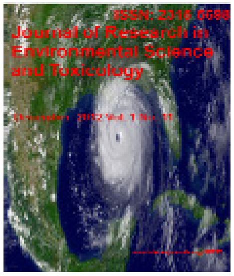

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Full Length Research Paper

Mitigation of the impacts of extrusion wastewater by microorganisms

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The microbiological and physico-chemical qualities of Aluminium extrusion wastewater were assessed to determine the extent of pollution using standard methods. Prior to treatments, total coliform counts ranged from 1.13×10^{3} – 1.8×10^{3} cfu/ml while physico-chemical analysis of the wastewater samples showed ammonia (1.6 – 2.0mg/l), nitrite (32 – 40mg/l), nitrate (23 – 28mg/l), dissolved oxygen (1.8 – 2.8mg/l) and pH (3.5 – 3.8) levels were not in compliance with the limits set by the Federal Environmental Protection Agency (FEPA). On treatment with Pseudomonas aeruginosa, Acinetobacter sp., Aspergillus flavus and Fusarium sp. at various combinations for 24 days, most of the parameters fell within the tolerant limit with the exception of nitrate level in most samples. It was shown that pure cultures of Fusarium as well as a consortium of the microbial isolates showed improvement in terms of nutrient reduction.

Keywords: Aluminium extrusion effluent, wastewater, bioremediation, physico-chemical parameters.

INTRODUCTION

Wastewater from industries include employee's sanitary waste, process wastes from manufacturing, wash waters and relatively uncontaminated water from heating and cooling operations (Emongor et al., 2005). Various industrial activities contribute significantly to environmental pollution. Wastes generated from these activities are discharged either directly or indirectly into the environment through public sewer lines (Asia and Akporhonor, 2007). According to a survey carried out by Ogbuagu and Ajiwe (1998), Aluminium extrusion and five other industries topped the list of manufacturing industries contributing to environmental pollution in Anambra State, Nigeria. These industries discharged their effluents into the environment with little or no treatments.

Effluents discharged from wastewater treatment plants have major detrimental effects on aerobic biota and the health of aquatic ecosystems. Increased nutrient load can lead to eutrophication (Gucker et al., 2006) and temporary oxygen deficit (Rueda et al., 2002). Ekhaise and Anyasi (2005) found that ammonium oxidation and decomposition of organic matter within receiving waters can have a significant drawdown effect on dissolved oxygen. Heavy metals contained in effluents (either in free form in the effluents or adsorbed in the suspended solids) from industries have also been found to be carcinogenic (Tamburlini et al., 2002).

In conventional wastewater treatment, some organic nitrogen, organic phosphorus and heavy metals associated with solids are removed during primary sedimentation while colloidal and dissolved constituents are usually not affected (Al-Rekabi et al., 2007). A sustainable alternative to the conventional methods in dealing with environmental waste is widely reported (Ugoji and Aboaba, 2004; Asamudo et al., 2005). According to Ahmedna et al. (2004), the use of natural biota and their processes in cleaning up wastewater is cost effective and the end products are non-harzardous.

Heterotrophic bacteria have been used in research on bioindicators in aquatic systems as a measure of sewage pollution (Ward, 2006). Their ability to utilize a given compound or various inorganic materials depends on the presence of a series of enzymes (Olutiola et al., 2000). While some bacteria have the capability to accumulate excess amounts of phosphorus as polyphosphates within their cells (Tchonobanoglous et al., 2003), competetion, predation and mutualism among the organisms also

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Contribute to the removal of organic matters from polluted water (Sudo and Aiba, 2006).

This study attempts to examine the pollutional strength of aluminum extrusion effluent and then evaluate the effect of microbial cultures on the microbiological and physico-chemical parameters of the wastewater.

MATERIALS AND METHODS

Collection of Samples

The wastewater samples were obtained from Aluminium Extrusion Company along the Lagos-Abeokuta expressway, Lagos. The samples were aseptically collected from two different discharge outlets (designated A and B – for the present study) into two sterile 2-litre plastic containers and immediately transferred to the laboratory in the Department of Microbiology of the University of Lagos, Nigeria. They were analyzed within 7 h of collection. Samples for BOD and DO were collected separately in pre-sterilized bottles. Where analysis could not be carried out immediately, samples were stored in a refrigerator at 4°C to inhibit biodegradation.

Isolation and Identification of Bacteria and Fungi

Bacteria and fungi were isolated by serial dilution and identified using Bergey's manual of Determinative Bacteriology (Holt, 1994), API –20E system (bioMerieux sa 62980, www.biomerieux.com) and methods described by Larone (2002) for bacterial and fungal isolates respectively. Pure colonies were stored at 4°C using nutrients agar slants for bacterial isolates and potato dextrose agar slants for moulds.

Isolation and Identification of Algae

Aliquot of each sample was placed in a haemocytometer counting chamber using a 1ml Pasteur pipette. Cells were viewed using the ×40 objective of a light microscope and enumerated. The process was repeated three times and the average determined. Algal cells were identified according to the method described by Wehr and Sheath (2003).

Isolation and Identification of Protozoa

This was done according to the method described by Rehman et al. (2007). Algae were excluded from the samples by keeping the culture in semi-darkness. Counting was done directly under the microscope at a magnification of ×100 and cells identified by observing their morphological features and movement (Minchin, 2003).

Treatment of Wastewater Sample

Four of the identified isolates *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Aspergillus flavus* and *Fusarium* sp. Were used for treatment at various combinations for 24 days. Each of the isolates was stabilized in sterile phosphate buffer and maintained in broth culture. Aliquot (2ml) of each culture or consortium was aseptically inoculated into 300ml of wastewater. Total viable counts (TVC/ml) and optical densities were taken every two days to monitor microbial growth in the effluent.

Physico-chemical and Statistical Analysis

The physico-chemical parameters of the wastewater samples were examined and recorded before and after treatment of the wastewater samples in accordance with standard methods (APHA, 1993). Results were analysed using one way analysis of variance (ANOVA) and treatment means compared using least significant difference (LSD $p<0.05$).

RESULTS

The physico-chemical characteristics of sample A is shown in Table 2. Values for nitrate, nitrite, ammonia, dissolved oxygen (DO) and pH were 23mg/l, 40mg/l, 2.0 mg/l and 3.5 respectively. They were not within the Federal Environmental Protection Agency (FEPA) standard for effluent discharge to surface water in Nigeria. Other parameters were within the range recommended by FEPA (1991) and EPA (2002). After treatments, while the nitrate and DO level still fell short of FEPA standard, other parameters examined were within the recommended range. There was a reduction in total coliform count from 1.13×10^3 cfu/ml to a range of 7.7×10^2 cfu/ml – 1.0×10^3 cfu/ml. Microbial growth in samples treated with *As*, *F*, *BF* and *AsF* were significantly different from the control while those treated with *A*, *Ps*, and *Aps* were not at $p<0.05$.

Similarly, the physico-chemical characteristics of sample B showed the pH (3.8), nitrate (28mg/l), nitrite (32mg/l), ammonia (1.6mg/l) and DO (1.8mg/l) levels were not within recommended range. After treatments, there was reduction in ammonia and nitrite level in the wastewater samples. The level of DO (1.1 – 1.8mg/l) was unacceptable. Samples treated with *F*, *BF* and *AsF* as well as the control had nitrate level reduced to recommended range while other treatments slightly exceeded the set limit of 20mg/l. Total coliform count ranged from 9.9×10^2 cfu/ml to 1.6×10^3 cfu/ml in the

Table 1. Total microbial count of Organisms before treatments

	Bacteria ($\times10^6$cfu/ml)	Fungi ($\times10^3$cfu/ml)	Coliform ($\times10^3$cfu/ml)	Algae ($\times10^3$cells/ml)	Protozoa ($\times10^3$cells/ml)
Sample A	5.6±0.4	3.4±0.5	1.13±0.4	3.0±0.4	6.6±0.7
Sample B	9.4±0.4	8.2±0.8	1.8±0.5	7.0±0.6	4.3±0.4

Values are mean of triplicate determinations ± SD

Table 2. Physico-chemical characteristics of wastewater before treatment

Parameter	Sample A	Sample B
Temperature (°C)	29.5	27.2
pH	3.5	3.8
Turbidity	132	50
TS (mg/l)	130	127
TSS (mg/l)	20	9.0
TDS (mg/l)	110	118
Total Alkalinity (mg/l)	22	15
Total Acidity (mg/l)	300	650
Calcium (mg/l)	50	42
Magnesium (mg/l)	98	91
Potassium (mg/l)	0.214	0.226
BOD (mg/l)	20	8.0
COD (mg/l)	35	12
DO (mg/l)	2.8	1.8
TOC (%)	0.12	0.06
Phosphate (mg/l)	ND	ND
Ammonia (mg/l)	2.0	1.6
Nitrate (mg/l)	23	28
Nitrite (mg/l)	40	32
Sulphate (mg/l)	38	52
Aluminium (mg/l)	ND	ND
Manganese (mg/l)	0.121	0.240
Iron (mg/l)	0.015	0.006
Zinc (mg/l)	0.656	0.026
Copper (mg/l)	0.026	0.102
Chromium (mg/l)	0.197	0.016

ND: Not Determined

treated samples - indicating a reduction from the initial 1.8×10^3 cfu/ml.

Generally, results of physico-chemical parameters obtained from treated samples were not significantly different from the control samples ($p<0.05$).

DISCUSSION

As shown in Table 3, these groups of organisms isolated are typical of industrial wastewater (Daims et al., 2006). The relatively high microbial population in sample B can be attributed to high nutrient load which supports their growth. The ciliates *Opercularia* spp. were abundant in the wastewater sample studied. According to Madoni et al. (1993), the presence of these organisms is highly associated with high effluent BOD and ammoniacal N concentrations.

In this study, the two bacterial species used in treatment (*Pseudomonas aeruginosa* and *Acinetobacter* sp.) exhibited different trends with respect to their growth patterns and utilization of nutrients. This is probably in line with the findings of Guest and Smith (2002) who reported that the bacteria responsible for the removal of nitrogen are susceptible to the influence of many unfavourable factors resulting in significantly reduced

Table 3. Organisms isolated from the wastewater

Organisms	Sample A	Sample B
Acinetobacter spp.	+	+
Pseudomonas aeruginosa	+	+
Bacillus spp.	+	+
Micrococcus sp.	-	+
Staphylococcus aureus	-	+
Aspergillus flavus	+	+
Fusarium sp.	+	+
Saccharomyces spp.	+	+
Geotrichum sp.	-	+
Penicillium sp.	+	-
Diatoms	+	+
Cryptomonads	+	+
Opercularia spp.	+	+
Paramecium sp.	-	+
Aspidisca spp.	+	+

+ : Present - : Absent

Table 4. Characteristics of Sample A after 24 days of treatment

Parameter	*C*	*As*	*A*	*F*	*Ps*	*BF*	*AsF*	*APs*
Ammonia (mg/l)	0.58	0.38	0.31	0.42	0.26	0.22	0.38	0.58
Nitrite (mg/l)	0.639	0.812	0.69	0.801	1.902	0.667	0.221	0.639
Nitrate (mg/l)	22.5	62.1	61.3	30.5	65.2	35.0	42.6	22.5
Phosphate (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND
pH	8.7	8.7	8.4	8.6	8.5	8.5	8.5	8.7
DO (mg/l)	2.4	2.3	2.1	2.1	2.0	2.3	1.8	2.4
Turbidity (NTU)	90.6	70.1	80.1	73.4	70.9	77.0	68.9	75.0
Sulphate (mg/l)	29.5	21.3	23.2	20.6	24.1	20.0	19.8	25.2
TSS (mg/l)	16.0	13.3	14.8	15.0	14.2	13.5	14.7	12.0
Zinc (mg/l)	0.092	0.083	0.111	0.040	0.081	0.038	0.051	0.101
Mn (mg/l)	0.029	0.012	0.024	0.008	0.011	0.015	0.002	0.020
Aluminium (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND
TC (cfu/ml)	1.0×10^3	9.0×10^2	8.1×10^2	9.1×10^2	8.6×10^2	7.7×10^2	9.7×10^2	8.2×10^2

C = control; *As* = *Aspergillus flavus*; *A* = *Acinetobacter* sp.; *F* = *Fusarium* sp.; *Ps* = *Pseudomonas aeruginosa*; *BF* = Bacteria (*Acinetobacter* sp. and *Pseudomonas aeruginosa*) + Fungi (*Aspergillus flavus* and *Fusarium* sp.); *AsF* = *Aspergillus flavus* + *Fusarium* sp.; *APs* = *Acinetobacter* sp. + *Pseudomonas aeruginosa*

TC = Total Coliform ND: Not Determined

performance or complete failure of the system. These factors according to them include, dilute wastewater, inhibitory chemical compounds, low temperature and low nutrients.

After 24 days of treatment (Tables 4 and 5), samples treated with pure *Fusarium* cultures (*F*) and mixed cultures of the moulds (*AsF*) had lower concentration of nitrate than other treatment options in both samples A and B (with the exception of those treated with *APs* in sample A). This suggests that some biological processes that culminate in nutrient reduction depend on the microbial species involved. Although partial denitrification by *Pseudomonas aeruginosa* and *Acinetobacter* sp. have been reported, many heterotrophic bacteria are incapable of converting nitrates to nitrites (Tchobanoglous et al., 2003).

The ratio of COD:BOD was found to be less than 2 before treatment. According to Quano et al. (1978), organisms have about 50 – 90 percent substrate biodegradation if the COD:BOD ratio ranges between 2 and 3.5. Therefore, there is the likelihood the wastewater may not be sufficiently treated using only biological method (Asia and Akporhonor, 2007).

About 71 – 99% ammoniacal nitrogen was removed after treatment. It is the preferred source of nitrogen for bacteria (Strous and Jetten, 2004). Dissolved oxygen (DO) level was not significantly improved after treatment. Low concentration of DO is one of the most common

Table 5. Characteristics of Sample B after 24 days of treatment

Parameter	*C*	*As*	*A*	*F*	*Ps*	*BF*	*AsF*	*APs*
Ammonia (mg/l)	0.38	0.16	0.03	0.16	0.07	0.08	0.18	0.02
Nitrite (mg/l)	0.053	0.050	0.045	0.077	0.003	0.034	0.064	0.02
Nitrate (mg/l)	8.9	22.0	23.0	12.6	27.4	19.0	10.3	25.3
Phosphate (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND
pH	8.0	7.8	7.6	7.8	7.9	7.8	7.8	7.9
DO (mg/l)	1.4	1.1	1.5	1.7	1.7	1.8	1.2	1.4
Turbidity (NTU)	20.3	20.0	22.1	20.2	19.5	17.0	17.8	18.5
Sulphate (mg/l)	31.4	25.0	28.0	22.7	25.9	25.2	23.9	26.3
TSS (mg/l)	4.4	3.9	4.5	4.0	4.8	3.3	3.7	2.8
Zinc (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND
Mn (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND
Aluminium (mg/l)	ND	ND	ND	ND	ND	ND	ND	ND
TC (cfu/ml)	1.6×10^3	1.2×10^3	1.1×10^3	1.5×10^3	1.4×10^3	1.4×10^3	9.9×10^2	1.3×10^3

C = control; *As* = *Aspergillus flavus*; *A* = *Acinetobacter* sp.; *F* = *Fusarium* sp.; *Ps* = *Pseudomonas aeruginosa*; *BF* = Bacteria (*Acinetobacter* sp. and *Pseudomonas aeruginosa*) + Fungi (*Aspergillus flavus* and *Fusarium* sp.); *AsF* = *Aspergillus flavus* + *Fusarium* sp.; *APs* = *Acinetobacter* sp. + *Pseudomonas aeruginosa*

TC = Total Coliform   ND: Not Determined

environmental disturbances which may detrimentally affect aquatic biota (Asia et al., 2006).

The TDS and TSS in these samples were within the range of FEPA recommendation. Suspended particles provide medium for microbial growth and heavy metal attachment. They adsorb nutrients and also form sediments by sedimentation (Inoue et al., 2009). Heavy metals in the effluent did not exceed their tolerant limits (Table 2). Heavy metal contamination of an area can be traced to industrial effluents (Sekhar et al., 2003). They must be removed if the wastewater is to be reused in order to prevent health hazards.

Treatment with *BF* had the highest coliform reduction (32%) in sample A and about 45% in sample B with *AsF*. Reduction in number of indicator organisms is brought about by the combined effects of separation of solid materials, predation, competition and inactivation due to changes in pH and temperature (Godfree and Farrel, 2005). They are often associated with faecal contamination.

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