antibiotic effluent. Throughout the study, four samples (two in each phase) were analyzed (Table 5). The variation in the feed tylosin levels was attributable to normal fluctuating levels in the stock tylosin wastewater used to make up the mixed antibiotic effluent (Table 5). The degree of reduction for tylosin on day 25 was not representative of the actual tylosin reduction at the time, because the reactor had not reached steady state. On day 43, the tylosin reduction efficiency was 86%, but the COD reduction was 68%. The acclimatization of the biomass to the Phase 2 feed was complete by day 59 (Fig. 3). Therefore, it can be assumed that the acclimatization period was not needed for the tylosin in the mixed antibiotic effluent, because this was already being degraded effectively before day 60. These observations suggest that tylosin was more readily degraded than some components of the COD present in the influent. The tylosin reduction (Table 5) was comparable to the levels achieved (95%) in a multistage anaerobic system (Chelliapan et al. 2006), meaning it is degradable under both conditions.

Table 5. Results from Tylosin Analysis

Phase	Day	Feed (mg/L)	Effluent (mg/L)	Reduction (%)
2	25	324	19	94
2	43	146	21	86
3	84	216	17	92
3	90	263	15	94

Conclusions

This study demonstrates that a thermophilic aerobic system can be used effectively to treat mycelia sludge alone, and combined with a mixed antibiotic wastewater, with minimal excess sludge formation. Mycelia sludge was found to be readily biodegradable, and mycelia solids did not build up during the experiment. Sludge waste was not required for stable operations during the 92-day experiment. Furthermore, chemical treatment controlled the increase of NVSS within the reactor and stabilized fluctuations in the reactor COD and solids. An acclimatization period of 37 days was required for effective treatment of the mixed antibiotic wastewater.

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