Microbial contamination and bacterial treatment of cutting fluids from selected metal workshops in Ibadan, Nigeria

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Abstract: Studies were carried out on the microbial contamination and biotreatment of cutting fluid samples collected from nine different metal workshops within Ibadan metropolis. Isolation was done using the pour plate technique while the heavy metal concentration of the cutting fluids was monitored using the Atomic Absorption Spectrophotometer. The Total Organic Carbon (TOC) and Biochemical Oxygen Demand (BOD) which were used as indices for the biotreatment of the fluids by the bacteria isolates were monitored using different analytical procedures. A total of thirty bacteria were isolated from the cutting fluids samples. They include *Bacillus* sp, *Proteus* sp, *Staphylococcus* sp, *Citrobacter* sp, *Corynebacterium* sp, *Enterobacter* sp, *Acinetobacter* sp, *Alcaligenes* sp, *Flavobacterium* sp, *Klebsiella* sp with *Pseudomonas* sp being the most prevalent. The highest concentration of Pb was observed in the cutting fluid sample from the Physics Department (7.02µg/g), while the sample from Maintenance Department had 1.30µg/g Ni. The Zn and Cr level were detected to be highest in the sample from Tat metal workshop with concentration of 0.91µg/g and 0.19µg/g respectively. The ability of the isolates to treat the fluids (singly or in consortium) was assessed using the TOC and BOD of the cutting fluid after treatment using the bacteria species. The heavy metal concentration was lowered by all the treatment, however the highest reduction of the metal levels was observed in the fluid treated with the consortium (*Pseudomonas aeruginosa* and *Bacillus* sp.). The same trend was observed in the TOC and BOD values after treatment with the bacterial isolates. The consortium reduced the TOC of the fresh cutting fluids by 43.80% compared to 39.31% and 31.15% by *Pseudomonas aeruginosa* and *Bacillus* sp. respectively. The BOD value of the fresh fluid was reduced by 62.01% using the consortium as against the 51.42% and 41.27% reduction by the singular organism. The BOD and TOC of the spent cutting fluid, in similar vein were reduced effectively by the consortium with percentage reduction of 64.35% and 61.00% respectively. These bacteria can prove to be very effective in the biotreatment of environment contaminated with pollutants especially heavy metals.

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Key words: cutting fluids, heavy metal, BOD, TOC.

1.0 INTRODUCTION

Metalworking fluids, otherwise known as cutting fluids is a generic term covering a wide variety of fluids that are used as lubricants and coolants during the machining or treatment of metal components. These fluids are used in grinding, cutting, boring, drilling, and metal turning (Simpson *et al*., 2003). The cooling function is accomplished by the ability of the fluid to carry off the heat generated by the frictional contact between the tool and the work piece and/or any heat resulting from the plastic deformation of the work. Cooling aids tool life; preserves tool hardness and helps to maintain the dimensions of the machine parts. The second function is accomplished by the ability of the fluid to lubricate the tool-work piece interface in order to reduce tool wear, frictional heat generation and power consumption (Mackerer *et al*., 1995).

Traditionally, cutting fluids have been seen as a solution rather than a problem in metal cutting. They prevent overheating of the work piece, increase tool life, improve surface finish, help to remove swarf from the cutting area, reduce cutting forces, enhance size control and afford some corrosion resistance to work and machine tool (Trent and Wright, 2000). This set of attributes represents a significant benefit to the manufacturing process and, until recently, any problems associated with the use of cutting fluids have been accepted as the price of increased productivity.

Cutting fluids fall into two categories; namely the water miscible and non-water miscible fluids; although the former can also be sub-categorised into soluble, semi-synthetic and synthetic fluids. Each category has its advantages and disadvantages, and the choice is decided by the type of activity the oil is

to be used for and the quality output expected (Tolbert, 1997; Passman, 1988; Olds, 1973).

Cutting fluids are extremely complex lubricant which may contain eighteen or more components in addition to secondary reaction products which occur in the manufacturing process. They commonly contain hydrocarbon, fatty acids, emulsifiers, organo-corrosion inhibitors, amines, amides and glycols, but they may include organo-boron complexes, copolymer esters, alcohol, halogenated hydrocarbon, organophosphates and phosphate (Gannon *et al*., 1981). These fluids are complex mixtures of oils, detergents, surfactants, biocides, lubricants, anti corrosive agents, and other potential toxic ingredients. These fluids also serve to carry away debris from the work area and as such serve as a vehicle for the transmission of toxic materials e.g. heavy metals and other toxicants into the environment.

According to Mackerer *et al.* (1995), cutting fluids apart from their primary functions to serve as coolants and lubricants, should also protect the machine surfaces, tools and other equipment from rust and corrosion; should not themselves corrode, discolour or form deposits in or around the work area, should not produce undesirable fumes or smoke. And, in those instances where skin contact is unavoidable, the cutting fluid compositions should be non-toxic and dermatologically safe.

Microorganisms require a wide array of nutrient sources to flourish at maximum capacity. If the cutting fluid system does not provide one or more of the essential substrates, microbial growth will be limited by the absence of the missing component(s). However, with the addition of tramp oil; which originates from the various types of substances used to fulfil the lubrication requirements of the machine tool system, a new set of nutrients may be provided for the microorganisms, including those that the organisms may have been lacking. Sulfurized materials found in tramp oils have the potential to act as substrates for microbial growth (Abanto *et al*., 1994).

The aerobic flora of metal cutting fluid is now known to be composed of many different species. It is important to know which species grow in these products since they are implicated in cutting fluid deterioration. Growth studies of bacteria in cutting fluids have been reported by several researchers. Van der Gast *et al.* (2002) reported the isolation of several bacteria species from metal working fluids. Furthermore microbial enzymes may cause changes in viscosity, altering the lubricating and cooling properties. Acid products of microbial metabolism may lower the pH, causing corrosion. Excessive growth may clog filters and nozzles and may interfere with metalworking operations (Lonon, 1995). However, due to the diverse range of work, tool and material combinations, cutting fluids have evolved into a complex blend of oils and compounds; the evolutionary process demanding contemporary research into their effectiveness and environmental suitability (Schenach, 1999; Williams and Tabor, 1977; Howes *et al*., 1991).

This study aims at ascertaining the bacteria flora present in the cutting fluids and evaluates the potentials of these microbial species in the biotreatment of spent cutting fluids, with a view to generating an effluent that will be environmentally friendly.

2.0 MATERIALS AND METHODS

2.01 Sampling locations

The sampling locations are selected metal-cutting workshops in Ibadan, Oyo State, Nigeria as shown in Table 1.

TABLE 1: Sampling locations and mode of operation

WORKSHOP	LOCATION	OPERATION
Automech, metal workshop	Oke-Ado	Grinding
Inter-raymond, metal workshop	Iwo-Road	Milling
Maintenance Department	U.I.	Turning
Mak-Precision, metal workshop	Barika	Turning
Mechanical Engineering Dept	Ibadan Poly	Turning
Mechanical Engineering Dept	U.I	Turning
Physics Department	U.I.	Turning
Reliance, metal workshop	Iwo-Road	Milling
Tat metal workshop	Shasha	Milling

2.02 Sample collection and transport

A total of 18 samples of undiluted, diluted and spent cutting fluids (soluble oil) (2 replicates from each workshop) were collected from 9 different sources in Ibadan. The samples were collected in sterile sample bottles and transported to the Environmental Microbiology and Biotechnology

laboratory, Department of Microbiology, University of Ibadan, Nigeria on ice packs.

2.03 Physicochemical properties of the metal-cutting fluids

2.031 Determination of the Biochemical Oxygen Demand (BOD) of the cutting fluid samples.

The Biochemical Oxygen Demand (BOD) determination of the cutting fluid samples was carried out using the procedure of APHA (1985).

2.032 Determination of the Total Organic Carbon (TOC)

This was done using the procedure of APHA (1985). The Total Organic Carbon (TOC) for the cutting fluid samples were carried out in two steps: the oil was first concentrated using the oil and grease procedure, after which Total Organic carbon analysis was carried out.

2.034 Determination of the Heavy Metal Content of the cutting fluid samples

This was done using the method of ASTM (2000).

2.05 Preparation of culture media

All culture media were prepared following the manufacturers' specification. They were sterilized by autoclaving at 121^0C for 15 minutes.

2.06 Isolation, sub culturing and culture preservation

The isolation was done using the pour plate technique of Harrigan and McCance (1966). Appropriate dilution of the sample was plated out on the culture media, after serial dilution was carried out on the initial cutting fluid. The set up were then incubated at 37^0C for 18-24 hours. Distinct colonies were picked and sub cultured repeatedly to obtain pure cultures. The isolates were then preserved in 15% glycerol broth in freezing condition for subsequent investigation.

2.07 Screening of the isolates for cutting fluid utilization

The isolates were then screened for their ability to utilize metal-cutting fluid as the sole source of carbon. The mineral salt medium of Zajic and Supplison (1972) and Jensen (1975) with slight modification were used. The basal medium with 2% bacteriological agar, trace elements and carbon source were sterilized separately. 1% of the trace element was added to the basal medium after sterilization before addition of the metal-cutting fluid which served as the carbon source. The isolates were exposed to increasing metal-cutting fluid concentrations (up to 3%). Based on the luxuriance of growth on solid medium, two isolates were selected for further study.

2.10 Characterization of the bacteria

The isolates were characterized using Morphological, Biochemical and Sugar fermentation tests, and the results compared with schemes in the Bergey's Manual of Systematic Bacteriology 9th Edition (Holt, 1994) and Cowan and Steel's Manual for Identification of Medical bacteria 3rd Edition (Barrow and Feltham, 1993).

2.11 Bioremediation set up

Ninety millilitres (90ml) of the spent and the unused cutting fluids were dispensed into sterile flasks. The samples were sterilized at 121^oC for 15 minutes and then allowed to cool. The samples were then inoculated with 10ml aliquot of the each test organism and the consortium (ratio 1:1). This was incubated at 30^oC for a period of 5 days in a rotary shaker incubator at 150rpm. The Total Heterotrophic count, Biochemical Oxygen Demand (BOD), Total Organic Carbon of the materials recovered and the heavy metal content were determined after the set up was centrifuged at 10,000rpm for 10 minutes at 4^0C in the cold centrifuge.

RESULTS

The frequency of occurrence as given in Table 2 showed that *Pseudomonas* sp was the predominant organisms (36.7%) isolated from the metal-working fluids from all the locations employed in this study.

The physicochemical parameters analysed for the metal-working fluids included the pH, BOD and the TOC (Table 3) and the Heavy metals (Table 4) . The pH ranged from 6.70-8.30. However the BOD value was lowest in the sample obtained from the Physics Dept (0.11×10^3mg/l), and highest in the fresh metal-working fluid (16.83×10^3mg/l). The Total Organic Carbon (TOC), ranged from 0.49×10^5mg/g -3.94×10^5mg/g. Tables 5 and 6 showed the BOD and TOC values of the bio-treated Fresh and Spent cutting fluid.

Lead (Pb) was not detectable in the fresh metal-working fluid, and the sample obtained from the Automech workshop while the highest Lead value was detected in the sample from Physics Dept metal workshop (7.02 ug/g). The range of the Zinc (Zn) was between 0.03-0.91 ug/g; Nickel (Ni), was between 0.17 ug/g in the fresh sample to 1.30 ug/g in the sample from Maintenance department workshop. The Chromium concentration in the fluid samples ranged from 0.01-0.19 ug/g (Table 4).

The growth of the two isolates selected and the consortium on 3% fresh and spent metal –cutting fluids as enumerated by the Total Heterotrophic count is shown in Figures 1 and 2. In the fresh fluid, there was a noticeable rise in the THC for *Pseudomonas aeruginosa* throughout the 120 hour of incubation, this was not observed for *Bacillus* sp. as there was a drop in the THC from 0-72 hours, there was however a sharp rise from the 72nd hour onward. The consortium showed a drop in THC until the 48th hour when there was a rise till the 120th hour. For the spent fluid, a drop in THC was noticed for both

Pseudomonas aeruginosa and the consortium. The drop continued for the consortium before a rise was noticed, however from the 48th hour onward, the THC of *Pseudomonas aeruginosa* in the fluid rose till the end of the incubation period. This is in sharp contrast with the THC of *Bacillus* sp. which dropped throughout the period.

There was varying degree of reduction in the heavy metal constituent of the fluids after treatment with the bacteria isolates singly and in consortium. The metal working fluid treated with the consortium removed a higher percentage of all the metal species compared to the single bacteria as shown in Figure 3.

Table 2: Percentage occurrence of bacteria in the metal-working fluids

Isolates	Number of occurrence	Percentage occurrence (%)
Pseudomonas sp	11	36.7
Acinetobacter sp	3	10
Enterobacter sp	3	10
Serratia sp	1	3.3
Bacillus sp	2	6.7
Proteus sp	1	3.3
Citrobacter sp	2	6.7
Klebsiella sp	1	3.3
Corynebacterium sp	1	3.3
Staphylococcus sp	1	3.3
Alcaligenes sp	1	3.3
Flavobacterium sp	1	3.3
Unidentified	2	6.7
Total	30	100

Table 3: The Physicochemical parameters of metal-cutting fluids.

Sample	pH	BOD ($\times 10^3$mg/l)	TOC ($\times 10^3$mg/g)
Fresh oil	8.30	16.83	2.81
Automech	7.57	2.39	2.24
Inter-raymond	6.70	11.35	3.94
Maintenance Dept, UI	6.91	0.21	1.83
Mak-Precision, workshop	6.97	1.76	0.49
Mech Eng. Dept, Poly	6.78	0.61	1.84
Mech Eng. Dept, U.I	7.11	0.35	1.37
Physics Dept, U.I	7.04	0.11	3.20
Reliance metal workshop	6.98	0.78	6.40
Tat metal workshop	7.21	2.94	2.05

Table 4: Heavy metal concentration (ug/g) of metal-cutting fluid samples.

SAMPLES	Pb	Zn	Ni	Cr
Fresh oil	0.00	0.03	0.17	0.01
Automech	0.00	0.79	0.48	0.05
Inter-raymond	1.43	0.58	0.38	0.08
Maintenance	3.71	0.88	1.30	0.17
Mak-Precision	2.97	0.85	0.41	0.16
Mech Eng, Poly	4.16	0.45	1.06	0.09
Mech Eng, UI	ND	ND	ND	ND
Physics	7.02	0.75	0.49	0.07
Reliance	0.05	0.62	0.72	0.16
Tat workshop	1.34	0.91	1.00	0.19

ND: Not determined

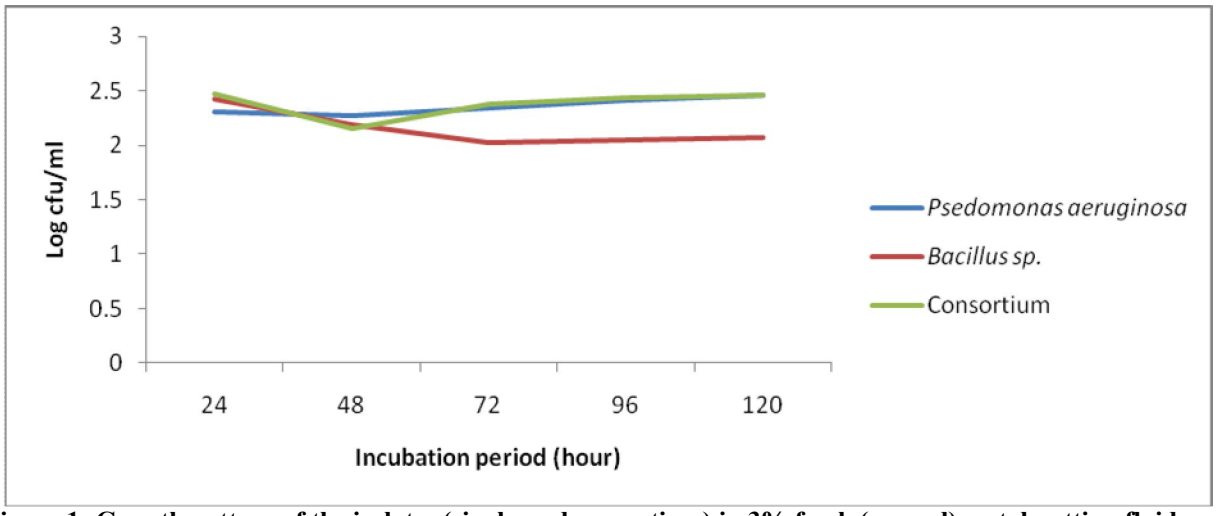

Figure 1: Growth pattern of the isolates (singly and consortium) in 3% fresh (unused) metal-cutting fluid.

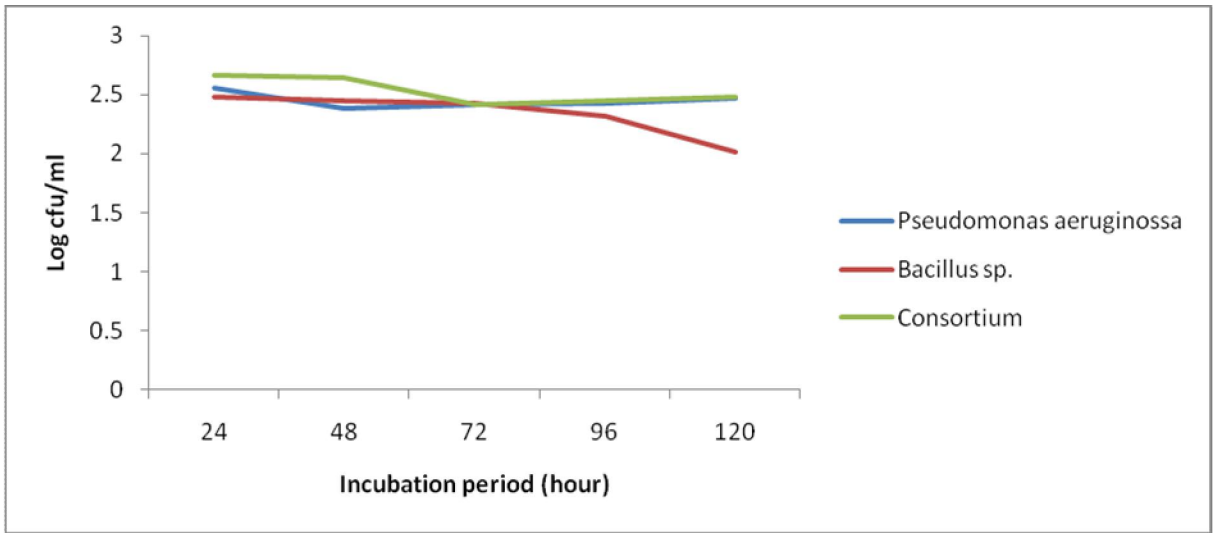

Figure 2: Growth pattern of the isolates (singly and consortium) in 3% spent metal-cutting fluid.

Table 5: Changes in the BOD of the biotreatment set up ($\times 10^3$ mg/l)

Samples	Fresh cutting fluid				Spent cutting fluid		
Duration	Day 1	Day 3	Day 5		Day 1	Day 3	Day 5
Control	0.0916				0.04320		
Ps	0.0652	0.0515	0.0445		0.0186	0.0168	0.0160
Bc	0.0673	0.0623	0.0538		0.0195	0.0175	0.0165
Consortium	0.0534	0.0418	0.0348		0.0175	0.0162	0.0154

Table 6: Changes in the TOC of the biotreatment set up ($\times 10^3$ mg/g)

Samples	Fresh cutting fluid				Spent cutting fluid		
Duration	Day 1	Day 3	Day 5		Day 1	Day 3	Day 5
Control	0.1104				0.0621		
Ps	0.100	0.075	0.067		0.0518	0.0345	0.0276
Bc	0.093	0.089	0.076		0.0522	0.0380	0.0311
Consortium	0.082	0.074	0.062		0.0414	0.0328	0.0242

Table 7: Percentage reduction in the TOC and BOD values after the 5- day treatment with the isolates (singly and in consortium).

	Fresh cutting fluid		Spent cutting fluid	
Treatment	**TOC**	**BOD**	**TOC**	**BOD**
Ps	39.31	51.42	55.50	62.96
Bc	31.15	41.27	49.90	61.81
Consortium	43.80	62.01	61.00	64.35

Key
BOD: Biochemical Oxygen Demand TOC: Total Organic Carbon
Ps: *Pseudomonas aeruginosa* Bc: *Bacillus* sp.
Consortium: *Pseudomonas aeruginosa* + *Bacillus* sp. (1:1)

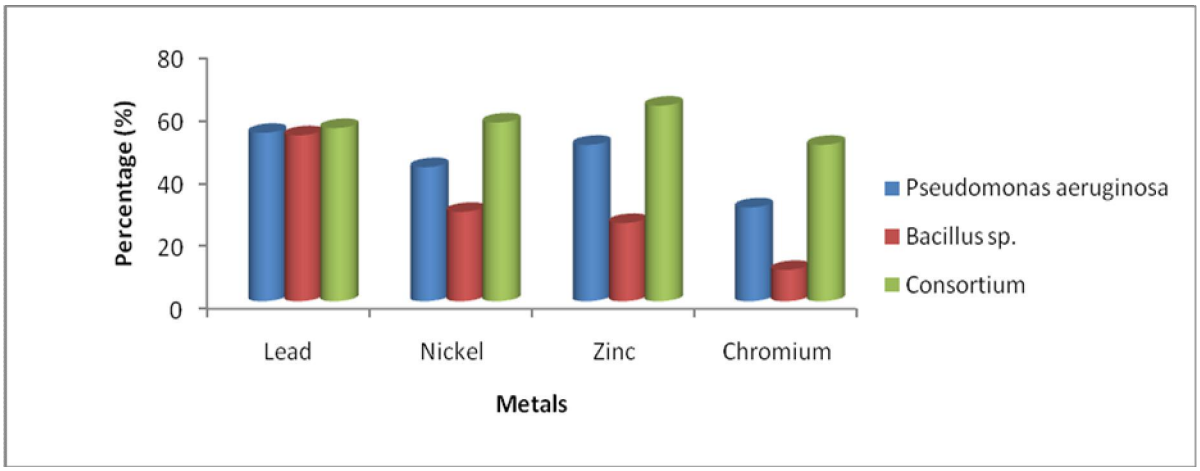

Figure 3: Percentage reduction in the metal composition of the metal-cutting fluids after treatment with the bacteria (singly and in consortium)

DISCUSSION

A total of thirty isolates were obtained from the metal-cutting fluids from all the sources (Table 2). They belong to the genera *Pseudomonas, Enterobacter, Flavobacterium, Bacillus, Acinetobacter, Corynebacterium, Staphylococcus, Alcaligenes, Klebsiella, Citrobacter, Serratia, Proteus*; this is similar in trend to the findings of Tant *et al*. (1956) and Lonon, 1995).who reported the growth studies of bacteria in metal-cutting fluids. *Pseudomonas* had the highest frequency of occurrence (36.7%) and this might be due to the fact that *Pseudomonas* sp has a generic penchant for nutritional adaptation and is structurally suited to be the pioneers in the cutting fluid ecosystem (Rossmoore, 1986). McCoy *et al*. (1981) also reported that *Pseudomonas* sp have external polysaccharide fibrils which allows them to initiate attachment to the tool surfaces, metal fines and begin biofilm formation and become dispersed throughout the system benefiting from extensive aeration produced by fluid flow. During growth, they can utilize all the organic components of the fluid, oxidizing alkanes to fatty acid, *B*- oxidizing fatty acids, deaminating amines, hydrolyzing petroleum sulfonates and degrading corrosion inhibitors. By their activities, they tend to discolour the fluids, cause separation of emulsions, and a drop in the pH.

The physicochemical parameters of the fluids as shown in Table 3, revealed that the fresh/unused oil has the highest pH and BOD compared to the spent samples obtained from the different sources (16.83 mg/l). This is because the sample is a freshly prepared emulsion and so the amount of oxygen required by the organism to break down the organic matter present in the sample is expected to be higher than those of the spent cutting fluid samples, since the sample has not undergone any form of microbial deterioration. The sample from Inter Raymond metal workshop also had a high BOD value of 11.35 x 10^3mg/l. This may be due to the fact that the workshop does not carry out much machining operations therefore the sample has not under gone much use and so the level of microbial contamination is low. The other samples had low values for Biochemical oxygen demand ranging from 0.11-2.39 x 10^3mg/l. This is because the samples had undergone deterioration during machining operations, indicating that some of the organic matter of the fluid has been used up by microorganisms (Lonon, 1995).

The TOC values suggest that the metal-working fluids from some of the workshops were high in

organic carbon which can serve as a potential nutrient source for the proliferation of microorganisms. The TOC which is a measure of the organic carbon available is higher in the fresh sample than that for most of the sampling sites and this might be due to the fact that fresh metal working fluids contain more carbon and phenolics compared to the spent oil (Van der Gast (2002), Also the metabolic activities of the microorganisms introduced into the oil during the machining processes might have greatly influenced the TOC values of the spent cutting fluids. However the same was not observed for three of the samples collected (Reliance, Physics Dept and Inter-raymond) and this might be attributed to the composition of fluids and the extent and length of the machining routine being carried out in the workshops.

There was a sharp drop in the TOC values of both the fresh and spent metal-cutting fluids treated with the bacterial isolates, singly and in consortium monitored over a 5-day period. Although the trend as shown in Table 7, depicts that the combination of both organisms achieved more reduction of the TOC values, showing that there might be a synergistic effect of both organisms in the biodegradation of the metal-cutting fluids, which according to Gannon *et al*. (1981), is a reliable tool to assess the potential biodegradative ability of isolates on metal-cutting fluids.

The heavy metal analysis showed the presence of heavy metals in both the used and unused samples. The very low levels of heavy metals found in the fresh oils indicates the non-addition of metals into the fluids because it has not been used in any machining operation, however the addition of heavy metals into the fluids during the machining process as a result of dissolution of metals in the fluids might be responsible for the observable level of metal species in the spent samples of the cutting fluids (Simpson *et al*., 2003; Chakrabati, 1981; Gannon *et al*., 1981; Njue *et al*., 1999). Another possible source of heavy metals is the corrosion of storage tanks holding the metal-cutting fluids before their use in machining. The bacteria from this study were able to reduce the metal composition of the metal-cutting fluids suggesting that they might have very good application in the bioremediation of heavy metal laden environment.

Shumate and Strandberg (1985); Andres *et al.* (1992); Fourest and Roux (1992); Hussein *et al.* (2001); Hussein *et al.* (2003) reported that microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) in their environment and this might be because microbial population in metal polluted environments contain micro-organisms which have adapted to toxic concentrations of heavy metals and become "metal-resistant". The metal concentration of the cutting fluid treated with the bacteria isolates reduced in different percentages with respect to the untreated cutting fluids, although the consortium removed more metal species than the individual isolate.

This study affirms the relative tolerance or resistance of the bacteria to the biocides present in the fluids or possible low-level of the biocide agent in the cutting fluids as outlined in the previous reports of Bennet (1972), hence there might be need to research into the response of the bacteria flora in the metal cutting fluids to antibiotics, because it is very possible that they might have acquired some form of resistance genes that will make them resist antimicrobial agents through their exposure to the biocide in the cutting fluids and therefore contribute to antibiotic resistance in the environment.

Recommendation

We will recommend the utilization of the inherent potentials of the indigenous microflora of the metal-cutting fluids in the bioremediation of spent fluids generated by the metal-working workshops to safeguard the environment against the influx of heavy metals and other toxicants generated by these workshops. Further studies should be geared towards the enhancement of the ability of these isolates to biotreat the metal cutting fluids.

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