Physicochemical Characteristics and Biodegradation of Pharmaceutical Effluent.

IBEGBULAM-NJOKU, P.N[1*], CHIJIOKE –OSUJI C.C[2] and IMO, E. O[3]

Abstract-Pharmaceutical and personal care products (PPCPs) industries suffer inadequate effluent treatment due to the presence of recalcitrant substances, insufficient carbon source and nutrients. Biodegradation is the ability of microorganisms to metabolize pollutants in soil, sediment and water environment. There is great awareness in the positive impact that microorganisms play in protecting environmental and human health. A large number of pre treatment systems are employed to remove these pollutants to prevent a host of problems that may arise in the biological process thereby reducing the efficiency of the treatment plant. Pharmaceutical effluent collected was characterized for their pollution characteristic and resultant analysis showed that the total suspended solid (TSS) and total dissolve solid (TDS) were 150.4 and 120.7 mg/l respectively while biochemical oxygen demand (BOD) and Chemical oxygen demand (COD) were 1720 and 5680 mg/l respectively. These values are high when compared to World Health Organization maximum permissible limits (Chikogu *et al*., 2012).Initial treatment of this effluent with selected organisms yielded slight reduction in the solid concentration, BOD and COD, hence optimization of biodegradation of the selected organisms. Optimization of biodegradation with 2% carbon source and nitrogen respectively showed improved biodegradation of pharmaceutical effluent with maltose showing 78.5% TDS reduction, cassava starch 95.8% BOD and 97% COD reduction respectively in *Saccharamyces cerevisea* treated pharmaceutical effluent. Similarly yeast extract was best utilized by *Saccharamyces cerevisea* with 99% BOD, 82 % TDS reduction and 90.5% growth in the treated effluent while it reduced COD to 98.2% in *Pseudomonas aeruginosa* treated pharmaceutical effluent. However optimization of biodegradation with carbon and nitrogen sources was not properly utilized in *Aspergillus niger* treated effluent.

Keywords: Pharmaceutical effluent, Pollution, Biodegradation, Physico-chemical characteristics , Recalcitrant.

INTRODUCTION

The increasing contaminants of pharmaceuticals and personal care products (PPCPs) have drawn serious attention due to their hazardous effect on environment and human (Yu and Wu, 2012).

Their occurrences are reported globally in a range of aquatic environments and land, sludge (Thomaidis *et al*.,2012; Calafat *et al*.,2008; Giokas *et al*.,2007; Buser *et al*.,2006, Balmer *et al*.,2005).

Pharmaceutical and personal care products (PPCPs) effluents are wastewater generated by these industries during the process of drug manufacturing. Their effects to environment are so enormous. The increase in demand for keeping a healthy life has resulted in the establishing of more pharmaceutical and PPCPs manufacturing companies in Nigeria. The risks of toxic substances in pharmaceutical effluent cannot be over emphasized as it causes danger to fish

(feminization of male fish), frogs, wildlife and increased levels of resistance of antibiotics by microorganisms since most antibiotics turn up in the environment whether through excretion, dumping, washing activities of the equipment or wash off discharges although its danger is reported to be

- Ibegbulam-Njoku P.N* has Ph.D (Microbiology) from Michael Okpara University of Agriculture, Umudike, Nigeria. E-mail: peacennem@gmail.com Mobile: +2348033603237, +2348027425013.
- Chijioke-Osuji C.C is currently pursuing Ph. D (Microbiology) program at Kwame Nkrumah University of Science and Technology, Kumasi, Ghana(e-mail: : Chinenyechijioke_osuji@yahoo.com).
- Imo E.O is currently pursuing Ph.D (Microbiology) program at FUTO ,Nigeria (e-mail : ejeagba@yahoo.com)

lesser in man given the very low concentrations of these contaminants in groundwater and waterways (Johnson and Sumpter, 2001, Bhatnagar *et al*, 2002). Level of wastewater pollution varies from industry depending on the type of process and capacity of the industry (Garcia *et al*., 1995).

Wide variety of chemicals produced by pharmaceutical industries include antibiotics, analgesic, water soluble salt, disinfectants, anti-flammatory sunscreen agents, steroids, anaesthetics, vitamins in form of tablets, capsules, creams , preservatives such as triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol used in antiperspirants, triclocarban ibuprofen, sulfamethoxazole, wax, glycerin, oil and grease, naphthalene, diazepam, fragrances, dyes, etc.

The organic pollutants are mainly from the raw materials and finished products. They are mostly polar and potentially resistance to biotransformation. Pharmaceutical compounds have been detected in treated effluent in concentrations ranging from µg/L to ng/L, possibly due to incomplete removal during the treatment process; this subsequently reach wider water and soil environment through effluent discharge or sludge use (Daughton *et al* 1999). Commonly reported pharmaceutical compounds in effluents include estrogens (estradiol and estrone), contraceptive drugs, and surfactants degradation products nonylphenol (NP), octylphenol (OP) and their mono- and di-ethoxylates, antibiotics, nonsteriodal anti inflammatory drugs, anti epileptics and disinfectants such as triclosan. (Ternes 1998, khan and Origerth, 2002). Most of the above mention pharmaceutical compound have shown pyrogenic activities and can cause feminization of male fish at elevated concentrations in aquatic environment (Purdom *et al*., 1994.).

Phenol at lower concentration is one of those recalcitrant from pharmaceutical effluents that adversely affect aquatic as well as human life. Although, these compounds form complexes with metal ions discharged from other industries, it can be carcinogenic in nature. It is soluble, highly mobile, imparts medicinal taste and odour even at minute concentration of 2 µg/l and it is lethal to fish at concentrations of 5-25mg/l (Kumar *et al* 2004). Those recalcitrant and xenobiotic compounds pose problems in conventional wastewater treatment, due to their resistance to biodegradation.

Considering efforts made to assess the presence of pharmaceuticals in effluent, their chemical properties and biodegradability, number of reports are found using biological process (La Para *et al*.,2001, Carballa *et al*., 2004, 2005, Clara *et al*., 2005, Huber *et al*., 2005, Joss *et al*., 2005). Pharmaceuticals like estradiol and nonyl phenol have been found to be biodegradable under aerobic condition while some others like DDT and PCBs and their metabolites are said to persist even its minute levels. It has also been revealed that acetaminophen, one of the most abundant drugs in wastewater- streams and active ingredient in the fever reducer Tylenol, reacts readily with the chemicals in standard chlorine base water treatment (Bedner and MacCrehen ., 2006). Many drugs such as Clofibric acid are polar and can cause leaching into ground water due to their high mobility in the environment (Heberer and Stan., 1997). Recently studies have shown that efficiencies for removal of polar compounds are between 60-90%. Their removal is not only attributed to biodegradation but to attachment to solid surfaces. However, the removal efficiencies of these materials are influenced either by chemical properties of specific compounds, microbial activities or environmental conditions. The reuse of pharmaceutical effluent in agricultural land may result in seepage of such compounds into the soil environments even though little is known about their fate in these environments.

Generally, the fate of pharmaceutical effluents depends on the physicochemical properties of individual compounds contained in the effluent and the nature of the receiving environment (pH, redox condition etc). The major factor influencing the efficiency of pollutants removal from effluents is their inability to interact with solid particle (both natural (clay, sediments, micro organisms) or added to medium (active carbon, coagulants), because this facilitates their removal by physicochemical (settling, floatation) or biological processes (biodegradation). However, compounds with low adsorption coefficient tend to remain in the aqueous phase, which favours their mobility through sewage treatment plant and receiving environment (Ohlenbush *et al*., 2000). The effluent discharges from pharmaceutical industry have high organic load and treatment is mainly carried out using biological methods either aerobic or anaerobic (Shreeshivadasan and Sallis., 2011). Bioremediation is the process of using microorganisms to clean up harmful chemicals in the environment. When the microbes completely digest these chemicals, they change them into non-toxic products such as water and carbon dioxide (M Alexander 1994). The aim of this study is to evaluate the use of screened isolates of bacteria and fungi in biodegradation process of pharmaceutical effluent in order to reduce the following parameters COD, BOD, TDS, TSS, and Growth (OD), also determine the cultural

conditions for the degradation of pharmaceutical effluent.

Material and Methods.

Sample Collection.

Samples of pharmaceutical effluent were obtained from Petsow Laboratory a division of Starline Group of Companies in Aba, Abia State, Nigeria, which produce approximately 165m^3 of effluent per day. The company produces antibiotic (tetracycline), analgesic (Petsow strong, starcimol, starcimol extra and starbumol), multivitamins in form of tablets(Petsow vitamin B complex and Petsow-Vitamin C), capsules, triclosan. The pharmaceutical effluent was a mixture of wastewater from mixing vessels, other equipment and run- off from the factory. Samples were collected from the point of discharge into the environment. The samples were collected in clean sterile, dry glass 5Lbottles, transferred immediately after collection to the laboratory, and kept at 4℃ prior to analysis.

Physicochemical properties of pharmaceutical effluent.

Physicochemical properties of the pharmaceutical effluent were assessed before and after the treatment to determine the efficiency of the treatment. The samples were collected analyzed using standard methods (APHA, 1998) in order to monitor the biodegradation process. In this study the parameters analyzed were TDS, BOD, TSS, Growth (OD) and COD to evaluate the efficiency of degradation.

Microbial isolation and identification.

The bacteria isolates in pharmaceutical effluent were determined by a pour plate method, cultured in Nutrient agar (NA) and mineral salt medium (MSM) while the mycological count was measured by the spread plate method using PDA containing 50 mg/ml chloramphenicol in order to prevent bacterial contamination.

All fungal isolates were grown on Potato dextrose agar (PDA) by adding 1ml of pharmaceutical effluent into separate empty sterile Petri dishes while autoclaved PDA, cooled to 50^{o}C was poured onto the samples in the Petri dishes with immediate swirling of the petri dishes to ensure adequate mixing. Five replicates were prepared for each sample and incubated at 27^{o}C for a period of four days after which fungal growth was observed. Each pure fungal isolates was then sub cultured onto fresh PDA plates and incubated at room temperature (28^{o}C) for 120 h respectively before identification. All isolated fungal strains obtained from the plates were identified by visual observation and micro-morphological techniques (Molla *et al.,* 2002) and according to the general principles of fungal classification based on their mycelium colours and growth patterns as described by Barnett and Hunter (1987) and Samson *et al*. (1984).

The bacteria isolates were characterized using both morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology, (Garrity., 2005).

Screening for organic degrading activity of the isolates from pharmaceutical effluent.

Fungal isolates were screened for their ability to degrade the organic constituents of pharmaceutical effluent based on their average growth rate by calculating the diameter of radial extension of the fungal mycelium, on minimal salt medium containing as follows (g/l): KH_2PO_4 0.05, $(NH4)_2SO_4$ 0.05, MgSO $2H_2O$ 0.05, agar 15 amended with 1% (v/v) filtered sterile pharmaceutical effluent as the sole carbon source. The control plate was prepared using the same compositions excluding the 1.0(v/v) of filtered sterile pharmaceutical effluent. The effluent-agar plates were then inoculated with 1 cm^2 mycelial plug from each of the pure cultures prior to the incubation at 27^{o}C for a period of 7 days. All the experiments were carried out in triplicates. The growth rates of the fungal isolates were recorded daily by measuring the diameter of the radial extension of the mycelium. The average of the diameter of growing colony was determined by measuring at least two diameters per plate. The average growth of the diameters was used as the

colony diameter at that particular time of measurement. Colony growth rates (cm/day) were calculated by the regression of the colony diameter against the days after inoculation (Santos *et al.*, 2008). The fungal isolates that yielded heavy sporulation, greater colony diameter, or more abundant aerial mycelium on plates containing used pharmaceutical effluent were selected for further examination.

The average growth rate varied from 0.19 -0.85cm /day with *Saccharamyces cerevisea* showing the highest average growth rate diameter of 0.85cm/day and *Penicillium digitatum* with least average growth rate of 0 .19cm/day (Table 1).

The ability of the bacterial isolates to utilize the pharmaceutical effluent as sole source of carbon and energy was determined using the method of El-Bestawy *et al.* (2004) for vegetable oil wastewater degradation studies. The isolated bacterial species were individually grown in 300 ml minimal broth medium in pre-sterilized conical flasks supplemented with 1% v/v pharmaceutical effluent. The pharmaceutical effluent-supplemented medium was then sterilized by autoclaving at 121 ^{o}C for 15 min. 0.1 ml (about $4.5 \cdot 10^4$ cells per ml) of 18 h Nutrient broth culture of each test organism was aseptically seeded into the supplemented MSM and then incubated at 28^{o}C under static condition for 7 days while the unseeded tubes served as controls. The population dynamics of the inocula in the MSM was determined at 8 hourly intervals by cultural technique as an index of their ability to utilize the pharmaceutical effluent as their sole source of carbon and energy. Growth of the test organisms was also scored as abundant or high (+++), moderate (++) and minimal (+) depending on the degree of turbidity. *Psuedomonas aeruginosa* showed the highest degree of turbidity (+++) and *Proteus vulgarius* showed the least the degree of turbidity (Table 2) .The bacterial isolate with high growth was used for further studies.

Biodegradation experiments.

The three isolates used were those that utilized pharmaceutical effluent as sole carbon source during screening process as shown on Table 1.and 2. The isolated cultures at 0.1ml (10^6cells/ml) inoculum were inoculated into three different 250ml conical flasks containing 100 ml pharmaceutical effluents and incubated for 144h at 30^{o}C respectively. The biodegraded pharmaceutical effluent samples were drawn and determined for growth as OD_{540nm}, BOD_5, COD, TDS and TSS.

Optimization of biodegradation using Carbon sources.

The method used was as described by Wu *et al.* (2006) with little modification in pharmaceutical effluent. Experiments were conducted to determine the effect of the type of carbon source on the effluent degradation. The sources were glucose, maltose, cassava starch and corn starch. Carbon sources at 2% (w/v) were each added to respective 250ml conical flask containing 100ml pharmaceutical effluent, inoculated with 0.1ml (10^6cells/ml) inoculum and incubated at 28^{o}C for 144h on a rotary shaker at 180rpm.Samples were drawn and tested for OD_{540nm}, BOD_5, COD, TDS and TSS.

Optimization of biodegradation using Nitrogen sources.

The method used was as described by Wu *et al.* (2006).Different nitrogen sources (soybean meal, yeast extract and NH_4Cl) were used. Each nitrogen source was separately incorporated at a level of 1% (w/v) into the basal medium. For fungal incubation the broth was inoculated with agar plug of a four days old culture of the isolates, and incubated at 30^{o}C for seven days. Samples were drawn and tested for OD_{540nm}, BOD_5, COD, TDS and TSS.

Table 1. The average growth rate (cm/day) of 7 fungal isolates on minimal media amended with 1.0% (v/v) pharmaceutical effluent for 7 days at 27°C.

Organisms	Average growth rate(cm/day
Aspergillus niger	0.68 ± 0.005
Penicillium digitatum	0.19 ± 0.050
Mucor racenosu	0.24 ± 0.038
Saccharamyces cerevisea	0.85 ± 0.360
Geotricum candidium	0.48 ± 0.030
Candida rugosa	0.32 ± 0.036

Table 2. Growth of organisms in pharmaceutical effluent medium.

Organisms	degree of turbidity
Bacillus subtilis	++
Bacillus cereus	++
Pseudomonas aeruginosa	+++
Proteus vulgarius	+
Staphylococcus aureus	+ +

- Measured as degree of turbidity

Table 3. Biodegradation of pharmaceutical effluents by the different microbial isolates

Isolates	COD (mg/l)	reduction %	TDS (mg/l)	reduction %	BOD (mg/l)	reduction %	TSS (mg/l)	reduction %	Growth (OD_{540nm})	Increase %
Control	5680	-	120.7	-	1720	-	150.4	-	168	-
Pseudomonas aeruginosa	3124	45	56.7	53	901.3	47.6	61.7	59	262.1	56
Saccharamyces cerevisea	3652.7	35.7	66.4	45	951.2	44.7	64.7	57	253.7	51
Aspergillus niger	3885.1	31.6	74.8	38	1039	39.6	77.5	49	248.6	48

The treated pharmaceutical effluent samples revealed that *Pseudomonas aeruginosa* showed best degrading capacity with 45% COD, 47.6%BOD reduction, 59%TSS,56.7% TDS reduction and 56%OD_{540nm} (growth) while *Aspergillus niger* showed the least BOD and COD reduction of 39.6 %and 31.6 % respectively.

Table 4. Optimization of biodegradation using Carbon sources

Organisms	Carbon Source	Growth (OD_{540nm})	TDS (mg/l)	TSS (mg/l)
Control	-	168±.03	120.7±56	150.4±12
Pseudomonas aeruginosa	Glucose	269 ±.01	59±21	44±.33
	maltose	374±.01	33±11	36±25
	Cassava starch	251 ±.01	53±13	42±26
	Corn starch	286±.03	34±18	50±25
Saccharamyces cerevisea	Glucose	386±.02	48±21	24±.33
	maltose	284±.04	26±11	46±25
	Cassava starch	354 ±.01	33±13	25±26
	Corn starch	308±.03	41±18	28±25
Aspergillus niger	Glucose	274±.06	49±19	49±44
	maltose	261±.08	44±17	48±34
	Cassava starch	321±.03	42±11	39±26
	Corn starch	313±.13	50±15	52±33

*Values are the means of replicate determinations ± SD

The use of maltose in optimization of biodegradation of pharmaceutical effluent supported the growth of P*seudomonas aeruginosa* and *Saccharamyces cerevisea* with TDS reduction of 72.5%and 78.5% respectively in Table 4 while glucose best supported the TSS removal by *Saccharamyces cerevisea* in pharmaceutical effluent.

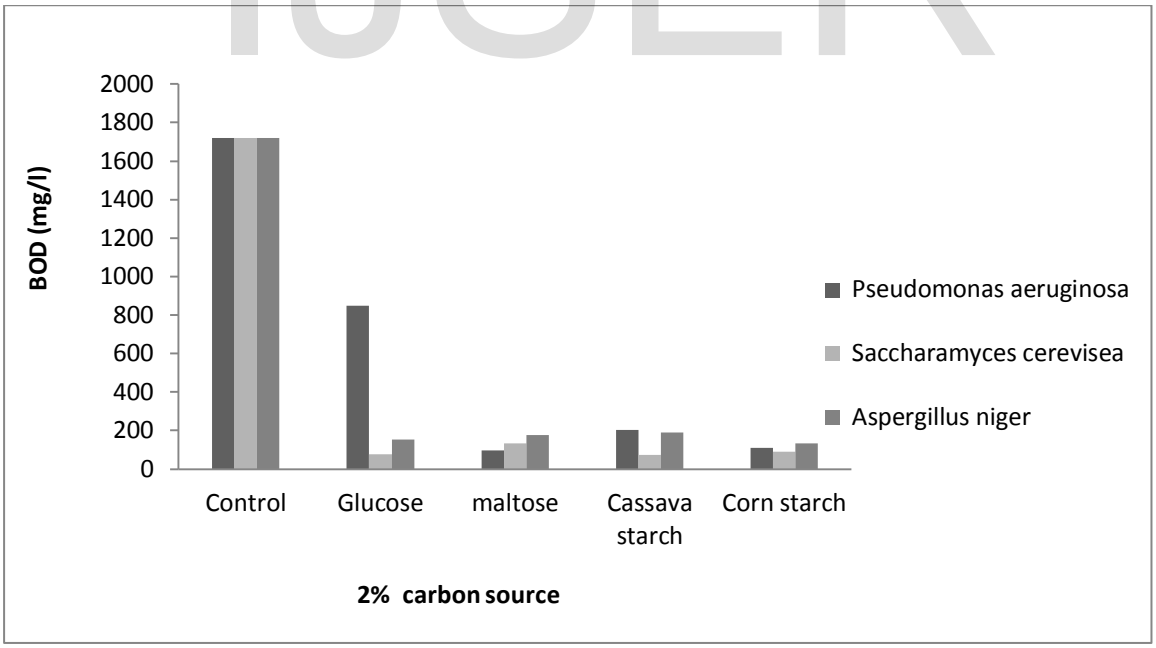

Fig 1. **Effect of carbon source in BOD reduction of pharmaceutical effluent**

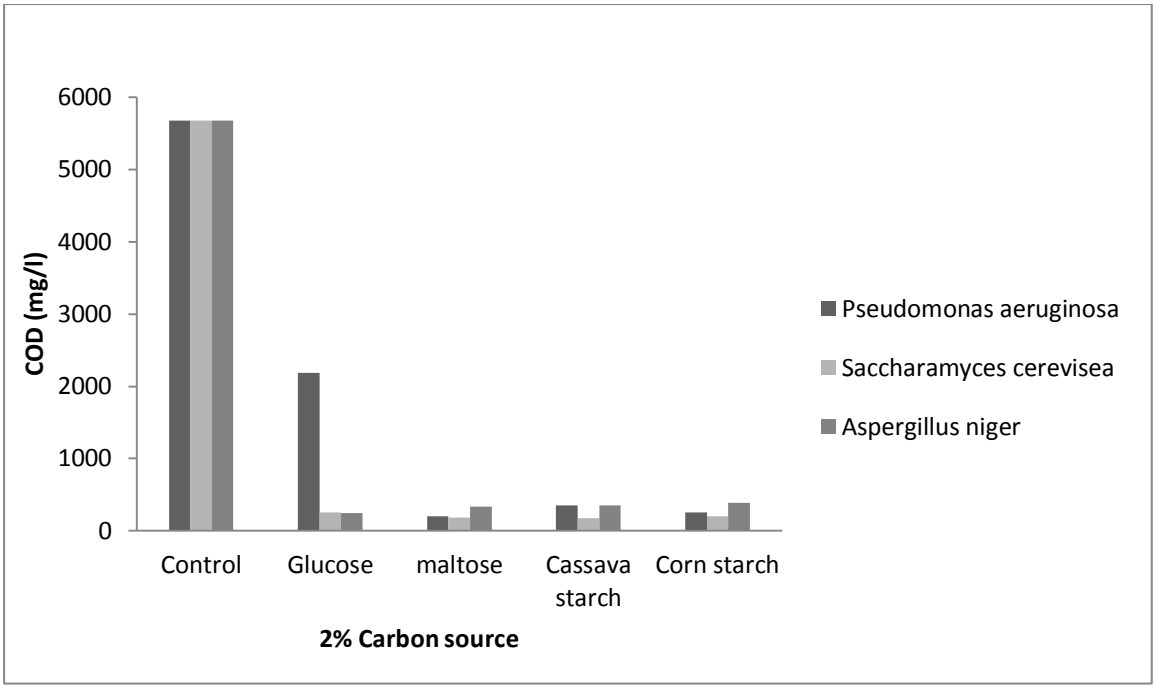

Fig 2. **Effect of carbon source in COD reduction of pharmaceutical effluent.**

The different carbon sources supported both COD and BOD reduction in pharmaceutical effluent with the three organisms used. Fig 1 and 2 revealed that *Saccharamyces cerevisea* best utilized cassava starch in the biodegradation of pharmaceutical effluent which resulted in 95.8% BOD and 97% COD reduction respectively while *Aspergillus niger* showed the least utilization of all carbon sources amongst the three organisms used in this study.

Table 5**. Optimization of biodegradation using nitrogen sources**

Organisms	Nitrogen	Growth	BOD	COD	TDS	TSS
	source	OD_{540nm}	(mg/l)	(mg/l)	(mg/l)	(mg/l)
Control	-	168±.016	1720±03	5680 ±018	120.7±56	150.4±12
Pseudomonas aeruginosa	Soy bean	370±.002	24±02	106±08	23±09	41±02
	Yeast extract	254±.002	43±05	100±09	59±07	63±07
	NH_4Cl	321±.013	50±09	250±06	53±03	33±06
Saccharamyces cerevisea	Soy bean	320±.002	26±20	337±07	37±15	29±07
	Yeast extract	386±.003	14±40	270±78	21±26	41±09
	NH_4Cl	286 ±.005	28±02	364±71	56±07	34±04
Aspergillus niger	Soy bean	320±.007	43±68	184±36	49±07	32±02
	Yeast extract	298±.001	43±83	230±97	44±15	64±06
	NH_4Cl	322±.005	30±91	284±98	37±21	42±03

*Values are the means of replicate determinations ± SD

Yeast extract best supported biodegradation amongst the organisms with 99% BOD, 82 % TDS reduction and 90.5%growth increase in *Saccharamyces cerevisea* treated effluent while the highest 98.2% COD reduction was observed with *Pseudomonas aeruginosa* treatment. However, soy bean source supported 80.7% TSS removal in *Saccharomyces cerevisea* treated effluent while the least utilization of the nitrogen sources was

observed in the *Aspergillus niger* treated pharmaceutical effluent.

DISCUSSION.

Most substances found in a pharmaceutical industrial wastewater are structurally complex organic chemicals that are resistant to biological degradation (Cokgor *et al*., 2004 Ren *et al*, 2008), thus the need for segregation and collection of particularly toxic materials in a conventional biological treatment of pharmaceutical industrial wastewater (Gallely *et al*., 1977). The pollutant loads in terms of biological oxygen demand (BOD) may be negligible hence higher chemical oxygen demand (COD) than BOD (Bitton., 2005, Huseyin *et al*., 2006). Aerobic condition is said to accelerate biodegradation at faster rate and to a greater extent than anaerobic conditions within a specified period (Murphy *et al.,* 1995). However the presence of certain raw material like starch tends to enhance biodegradation of pharmaceutical effluent using *Saccharomyces cerevisiae* . Many studies have reported the use of *Saccharomyces cerevisiae* treatment of industrial effluent (Sun *et al*., 2009).

CONCLUSION

This study reveals the need for enforcing adequate effluent treatment methods before their discharge to surface water; this is to reduce the potential environmental hazards mainly caused by these recalcitrant complex organic chemicals. This study has demonstrated that optimization with carbon and nitrogen sources, most organic constituents in pharmaceutical effluent can be reduced to acceptable level using yeast *Saccharomyces cerevisiae.*

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