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Enzymatic biodegradation of pharmaceutical wastewater

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Abstract
The present effort is an attempt to reduce pollution caused by the discharge of untreated wastewater (effluents) to the environment by using a low cost method. The effluent was bio-remediated using yeast and amylase as the active agents. The greater the decomposable matters present in an effluent, the greater the oxygen demand; the greater the Biological Oxygen Demand (BOD) and Chemical Oxygen Demand(COD) values, the less Dissolved Oxygen(DO) values. 10g of yeast and amylase were added to 1000ml each of pharmaceutical effluent. 150 ml of the effluent (from the yeast and amylase) dosed was withdrawn weekly for analysis alongside with the effluent without enzymes for turbidity, DO, BOD and COD. After a period of six weeks the effluent dosed with yeast gave the highest performance followed by that dosed with amylase. The result shows that as time increases, the amount of oxygen demand reduces while the dissolved oxygen content of the effluent increases. This indicates that the yeast enzyme was able to aid remediation of the pharmaceutical effluent.
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Keywords: Bio-remediation; Effluent; Environment; Enzyme; Pharmaceutical; Pollution.

1. Introduction
In view of high cost of conventional wastewater treatment systems there is an increasing need to develop low cost methods of treating wastewater particularly that of municipal and industrial origin. Rapid industrialization has resulted in the rise of pollution. Traditional pollution abatement at the discharge point, so called "*End-of-pipe treatment*", is being seriously questioned, since end-of-pipe treatment only transfers the pollution from one form to another [1]. To counter the above shortcoming and to preserve the high quality of the environment new concept so called "*Cleaner Production*" for waste minimization is being introduced, technology designed to prevent waste emission at the source of generation itself [2].
Developing low cost technology for wastewater treatment offers an alternative, most effective for treatment of domestic and industrial wastewater, particularly for those situated in the tropical and subtropical regions [3].
Many other studies identified many products, including analgesics, antiinflammatories, antibiotics, antiepileptics, beta-blockers, blood lipid regulators, antidepressants, contrast media, oral contraceptives, and cytostatic and bronchodilator drugs in sewage, surface water, groundwater, and drinking water [4-10]. Technologically because of the simplicity of waste stabilization ponds affluent nations, which can afford the luxury of expensive wastewater treatment, are planning to use more and more low cost treatment technologies. Environmental degradation is an escalating problem owing to the continual expansion of industrial production and high-levels of consumption. A renewed dedication to a proven strategy to resolve this problem is needed. Cleaner Production is one such strategy, which can address

this problem. It is a preventive environmental management strategy, which promotes eliminating waste before it is created to systematically reduce overall pollution generation, and improve efficiencies of resources use. Several countries have turned down opportunities to implement wastewater recycling to supplement potable water needs during drought conditions [11, 12], but it is hard to conceal the fact that many people in those countries have been consuming wastewater-impacted drinking water for many years without any evidence of negative exposure-related health outcomes [13].

Developing low cost technology for wastewater treatment offers an alternative and has been found to be most effective for treatment of domestic and industrial wastewater, particularly for those situated in the tropical and subtropical regions [3, 14-17]. Technologically because of the simplicity of waste stabilization ponds even affluent nations, which can afford the luxury of expensive wastewater treatment, are planning to use more and more low cost treatment technologies [18, 19].

The objective is to evaluate the constituents of a typical production waste-water from a pharmaceutical industry and designing suitable treatment technologies to reduce the environmental impact of waste water, and to also minimize the amount of water wasted in the production unit. This study will provide a viable means of minimizing environmental pollution problems arising from the effects of pharmaceutical waste water, and also provide an information database for future researchers, who may be interested in issues concerning pharmaceutical waste water, and its treatment.

2. Experimental

2.1 Materials and method

The equipments used in this work shown in Table 1.

2.2 Preparation of reagents

Chemicals for dilution water preparation Phosphate buffer solution: 8.5g KH_2PO_4, 21.75g K_2HPO_4, 33.4g $Na_2HPO_4.7H_2O$ and 1.7g NH_4Cl was dissolved in 500ml distilled water and then diluted to 1 litre. It was preserved in stock bottles in which there must be no biological growth.

Magnesium sulphate solution: 22.5g $MgSO_4.7H_2O$ was dissolved in distilled water and marked up to 1 litre.

Calcium chloride solution: 27.5g anhydrous $CaCl_2$ was dissolved in distilled water and marked to 1 litre.

Iron (III) chloride solution: 0.25g $FeCl_3.6H_2O$ was dissolved in distilled water and marked up to 1 litre.

Standard potassium heptaoxochromate (VI), $K_2Cr_2O_7$ solution, 0.125M**.** 12.259g $K_2Cr_2O_7$, previously dried at 103^OC for 2 hr, was dissolved in distilled water and marked up to 1 litre mark.

Ag_2SO_4-H_2SO_4 solution: 11g Ag_2SO_4 crystals was dissolved in a Winchester bottle 2.5 litters of concentrated H_2SO_4, (s.g.1.84).

Mercury (11) sulphate: 0.4g powder is needed for each determination.

Sulphonic acid; this is needed if $N0_2$-N is known to be present in the sample. About 2mg sulphonic acid crystals are needed for each determination.

Standard iron (11) ammonium sulphate (A.R) solution, 0.05M**:** 39g $FeSO_4.(NH_4)_2SO_4.6H20$ was dissolved in distilled water.20ml conc. H_2SO_4, was then added, cool, and diluted to 1 litre mark. It was shaken properly and then Standardized against standard $K_2Cr_20_7$ solution.

2.3 Standardization

10 ml standard $K_2Cr_20_7$ solution was diluted to about 100 ml. 30 ml conc. H_2SO_4 was then added and allowed to cool. The solution was titrated against iron (11) ammonium sulphate solution using 2 drops of ferroin solution as indicator to red brown end point.

Ferroin indicator solution: 1.458g 1,10-phenanthroline monohydrate and 695mg iron (11) sulphate heptahydrate, $FeS0_4.7H_20$,was dissolved in distilled water and diluted to 100 ml mark and was shook properly.

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Table 1. Apparatus / Equipment

Equipment/ Material	Source	Properties/ Specification
Spectrophotometer (DR 2000)	Hach, USA, DR2000, 44863-00 44863-00	DIRECT READING
DO meter	DO 970903016356, DELTA OHRMS, ITALY,2003	DATA LOGGER
COD reactor	BIOHIT BIOTRATE	Digital Burette
Yeast	Biochemika	Powder slightly brown
Amylase	Biochemika	Powder 30units/mg slightly brown, pH 6.0
Pharmaceutical waste water	Service Pharmaceutical, Benin city, Nigeria	
Phosphate buffer solution	Biochemika (APHA)	pH at 25°C is 7.2
Magnesium sulphate solution	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Calcium chloride solution	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Iron (III) chloride solution	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Potassium dichromate	BDH Laboratory, Supplies poole. BH151D England	Minimium assay 99.8% pH (5% aqueous) 6.5 – 7.5
Ag_2SO_4-H_2SO_4 solution	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Mercury (II) sulphate	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Sulphuric acid	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Iron (II) ammonium sulphate solution (0.05M)	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Hydrazine sulphate	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Hexa-methylene tetramine	Graffine and George 285 Ealine Rd. Wembley Middlesex	Minimum assay 99.5% pH(5% aqueous) 8.5-9.5
Sodium hydroxide	Park Scientific Ltd. Northampton, UK	Minimum assay 99.5%
Sodium iodide	Park Scientific Ltd. Northampton, UK	Minimum assay 99.5%
Sodium azide	Park Scientific Ltd. Northampton, UK	Minimum assay 99.5%
Sodium thiosulphate	BDH Laboratory, Supplies poole. BH151D England	Minimum assay 99.8% pH (5% aqueous) 6.5 – 7.5
Trichloromethane	Graffine and George 285 Ealine Rd. Wembley Middlesex	Minimum assay 99.5% pH(5% aqueous) 8.5-9.5
Potassium iodide	Graffine and George 285 Ealine Rd. Wembley Middlesex	Minimum assay 99.5% pH(5% aqueous) 8.5-9.5

2.4 Preparation of stock turbidity suspension

Solution 1: 1g hydrazine sulphate was dissolved in distilled water and diluted to 100 ml mark in volumetric flask.

Solution 2: 10g hexa-methylene tetramine was dissolved in distilled water and was diluted to 100 ml mark in a volumetric flask.

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Stock turbidity suspension: Using clean pipettes, 5ml of solution 1 and 5 ml of solution 2, was withdrawn into a 100 ml volumetric flask and was mixed then thoroughly corked and allowed to stand for 24hrs in an incubator pre-set to $25^{O}C$. It was taken out, swirled gently and allowed to assume room temperature. And once again, swirled gently and diluted to 100 ml mark. The turbidity of this suspension was 400 NTU.

Manganese (II) sulphate solution: 480g $MnSO_4$ was dissolved in distilled water, filtered and diluted to 1 Litre.

Alkali iodide azide reagent: 500g NaOH and 135g NaI was dissolved in distilled water and diluted to 1 liter. Dissolved 10g NaN_3 in 400ml distilled water was then added to the alkali solution.

Sodium thiosulphate solution (0.05M): 24.82g $Na_2S_2O_3.5H_2O$ was dissolved in boiled and then cooled distilled water, and diluted to 1 litre. The solution was then preserved by adding 5ml trichloromethane per litre.

0.0125M sodium thiosulphate solution was prepared by diluting 250ml of the 0.05M solution to 1 litre and standardized by titrating against potassium dichromate solution.

Starch: 5g of starch was weighed into a 100ml beaker and little water was added to make a suspension. The suspension was then added to about 800ml of boiling water while stirring. It was then Diluted to 1 litre and allowed to boil for a few minutes and left over night to settle. The supernatant was used as indicator for the titration.

2.5 Methodology

10g of yeast and amylase were added to 1000ml each of pharmaceutical effluent. 150 ml of the effluent (from the yeast and amylase effluent) was withdrawn weekly for analysis alongside with the effluent without enzymes.

2.5.1 Determination of turbidity (Nephelometric method)

Procedure**:** The sample was shaken to dispense the solid contents thoroughly. The sample was then poured into the turbidimeter tube and read directly from the instrument scale

2.5.2 Determination of dissolved oxygen by DO meter (Iodometric titration method)

Procedure: The water sample was put in a 300 ml bottle and then Added 2 ml $MnSO_4$ solution and 2 ml alkali-iodide-azide reagent well below the surface of the liquid, stopped with care to exclude air bubbles and mixed by inverting the bottle a number of times until a clear supernatant water was obtained. It was allowed to settle for about 2min. 2ml conc. H_2SO_4 was added by allowing the acid to run down the neck of the bottle, re-stopped and mixed by gentle inversion until dissolution was completed. 20ml was used for titration. This was titrated with 0.0125M sodium thiosulphate solution to a pale straw colour. 2ml starch solution was then added, the colour became blue, and the titration was continued by adding the thiosulphate solution drop-wise until the blue colour disappeared.

2.5.3 Determination of biological oxygen demand (BOD) (By non-dilution method or direct method) APHA-508

Procedure: The effluent was thoroughly aerated and then filled into a screw tapped incubation bottle to the brim. The bottle was sealed and incubated in the dark for 5days at $20^{O}C$ and then a DO determination was carried out on a suitable portion of the incubated sample. In the direct method used, the dilution factor that appeared in the formulation will be equated to zero. BOD is the difference between the two determined DO levels (DO_1 and DO_5). The formula for the calculation of BOD_5 is stated in appendix I.

2.5.4 Determination of chemical oxygen demand (COD) APHA-422B

Procedure: 0.4g mercuric sulphate was placed in a reflux flask and 20ml sample or aliquot was added and diluted to 20ml. 2 mg sulphamic acid, 10ml of $K_2Cr_2O_7$ solution and then several glass beads previously dried at $600^{O}C$ for 1hr were also added. While swirling slowly and gently, 30ml silver sulphate – sulphuric acid solution was then added and the flask was connected to the condenser and a blank mixture was also prepared. The mixture was Refluxed for 2hrs and then cooled. The condenser was washed with distilled water into Erlenmeyer flask and diluted to about 150ml. It was cooled to room temperature and then the excess dichromate was titrated with standard ferrous ammonium sulphate (FAS) using 2drops of ferroin as the indicator. The formular for the calculation of COD is stated in appendix II.

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3. Results and discussion

In this study, yeast and amylase were used as enhancer to degrade pollutants in pharmaceutical effluent. Same amount of enzyme (10g) was added to same volume of effluent (1L) and left to degrade for six weeks. Weekly, 150ml of effluent (with and without) enzyme were collected for analysis. The following parameters, turbidity, dissolved oxygen (DO), biochemical oxygen demand (BOD) and chemical oxygen demand (COD were measured to evaluate rate of degradation enhancement by the enzymes. The results obtained for the enzyme enhanced degradation was compared with that obtained for the non-enzyme enhanced degradation.

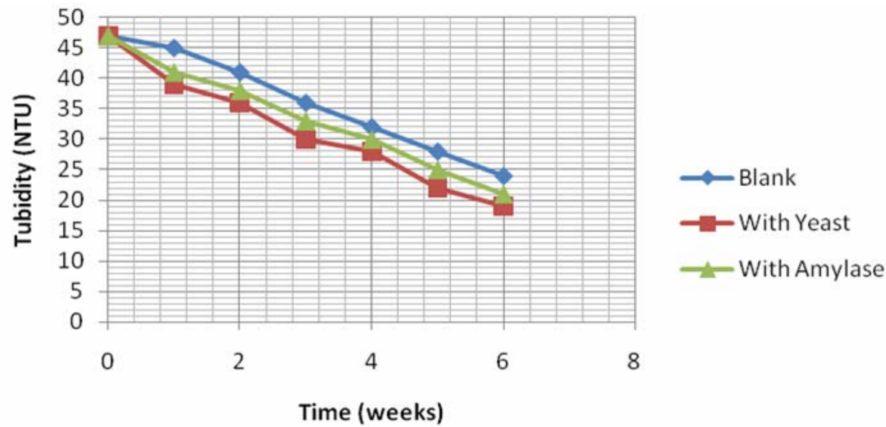

Figure 1. Effect of enzyme on turbidity of Pharmaceutical effluent

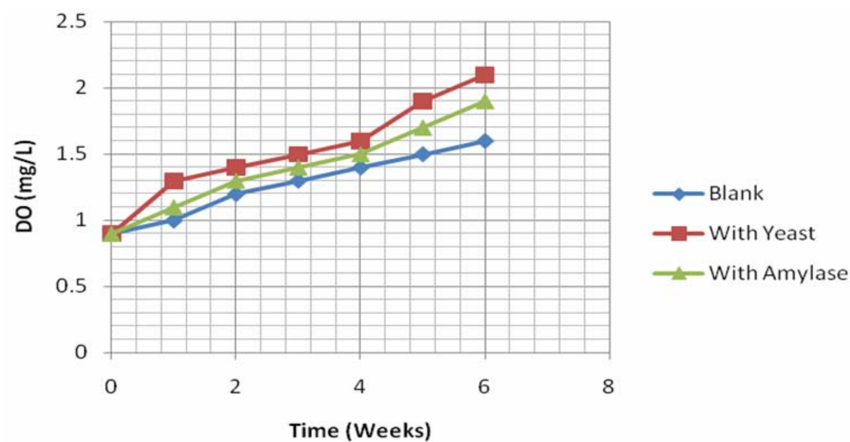

Figure 2. Effect of enzyme on the amount of DO in Pharmaceutical effluent

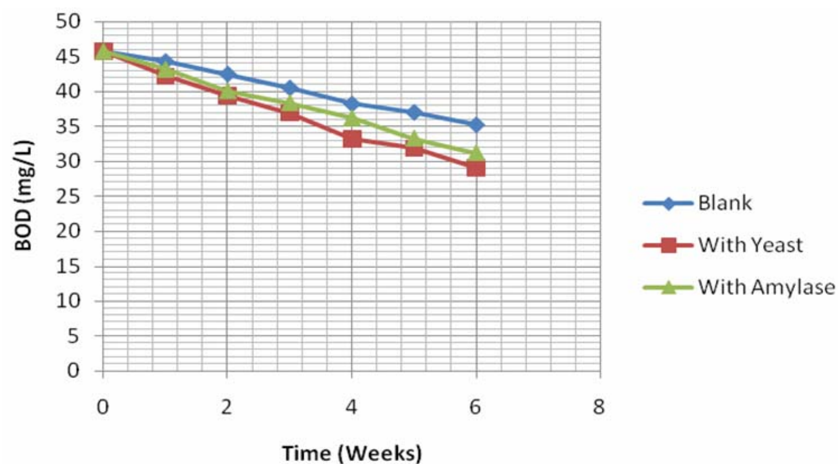

Figure 3. Effect of enzyme on the amount of BOD in Pharmaceutical effluent

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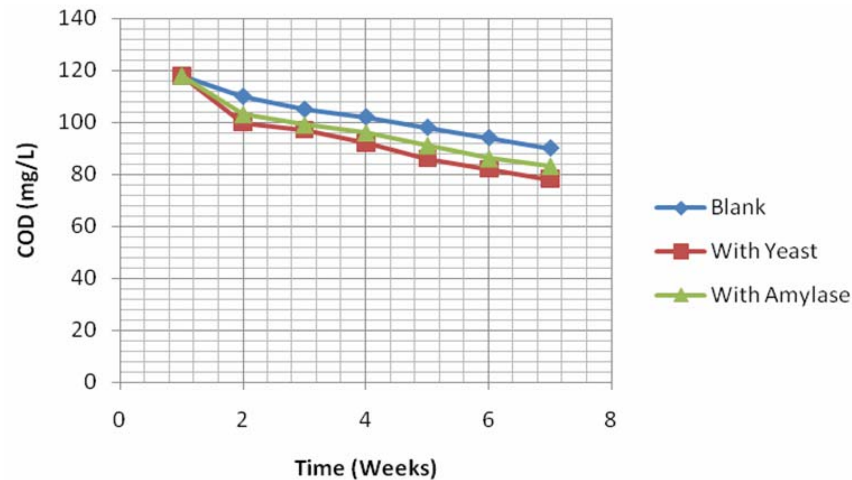

Figure 4. Effect of enzyme on the amount of COD in Pharmaceutical effluent

From Figure 1, at the first day (week 0) of the experiment, all samples had the same turbidity value as the enzymes had not started the degradation process. The turbidity values were observed to decrease with time of remediation. After the six weeks study the turbidity value found in the three samples were 24 NTU for without enzyme, 19NTU for yeast and 21 NTU for amylase. The enzymes were able to reduce the turbidity of the effluent by more than 50%.

Figure 2 shows the graph of the effect of enzyme on the amount of DO in the pharmaceutical effluent. The amount of DO in the effluent increased with time of remediation. The amount of oxygen in the effluent in the sample containing yeast is seen to have the highest amount of DO in the effluents analyzed after each week analysis. This was followed by the sample containing amylase. The sample without enzyme had the lowest amount of DO after each week analysis. After six weeks of degradation process, none of the samples was found to comply with the federal ministry of environment (FMENV) permissible values of between (4.0 – 5.0)mg/l due to time constraint.

BOD (5-day) is the widely used organic pollution parameter applied to waste water. Its determination involves the measurement of the dissolved oxygen used by micro-organisms in the biochemical oxidation of organic matter. In this study, the amounts of BOD in the three samples were found to decrease with time of remediation. The decrease was more in the sample containing yeast than that containing amylase and the Blank (without yeast or amylase). At the end of the six weeks enzyme bioremediation study, yeast was able to reduce the amount of BOD in the effluent below the FMENV permissible value of 30mg/l. This study shows yeast effectiveness in enhancing the activities of microorganisms in the effluent in the breakdown of the organic matter present in the effluent. The graph of the effect of enzyme on the amount of BOD in the pharmaceutical effluent is shown in Figure 3.

COD is the measure of the amount of oxygen required for complete oxidation of the organic matter present in a sample of effluent. In this study, COD analysis was conducted to evaluate the amount of organic matter left in the pharmaceutical effluent after each week remediation. The study revealed that the COD values of the sample treated with yeast had the lowest amount of COD in the effluent. This was followed by the COD values in the sample treated with amylase. The sample without yeast or amylase (Blank) had the highest amount of COD. It shows that the enzymes can enhance the activity of microorganism thereby increasing the rate of decomposition of organic matter in effluent by microorganisms. After the six weeks remediation study, the amount of COD in the sample was below the FMENV permissible limit of 30 mg/l for effluent as shown in Figure 4. The COD values were generally found to decrease with increase in time of remediation.

4. Conclusion

The result of the enhanced bioremediation experiment using yeast and amylase as catalyst, for the breakdown of the organic matter present in pharmaceutical effluent by microorganism shows the enzymes effectiveness in the enhancement. The result shows that as time increases, the amount of oxidizable oxygen demand reduces while the dissolved oxygen content of the effluent increases. This shows that the yeast enzyme was able to aid remediation of the pollution effluent. The greater the

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decomposable matter present in an effluent, the greater the oxygen demand, the greater the BOD and COD values and the less DO value.
Yeast will be recommended for use as catalyst for the breakdown of organic matter present in the pharmaceutical effluent by micro-organism, since from all results derived using the four parameters; Turbidity, Dissolved oxygen, chemical oxygen demand and Biochemical oxygen Demand, it shows a higher remediation effect than amylase. Also more time should be allowed for bioremediation to be complete.

Appendix I
DO and BOD Calculation

$$\text{DOmg/l} = \frac{16000 \times M \times V}{V_2 / V_1 (V_1 - 2)} \quad \text{(A1)}$$

where M = molarity of the thiosulphate solution
V = volume of thiosulphate used for titration
V_1 = Volume of the bottle
V_2 = Volume of aliquot taken for titration

BOD_5(mg/l)=DO_1-DO_5 (A2)
DO_1= Initial DO of sample
DO_5 = Final DO of sample after incubation period

Appendix II
COD Calculation

$$\text{COD(mg/l)} = \frac{(V_b - V_s) \times M \times 16000}{ml_{sample}} \quad \text{(A3)}$$

where V_b = ml FAS used for blank
V_s = ml FAS used for sample
M = molarity of FAS.

References

[1] Hashmi Imran. Wastewater monitoring of pharmaceutical industry: treatment and reuse options. Electron. J. Environ. Agric. Food Chem.2005, 4 (4), 994-1004
[2] Freitos dos santos, L.M and Livingston,A.G .Novel Membrane Bioreactors for Detoxification of Voc Wastewaters : Biodegradation of 1,2 Dichloroethane Water Research , 1995,pp.179-194.
[3] El-Gohary, F. A., Abou-Eleha, S. I. and Aly, H. I. Evaluation of biological technologies for waste water treatment in the pharmaceutical industry. Water Science and Technology,1995,32(11): 13-20.
[4] Costanzo S, Murby J, Bates J. 2005. Ecosystem response to antibiotics entering the aquatic environment. Mar Pollut Bull.2005, 51:218–223
[5] Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ Health Perspect 107: suppl.1999,(6):907–938.
[6] Glassmeyer S, Koplin D, Furlong E, Focazio M. Environmental presence and persistence of pharmaceuticals: an overview. In: Fate of Pharmaceuticals in the Environment and in Water Treatment Systems (Aga DS, ed). Boca Raton, FL: CRC Press, Taylor and Francis Books.2008, pp. 3–51.
[7] Heberer T.Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data. Toxicol Lett.2002, 131:5–17
[8] Stackelberg PE, Gibs J, Furlong ET, Meyer MT, Zaugg SD, Lippincott RL. Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. Sci Total Environ,2007, 377:255–272
[9] Ternes TA. Occurrence of drugs in German sewage treatment plants and rivers. Water Res.1998, 32:3245–3268.

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[10] Ye Z, Weinberg HS, Meyer MT. Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry. Anal Chem.2007, 79:1135–1144.
[11] Casani S, Rouhany M, Kn?chel S. A discussion paper on challenges and limitations to water reuse and hygiene in the food industry. Water Res.2005, 39:1134–1146.
[12] Wilson Z, Pfaff B. Religious, philosophical and environmentalist perspectives on potable wastewater reuse in Durban, South Africa, Desalination, 2008, 228:1–9.
[13] Rodriguez-Mozaz S, Weinberg HS. Meeting Report: Pharmaceuticals in Water—An Interdisciplinary Approach to a Public Health Challenge. Environ Health Perspect,2010, 118:1016-1020
[14] NgMiranda, W. J., Yap, G. S. and Sivadas, M. Biological treatment of a pharmaceutical wastewater. Biological Wastes,1989, 29(4): 299-311
[15] Puskas, K., Essen, I. I., Banat, I. and Al-Daher, R. Performance of an integrated ponding system operated in arid zones. Wat. Sci. Tech.1991, 23:1543-1542.
[16] Rosen, M., Welander, T., Lofqvist, A. and Holmgren, J. Development of a new process for treatment of a pharmaceutical wastewater. Water Science and Technology, 1998,37(9): 251-258.
[17] Larsen, T. A., Lienert, J., Joss, A. and Siegrist, H. How to avoid pharmaceuticals in the aquatic environment. Journal of Biotechnology.2004, 113(1-3): 295-304.
[18] Khan, M. A. and Ahmad, S. I. Performance evaluation of pilot waste stabilization ponds in subtropical region. Wat. Sci. Tech. 1992, 26: 1717-1728.
[19] Junico, M. and Shelef, G. Design operation and performance of stabilization reservoir for waste water irrigation in Israel. Wat. Res. 1994, 28: 175-186.

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