REMOVAL OF AN ANTIBIOTIC IN ALGAL WASTEWATER TREATMENT SYSTEMS: TETRACYCLINE

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ABSTRACT

To determine if the conditions present in algal WWT systems enhance the removal of emerging pollutants, the fate of tetracycline is being evaluated in lab-scale high rate algal ponds (HRAP) continuously fed with clarified domestic wastewater. Results thus far show up to 90% removal of tetracycline when fed at an influent concentration of 2 mg/L. A current major difficulty is ensuring the lab-scale HRAPs maintain a steady representation of full-scale conditions. Prior research on lab-scale HRAP semi-continuously fed with synthetic wastewater (retention time 7 days) suggested that sorption and photodegradation were the main tetracycline removal pathways, with an overall tetracycline removal of 69 ± 1 % (influent concentration 2 mg L^{-1}). However, this presentation will show results from batch tests conducted under dark conditions which reveal sorption was insignificant but that biodegradation caused 50 ± 8% removal over 14 days. Batch photodegradation tests in phosphate-buffered water showed an interaction of pH with photolysis, with pseudo-first order kinetic rates of 0.051 ± 0.005 h^{-1} at pH 8 and 0.0228 ± 0.0004 h^{-1} at pH 7. Further work is needed to narrow down kinetics of removal mechanisms to enable modelling and prediction of algal systems.

KEYWORDS

Antibiotics; Emerging pollutants; Algae; Wastewater; Tetracycline; High rate algal ponds

1 INTRODUCTION

Among the multitude of emerging contaminants, antibiotics are of particular interest due to their ubiquity, broad use as human medicines and veterinary drugs (van den Bogaard & Stobberingh, 1999; Allen et al., 2010; Lupo et al., 2012), and involvement in the spread of antibiotic resistance (Aminov, 2009; Blázquez et al., 2012). Effluent from wastewater treatment systems (WWT) is a major source of antibiotics released into the environment, and many studies have therefore investigated the fate of these pollutants during conventional and advanced wastewater treatment (Michael, 2013; Monteiro & Boxall, 2010). This research has shown that the removal of antibiotics is highly dependent on antibiotic structure, wastewater matrix, environmental conditions, and the treatment process design and operation. By contrast, little research has been conducted on the fate of antibiotics during algae-based wastewater treatment despite the widespread application of these systems around the world. Specific research in this field is needed because of the unique environmental conditions in these systems: In particular, photodegradation pathways may be favoured by their high illuminated-surface/volume ratio and the presence of algal biomass may impact biodegradation and sorption mechanisms. Algal WWT processes are also operated at relatively long hydraulic retention times (HRT, 3-40 days), which may allow slow removal mechanisms to become quantitatively significant.

2 DISCUSSION

2.1 BATCH TETRACYCLINE DEGRADATION UNDER DARK CONDITIONS

To assess the biodegradation capabilities of algae-based biomass under near ideal conditions, a biodegradability assay was conducted. By screening a number of biomass sources, the inherent biodegradability of the tetracycline could be tested. If biodegradation was found to be significant, then major differences between biomass sources would indicate tetracycline biodegradation requires specific enzymatic capabilities, whereas little differences between biomass sources would indicate a general capacity for microbes to degrade tetracycline. Sorption was quantified to determine its significance during the experiment, and to differentiate the quantitative impact of sorption from biodegradation.

Synthetic wastewater was provided as a nutrient and food source. Biomass concentration was 200mg/L. Controls with autoclaved biomass or no biomass were used to assess sorption without biological activity and removal via abiotic mechanisms. Six biomass sources were used, two from dominant algae cultures maintained in open lab-scale HRAPs, and the others sourced from primary and secondary facultative ponds at Rongotea (a rural NZ town), pre-treated by aerating for 1 week in the light or dark (pre-treatment in the light should favour algae).

Samples were incubated in the dark (to prevent photo-based mechanisms), at 25°C. Samples were prepared in duplicate, with each flask aqueous phase measured at 7 and 14 days, and the sorption quantified for one of the two replicates at 14 days.

Results are summarised in Figure 1 and Figure 2 (full data set not shown). Figure 1 shows the overall removal of tetracycline, and the significance of biodegradation (difference between alive and dead biomass samples). Figure 2 identifies some pathways that contribute to the removal of the original tetracycline concentration.

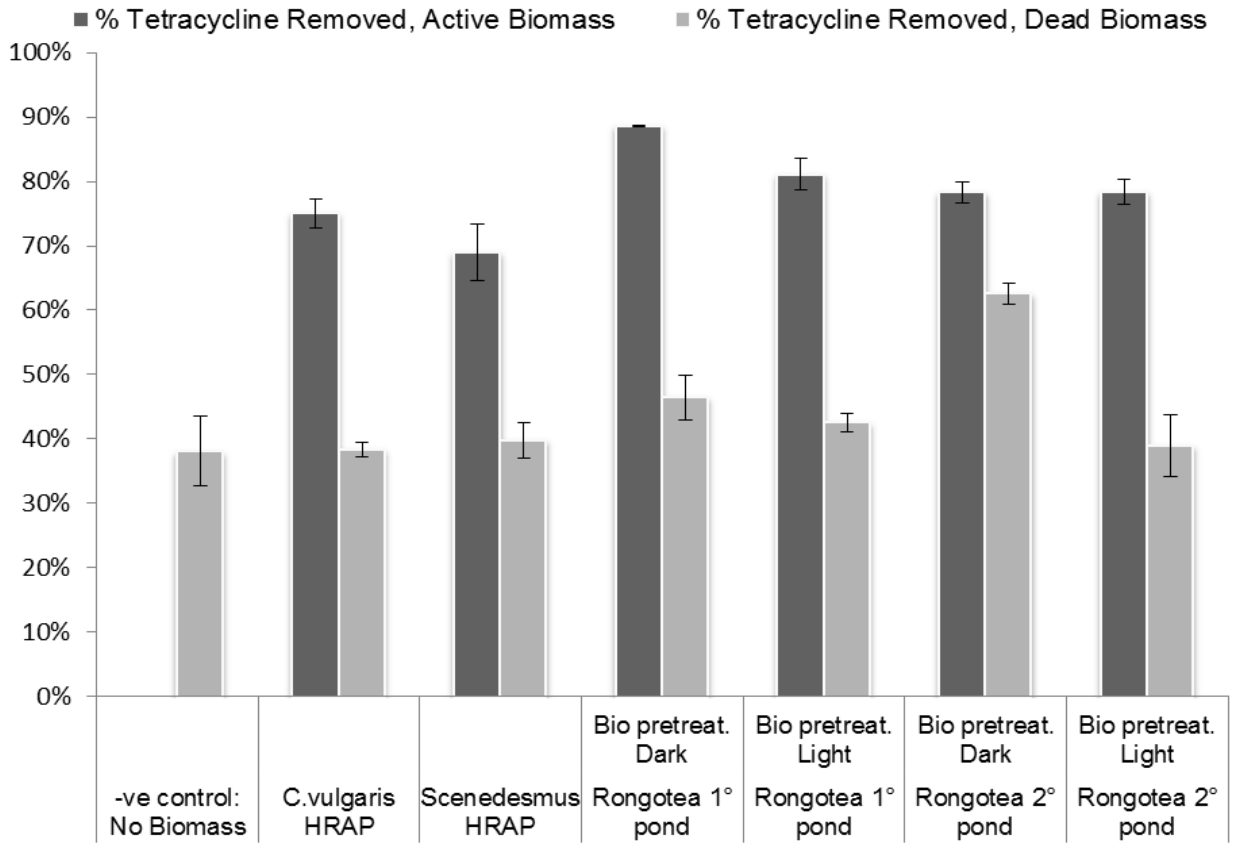
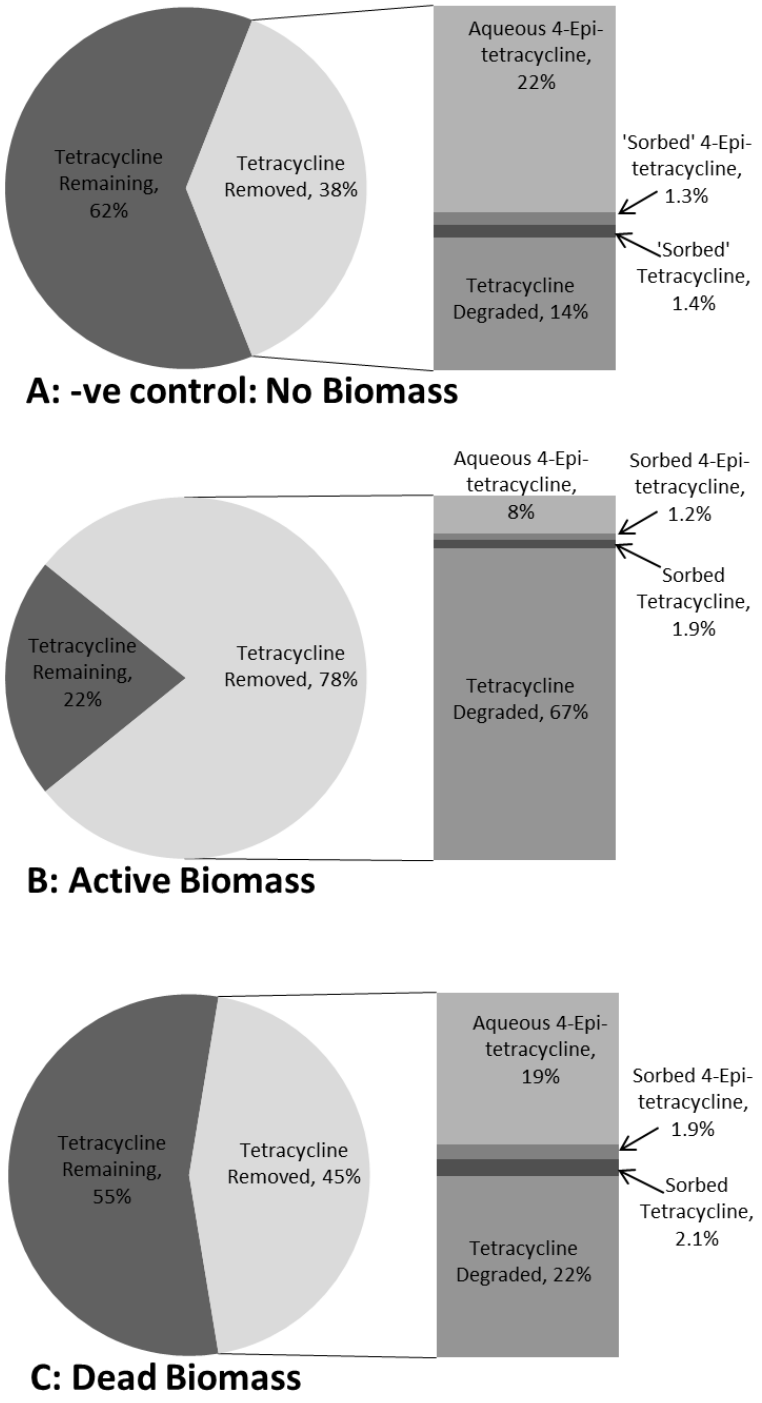


Figure 1: Difference in tetracycline degradation between the biomass types over 14 days, with controls having autoclaved (dead) biomass shown. Error bars show max/min values for duplicates.

The abiotic controls showed significant removal of aqueous tetracycline. Tetracycline removal in the dead biomass controls was slightly greater (and significantly greater in one case), indicating that the autoclaved biomass may have assisted hydrolysis or sorption (Figure 13). The reason for the one outlier is uncertain. From the data in Figure 2, sorption is shown to be low (< 2%), indicating the biomass may assist hydrolysis. Also from Figure 2, we can see that the equilibrium between tetracycline and its isomer 4-epi-tetracycline is responsible for most of the tetracycline removal.



The significant increase in degradation in the presence of active biomass shows that tetracycline can be biodegraded by both mono-cultured algae, and algae/bacterial biomass. The biodegradation is slow (14 day trial), but since algal WWT processes have a long retention time, the results suggest biodegradation may be a significant removal pathway of tetracycline in algal WWT. No conclusive differences are seen between the biomass samples (Figure 1). Some are statistically different, but all biomass samples show significant biodegradation. Because 4-epitetracycline is reduced along with the original tetracycline, biodegradation is responsible for an average 45% tetracycline removal (Figure 2, Tetracycline degraded [Active Biomass] – [Dead Biomass]).

Sorption appears a minor contribution to tetracycline removal at this pH, temperature, and biomass concentration, with sorption of both tetracycline and the isomer 4-epitetracycline forming only 3% of removal based on detection by extraction (see Figure 2). However, this method also detected similar sorption in samples with no biomass, so true sorption may be even less. The low sorption may be due to use of synthetic wastewater rather than true wastewater. Autoclaved samples showed more sorption than their active biomass counterparts, possibly due to cell damage or denatured proteins.

Figure 2: Breakdown of the tetracycline removed during the biodegradation experiment. Data shown as an average across all types of biomass tested. 'Tetracycline degraded' includes degradation by biodegradation and hydrolysis. 'Sorption' for control with no biomass was detected using the extraction

method, despite no biomass present, therefore true sorption values may be lower for the active and dead biomass samples.

2.2 BATCH TETRACYCLINE DEGRADATION UNDER ABIOTIC LIGHT CONDITIONS

100mL solutions were incubated at 27°C, at pH 7 or pH 8, with triplicate samples exposed to light (32 W m^{-2} PAR, 6 W m^{-2} UVA/B) and duplicate control samples kept in the dark. 0.1 M phosphate buffer was used to control pH. Beakers were left open.

Exposure to light was expected to increase degradation due to photolysis pathways, and because tetracycline is more fluorescent at high pH, photolysis (and thus degradation) was expected to increase at high pH. For the dark controls, neutral pH was expected to have more degradation than pH 8, based on the observations of tetracycline hydrolysis by Kang et al. (2012).

Results are shown in Figure 3. Good consistency was obtained across triplicate samples in the light and duplicate samples in the dark. Tetracycline degraded fastest in pH 8 phosphate buffer exposed to light, with significant degradation also occurring at pH 8 in the dark and pH 7 in the light. The pH 7 samples in the dark showed little degradation. First order kinetic degradation models fit all the data except the pH 8 sample in the light, which showed distinct trends in the residuals to a first order fit.

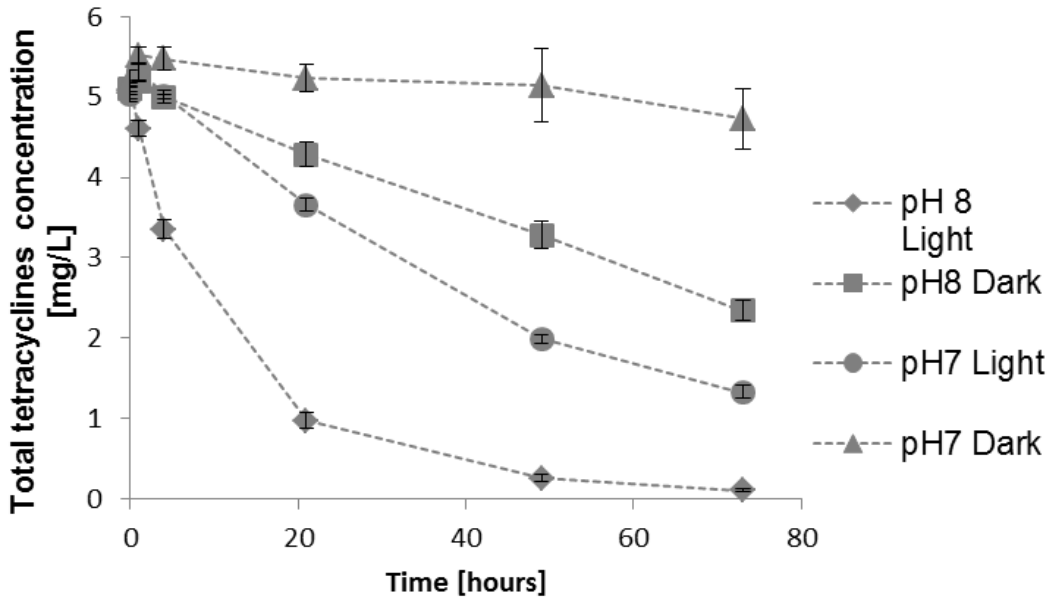


Figure 3: Detected tetracycline concentrations over the 3 day experiment. Error bars show max/min values. Triplicate samples in the light, duplicate samples in the dark. The graph shows the combined total of tetracycline and its isomer 4-epi-tetracycline.

Spectral analysis showed that the degradation was incomplete. One tetracycline absorbance peak (~360nm) decreased in correlation with tetracycline concentrations measured in the HPLC. However, the second tetracycline absorbance peak (~290nm) was not reduced during the experiment.

2.3 TETRACYCLINE REMOVAL IN CONTINUOUS HRAPS

Two identical lab-scale stainless steel HRAPs (A & B) situated side-by-side were used to simulate a continuous wastewater treatment HRAP. Each pond had dimensions 0.5 m long, x 0.3 m wide with an island in the centre 5 cm wide and a 0.065 m liquid depth, resulting in a total illuminated area of 0.108 m^2. The pond depth is less than a typical outdoor pond depth (0.2-0.5 m, therefore the lab pond is 3-8 times shallower than outdoor depth), because of the difficulty of providing high light intensities in the lab (pond depth is designed roughly on light intensity/penetration). Illumination was provided by 9 x full spectrum T8 fluorescent lights (Vivalite®) with an average measured intensity of 30 ± 2 W m^{-2} at the pond surface. This light intensity is similar to an overcast day in winter (20 - 40 W/m^2) (http://www.skyeinstruments.com/), but around 75% of summer overcast measurements, and 10 ± 3 times lower than clear sky irradiance, hence the design for reduced depth to compensate for the lower light intensities.

Wastewater influent was introduced by peristaltic pump every 5 min every 103 min (14 times daily) to approximate a continuous influent flow. Influent wastewater was pumped from a fridge at 4°C. Primary clarified wastewater was collected from Palmerston North Wastewater Treatment Plant and stored at -10°C in 5 L jerry cans, defrosting the day prior to requirements. Purified (RO) water was added to the primary clarified wastewater before addition to the ponds, to compensate for observed evaporation.

Mixing in the HRAPs was maintained by paddle-wheels rotating at 42 rpm, for a fluid velocity of 0.15m/s. This is similar to other researchers, who have used 0.09-0.15 m/s in pilot scale HRAP, (Park et al. 2013, García et al. 2006).

Biomass for the ponds was obtained by concentrating biomass collected from a secondary facultative wastewater treatment pond at Rogotea by centrifuging to obtain a concentration factor of 3. This biomass was grown for two weeks in the lab-scale HRAPs in order to acclimatize them to the new conditions, before adding the tetracycline to one pond, leaving the second pond as a control.

When tetracycline addition began, the influent was spiked to result in a 2mg/L tetracycline concentration. It was expected the tetracycline concentration would slowly build to reach a steady state concentration after three to four weeks operation.

Contrary to expectations, the tetracycline concentration in the HRAP seemed to reach a steady concentration after only a day. The concentration generally remained at 0.15-0.22 mg/L, which was an effective removal of approximately 90% at the end of this period, as seen in Figure 4. During this period, fluctuations in nitrate particularly were noticed in the pond, and the biomass appearance changed visually. Despite these changes, there was little effect on the effluent tetracycline concentration. Much higher removal occurred than the only known previous study by de Godos et al. (2012) of tetracycline removal in lab-scale HRAP, which observed degradation of 69% observed. This difference is attributed to key differences in the pond design, where the ponds in de Godos' study operated with 0.2 m working depth, and had a lower light intensity of 10 W m^{-2} PAR, resulting in less energy received from the light, and lower biomass concentrations. Other notable differences were the continuous supply of light (rather than cyclic), once-daily feeding, and use of synthetic wastewater rather than real wastewater. There is currently insufficient information to conclude precisely what effect each of these differences had.

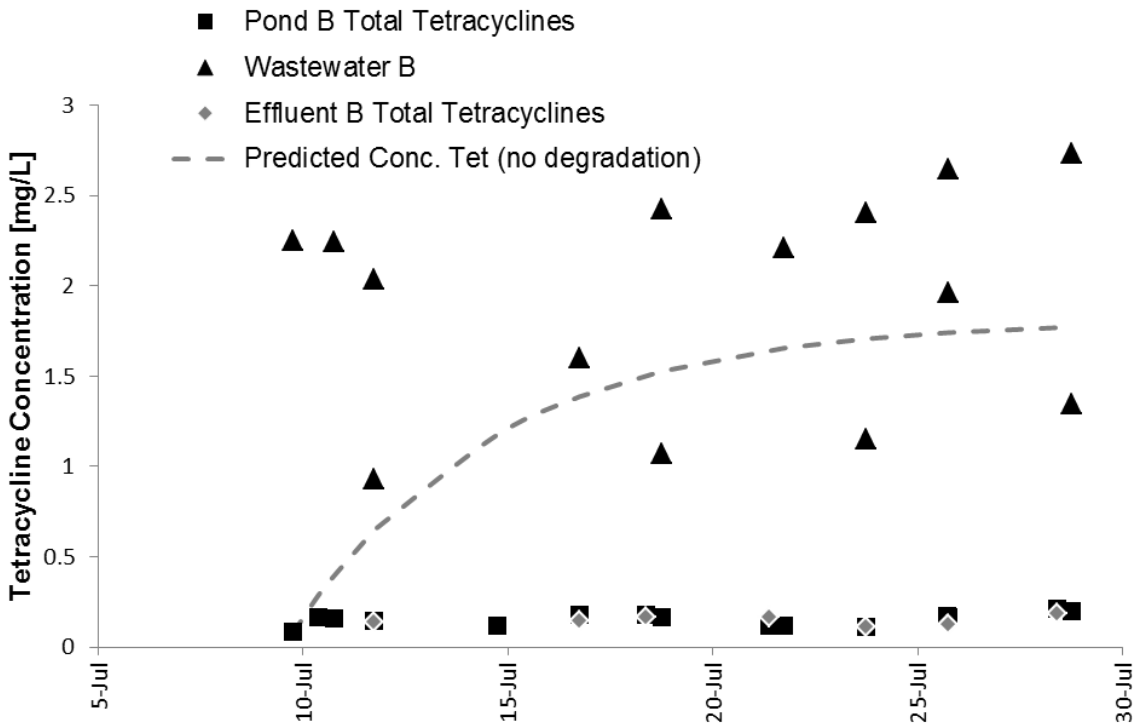


Figure 4: Tetracycline concentration over time. 90% tetracycline removal was recorded, compared with the predicted tetracycline concentration if no removal occurred. Influent tetracycline concentration varied due to storage times in the feed container.

3 CONCLUSIONS AND RECOMMENDATIONS

Batch tests conducted under dark conditions reveal sorption to be insignificant but that biodegradation caused 50 ± 8% removal over 14 days. Batch photodegradation tests in phosphate-buffered water showed an interaction of pH with photolysis, with pseudo-first order kinetic rates of 0.051 ± 0.005 h^{-1} at pH 8 and 0.0228 ± 0.0004 h^{-1} at pH 7. In a continuous lab-scale HRAP, 90% removal of tetracycline was achieved when fed at an influent concentration of 2 mg/L, with little fluctuation in effluent tetracycline concentration despite changing conditions in the pond.

A current major difficulty is ensuring the lab-scale HRAPs maintain a steady representation of full-scale conditions. Further work is needed to narrow down kinetics of removal mechanisms to enable modelling and prediction of algal systems.

Removal kinetics are now being studied under various environmental conditions in order to formulate a simple predictive kinetic model for tetracycline removal during algal WWT. This model will then be validated outdoors.

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