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ENHANCED BIOLOGICAL TREATMENT OF SPENT METALWORKING FLUIDS BY PRIOR REMOVAL OF A POLYMER

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ABSTRACT

The biological treatment of spent synthetic metalworking fluid (MWF) has been investigated in many studies, with most reporting the presence of un-degraded components in the residual waste.[1-7] In this study, we investigated a hybrid approach to waste treatment of spent MWF, involving both physical treatment and microbial degradation. The effect on biological degradation, of the removal of two synthetic polymers, was investigated in a bioreactor system. Polymer removal was found to have little effect on degradation efficiency of the remaining components, but resulted in a waste product with a 14 % lower chemical oxygen demand (COD) resulting from the physical removal of the polymers. An additional benefit of this approach is that it enables the possibility of recycling of the recovered components, with the aim of economising on future product formulation and reducing waste discharge.

INTRODUCTION

Metalworking fluids (MWFs) are a vital and widespread component of the metal manufacturing industry. They are required as both coolants and lubricants to prevent damage of metal parts in working processes and ensure effective cutting and drilling.[8] Microbial contamination of in-use MWF is a common problem, which typically originate from machine workers, water (fluid diluent) and factory air.[9] This contamination is undesirable as it can lead to partial bio-deterioration of the MWF, whereby emulsifiers are degraded resulting in a reduction of

metal-processing properties. Synthetic MWFs are more resistant to in-use microbial biodeterioration, because of their complex mix of components, some of which are recalcitrant (non-biodegradable) and toxic.[10]

If contamination occurs and the MWF is spoiled, then disposal and replacement is necessary.[11] This is common place for the oil-based MWFs, whereas synthetic MWFs do not readily bio-deteriorate and therefore do not need to be so regularly replaced for this reason. However, routine periodic factory shutdown and unforeseen events inevitably results in the requirement for disposal. Consequently, over 400,000 tons of waste MWF is disposed of annually in the UK, which is usually achieved using chemical or physical treatment methods.[12] However, synthetic fluids are much harder to treat using these methods, due to the complex nature of their formulation, resulting in a more toxic aqueous waste that has to be disposed.[8]

Because of these difficulties there is growing interest in employing biological treatment systems for treating operationally exhausted MWF. Indeed, biological treatment systems have been successful in reducing the toxicity of waste MWF.[12] Recent developments have seen the exploitation of the indigenous MWF microbial communities to produce commercial inocula for bioprocessing of waste fluids.[13] However, some MWF components are difficult to degrade biologically (recalcitrant), particularly within synthetic formulations.[10]

The aim of this study was to enhance the potential for biological degradation, by identification and removal of recalcitrant components using physical approaches, using a specific synthetic MWF formulation as the model. The removal of a synthetic reverse-solubility polymer was investigated, as this component is known to constitute over 50 % COD of the MWF, but can be easily removed using chemical methods. This treatment results in a lower pollution load within the MWF to be degraded biologically, the investigation of which is presented in this study, and a portion of polymer. The possibility of recycling the polymer will be investigated in future studies.

MATERIALS AND METHODS

Metalworking Fluid

The synthetic MWF used in this study (supplied by Castrol Industrial Lubricants) was chosen because of its inherent recalcitrance. Such recalcitrance would pose a problem during biological waste treatment processes,

and so was selected as a model to represent those MWF that are difficult to treat biologically. The MWF selected for study comprises of twelve components, three of which are synthetic. Due to commercial sensitivities the precise identity of the components cannot be revealed, but are generically referred to as: amine 1 & 2, benzene derivative, organic acid 1 – 5, boron compound, biocide and polymer 1 & 2. The MWF is prepared from a fresh concentrate for industrial use at concentrations of 3 to 10 % (v/v). For the biodegradation studies described here, the MWF was prepared at a concentration of 1 % (v/v), with the addition (post polymer removal) of minimal media M9 to support microbial growth.[14]

Removal of Polymer

The polymer component of the MWF formulation studied represented 37 % (v/v) of the total organic content, with the property of reverse solubility, and precipitate out of solution at temperatures above 60 ^{o}C. In order to remove the polymer, MWF samples were incubated at 65 ^{o}C for 24 hours in a static oven, resulting in its precipitation and collection in the bottom of the solution. The polymer was rapidly collected by pipetting, so as not to allow the solution to cool and so dissolve back into solution. The MWFs were subsequently referred to as MWF +P (with polymer) and MWF -P (without polymer).

Bioreactor Operation

The study was undertaken in four open-top trickle filter reactors containing a solid matrix, and one sealed abiotic reactor, all of 4.8 l fluid volume. The MWF was continuously circulated using a peristaltic pump (Fisher Scientific, UK), at a rate of 0.12 l/min. The reactors were aerated using pumps and air spargers (Fisher Scientific, UK), at a flow rate of 200 l/hour, using a 0.2 μm filter to sterilise incoming air. The temperature was maintained at 28 ^{o}C using 200 W aquarium heaters. Hydraulic retention time of the MWF was 21 days. Samples (35 ml) were taken every 24 hours for 7 days, then approximately every 72 hours for the remaining 14 days. Prior to chemical analysis, MWF samples were filtered through a 0.2 μm membrane and stored at 4 ^{o}C.

Inoculum Preparation

The inoculum comprised of bacteria that were isolated from enriched in-use MWF samples in a previous study (Connolly H, unpublished). The eight bacteria included: *Flavobacterium* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Comamonas* sp., *Alcaligenes* sp., *Variovorax* sp. and *Bacillus* sp. Each bacterium was inoculated into two 250 ml conical flasks containing 100 ml tryptic soy broth (1:10 w/v, Difco UK), and incubated at 28 ^{o}C for 12

hours on an orbital shaker at 180 rpm. All 16 cultures were mixed thoroughly, divided into four portions and centrifuged at 10000 rpm for 10 min. The three pellets were resuspended in 1 % MWF B (v/v)/minimal solution (M9) and the remaining pellet was resuspended into 1 % MWF A (v/v)/minimal solution (M9). Each suspension was inoculated into its respective reactor.

Analysis of Amines

The two amines were analysed using a previously described method.[15] Briefly, cells were removed from the MWF by centrifugation followed by 0.2 μm filtration. A 0.5 ml aliquot of MWF was diluted with 0.5 ml distilled water and 0.02 ml of sodium hydroxide (9 M; BDH, UK) added. To the solution, 0.1 ml of copper (II) nitrate (20g in 100 ml distilled water; BDH, UK) was added and samples centrifuged at 14 000 rpm for 2 min to pellet the precipitate. A 1 ml aliquot of supernatant was removed and the absorbance measured at 650 nm using a UV spectrophotometer.

Analysis of Organic Acids

The five organic acids were detected by gas chromatography/mass spectroscopy (GC/MS), using a modified protocol previously described for the analysis of fatty acid methyl esters in cell membranes.[16] All chemicals were HPLC or ACS grade where applicable and supplied by BDH, UK. MWF samples (2 ml) were methylated by addition of 2 ml 6N HCl/methanol (1.18:1 v/v ratio), and 10 min incubation in a water bath at 80 ^{o}C (± 1 C) followed by rapid cooling. Fatty acid methyl esters were extracted by addition of 1.25 ml hexane/methanol (1:1 ratio) and 10 min turning on a clinical rotator. The lower aqueous layer was discarded, and samples washed by the addition of 3 ml NaOH (12g/l) followed by 5 min turning on a clinical rotator. The organic phase was analysed using Perkin Elmer GC/MS. For calibration, standards of 50 to 250 ppm were run for each acid and all unknown peaks quantified with respect to these standards.

Analysis of Benzene Derivative

MWF samples were subject to high performance liquid chromatography (HPLC) analyses using a Dionex HPLC system comprising P680 HPLC pump, ASI-100 automated sampler and UVD170U 4-Channel UV-Vis Detector. For calibration, benzene derivative (Castrol, UK) standards of 25 to 250 ppm were run and all unknown peaks quantified with respect to these standards. Samples were run for 10 min at a flow rate of 1.0

ml/min, using 15 mM acetic acid, 45 % (v/v) acetonitrile (BDH, UK), 55 % water (v/v) solution as the mobile phase, with detection at 254 nm. Peaks were analysed using Chromeleon 6.5 software.

Chemical Oxygen Demand

Chemical oxygen demand (COD) was used as a measure of total carbon remaining in the MWF. The COD was measured using Dr Lange COD cuvette tests, 5-60 g/l (914) and 1000-10000 mg/l (014), measured on a LASA 100 colorimeter (HACH LANGE, UK).

Inductively Coupled Plasma (ICP) Analysis

ICP analysis was carried out by BP-Castrol UK Ltd (Hyde, UK), using a Thermo Electron ARL 3580 optical emission spectrometer to determine the levels of boron within the MWF.

Toxicity testing using *Pseudomonas fluorescens* SBW25R::*luxCDABE*

Pseudomonas fluorescens SBW25R::*luxCDABE* was used a luminescent biosensor for testing the toxicity of the MWF.[17] *Pseudomonas fluorescens* SBW25R::*luxCDABE* was incubated in LB Media (Difco, UK) for 8 hours and cells harvested and re-suspended in phosphate buffered solution (Fisher Scientific, UK). MWF samples (180 µl) were pipetted into 96 well plates, followed by re-suspended culture (20 µl) and incubated at room temperature for 20 min prior to analysis on a LUCY plate reader.

RESULTS

COD Removal

The COD was used as an indicator of overall degradation of the MWF. The initial COD values for the two fluid types, MWF +P and MWF -P were 13500 mg/l and 9980 mg/l respectively. Physical removal of the polymer from the MWF reduced the COD by over 3500 mg/l. The final COD reduction was 34 % for MWF +P and 30 % (± 4 %) for MWF -P (Fig 1). The final COD values for these MWFs were 8883 mg/l O_2 and 7028 mg/l O_2 respectively. The abiotic reactor revealed a reduction in COD of 1 % with a final value of 13500 mg/l O_2. The majority of COD reduction was observed within 10 days, after which degradation slowed down. Overall, a greater portion of COD (1655 mg/l O_2) was removed from MWF A than MWF B during the bioreactor studies.

Chemical Analyses

Amine Analysis

The degradation of the two amines (amine 1 & 2) over the 21 days is represented in Fig 2. The method does not allow distinction between the two amines, but provides an estimate of the overall ethanolamine content of the MWF. The ethanolamine content within MWF +P and -P was degraded by 28 % and 25 % (± 1 %) respectively. There was a small decrease in the ethanolamine content of the abiotic reactor, of 5 %, which could be due to evaporation of these components or their removal though sampling. Again, degradation of the amines was at its greatest within the first 10 days.

Organic Acid Analysis

The degradation of the five organic acids (OA1-5) was determined by GC/MS analysis. Within 14 days, OA1, 2 and 4 had completely degraded and OA3 had degraded by 86 % and 91 % (± 8 %) in MWF +P and -P respectively (Fig. 3, A). However, OA4 was only detected on day 0 in the inoculated reactors, and was assumed to be below detectable range of the GC, as it was not detected in further samples. OA5 was the second most abundant acid in the formulation, but only showed reductions of 25 % and 24 % (± 5 %) in MWF +P and -P respectively, over the 14 day period. The MWF in the abiotic reactor showed no change in OA concentration over the 14 day period (Fig. 3, B).

Benzene Derivative Analysis

The concentration of the benzene derivative was monitored using HPLC analysis. A reduction in concentration of 84 % in MWF +P and 86 % (± 4 %) in MWF -P was observed over the 21 day period (Fig. 4). Over the same time, the concentration in the abiotic reactor remained stable, showing only a 4 % reduction.

Boron Analysis

The boron content of the MWF was determined using ICP analysis, and was found to decrease by 27 % and 29 % (± 2 %) within MWF +P and -P respectively (Fig 5). The level of boron in the abiotic reactor fell by 12 % over the 21 days.

Toxicity Testing

The bioluminescent organism *Pseudomonas fluorescens* SBW25R::*luxCDABE* was used as a biosensor to monitor the toxicity of the MWF throughout the bioreactor treatment. The toxicity was expressed as relative light units (RLU, %) of luminescence emitted by the biosensor. The initial level of bioluminescence emitted by the biosensor, for MWF +P and -P, was found to be 38 % RLU. The levels of toxicity were observed to be reduced to zero, shown as 100 % RLU, for both MWF +P and -P within 10 days (Fig 6). The toxicity in the abiotic reactor was found to reduce slightly, with an increase in RLU of 12 % observed over the 21 day period.

DISCUSSION

The aim of this study was to determine the effect of a physical pre-treatment step, on the biodegradation of a synthetic MWF. The MWF was a commercial formulation, and known to be particularly difficult to degrade biologically, and this was reflected in its lack of in-use spoilage. There is an increasing demand for synthetic MWF products that provide a long shelf-life with fewer spoilage problems, such as the one tested in this study. If biological degradation is to be a viable option for disposal of such products once they become operationally exhausted, in-depth knowledge of the degradable components and identification of recalcitrant components is crucial in enhancing the process.

The efficiency with regard to percentage COD reduction, of both MWF +P (with polymer) and MWF -P (without polymer) showed little difference. The removal of the polymer did not appear to affect the overall degradation potential of the MWF. Degradation rates in both treatments slowed down at a similar point at around 10 days after inoculation, leaving over 7000 mg/l COD remaining, and the chemical analyses enabled identification of the degradable and recalcitrant components. Three of the organic acids degraded within 14 days in both inoculated treatments, and the fourth acid was detected in minute quantities at time 0. The fifth acid revealed low degradation potential, within both systems, and remained at around 75 % of its initial concentration after 14 days. This component is of a more complex structure than the previous four organic acids, and identified as one of the components that would be problematic to degrade in a biological disposal system. Also, both of the amines proved difficult to degrade, only displaying approximately 25 % degradation within both MWF formulations.

The benzene derivative degraded efficiently within both inoculated treatments, with final values of approximately 15 % of the initial concentration. The degradation of the three OA and the benzene derivative can be attributed to microbial degradation, as the levels within the abiotic reactor did not significantly change throughout the same time period. The levels of boron appeared to decline over the 21 day time period, but would not be expected to be degraded. The reduction of boron levels may have been due to sequestration by cells within the fluid, which would not have been detected by ICP as only the cell free extracts were analysed.

The toxicity of the MWF declined to zero within both formulations during the first 10 days of the bioreactor treatment. This duration corresponds to the degradation of four of the organic acids and the benzene derivative. The loss of toxicity could be attributed to the removal of one of these compounds alone, or possibly a combination of the reduction of many components to levels that are no longer toxic to the biosensor. The toxicity of the individual components will be investigated in future studies to determine their effects on the biosensor.

The remaining 7000 mg/l COD, which accounted for approximately 50 % of the overall carbon content of the MWF, is likely to contain OA5, the two amines, and residual polymer. The biocide was present in this portion, but is known to be in minute quantities within the formula and will not constitute a great portion of the remaining COD. Therefore, any future biological degradation system will need to focus on strategies to overcome the recalcitrance of these components.

The removal of the polymer from the MWF, in a pre-treatment step, had no impact on the degradation of the MWF components. However, the inclusion of this phase allowed removal of an extra 2000 mg/l O_2 COD that could not be degraded in the pre-treatment fluid. Alongside the benefit of possible purification and recycling of the polymer, this pre-treatment strategy could promote the possibility of using biological disposal methods in place of conventional treatment processes. The results of this study demonstrate that a hybrid approach for treating industrial waste, including biological and physical separation, could lead to a more effective disposal method requiring little additional cost or time.

REFERENCES

1 Sutton PM, Kothari D, Mishra PN and Hachigan L, Biological treatment of metalworking fluids: a new application for fluidized bed technology. *Water Pollution Control Federation Conference*, Kansas City, Missouri 1-12 (1985).

2 Sutton PM and Mishra PN, Fluidized bed biological wastewater treatment: effects of scale up on system performance. *Wat. Sci. Tech.* **22**: 419-430 (1990).

3 Roberts JA, Sutton PM and Mishra PN, Application of the membrane biological reactor system for combined sanitary and industrial wastewater treatment. *Int. Biodeter. Biodegr.* **46**: 37-42 (2001).

4 Kim BR, Matz MJ and Lipari F, Treatment of a metal-cutting-fluids wastewater using an anaerobic GAC fluidized-bed reactor. *J. Water Pollut. Control Fed.* **61**: 1430-1439 (1989).

5 Kim BR, Anderson SG and Zelma JF, Aerobic treatment of metal-cutting-fluid wastewater. *Water Environ. Res.* **64**: 258-262 (1992).

6 Kim BR, Rai DN, Zelma JF, Lipari, F and Harvath PV, Biological removal of organic nitrogen and fatty acids from metal-cutting-fluid wastewater *Wat. Res*. **28**: 1453-1461 (1994).

7 van der Gast CJ, Whiteley AS and Thompson IP, Temporal dynamics and degradation activity of an bacterial inoculum for treating waste metal-working fluid. *Environ. Microbiol.* **6**: 254-263 (2004).

8 Buers KLM, Prince EL and Knowles CJ, The ability of selected bacterial isolates to utilize components of synthetic metal-working fluids as sole sources of carbon and nitrogen for growth. *Biotechnol. Lett.* **19**: 791-794 (1997).

9 van der Gast CJ, Knowles CJ, Wright MA and Thompson IP, Identification and characterisation of bacterial populations of an in-use metal-working fluid by phenotypic and genotypic methodology. *Int. Biodeter. Biodegr*. **47**: 113-123 (2001).

10 Anderson JE, Kim BR, Mueller SA and Lofton TV, Composition and analysis of mineral oils and other organic compounds in metalworking and hydraulic fluids. *Crit. Rev. Env. Sci. Tec.* **33**: 73-109 (2003).

11 Mattsby-Baltzer I, Sandin M, Ahlstrom B, Allenmark S, Edebo M, Falsen E, Pederson K, Rodin N, Thompson RA and Edebo L, Microbial growth and accumulation in industrial metal-working fluids. *Appl. Environ. Microb.* **55**: 2681-2689 (1989).

12 Department of Trade and Industry, A Guide to Biological Treatment for Metalworking Fluids Disposal. BIOWISE Biotechnology at work DTI/BW/25/2000/NP (2000).

13 van der Gast CJ, Whiteley AS, Lilley AK, Knowles CJ and Thompson IP, Bacterial community structure and function in a metal-working fluid. *Environ. Microbiol.* **5**: 453-461 (2003).

14 van der Gast CJ, Microbial dynamics of metal-working fluids [Thesis]. Oxford: University of Oxford; 2001.

15 Buers KLM, A treatment process for the degradation of metal-working fluids using mixed microbial cultures [Thesis]. Kent: University of Kent at Canterbury; 1997.

16 Thompson IP, Bailey MJ, Ellis, RJ and Purdy, KJ, Subgrouping of bacterial populations by cellular fatty acid composition. *FEMS Microbiol. Ecol.* 102: 75-84.

17 Wiles S, BIOMATE: development of custom-designed bioluminescent sensors for toxicity testing [Thesis]. Edinburgh: Napier University, 2001.

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Figure 1. Reduction of chemical oxygen demand (COD, mg/l O_2) within the bioreactors over 21 days: MWF +P (with polymer), inoculated; MWF -P (without polymer), inoculated; MWF +P (with polymer), abiotic. Error bars represent standard deviation of the mean (n=3).

Figure 2. Reduction (%) of ethanolamine content of the MWF within the bioreactors over 21 days: MWF +P (with polymer), inoculated; MWF -P (without polymer), inoculated; MWF +P (with polymer), abiotic. Error bars represent standard deviation of the mean (n=3).

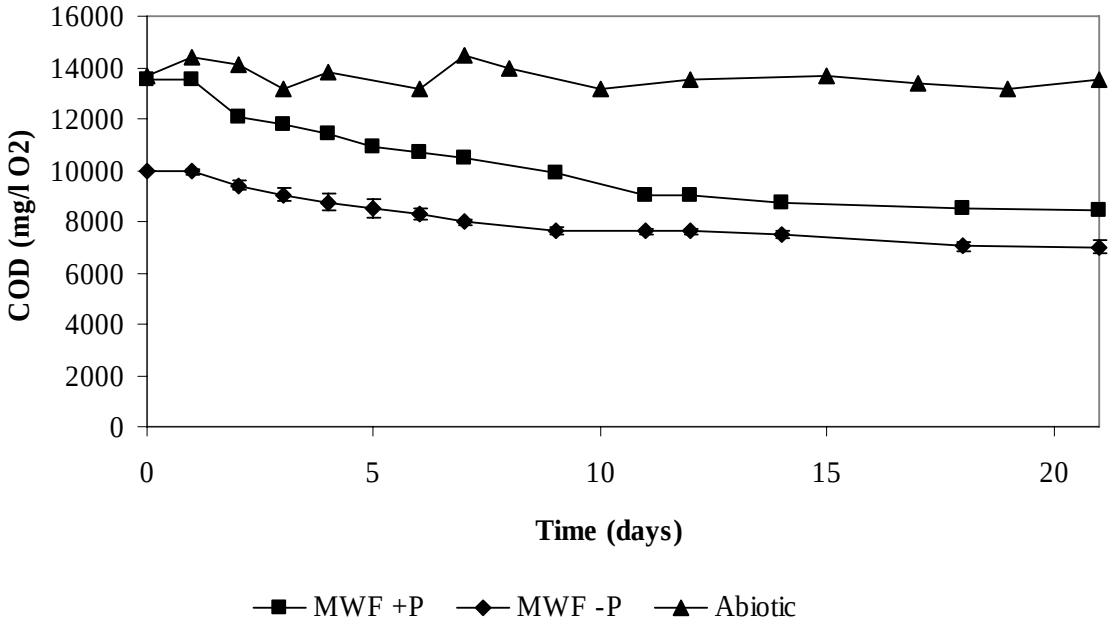
Figure 3. Reduction (%) of the five organic acids (OA 1 to 5) within the bioreactors over 21 days: (A), MWF +P (with polymer), inoculated; MWF -P (without polymer), inoculated; (B), MWF +P (with polymer), abiotic. Error bars represent standard deviation of the mean (n=3).

Figure 4. Reduction (%) of benzene derivative over 21 days in the bioreactors: MWF +P (with polymer), inoculated; MWF -P (without polymer), inoculated; MWF +P (with polymer), abiotic. Error bars represent standard deviation of the mean (n=3).

Figure 5. Reduction (%) of boron over 21 days in the bioreactors: MWF +P (with polymer), inoculated; MWF -P (without polymer), inoculated; MWF +P (with polymer), abiotic. Error bars represent standard deviation of the mean (n=3).

Figure 6. Toxicity (expressed as relative light units emitted) of MWF over 21 days, using bioluminescent biosensor *Pseudomonas fluorescens* SBW25: MWF +P (with polymer), inoculated; MWF -P (without polymer), inoculated; MWF +P (with polymer), abiotic. Error bars represent standard deviation of the mean (n=3).

1 Figure 1



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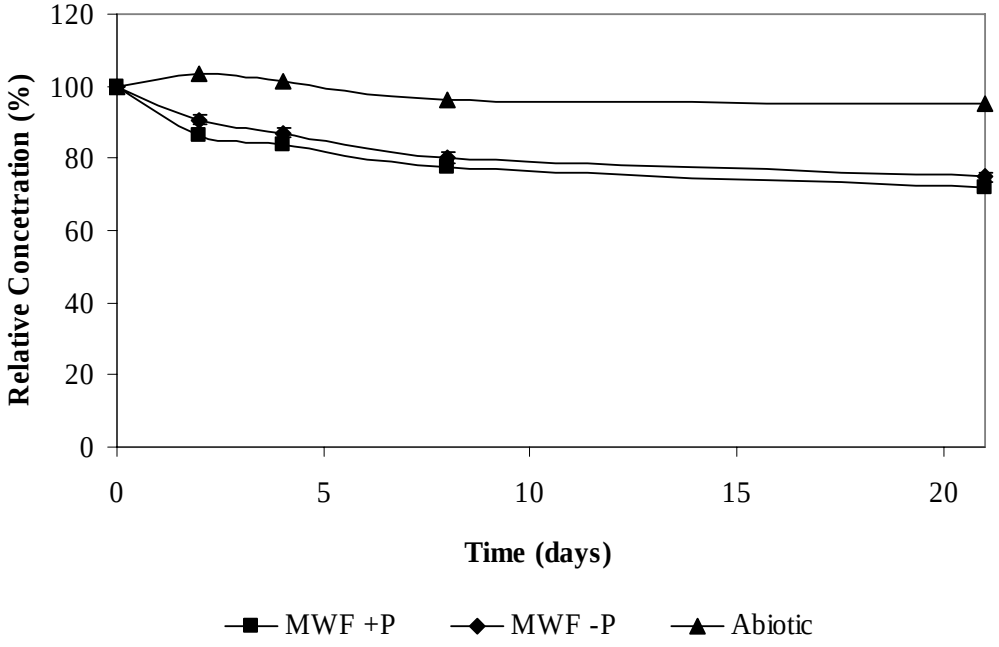

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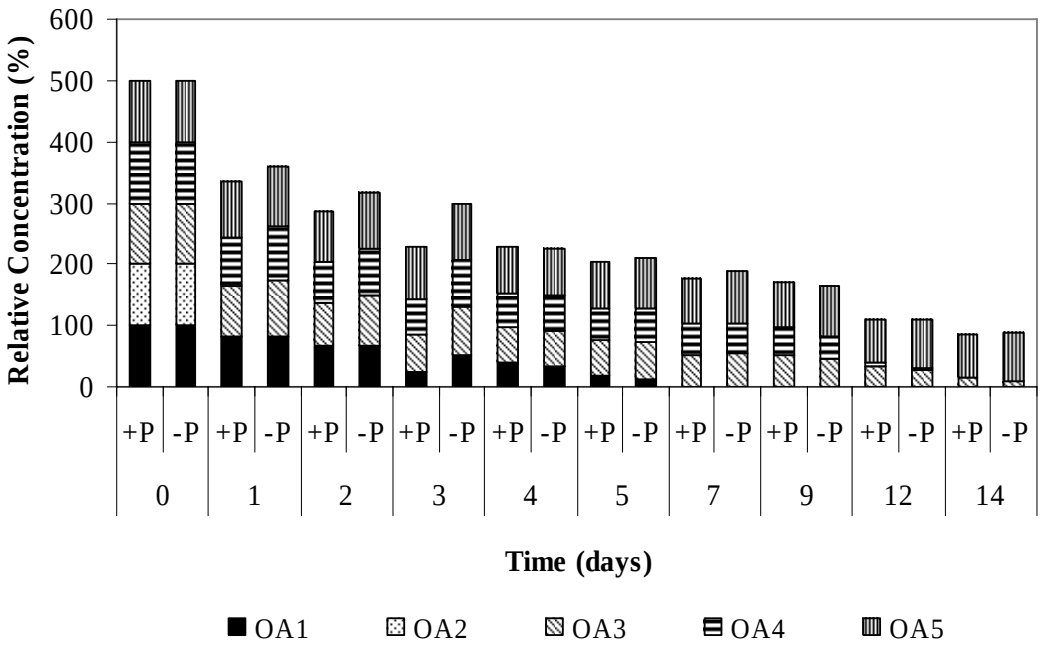
Figure 3A



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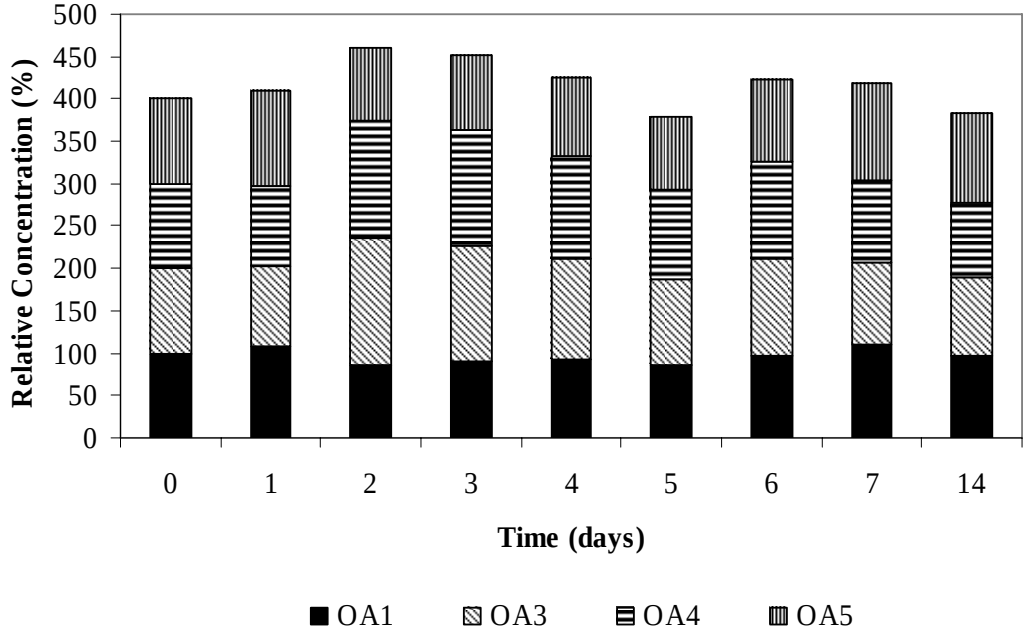

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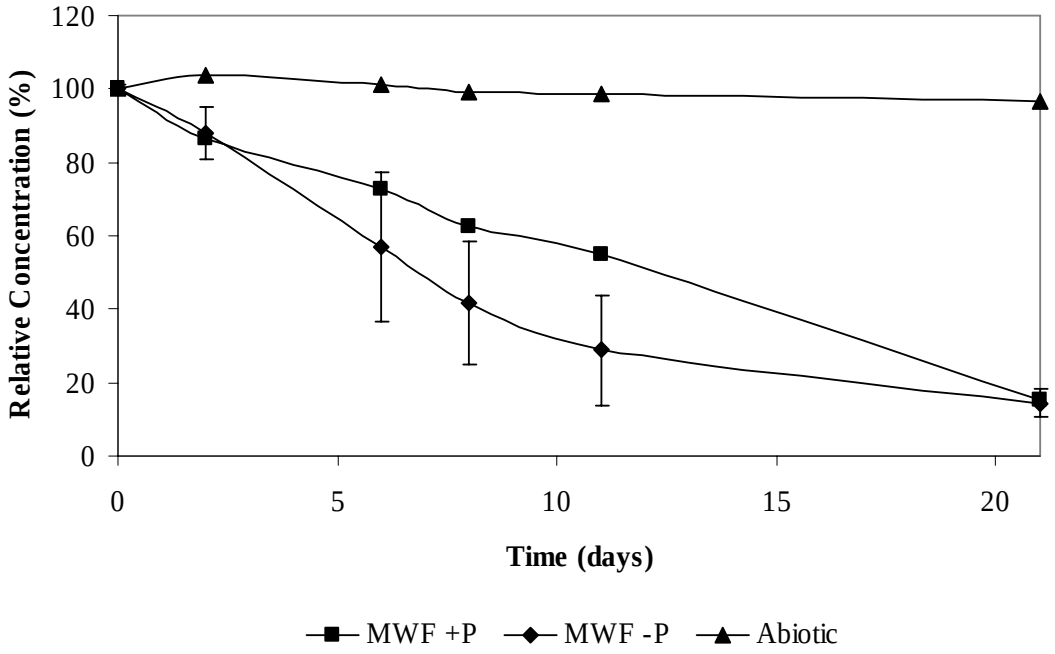

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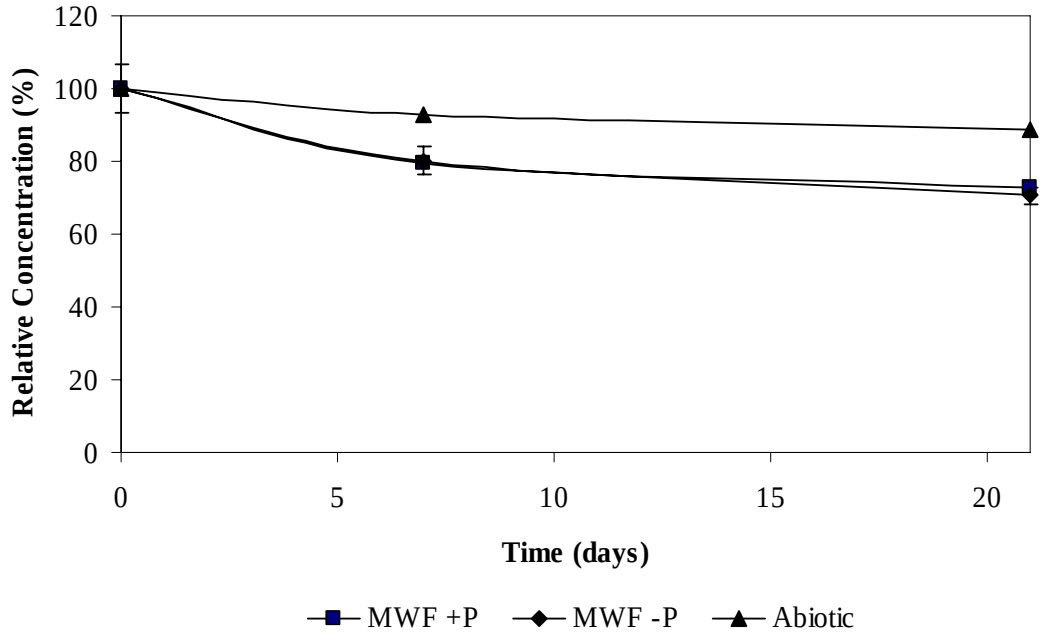

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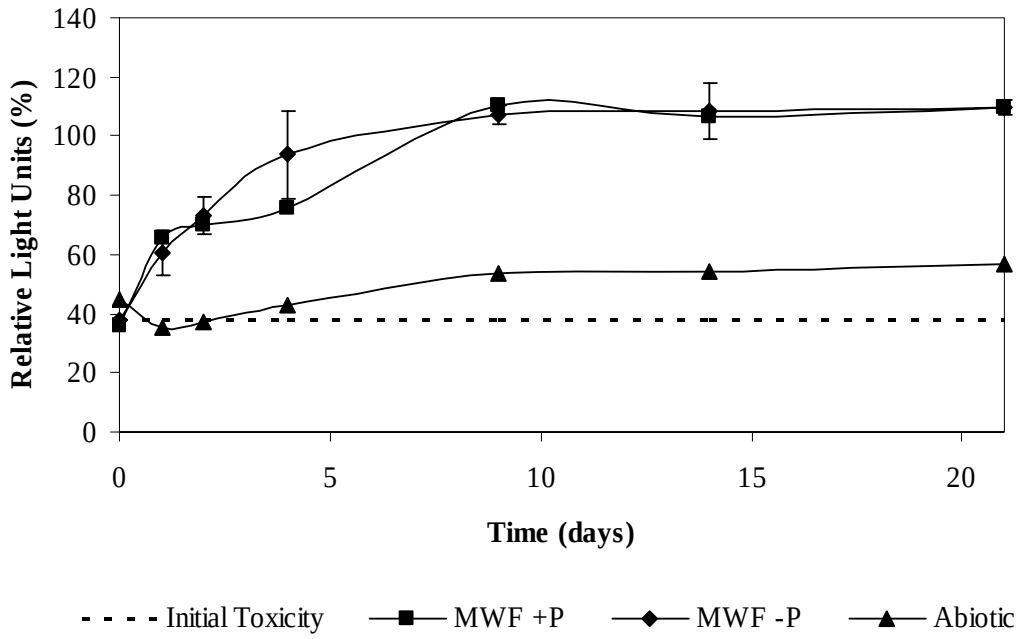
140
120
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Relative Light Units (%)
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5
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Time (days)
Initial Toxicity
MWF +P
MWF -P
Abiotic

2