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Assessment and biological treatment of effluent from a pharmaceutical industry

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ABSTRACT

The assessment of effluent collected from a Pharmaceutical Company in Ilorin, Kwara State, Nigeria was carried out. The effluent was analyzed for biochemical oxygen demand (BOD), Chemical oxygen demand (COD), total solids (T.S), suspended solid (SS), colour intensity and heavy metals, prior to being inoculated (treated) with pure and mixed culture of Saccharomyces (Fungi) and Pseudomonas aeruginosa (Bacteria) isolated from the pharmaceutical effluent. The bacteria and fungi were isolated through Pour plate method. The product of biological treatment was analyzed after five days of treatment. The result revealed that the effluent was initially of high BOD, COD, TS, DS, SS and pH. The method has significantly reduced COD to well below 250mg/L, BOD below 30mg/l and TS below 30mg/l which are upper limits for effluent's disposal into water. The heavy metals in the effluent were also drastically reduced with some completely eliminated from the sample.

Key words: Pharmaceuticals effluent, assessment, Pour plate method Saccharomyces, Fungi, Pseudomonas aeruginosa, Bacteria

INTRODUCTION

Effluent discharged from pharmaceutical company can be classified based on the type of pharmaceutical compounds such as antibiotics, prescription and non-prescription pharmaceuticals present in it (Roth and Etho, 2005). These effluents are of great environmental concern due to wide usage. For example, the result of effluent that contains Fluoroquinone antibiotics, when discharged to a river has led to the ability of bacteria to mutate into strains that are resistant to the widely spread antibiotics paving way for infections that cannot be cured (Benotti and Etho, 2005).

According to Benotti and Etho (2005), the understanding of the fate and effects of pharmaceuticals in the environment has progressed significantly in the last few years, but there is

still much work to do for a complete evaluation of the risks associated with the ubiquitous presence of these compounds. The existing methods of purifying effluents of industrial origin are divided into mechanical, physiochemical, thermal and biological processes. However, owing to the great variety of impurities and their complex composition, a combination of the effluent purification methods is generally used.

The biological or biochemical treatment, unlike other forms, is in a large scale and the most widespread method of treating sewage. The method is based on the biochemical oxidation of organic and inorganic substances due to the activity of microorganisms using the impurities such as a nitrite substrate and forming harmless oxidation products; water, CO₂, NO₃⁻ and SO₄²⁻ ions and also biochemical matter. The degree of decomposition of organic compounds in the biochemical treatment is characterized by the ratio BOD/COD. The greater the biological oxidation of a given waste, the higher is this ratio (Nsi, 2007).

In biochemical treatment of effluents, bacteria constitute the most numerous organisms. A very wide range of bacteria has been recorded, but the dominant aerobic genera appear to be gram-ve rods; *pseudomonas*, *achromobacter*, *alcaligens* and *flavobacterium*. Fungi are normally out numbered 8:1 by bacteria. Autotrophic bacteria tend to be more predominant in the lower layers of the biofilms with *Nitrosomonas* oxidizing nitrite to nitrate (Christopher, 2002). Protozoa, according to Lester (1996) and Algae, according to Laliberte *et al.*, (1994), have also been implicated in the biological treatment of effluent from pharmaceutical companies.

The efficiency of the treatment of effluents from pharmaceutical companies discharged into water networks can be assessed by the variation in the physiochemical properties, color, turbidity, pH, temperature, BOD, COD, heavy metals, dissolved oxygen etc, observed before and after the treatment (Nsi, 2007).

This study aimed at examines the efficiency of the biological treatment method, using the indigenous organisms isolated from the effluent of a pharmaceutical industry in Ilorin, Nigeria.

MATERIALS AND METHODS

Sampling area and Collection:

The effluent was collected from the pharmaceutical industry (Sam Pharmaceutical Limited located in Geri Alimi, Ilorin.) in a sterile 4 litre plastic container carried in an ice-chest and stored in a refrigerator until used for the analysis. The sample was collected from the channel of flow into the river and was passed through a sieve in order to avoid debris, paper twigs as well as silt or other small floating materials.

Physico and Biochemical analysis of the Effluent

The physicochemical and biological properties of the effluent were analyzed before and after treatment with isolated microorganisms as described by Nsi (2007), Alpha standard method (1989) and Aderomoti (1989).

Microbiological Analysis of the Effluent

A sterile Nutrient Agar plate was inoculated with 10⁻¹ml dilution of the effluent using the Pour plate method and incubated upside down at 35⁰C for 24hrs for the bacteria isolation. This was followed by the total bacterial count multiplied by the dilution factor. A sterile PDA plate was also inoculated with 1ml of the sample using the Pour plate method and incubated upside down at 37⁰C for 5 days, so that viable growth could be seen. Characterization and Identification

of Isolates were done as described by Fawole and Oso(1988) and Bergey's Manual of Systematic Bacteriology (Lacey and Cross 1986).

Biological treatment of the Effluent

The sample was divided into three and labeled A, B and C. the bacteria of interest which outgrows other bacteria species was singly inoculated in sample A. Sample B was inoculated with the Fungi while sample C was inoculated with bacteria and only isolated fungi. These samples were left for 5 days under normal laboratory conditions, and observed daily until a clearer mixture was obtained.

Determination of the concentration of heavy metals:

Digestion of the sample was done in triple acid mixture (5:1:1 HNO₃:HClO₄:H₂SO₄) (Kalesh et al, 2005). The concentrations of the metal in the digested samples were determined by Atomic Absorption Spectrophotometry (AAS) after appropriate dilutions.

RESULTS AND DISCUSSION

Identification of isolates:

The colonial morphological and biochemical characteristics of the isolates, in conformity with the Bergey's manual of systematic Bacteriology (Lacey and Cross, 1986) and Barrow and Feltham (1992), indicated that the organisms are; *Pseudomonas aeruginosa* and *saccharomyces spp* respectively.

Physicochemical parameters of the Effluent:

The results obtained from the parameters determined revealed overall percentage (with exception of turbidity, pH, electrical conductivity), reduction in the physicochemical, biochemical parameters and heavy metals in samples A, B and C respectively after treatment for five days. Table 1 show the initial high levels of BOD, COD, SS, and other parameters, as they were greatly reduced after treatment with each and combination of the organisms. Sample A, which was treated with only the bacteria appeared to be more remediated than sample B which was treated with the only isolated fungi. It was however less remediated than Sample C which was treated with the combination of bacteria and fungi.

Removal of Heavy Metals:

Table 2 revealed the activity of the microorganisms on the heavy metals in the effluent. It was discovered that the concentrations of the metals which were relatively high were reduced to the barest minimum most especially by the combination of the Bacteria and Fungi (94.3-100% reduction). The implication is that these two organisms can conveniently be used to bring the level of pollution resulting from the pharmaceutical effluent to a level that can be accommodated by the environment. Some of these metals(Zn,Cu and Mn) were even completely removed from the effluent within the period of the experiment

Efficiency of the Treatment methods:

Figure 1 shows the efficiency of the treatment methods in Samples A, B and C. The efficiency of the treatment ranges between 85 to 99% . It is observed that sample C with combination of Bacterial and Fungi yielded very good results (95-100% efficient). This was followed by sample A which was treated with Bacteria alone (about 92- 99% efficient) and Sample A with only Fungi least efficient (85- 97%).

Table 1: Overall Percentage Reduction of the physical and Biochemical Parameters in Samples A, B and C

Parameters	SAMPLE A		Remarks	SAMPLE B		Remarks	SAMPLE C		Remarks
	Initial	Final	% Reduction	Initial	Final	% Reduction	Initial	Final	% Reduction
Colour (HU)	20.0	15.0	25.0	20.00	18.00	10.0	20.0	12.0	40.0
Turbidity (FTU)	165	230	Increase	165	2500	Increase	165	220	Increase
pH	4.9	7.2	Increase	4.90	8.20	Increase	4.9	7.0	Increase
Electrical Conductivity μsm	159	530	Increase	159	392	Increase	159	540	Increase
Biological Oxygen Demand B.O.D. (mg l^{-1})	1120	340	96.9	1110	60.48	94.6	1120	17.92	98.4
Chemical Oxygen Demand C.O.D (mg l^{-1})	90.0	4.4	95.1	90.0	6.93	92.3	90.0	2.88	96.8
Total Solid (mg/l)	63.3	4.6	92.7	63.3	6.20	90.2	63.3	2.22	96.5
Suspended solid (mg l^{-1})	20.0	1.7	91.5	20.0	2.26	88.7	20.0	0.84	95.8
Total dissolved solid (mg l^{-1})	43.3	2.9	93.3	43.3	3.98	90.8	43.3	1.08	97.5
Volatile solid (mg l^{-1})	340	10.0	97.1	340	1.87	94.5	340	0.27	99.2

Note: Sample A: Bacteria + Effluent, Sample B: Fungi + Effluent, Sample C: Bacteria + Fungi + Effluent.

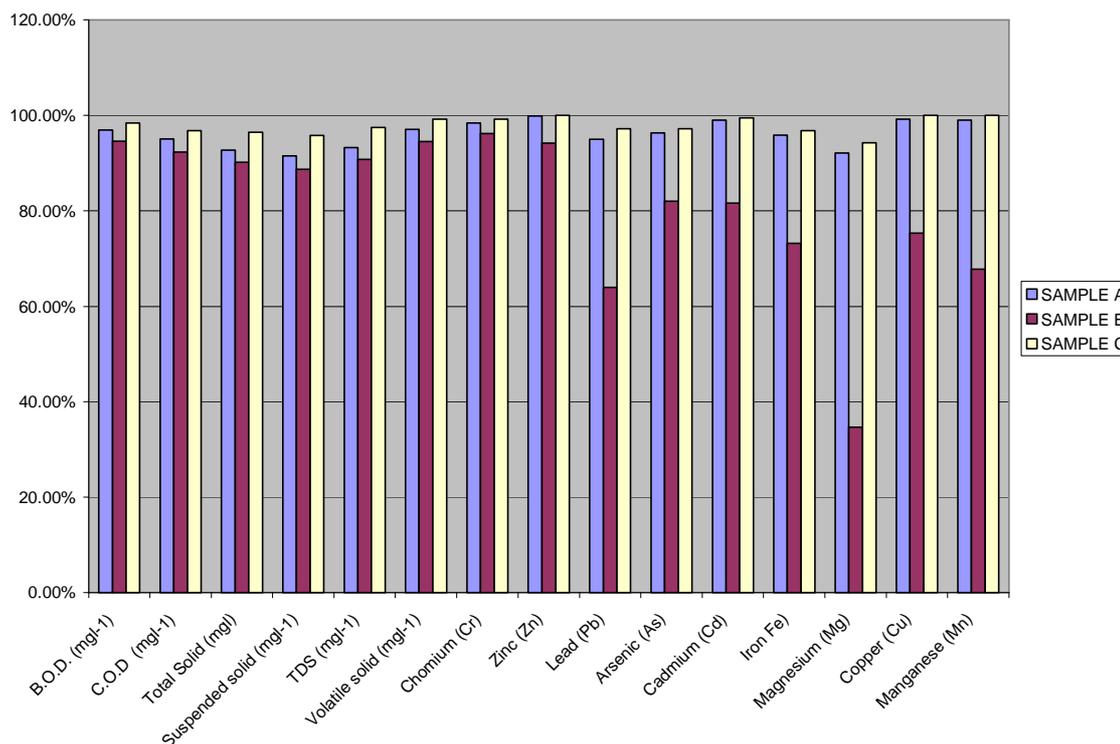
Table 2: Overall Percentage Reduction of Heavy Metals in Samples A, B and C

Heavy Metals (PPM)	SAMPLE A			SAMPLE B			SAMPLE C		
	Initial	Final	% Reduction	Initial	Final	% Reduction	Initial	Final	% Reduction
Chromium (Cr)	18.90	0.295	98.40	18.90	0.72	96.2%	18.90	0.15	99.2%
Zinc (Zn)	200.20	0.200	99.90	200.20	11.61	94.2%	200.20	-	100%
Lead (Pb)	11.80	0.589	95.00	11.80	4.25	64.0%	11.80	0.33	97.2%
Arsenic (As)	6.00	0.225	96.30	6.00	1.08	82.0%	6.00	0.17	97.2%
Cadmium (Cd)	2.40	0.025	99.00	2.40	0.47	81.6%	2.40	0.01	99.5%
Iron (Fe)	148.00	6.034	95.90	1480.00	39.66	73.2%	148.00	4.74	96.8%
Magnesium (Mg)	93.00	7.325	92.10	93.00	60.73	34.7%	98.00	5.30	94.3%
Copper (Cu)	115.80	0.910	99.20	115.80	28.60	75.3%	115.80	-	100%
Manganese (Mn)	60.30	0.594	99.00	60.30	19.42	67.8%	60.30	-	100%

Note: Sample A: Bacteria + Effluent, Sample B: Fungi + Effluent, Sample C: Bacteria + Fungi + Effluent.

1: Comparism of the Treatment Efficiency in Samples A, B and C

The increase in turbidity could be attributed to the presence of the particles resulting from the dead bacteria and fungi cells, due to nutrient depletion. The increase in the electrical conductivity and pH up to the neutral point in Sample C are very good indicator of efficiency of the method. The result obtained thus however, provides a giant stride to complement previous works in this area.



Figure

CONCLUSION

The high percentage efficiency of *Pseudomonas aeruginosa* and *Saccharomyces spp* in the treatment of effluent from pharmaceutical industries is a clear requirement for their inclusion and use for bioremediation. Their economic advantage could however be more harnessed when the combination of these two organisms is used.

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