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Continuous-flow laboratory simulation of stream water quality changes downstream of an untreated waste water discharge

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SHORT TITLE: Simulation of chemical fate under direct discharge

ABSTRACT

In regions of the world with poor provision of waste water treatment, raw sewage is often discharged directly into surface waters. This paper describes an experimental evaluation of the fate of two organic chemicals under these conditions using an artificial channel cascade fed with a mix of settled sewage and river water at its upstream end and operated under continuous steady-state conditions. The experiments underpin an environmental risk assessment methodology based on the idea of an "impact zone" (IZ) - the zone downstream of waste water emission in which water quality is severely impaired by high concentrations of unionised ammonia, nitrite and biochemical oxygen demand (BOD). Radiolabelled dodecane-6-benzene sulphonate (DOBS) and aniline hydrochloride were used as the model chemical and reference compound respectively. Rapid changes in ^{14}C counts were observed with flow-time for both these materials. These changes were most likely to be due to complete mineralisation. A dissipation half-life of approximately 7.1 h was observed for the ^{14}C label with DOBS. The end of the IZ was defined as the point at which the concentration of both unionised ammonia and nitrite fell below their respective predicted no effect concentrations for salmonids. At these points in the cascade, approximately 83 and 90 percent of the initial concentration of ^{14}C had been removed from the water column, respectively. A simple model of mineral nitrogen transformations based on Michaelis-Menten kinetics was fitted to observed concentrations of NH_4, NO_2 and NO_3. The cascade is intended to provide a confirmatory methodology for assessing the ecological risks of chemicals under direct discharge conditions.

Key words: Direct discharge, LAS, degradation, laboratory simulation study, ammonia, nitrite, river

INTRODUCTION

In many parts of the world, particularly in developing regions such as Asia, South America and Africa, untreated waste water is routinely emitted directly into surface water bodies (e.g. Whelan *et al.*, 1999; Eichhorn *et al.*, 2001; Eichhorn *et al.*, 2002; McAvoy *et al.*, 2003; Whelan *et al.*, 2007). This scenario is typically associated with high levels of suspended solids, biochemical oxygen demand (BOD), nitrite and unionised ammonia in receiving waters, resulting in significant ecological impairment. This presents a number of difficulties for conventional risk assessments for 'down-the-drain' chemicals. Most fundamentally, in the absence of removal by secondary sewage treatment, the predicted environmental concentration (PEC) will often exceed the predicted no-effect concentration (PNEC). However, since the ecosystem in the receiving environment will already be significantly impacted by other constituents present in raw waste water, the significance of this simple assessment for the risks posed by synthetic chemicals is questionable.

An alternative risk assessment model, based on the "impact zone" (IZ) concept, has been proposed for direct discharge conditions (Limlette III Workshop, 1995; McAvoy *et al.*, 2003). In this model, chemicals are assessed in terms of their potential impact on river recovery (self purification) processes (e.g. microbial respiration and nitrification) and in terms of their predicted concentration at the end of an "impact zone", within which the ecosystem is impacted by pollutants such as unionised ammonia, nitrite and BOD and beyond which, it is not. In the work presented here we only consider the latter assessment: i.e. the concentration of a synthetic organic pollutant at the end of the IZ. For a given scenario (river flow and pollutant loading),

the length of the IZ is defined, independently of the chemicals under consideration, as the point at which the concentration of unionised ammonia or nitrite decreases below the respective (nitrite or ammonia) predicted no effect concentration (PNEC) or the point at which dissolved oxygen (DO) concentrations increase above a given threshold. When assessing the impact of a synthetic organic chemical discharged in waste water, the change in chemical concentration within the IZ is estimated and the concentration estimated at the end of the IZ is compared with the chemical PNEC, as it would be in a conventional ecological risk assessment. Note that the length of the IZ is defined only in terms of the behaviour of nitrite, ammonia or dissolved oxygen. For a given scenario it will be the same for all organic chemicals. The extent of organic chemical transformation and loss within the IZ is needed in order to estimate the concentration of the chemical at the end of the IZ. This has been estimated in field monitoring studies using the anionic surfactant, linear alkylbenzene sulphonate (LAS) as a model substance (McAvoy *et al.*, 2003; Whelan *et al.*, 2007). However, such studies are often difficult and expensive to conduct and it is not feasible to assess the behaviour of new chemicals using these methodologies. A laboratory-based simulation system is, therefore, required which will enable the realistic behaviour and associated ecological risks of a range of different chemicals to be evaluated under direct discharge conditions. *In vitro* batch tests, such as those described by Peng *et al.* (2000), undoubtedly have some screening-level value but they are unlikely to generate rate constants which are comparable with those observed in the field. In addition, many batch studies (e.g. Peng *et al.*, 2000) have not compared the rate of loss of the test chemical against the dynamics of mineral nitrogen which controls the length of the IZ.

In this paper, we describe a laboratory simulation study using a continuous flow river model with attached biomass, under direct discharge conditions with a single point source at the upstream end. The experimental system used is loosely based on apparatus described by Boeije *et al*., 2000 and in ISO 14592 (2002). Note that both the system described in the IS0 14592 (part 2) guidelines and the system described by Boeije *et al.* (2000) have, thus far, only been applied to relatively clean systems (i.e. without significant fraction of raw waste water). Although an assessment of the inhibitory effect of the substance of interest on key ecosystem functions, such as microbial respiration or nitrification, is integral to the IZ risk assessment approach, we concentrate here on assessing the rate of loss of the test substances compared with the rate of loss of ammonia and nitrite. In order to allow a comparison with field observations (McAvoy *et al.*, 2003; Whelan *et al.*, 2007), a single LAS isomer: dodecane-6-benzene sulphonate (commercial LAS properties: dimensionless Henry's law constant = 7.66 x 10^{-10}; calculated log K_{OW} = 3.32: HERA, 2007) was selected as the model chemical. LAS is readily biodegradable under aerobic conditions but is not degraded anaerobically (HERA, 2007). In addition, aniline hydrochloride (dimensionless Henry's law constant = 7.76 x 10^{-5}; log K_{OW} = 0.9: USEPA, 1994) was employed as a reference compound. Although aniline is not expected to be found in waste water at high concentrations it is commonly used as a reference compound in laboratory biodegradation studies because it is known to degrade rapidly (e.g. Nyholm and Kristensen, 1992; Battersby, 1997; Toräng *et al.*, 2002; ISO, 2001).

Several field studies have reported in-stream removal rates for LAS in rivers (e.g. Whelan *et al.*, 1999; Fox *et al.*, 2000; McAvoy *et al.*, 2003; Whelan *et al.*, 2007), wetlands (Inaba *et al.*, 1988) and estuaries (Amano *et al.*, 1991). Short half-life

values of typically less than three hours have been reported for shallow rivers and streams (e.g. Takada *et al.*, 1994; Fox *et al.*, 2000; McAvoy *et al.*, 2003). Typically, the water in such systems has a greater contact with the stream bed and bank surfaces, and with associated microbial communities (attached biofilms). Boeije *et al.* (2000) observed that a fixed biofilm was critical for significant removal of LAS and suggested that rates of biodegradation are likely to be highest in shallow rivers with a high surface area to volume ratio. Indeed, much longer surface water half-lives for LAS have been reported for deeper rivers in temperate zones (e.g. 15 h: Whelan *et al.*, 1999) and in the tropics (7 h: Whelan *et al.*, 2007).

MATERIALS AND METHODS

Artificial river system

The test system consisted of a cascade containing five shallow rectangular channels made from unplasticized polyvinyl chloride (1.8 m in length, 0.08 m in width) constructed to form an aquatic "staircase" (Figure 1). The volume of water in each channel was 2 L ± 0.2 L and a water depth of 0.02 – 0.03 m was maintained. The bottom of each channel was covered with glass beads (5 mm diameter) to act as an artificial substrate to enhance the development of a fixed-film biomass (after Boeije *et al.*, 2000). The average mass of each bead was 0.17 g. A total bead mass of 724 g was added to each channel with an approximate total surface area of 3345 cm^2.

The channels were separated by a vertical distance of 0.18 m and were connected via tubing linking the downstream end of one channel with the upstream end of the next in the cascade. The depth of the outlet at the downstream end of each channel was

adjusted so as to maintain a water depth of about 0.01 m above the glass beads. The system was operated in a temperature controlled environment (20^{o}C) under controlled illumination with 8 h of light per day (measured at 1800 lux). The average water temperature was 19.7 ± 0.2^{o}C. The flow rate was maintained via a peristaltic pump at 0.42 L h^{-1} (7 mL min^{-1}). A steady-state hydraulic retention time (HRT) of 26 h was measured using a NaCl tracer test prior to any dosing with waste water, but including the glass beads. Flow was recorded at regular intervals during the study. The storage vessel for the test medium was connected to the first channel of the cascade. The test medium consisted of one part settled sewage (i.e. post primary settler at the STP) : two parts river water, reflecting the common situation of direct discharge of sewage into river water. Both settled sewage and river water were collected regularly (every other day) from a local sewage treatment plant (Broadholme STP, Northamptonshire, UK) and river (River Great Ouse at Felmersham Bridge, Bedfordshire, UK), respectively. Although the use of these media can potentially introduce variability into the system, they have the advantage of more closely representing the true nature of waste water and receiving surface water quality. To help reduce variability in the test medium, excess medium on any day was mixed with freshly prepared medium on the next. Approximately 10 L of test medium were freshly prepared daily and mixed with surplus medium (ca. 5 L) from the previous day. In other words, 10 L was used over one HRT and 5 L was carried forward to the following day. No aeration was provided to the system, apart from across the air-water interface.

FIG 1 ABOUT HERE

Acclimation of the test system

Prior to the introduction of the radiolabelled compounds, it was necessary to ensure that the cascade had reached a steady-state in terms of major water quality determinands (pH, DO, temperature, ammonium(NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-) and COD) and had developed a biofilm. From the start of the experiment, samples were taken from the inlet and outlet of each channel (i.e. at 0, 1.8, 3.6, 5.4, 7.2, 9.0 m) and analysed for NH_4^+, NO_2^-, NO_3^- and COD three times a week. All water quality analyses were performed using cuvette test methods on a Xion 500 Spectrophotometer (Hach-Lange GmbH, Dusseldorf, Germany). In addition, the channels were frequently monitored for temperature, pH and DO concentration. The water quality parameters and DO concentration were used to determine when the system reached steady-state conditions (i.e. after sufficient microbial biomass, including nitrifiers, had developed), at which point the radio-labelled materials were introduced to the feed.

Typically native LAS concentrations in waste water can range between 1 and 15 mg L^{-1} (Henze *et al.*, 2002). The average concentration of total native LAS in the test medium (measured by liquid chromatography electrospray ionisation mass spectrometry) prior to the addition of radiolabelled materials was 1.28 ± 0.15 mg L^{-1}.

Model chemical and reference compound

The behaviour of the following organic compounds was investigated: (1) Reference compound: [U-^{14}C] Aniline Hydrochloride supplied by Sigma-Aldrich, USA and (2) Model chemical: Radio-labelled C_{12} LAS: ^{14}C sodium dodecane-6-benzene sulphonate (Phenyl –^{14}C 6-DOBS), supplied by Scynexis, Ongar, UK.

The radiochemical purities of the [U-^{14}C]-aniline and Phenyl –^{14}C 6-DOBS were determined using Spectra Physics Radio HPLC (Prodigy C18 column, 250 x 4.6 mm, Phenomenex). The radiochemical purities of [U-^{14}C]-aniline and Phenyl –^{14}C 6-DOBS were both 99 percent, with specific activities of 77 and 19 mCi mmol^{-1}, respectively.

Stock solution of Phenyl – ^{14}C 6-DOBS was prepared by dissolving 7.4 mg of Phenyl – ^{14}C 6-DOBS in 2 mL of methanol (HPLC grade) to give a concentration of 3.7 mg mL^{-1}. Triplicate aliquots (50 µL) of the concentrated stock were made up to 10 mL with methanol to give three replicate solutions. Triplicate aliquots (100 µL) of each solution were mixed with Starscint (Perkin Elmer) for liquid scintillation counting. A working stock solution was prepared so that a count of approximately 5000 DPM mL^{-1}, for both the reference chemical and the test compound, was obtained after dilution in the cascade system. The target concentration for Phenyl – ^{14}C 6-DOBS was 30 µg L^{-1}.

Each radio-labelled compound was added continuously to the cascade as a secondary substrate after the establishment of steady-state conditions. The ^{14}C stock solution was mixed with the test medium (settled sewage / river water) from a syringe-controlled dosing unit in a stirred biometer flask (100 mL) prior to feeding the top of channel 1. The flask was changed regularly to prevent any biofilm development and degradation prior to injection into the cascade. The reference compound (^{14}C-aniline) was added to the influent on days 38-47 and the Phenyl-^{14}C 6-DOBS was added on days 95-104. Note that the system was clear of any radiolabelled aniline at this stage.

For both radiolabelled compounds, the analysis of samples collected at different points in the cascade represent total ^{14}C remaining, after sample acidification with H_2SO_4 (2 M) to expel dissolved CO_2. This does not differentiate between degradation products and parent molecules. Reductions in the concentration of ^{14}C, therefore, represent a net loss from the water column which is most likely due to complete mineralisation (conversion to CO_2) rather than primary degradation of parent molecules, although some loss from the water column by sorption to the substrate and the channel walls cannot be discounted.

MODELLING

During the experimental phase, it was assumed that the system behaved as a steady-state plug-flow reactor with complete vertical and cross channel mixing and with transport occurring exclusively by turbulent advection.

Organic Compounds

Single (pseudo) first order kinetics (SFO) have been successfully used to describe the degradation of a number of organic compounds, including LAS, in river systems (*e.g.* Fox *et al.*, 2000; McAvoy *et al*., 2003; Whelan *et al.*, 2007) and in artificial channels (e.g. Boeije *et al.*, 2000). The SFO model can be written

$$\frac{dC}{dt} = -k.C \qquad (1)$$

where C is the concentration of substance remaining (mg L^{-1}), t is time (h) and k is a biodegradation rate constant (h^{-1}) which is obtained via least squares fitting of the following solution to Equation (1):

$$C = C_0.\exp(-k.\tau) \quad (2)$$

where C_0 is the concentration at the start of the cascade and τ is the mean solute travel time (h), assumed to be the HRT derived from tracer tests with NaCl. Equation (1) will only apply where DO is not limiting to the degradation rate. The degradation half-life, $T_{1/2}$ (h) is thus:

$$T_{1/2} = \frac{\ln(2)}{k} \quad (3)$$

Mineral Nitrogen

Mineral nitrogen dynamics in the system were simulated using a simple three pool model system comprising of ammonium N, nitrite N and nitrate N. Although nitrification has frequently been described using first order kinetics (*e.g.* Chapra, 1997; Martin and Reddy, 1997; McAvoy *et al.*, 2003), Michaelis-Menten (MM) kinetics have also been used (*e.g.* Charley *et al.*, 1980). The use of MM has the advantage of allowing for the representation of the transition between zero order kinetics at high substrate concentration and first order kinetics at lower concentrations and was, therefore, adopted here. The following rate equations were applied:

$$\frac{dC_{NH4}}{dt} = -\frac{v_1.C_{NH4}}{k_1 + C_{NH4}} \quad (4)$$

$$\frac{dC_{NO2}}{dt} = \frac{v_1.C_{NH4}}{k_1 + C_{NH4}} - \frac{v_2.C_{NO2}}{k_2 + C_{NO2}} \quad (5)$$

$$\frac{dC_{NO3}}{dt} = \frac{v_2.C_{NO2}}{k_2 + C_{NO2}} - \frac{v_3.C_{NO3}}{k_3 + C_{NO3}} \quad (6)$$

where C_{NH4}, C_{NO2} and C_{NO3} are the concentrations of ammonium N, nitrite N and nitrate N, respectively (mg N L^{-1}), v_1, v_2 and v_3 are maximum rates (mg N L^{-1} h^{-1}) and k_1, k_2 and k_3 are half-saturation constants (mg N L^{-1}) for, respectively: (1) nitrification (ammonia to nitrite); (2) nitrification (nitrite to nitrate) and (3) nitrate loss (due to uptake by the microbial biomass or dentrification). Note that ammonium N will be formed in aqueous systems via the mineralisation of organic nitrogen and from the hydrolysis of urea. Urea hydrolysis is likely to be the main source for ammonium, but most urea will probably have hydrolysed before dosing to the cascade (Metcalf and Eddy, 1979). However, since there was no direct evidence of the formation of ammonium in the test system and since organic N and urea concentrations were not measured, an ammonium source term is not included.

Impact zone

The definition of the impact zone in the experiments was based on water quality criteria for concentrations of unionised ammonia and nitrite for toxicity to freshwater fish. A toxicity threshold (PNEC) of 25 µg L^{-1} has been reported by Alabaster and Lloyd (1980) for the protection of freshwater fisheries based on toxicity of unionised ammonia to salmonids (EC Freshwater Fish Directive 78/659/EEC). The fraction of total ammoniacal nitrogen which is unionised is highly pH-dependent. The

concentration of unionised ammonia (C_{NH3}) was calculated using the following equation:

$$C_{NH3} = \frac{C_{NH4}}{\alpha} \cdot \left(\frac{1}{1+10^{(pKa-pH)}} \right) \quad (7)$$

where α is the fraction of N in NH_4^+ (0.78) and where pKa is the temperature-dependent dissociation constant which was set at 9.42 (for a system temperature of 20 °C).

Nitrite toxicity is strongly dependant on the chloride concentration of surface waters, which tends to reduce toxicity (Eddy and Williams, 1994). There are existing EU standards for nitrite in surface waters under the Freshwater Fish Directive (78/659/EEC). The guideline PNEC value given under this Directive for salmonid waters is 10 μg L^{-1} (i.e. ~3 μg N L^{-1}).

RESULTS AND DISCUSSION

Test medium quality

The quality of the test medium was monitored over the course of the study. Overall results are shown in Table 1. In general, the variability was very low (CV <~10%) and there was no systematic temporal pattern to the variability of any of the measured variables.

TABLE 1 ABOUT HERE

The test medium was also analysed for native linear alkyl benzene sulphonate (LAS) already present in the waste water source using liquid chromatography / mass spectrometry (LC/MS) following solid phase extraction on six separate occasions over the course of the experiment. Details of the method used can be found in Whelan *et al.* (2007). The method showed good mean recoveries of total LAS (99.1 ± 2.5 %: mean ± standard deviation (SD), n=6), from spiked samples at 5 mg L^{-1}. Total LAS concentrations in the influent media ranged between 1.06 and 1.46 mg L^{-1} with a mean of 1.28 ± 0.15 mg L^{-1}. This indicates that the microbial biomass associated with the input medium, and hence the resident microbial community in the channel system, is likely to be well adapted to LAS and suggests that significant lag times (Shimp *et al*. 1989) should not be expected. The low variability in the LAS concentrations measured reflects (1) the fact that batches were collected from the STP at the same time of day each time; (2) the fact that the liquid collected was "settled" sewage – i.e. post primary settler and, therefore, already mixed and (3) the fact that the STP from which the sewage was collected is fairly large, integrating some of the variability which might be expected in smaller plants.

Dissolved Oxygen and COD

Observed DO measurements in the cascade are shown in Figure 2 for some selected days through the study period. These data show the development of a DO "sag" (see Adrian *et al*., 2004) with relatively low concentrations (*ca* 2 mg L^{-1}) at the upstream end of channel 1 and a gradual increase in DO concentrations further downstream. Based on our observations of LAS degradation under direct discharge conditions in Laos (Whelan *et al.*, 2007), the lowest DO concentrations observed in the cascade are

probably not low enough to inhibit LAS degradation – which is known to be a strictly aerobic process. Stepped increases in DO concentration are evident at the transition points between channel units. This is an artefact of the experimental system, which does not reflect expected processes in real rivers, with the exception of riffles and cataracts. The extent to which these discontinuities affect the behaviour of the nitrogen species used to define the IZ or that of the model chemical and reference compound is not known. However, corresponding step changes in the concentration of other determinands are not apparent, suggesting that the "steps" do not have a major impact – probably because oxygen was not limiting to degradation or nitrification.. The DO profiles on different days suggest that it took several days for steady-state conditions to develop. All measurements from about day 29 onwards show a very similar pattern, as illustrated for days 29 and 50.

FIG 2 ABOUT HERE

Changes in COD concentrations with distance downstream are shown in Figure 3 for different days after the start of the experiment. There was a gradual increase in the rate of COD reduction in the system during the first few days after the start of operation as the microbial biomass developed within the channels, followed by a period of quasi-steady-state. The rate of reduction in COD with distance was greatest in the first channel. This suggests that the conditions in channel 1 were more favourable to degradation – probably as a result of higher microbial biomass. For the overall cascade, first order kinetics were generally not applicable to describe COD. However, reasonable fits were obtained with a first order equation using data from channels 2-5 only (i.e. omitting data from channel 1).

FIG 3 ABOUT HERE

Reference Compound

The degradation pattern of radiolabelled reference material (^{14}C aniline hydrochloride) is shown in Figure 4. As with COD, the data suggest that the aniline is degraded very rapidly in the first channel of the cascade (i.e. after a travel time of 5.24 h), confirming the presence of a viable biofilm. However, degradation in subsequent channels was considerably slower. Overall the data could not be described well using first order kinetics, fitted using a least squares optimisation, with travel time estimated using an axial velocity of 0.34 m h^{-1} (see Figure 4 solid line). However, an acceptable fit was obtained by restricting the model fit to data from channels 2 to 5 only (i.e. optimising Equation (2) with C_0 set to the observed concentration at the start of channel 2). This is justified on the basis of clear visual evidence of a higher settled solids content in the first channel during the course of the experiments and the presence of a discontinuity at the downstream end as the flow is transferred to channel 2 via the connecting pipe. This suggests that aniline behaviour in the cascade can be described using first order kinetics provided that the nature of the fixed film biomass is approximately constant. The best-fit half-life of aniline in channels 2 to 5 was 21.7 h. The apparent half-life in the first channel derived from a first order best-fit to the first three data points was 3.02 h.

FIG 4 ABOUT HERE

Model Chemical

The observed pattern of ^{14}C reduction in the cascade after dosing with ^{14}C Phenyl -6-DOBS is shown in Figure 5. Concentrations are given in total ^{14}C remaining (i.e. including metabolites) and are, therefore, indicative of ultimate degradation (i.e. complete mineralisation). This is an important distinction between the results of this study and many field studies which often employ specific analysis (*e.g.* Fox *et al.*, 2000; McAvoy *et al.*, 2003; Whelan *et al.*, 2007) to measure primary degradation of the parent molecules.

The first order kinetic model best-fit is shown as a thick solid line in Figure 5. It is clear that the overall pattern of average ^{14}C reduction in the cascade does not follow first order kinetics. As with COD and ^{14}C aniline hydrochloride the rate of loss in channel 1 (i.e. upto a travel time of 5.24 h) is more rapid than in subsequent channels. In addition, there is considerable variability in the measured data. A best-fit second order kinetic curve for the average data is also shown in Figure 5 (dashed line), which clearly provides a superior description of the observed pattern. Nevertheless, it is more likely that the observed pattern of ^{14}C loss is due to discontinuities in settled solids and associated biofilm growth between channels in the cascade rather than due to the operation of non-first order kinetics. First order kinetics described the data in channels 2-5 reasonably well (thin solid line in Figure 5), yielding a best-fit half-life of 7.1 h. This is similar to the apparent half-life observed for primary degradation of LAS in Lao PDR by Whelan *et al.* (2007), although the primary degradation half-life of LAS in the cascade system is likely to be much less than the ^{14}C half-life reported above. The loss of metabolites (predominantly sulphophenylcarboxylates, SPCs) may limit the overall loss rate if the rate constant for these substances is slower than for the parent material. SPCs, which are less toxic than LAS, are formed by the

biodegradation of LAS mainly by ω-oxidation of the alkyl chain followed by β-oxidation which shortens the chain lengths by two carbon atoms respectively to give a wide range of homologues and isomers (Swisher, 1987; Gonzalez-Mazo *et al.*, 1997; Schleheck *et al.*, 2004). The rate of biodegradation of SPCs is generally believed to be significantly slower than for LAS (*e.g.* León, *et al.*, 2004). A shorter half-life for LAS would be expected in the cascade system due to low water depth and a high degree of contact between the water column and solid surfaces in the bed (Boeije *et al.*, 2000).

FIG 5 ABOUT HERE

LAS is non-volatile and ^{14}C removal was assumed to be completely due to biodegradation rather than sorption to solids or sedimentation. Since the glass beads were not extracted for ^{14}C, the significance of adsorption is unknown. Sorption to the unplasticized polyvinyl chloride channel walls is also possible (*cf* Antizar-Ladislao and Galil, 2006) but is likely to be minimised in our system by relatively rapid flow through and a low contact time. Confirmation of the potential for rapid biodegradation was obtained by using five glass beads with attached biofilm (taken from the first channel after the radio labelled analysis had been completed) as an inoculum source in a respirometric test with LAS in a mineral salt medium, using the OxiTop system (WTW, Weilheim, Germany). A control vessel containing beads from the cascade but no added LAS was operated in parallel to measure the background respiration rate. LAS was added to the test vessels at a concentration of 75 mg L^{-1} Theoretical Oxygen Demand. After 5 days, oxygen consumption in the test

vessels was > 57 mg L^{-1} higher than in the control vessels, confirming that biodegradation was a likely mechanism of removal (Finnegan, 2007).

Mineral Nitrogen Dynamics

The typical pattern of mineral nitrogen dynamics in the cascade is illustrated in Figure 6. Measured data from days 22-104 were used, when the system appeared to be operating under steady-state conditions. The data suggest a relatively rapid conversion of NH_4 to NO_2 and subsequently to NO_3 and a progressive build up of NO_3 concentrations to about 13 (± 1.3) mg N L^{-1}. Initial nitrite concentrations were about 2.3 (± 0.76) mg N L^{-1} and decreased progressively with residence time to about 0.03 (± 0.06) mg N L^{-1} at the end of the final channel.

It should be noted that before day 22, a gradual adaptation was observed in the pattern of mineral N concentrations in the system with an increase in NO_2-N concentrations observed through the cascade up to day 15, followed by a gradual levelling off and eventually a systematic decrease in NO_2-N concentrations after day 31 (data not shown). This reflects the slow development of a competent community of nitrifiers in the system.

FIG 6 ABOUT HERE

The N model (Equations 4-6) was solved numerically (Euler's method with a time step of 0.1 h). The parameters k_1, k_2, k_3, v_1, v_2 and v_3 were optimised using an iterative least squares fitting procedure against the observed data on mineral N in the cascade to yield the following values: 0.34 mg N L^{-1}, 0.26 mg N L^{-1}, 0 mg N L^{-1}, 0.638 mg N

L^{-1} h^{-1}, 0.8483 and 0.047 mg N L^{-1} h^{-1} respectively. Half-saturation constants for both nitrification steps (k_1 and k_2) are low compared with NH_4-N and NO_2-N concentrations in the first half of the cascade implying that kinetics are effectively zero-order over much of the channel length. This is in accordance with values for the half-saturation constant of nitrification reported by other workers (e.g. 0.59 mg N L^{-1} in activated sludge: Charley *et al.*, 1980). The low value for v_3 and the fact that the optimal value for k_3 is zero (i.e. zero order kinetics apply to nitrate losses) suggest that the loss rate for NO_3 is low. In fact, setting the value of v_3 to zero (i.e. assuming no losses for NO_3) results in an only marginally poorer fit. The data also imply that there was little significant ammonification in the system over the typical time-of-travel of the cascade. Although the concept of a half-life is not strictly applicable to Michaelis-Menten kinetics, the time taken for initial concentrations of NH_4-N and NO_2-N to reduce by half are approximately 7.8 and 9 h respectively. Which are both higher than the best-fit half-life for the ultimate degradation of ^{14}C Phenyl -6-DOBS. This is in agreement with reports from other workers (e.g. Fox *et al.*, 2000; McAvoy *et al.*, 2003; Whelan *et al.*, 2007) that primary degradation of LAS usually proceeds at rates which are significantly faster than ammonium oxidation.

Much of the literature on nitrification in aqueous systems suggests that, like biodegradation (e.g. Boeije *et al.*, 2000), it is performed predominantly by micro-organisms in fixed biofilms rather than by suspended colonies (e.g. Moreau *et al.*, 1994). Note that it is interesting that the discontinuities in the concentration data observed between the first and second channels for COD, ^{14}C aniline hydrochloride and ^{14}C Phenyl 6 DOBS are not apparent in the mineral nitrogen data. This could be due to the fact that nitrification appears to operate with zero order kinetics in the first

half of the cascade. At high concentrations, under zero order kinetics, the rate is limited to a maximum rate.

Implications for Risk Assessment

In our experimental system, the end of the IZ was assumed to be the point at which the concentration of unionised ammonia fell below the PNEC for salmonids. The PNEC for salmonids was arbitrarily chosen because fish are usually desireable animals to protect. However, toxic thresholds for other species may be more appropriate. Note that observed nitrite concentrations in the cascade were always above the nitrite PNEC. Under steady-state conditions, the mean modelled unionised ammonia concentration (Equation 7) in the cascade fell below the unionised ammonia PNEC (25 µg L^{-1}) at a travel time of approximately 16 h and the mean modelled nitrite concentration fell below the nitrite PNEC (~3 µg N L^{-1}) at a travel time of approximately 19 h. Thus the most conservative estimation for the end of the IZ is 16 h. At these points (16 and 19 h), the concentration of ^{14}C (after acidification) was observed to be about 17 % and 9.7 % of the concentration at the start (suggesting 83% and 90.3 % mineralisation), respectively. Based on an influent native LAS concentration of 1.28 mg L^{-1}, this implies that the total non-mineralised concentration (LAS + metabolites) should be approximately 218 and 124 µg L^{-1} respectively at these points in the system. These values are less than the LAS PNEC of 245 µg L^{-1} proposed by Dyer *et al*. (2003) based on a species sensitivity distribution. The use of ^{14}C-labelled material gives an indication of complete mineralisation, rather than just loss of the parent compound, so it is likely that the parent LAS remaining will be much less than these values. In a field study conducted on a waste-water-impacted river channel in Laos, Whelan *et al.* (2007) observed that the total SPC : total LAS

ratios near the end of the impact zone were as high as 200 percent. Such a ratio would, speculatively, put the total LAS end-of-IZ concentration at about 72 μg L^{-1} and 41 μg L^{-1} respectively at 16 and 19 h. However, in the absence of measured data on levels of native LAS in the cascade, such estimates should be viewed with caution.

It is important to note that both pollutant loading and flow regime are likely to vary in field situations. Although this variability may influence the extent of dilution provided by receiving water bodies, solute travel times and the length of the IZ, our experience in Laos (Whelan *et al.*, 2007) suggests that diurnal variability in LAS and ammonia concentrations under steady flow conditions are relatively constant in time. In such cases, the steady-state loading and river flow assumptions implicit in the IZ model are justifiable and lend validity to the steady-state laboratory cascade described here. That said, in cases where loading variability is more significant, the fact that the IZ approach is based on relative changes in organic pollutant concentrations compared with inorganic nitrogen concentrations should reduce the temporal and spatial dependency of the risk assessment, provided that loads of organic chemical and inorganic nitrogen change contemporaneously. In the same way, both mineral nitrogen dynamics and organic chemical degradation will be affected similarly by water depth and temperature. Thus, the fact that the laboratory cascade was operated at a lower temperature than that frequently observed in tropical systems and the fact that water depth in the cascade is relatively shallow are not believed to affect the risk assessment outcome significantly.

CONCLUSIONS

This paper presents experimental observations of downstream water quality changes in a laboratory-scale continuous-flow channel cascade subjected to direct discharge conditions. The system was able to reproduce expected changes in general water quality resulting from the emission of untreated waste water (e.g. high COD and BOD, associated DO sag, followed by recovery and high unionised ammonia and nitrite concentrations which decrease with distance downstream). When radiolabelled phenyl-6-DOBS and aniline hydrochloride were introduced continuously into the discharge rapid changes in ^{14}C counts were observed with flow-time, representing complete mineralisation. A mineralisation half-life of approximately 7.1 h was observed for ^{14}C Phenyl-6-DOBS (C_{12} LAS).

A simple model of mineral nitrogen transformations, based on Michaelis-Menten kinetics was fitted to observed concentrations of NH_4, NO_2 and NO_3. Approximately 83 and 90.3 percent of the initial concentration of ^{14}C Phenyl-6-DOBS were estimated to have been mineralised by the time unionised ammonia and nitrite concentrations fell below their respective PNECs. This is expected to represent a reduction in total native LAS concentration to well below the LAS PNEC and confirms similar observations made in the field under tropical direct discharge conditions reported by McAvoy *et al.* (2003) and Whelan *et al.* (2007).

The cascade system is intended to provide a confirmatory methodology for assessing the ecological risks of chemicals under direct discharge conditions, without the need for expensive field campaigns. Further work is required both in the field and in the laboratory to ascertain the extent to which the patterns observed for LAS can be generalised to other readily biodegradable and inherently degradable substances.

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Table 1. Average and SD of water quality variables measured in the test medium used in the study. In all cases $n = 21$.

	COD	NH4-N	TN	PO_4-P	Temp	pH	DO	TDS	EC
Units	mg L^{-1}	mg N L^{-1}	mg N L^{-1}	mg P L^{-1}	°C		mg L^{-1}	ppt	mS cm^{-1}
Average	90.3	9.0	24.8	2.1	19.9	8.14	4.8	0.6	1.2
SD	9	0.9	2.6	0.2	0.3	0.05	0.6	<0.1	<0.1

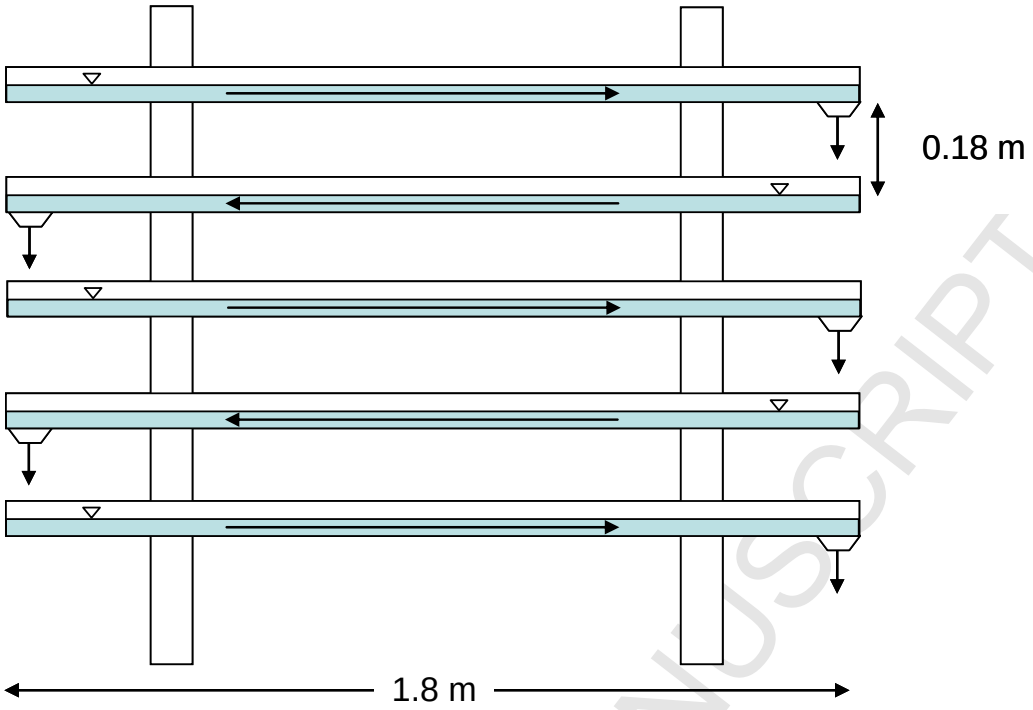

Figure 1 Schematic representation of the test apparatus illustrating direction of flow (not to scale).

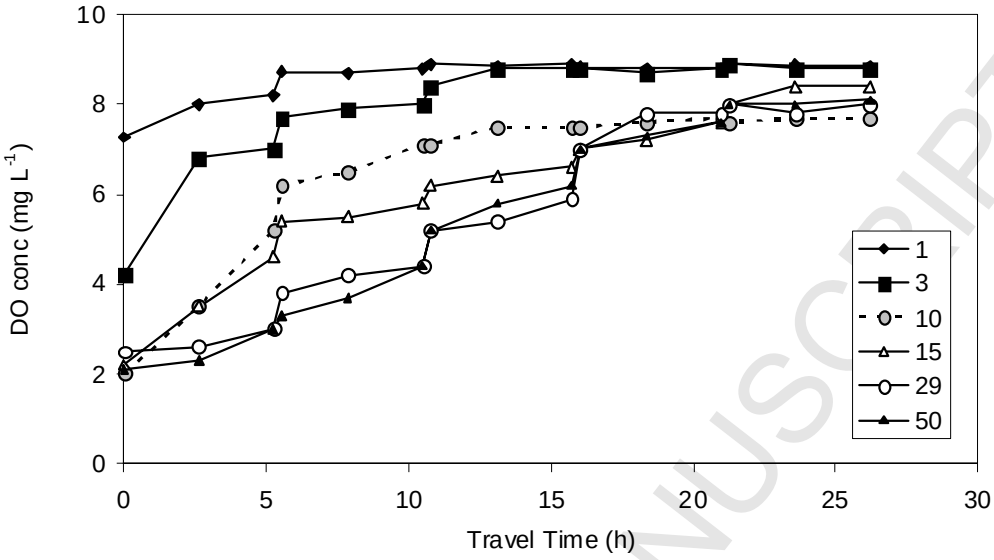

Figure 2 Observed dissolved oxygen concentrations with distance downstream through the cascade on different days (1, 3, 10, 15, 29, 50) of the study.

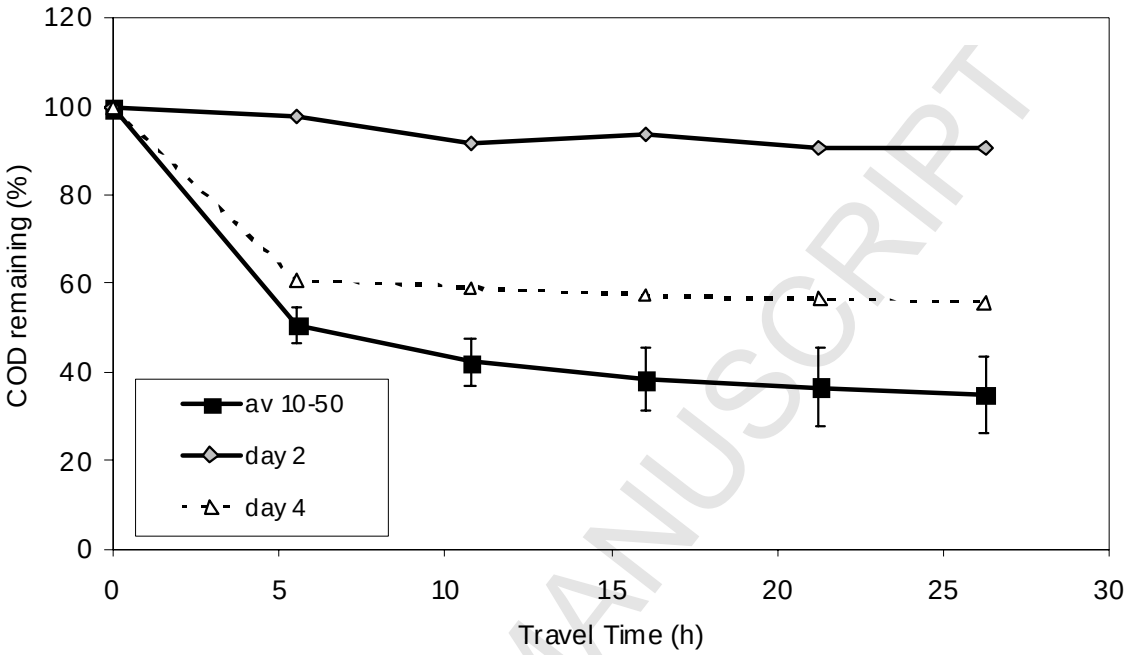

Figure 3 Observed changes in COD concentrations (normalised to the initial concentration) with travel time through the cascade on different days of the study (day 2, day 4 and the average ± SD of days 10-50).

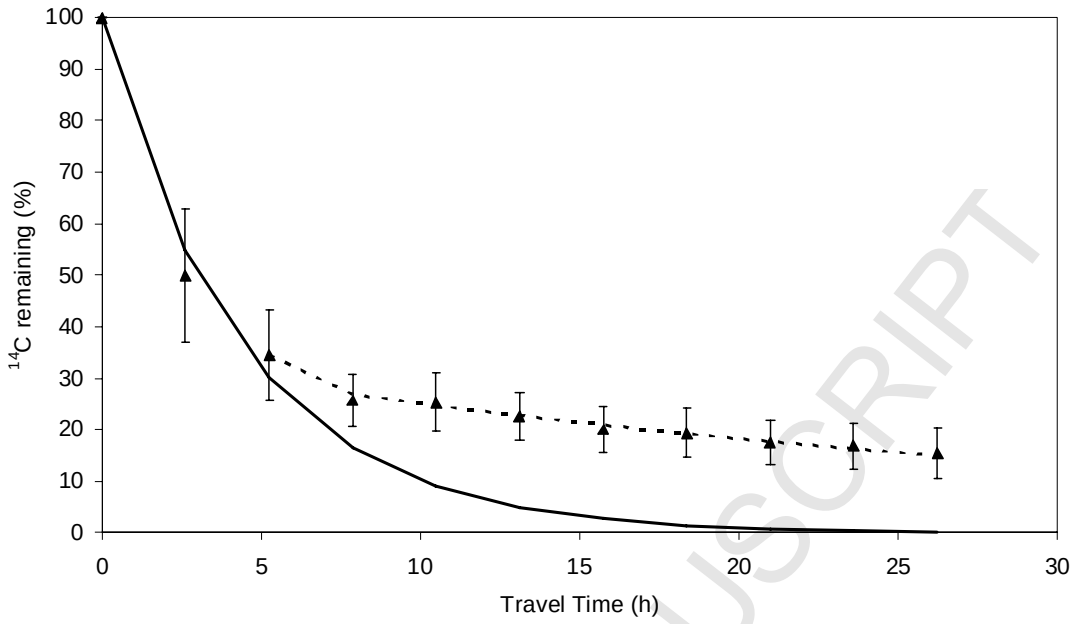

Figure 4 Fraction of aniline (counts) remaining with travel time along with best-fit solutions to first order kinetics for the first channel (solid line) and for channels 2 to 5 only (dashed line). Error bars show the measured mean 1 ± SD.

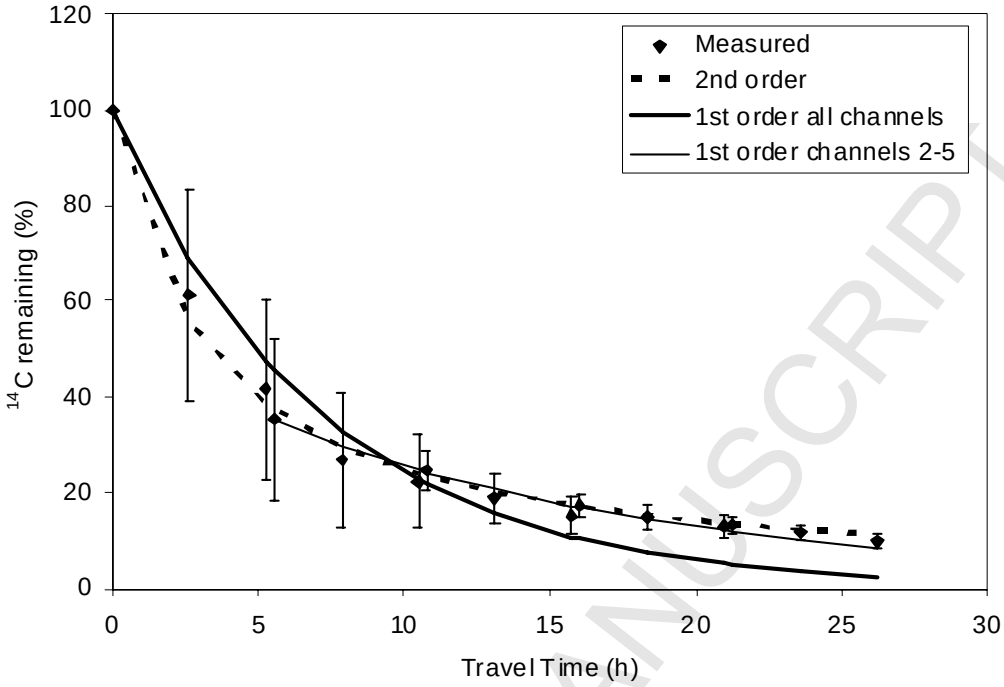

Figure 5 Observed and fitted pattern of the fraction of radiolabelled LAS (^{14}C Phenyl 6 DOBS) remaining with travel time in the cascade. Thick solid line shows the first order kinetic fit, the dashed line shows a second order fit and the thin solid line shows a first order fit to data from channels 2-5 only. Error bars show the measured mean 1 ± SD.

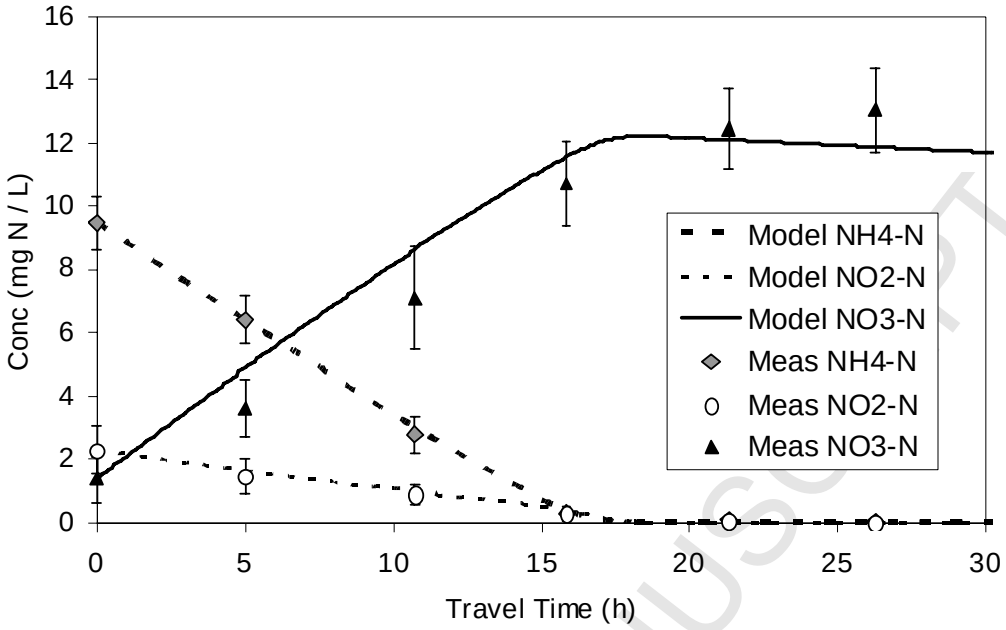

Figure 6 Mean measured and modelled mineral nitrogen concentrations in the cascade under steady-state conditions. Error bars show the measured mean ± 1 SD (*n* = 12).