

Comparative Analysis of Different Inocular Conditions on the Performance of a Bioreactor in the Treatment of Operationally Exhausted Metal Working Fluids (MWFs)

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Abstract

This work studied the biodegradation of the spent MWFs by using two inocular conditions: Indigenous microorganisms and seeding with Pseudomonas and Bacillus species (both earlier isolated from MWFs) both in a locally designed bioreactor. The performance of each inoculum condition was monitored by total viable bacteria counts, chemical oxygen demand (COD), biological oxygen demand (BOD), total organic carbon (TOC), oil and grease content and heavy metals residue over a 15-day period.

For indigenous organisms alone, the total viable counts ranged from 30×10^7 to 5×10^3 cfu/ml; COD, BOD & TOC showed reduction of approximately 34%, 37% and 24% respectively; oil and grease showed approximately 8% reduction and the results for heavy metal residues showed about 20% reduction for all heavy metals analyzed (Cu, Pb, Cd, Cr). Inoculation with the laboratory isolates showed high total viable counts throughout from initial 10×10^7 cfu/ml to 3×10^7 cfu/ml at the end of the period; COD, BOC, and TOC showed 44%, 51% and 37% reduction respectively; oil and grease content was reduced by 33% and result for heavy metals showed over 30% reduction for Pb and Cu while Cd and Cr were below 20% reduction.

The results showed that bio-augmentation with the laboratory isolates performed better than indigenous microorganism in the degradation of MWFs.

Keywords: Cutting fluids, heavy metals, microorganisms, biological oxygen demand and bio-augmentation.

Introduction

Metal working fluids are specially formulated fluid used as coolants and lubricants in a variety of industries such as automobile parts manufacture, in cutting and forming operations. Metalworking fluids typically are formulated to include chemicals that inhibit metal corrosion and microbial activity (biocides), whilst lubricating and cooling the metal cutting process (van der Gast *et al.* 2002). The fluid is comprised of eight main chemical constituents including a formaldehyde based biocide; benzotriazole (metal passivator), dodecanedioic acid, lauric

acid and sebacic acid (corrosions inhibitors); and amine propoxylate, glycerin and propylene glycol (lubrication agents) (van der Gast *et al.* 2003). While in use, metal working fluids accumulate foreign substances which include tramp oil, swarf, dissolved minerals and/or dirt from the process. In addition, the heat of operation and chemical reactivity of swarf fines initiates degradation of MWFs. The particles chemically interact with the oil in the emulsion weakening the emulsion and breaking the fluid down to its separate constituents. Swarf also facilitates the growth of microorganisms that feed on the coolant by providing a substrate for them to grow on. Microorganisms use nutrients containing carbon, hydrogen, nitrogen, sulphur,

traces of phosphorus, magnesium, calcium, and many other components normally found in emulsions used in the metal processing industry (Atlas 1988).

In order to use and dispose MWFs more effectively, the understanding of the indigenous microbial communities that colonize this product is significant. This knowledge may have two vital uses. First, identifying those microbial populations that spoil in-use MWFs would enable more targeted biocidal agents to be developed and a better assessment of the extent of the problem in order to control its biodeterioration. Second, identification of those communities that have the ability to colonize and catabolize in-use and exhausted MWFs provides realistic opportunities for developing inocula that can be exploited for disposal of waste fluids (Goddard *et al.* 2001).

At present, the majority of global waste MWF is incinerated, sent to landfill sites or treated at sewage works. However, with the imminent implementation of several proposed US Federal and European Union directives prohibiting incineration and landfill discharges. The present cost effective options for the waste management of spent MWF will no longer be viable (Environmental Protection Agency 1995, 2001; European Union 2000a,b).

One solution to this disposal problem is on-site biological treatment of waste MWF, using bioreactor systems. Bioreactor may refer to any device or system that supports a biologically active environment. From Petri dish, culture tube and to mechanically designed bioreactor. A bioreactor may also refer to a device or system meant to grow cells or tissues in the context of cell cultures. The bioreactor here refers to an engineering design that supports a biologically active environment. Such bioreactors are usually cylindrical, ranging in liters to cubic meters and are often made of stainless steel. The detail of its design is for the biochemical engineer.

Biological treatment of waste MWF by microorganisms in bioreactor systems has been investigated by several researchers (Baker *et al.* 1983; Kim *et al.* 1994; Roberts *et al.* 2000; Taylor 2001). Currently, bioreactors established for disposing of MWFs are commonly operated using a 'black box'

approach, typically inoculated with undefined microbial communities from sewage, a notoriously heterogeneous and potentially dangerous source because it is likely to harbour potential pathogens (Hamer 1997). As an alternative, bioaugmentation with carefully selected strains may improve the opportunity to create more reproducible systems that enhance degradation ability and by pre-emptive colonization, reduce invasion by opportunistic populations with little degradative ability. Evidence suggests mixed inoculation would be better suited for MWF treatment, as bioaugmentation of multi-substrate habitats (such as wastewater, ground water, soil or slurry) with pure cultures, has been typically proven ineffective (Goldstein *et al.* 1985; Bouchez *et al.* 2000).

This work sought to compare the performances of chance inoculation with bioaugmentation using two microorganisms, (*Pseudomonas* and *Bacillus* species earlier isolated from metal working fluid in the Environmental Microbiology and Biotechnology laboratory of the Department of Microbiology, University of Ibadan), in the biodegradability of operationally exhausted MWFs in a locally designed bioreactor. This is in a view to optimize condition that would make the working fluid environmentally friendly on final disposal.

Materials and Methods

Sample collection

Metal working fluids for the experiment were obtained from workshops unit in the Departments of Physics and Mechanical Engineering University of Ibadan. 15litres of fluid was used in each experiment.

Inoculation Conditions and Inocula

Two inocula conditions were investigated: indigenous microflora community of MWF (no addition inoculums) and bacterial consortium (indigenous community removed from MWF effluent by autoclaving). The consortium comprised of two bacterial genera earlier isolated from MWF in the

Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan. These were *Bacillus* and *Pseudomonas* species.

The two genera were inoculated separately into 250 ml conical flasks containing 50 ml of tryptic soy broth (Difco Uk) and fresh MWF concentrate prepared 6% v/v of distilled water and added as 10% v/v. The individual culture was incubated at 28^oC in an-orbital shaker, for 12hours. The suspension was removed and re-suspended in Minimal Salt Medium mixed together and added as 10% v/v inoculum into the bioreactor. The initial concentrations of microorganisms (zero hour) for indigenous organism alone was 30×10^7 cfu/ml and for inoculums was 10×10^7 cfu/ml.

Bioreactor Operation

Biodegradation study was performed in a bioreactor designed in the department of Mechanical Engineering Faculty of Technology, University of Ibadan. The reactor has 35 litres capacity. Air flow within the bioreactor was maintained through an electric blower. The bioreactor's temperature was set at 28^oC \pm 2. Samples were taken at the 0, 5th, 10th and 15th days for analysis.

Viable counts

Serial dilutions of sample from 10⁰ to 10⁻⁷ were prepared. 10⁻³, 10⁻⁵ and 10⁻⁷ dilutions were plated out on nutrient agar in duplicates. These were incubated and read after 24 and 48 hours.

Pollution Load Measurement

Biochemical oxygen demand (BOD), Chemical oxygen demand (COD), Total organic carbon (TOC), Oil and grease content determined using the standard method described by APHA (1985). The results obtained were expressed in percentages.

Heavy Metal Residue Determination

Heavy metal residue was determined according to Official standardized and recommended method of analysis. One

hundred milliliters of sample was taken and two milliliters (2 ml) of concentrated HNO₃ was added. The mixture was heated on a hot plate for two to three hours. The extract was filtered into a twenty milliliters standard flask and made-up to mark using deionized water. The extract was then stored in a plastic container for Atomic Absorbtion Spectrophotometer (AAS) analysis. The heavy metals assayed for were Lead (Pb), Copper (Cu), Cadmium (Cd) and Chromium (Cr). The data obtained were subjected to statistics analysis using ANOVA computer software by SAS Institute (2008).

Results

The result of the Logarithms of viable counts of the indigenous heterotrophic bacteria is shown in Fig. 1. It was observed that the growth of the bacteria population decreased continually as the day degradation increased. The Logarithms of viable count on the first day was 8.48 which reduced to 5.85 on the fifth day and 3.7 on the last day.

When the bioreactor was inoculated with the bacterial inoculum, there was a steady increase in the population of bacteria within the bioreactor as evidenced in the viable count until the tenth day after which it decreased. On the first day the Logarithm of the viable counts was 8.0 which increased to 8.18 on the fifth day and eventually decreased to 7.70 on the last day as shown in Fig. 2.

The result of percentage reduction in Biochemical Oxygen Demand (BOD) for indigenous heterotrophic bacteria and the inoculum is shown in Fig. 3. For the indigenous bacteria, it was observed that the BOD content continued to reduce as the days of degradation progressed. The percentage was reduced from 100% on the first to 71.3% on the fifth day which further reduced to 62.2% on the last day. While sample with the inoculum, the BOD reduced from 100% on the first day to 77.1% on the fifth day which later reduced to 49.4% on the final day.

The result of Chemical Oxygen Demand (COD) for indigenous heterotrophic bacteria showed that the COD reduced as the days of degradation increased. The percentage COD

reduced from 100% on the first day to 70.8% on the fifth day which reduced to 65.78% on the fifteenth day as shown in Fig. 4. The inoculated sample showed that similar pattern as the day progressed. The COD reduced from 100% on the first day to 82.96% on the fifth which reduced to 56.3% on the last day as shown in Fig. 4.

Analysis of Total Organic Carbon (TOC) content for indigenous heterotrophic bacteria showed reduction in TOC content as the day increased. The percentage COD reduced from 100% on the first day to 89.92% on the fifth day which declined to 75.33% on the final day as depicted in Fig. 5. TOC analysis for sample with inoculum showed reduction in TOC content as the day progressed. Percentage TOC reduced from 100% on the first day to 92.1% on the fifth day which reduced to 63.16% on the last day.

Oil and Grease analysis revealed that oil and grease content for sample with indigenous heterotrophic bacteria reduced marginally during the fifteen day period as the percentage oil and grease was reduced from initial 100% on the first day to 92% on the fifteenth day. Oil and Grease content's analysis when the bacteria inoculum was used in a bioreactor also showed reduction in oil and grease content. Percentage reduction was from 100% on the first day to 67% on the last day as shown in Fig. 6.

Figure 7 shows percentage reduction in heavy metal residue in bioreactor over fifteen day period for indigenous heterotrophic bacteria. It was observed that heavy metal contents of the MWFs were reduced to different degree by the organisms over 15-day period. The heterotrophic indigenous bacteria were able to reduce all heavy metals analyzed, having reduced Pb content from 100% to 80.07% on the fifteenth day, Cd content from 100% to 82.13%, Cu content from 100% to 79.97% on the fifteenth day and Cr content from 100% to 80% on the last day.

Heavy metal residue analysis for sample with bacteria inoculum showed reduction in Pb from 100% to 65.82%, Cd content reduced from 100% to 83.3% on the final day, Cu reduced 100% to 66.6% and Cr reduced from

100% to 82.5% on the fifteenth day as shown in Fig. 8.

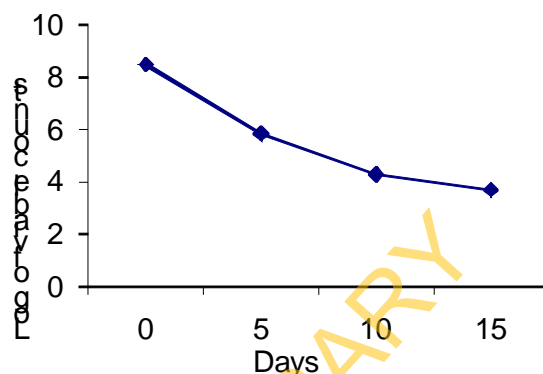


Fig. 1. The Logarithms of total viable count of bacteria (cfu/ml) within the bioreactor over 15-day period for indigenous microorganism alone.

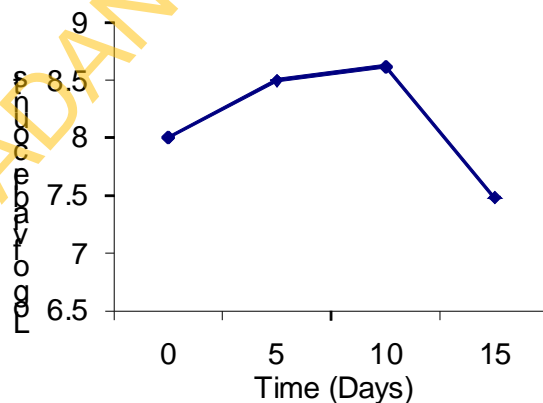


Fig. 2. The Logarithms of total viable count of bacteria (cfu/ml) within the bioreactor over 15-day period for the inoculum.

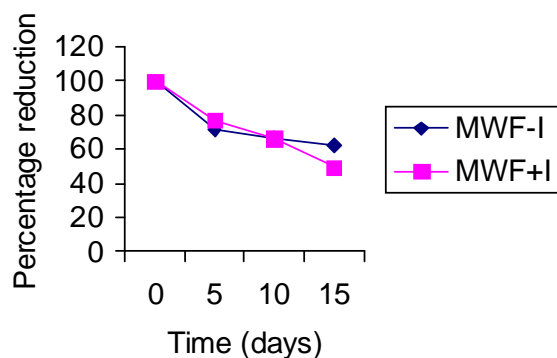


Fig. 3. Percentage reduction of biological oxygen demand (BOD, mg/lO₂) by indigenous microorganism (MWF-I) and inoculum (MWF+I) in the bioreactor.

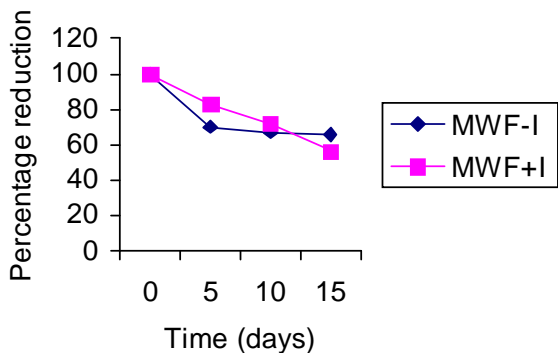


Fig. 4. Percentage reduction of chemical oxygen demand (COD, mg/l) by indigenous microorganism alone (MWF-I) and inoculum (MWF +I) in the bioreactor.

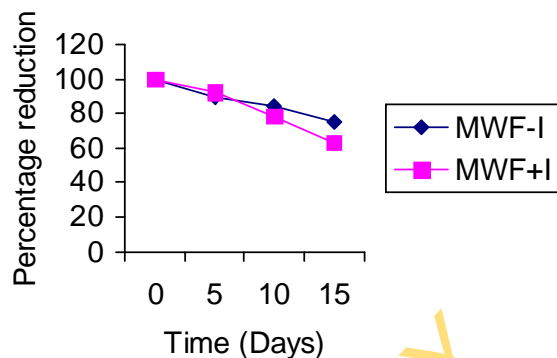


Fig. 5. Percentage reduction of chemical oxygen demand (TOC, mg/l) by indigenous microorganism (MWF-I) and inoculum (MWF +I) in the bioreactor.

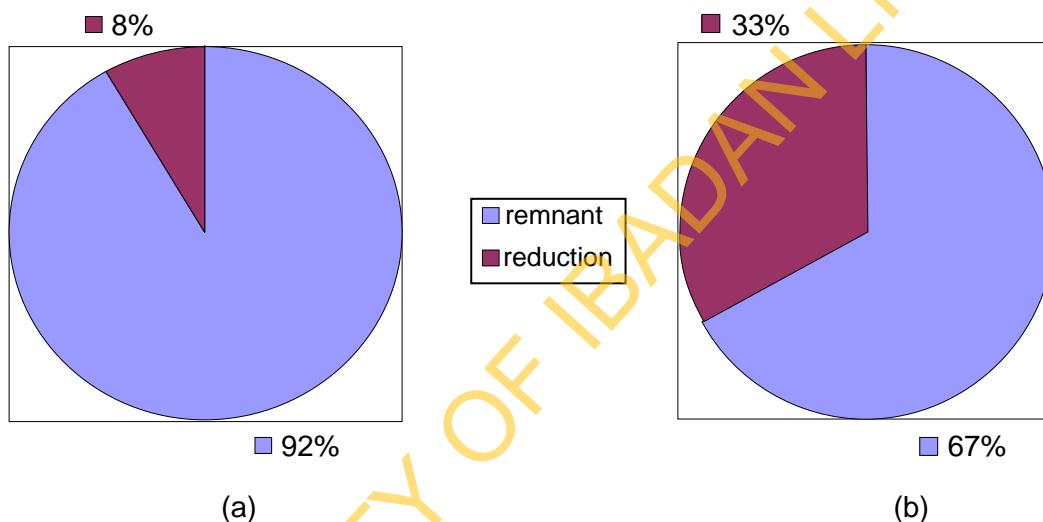


Fig. 6. Percentage reduction of Oil and Grease within the bioreactor over 15-day period by indigenous microorganism (a) and inoculum (b) in the bioreactor.

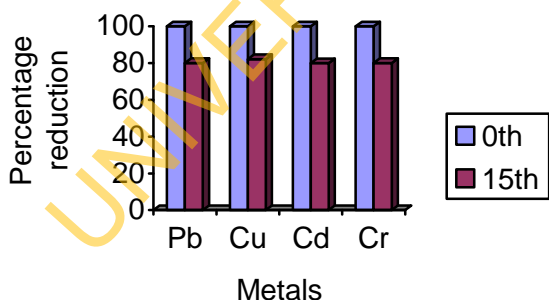


Fig. 7. Percentage reduction in Heavy Metal Composition of MWF over 15-day period by indigenous organisms in bioreactor.

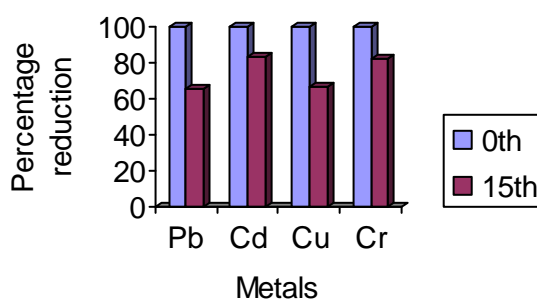


Fig. 8. Percentage reduction in Heavy Metal Composition of MWF over 15-day period by the inoculum in bioreactor.

Discussion

The results showed the heterotrophic indigenous organisms' population reduced from 30×10^7 cfu/ml on the first day to 5×10^3 cfu/ml on the last day during the period of experiment in the reactor. The results tend to suggest that the indigenous community must have comprised of many microorganisms contaminants which could not survive in the MWFs and thus led to the death rate observed (Jaksić *et al.* 1998). The bacterial inoculum population was shown to rise from the initial 10×10^7 cfu/ml to the maximum of 42×10^7 cfu/ml before declining to 3×10^7 cfu/ml at the end of the period. The bacterial growth was therefore high throughout this period. The inoculum population was able to stabilize and grow in the metal working fluids within the bioreactor because of survival advantage it has, having been isolated from such environment (van der Gast *et al.* 2002).

The BOD analysis of the inoculated sample showed continuous reduction throughout the period of the experiment. The inoculated sample gave a maximum reduction of approximately 51% in BOD while the uninoculated sample had reduction of 38% in BOD. This showed a strong relationship with the bacterial populations as the highest reduction in BOD tallied with highest bacterial growth. The inoculated sample had a reduction of 44% and the uninoculated had a reduction of 34% in COD which revealed that bacterial inoculum was better than indigenous microorganisms. The total percentage reduction in TOC was 37% in the inoculated experiment and 25% in the chance inoculated sample. These results tend to suggest that the indigenous organisms must have had as part of its population, organisms similar to the inoculum which was responsible for the reduction of the pollution load observed (van der Gast *et al.* 2002). Also, Atlas (1988) suggested that there has been utilization of organic matters present in metal working fluids as part of the chemical constituent for growth by the bacterial population for the observable reduction in analyzed parameters. This could

also be as a result of consumption of oxidizable compounds by the indigenous community of bacteria

The indigenous organisms showed marginal ability to degrade Oil and Grease (about 8%) as against that of inoculum which was 33%. The results tend to suggest the possible ability of the inoculum to secrete substances that are capable of solubilising oil and grease contents (emulsifiers), thus making such available for the bacteria use (Painter and King 1978).

The indigenous organisms were able to reduce all the heavy metals assayed for to approximately 20% whereas the inoculum gave between 17% and 34% reductions with the Cd being the smallest and the Pb being the highest. The reduction of the metals by the indigenous community of microorganisms must have been dictated by the need to use such metals as cofactors and part of cellular processes (Bruins *et al.* 2000). The inoculum having been isolated from MWFs environment must have acquired the capability to use the metals as either their sources of energy and or electron(s) as evident from the higher percentage reduction for the metals possibly because of acclimatization (Beaulieu *et al.* 2000; and Vogel and Walter 2001). The process of adaptation may also have conferred the capability to accumulate heavy metals in the inoculum (Wong and So 1993).

The results showed that inoculum performed better than the indigenous microorganisms for all parameters examined. This is in agreement with van der Gast *et al.*, (2004) that made case for the use of consortium of microorganisms for enhanced cutting fluid biodegradation. The result obtained in the study with combination two bacteria used in inoculating the cutting fluid for biodegradation is below that of van der Gast *et al.* (2004), who reported about 83% reduction in COD using consortium of four bacterial inoculums. Therefore, better result could be obtained if higher number of carefully selected organisms is coupled with optimization of environmental conditions of the bioreactor in terms of the regulation of the air flow rate, continuous agitation and temperature regulations.

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