07 A

# EFFECT OF ANTIMICROBIAL DISCHARGES INTO WASTEWATER TREATMENT PLANTS IN THE PHARMACEUTICAL INDUSTRY

by

### Manisha Y. Gunasekera

Conversity of Moraliuwa, Sri Lanka, Electra ක කාලය, Discertations ඉමාරවුව වශ්ව විදහාලය, ශුී ලංකාට ලෝරවූවා

Dissertation submitted to the Department of Civil Engineering University of Moratuwa Sri Lanka

for the partial fulfillment of

<u>624 "98 "</u> 628.3

Thesis coll.

7018C

Master of Engineering in Environmental Engineering and Management

November 1998

70180

\$

University of Moratuwa, Sri Lanka. Electronic Theses & Dissertations www.lib.mrt.ac.lk

This dissertation has not been previously prepared in whole or part to any University or Institution for a Higher Degree.

MI. y. Gunascher

(Manisha Y. Gunasekera) November 1998

### Acknowledgement

I acknowledge with gratitude the Chairman and the Management of State Pharmaceuticals Manufacturing Corporation, in giving me the opportunity to carryout this project at their Production facility at Ratmalana.

I am grateful to Dr (Mrs.) Indrika Abeyagunawardane and Dr.Ajith De Alwis, my co-supervisors whom very generously spared their precious time and provided every guidance and assistance to carry out this difficult task.

University of Moratuwa, Sri Lanka. Electronic Theses & Dissertations www.lib.mrt.ac.lk

٩

¥.

1

I extend my gratitude and thanks to Prof. (Mrs.) N.Ratnayaka, Head Environmental Engineering Division, Civil Engineering Department, University of Moratuwa and Dr. (Mrs.) Indrika Abeyagunawardena, Head, Department of Microbiology, University of Kelaniya for providing me with all necessary assistance in carrying out the analysis.

I am also thankful to the members of staff at SPMC, Microbiology Department University of Kelaniya and Environmental Engineering Division, Civil Engineering Department, University of Moratuwa for the assistance extended to me.

### Summary

5

ъÇ

۲

Pharmaceutical manufacturing industry is expected to generate effluents having a wide variety of antimicrobial substances. The biological wastewater treatment processes should thus be capable of handling effluents, containing these inhibitory materials, without being adversely affected.

In this investigation, the study of the wastewater treatment system of a Pharmaceutical Manufacturing facility is carried out with special reference to antimicrobial discharges. The manufacturing process studied dealt with formulation, filling and packaging of preparation for prescription products. Thus this facility falls into the 'dry formulation' category. A good clean supply of water is essential in this industry. The water used in the manufacturing areas is mainly for cleaning/washing of processing equipment and accessories. Water is also used for cooling the housing of the moving parts in machines. All liquid effluents generated pass through a wastewater treatment plant prior to discharge into an inland surface water body.

University of Moratuwa, Sri Lanka. Electronic Theses & Dissertations

The wastewater treatment plant in this facility consists of an activated sludge treatment process. For a primary understanding of this wastewater treatment plant a study of the general characteristics were done. The effect on the overall efficiency of the wastewater treatment plant was observed while studying the washing/cleaning activities carried out in the production zones on each sample collecting day. The findings of this study showed an overall efficiency less than the design efficiency of the wastewater treatment plant. It is seen that plant inputs and generated waste loads are low even when compared with typical waste characteristics of such facilities. A large fraction of the BOD/COD ratios determined showed low values. About 92% of the BOD/COD ratios determined in the equalization tank were less than 0.5. The analysis of BOD, with different dilutions was done in order to determine whether there is an effect by any antimicrobial substance on the biological activity.

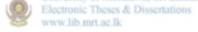
iv

The BOD test carried out for a series of wastewater sample dilutions showed decreasing BOD values as the dilution percentage increasing on certain days. In some days results showed 31% to 34% reduction in BOD value indicating the presence of an antimicrobial substance in the wastewater sample on that day.

Further, in order to study the antimicrobial activity of the wastewater discharged from this facility, *E. coli* was tested in liquid culture medium. The growth of *E. coli* was monitored as absorbance at 600nm in the presence and absence of waste samples.

The results indicated the growth of *E. coli* in wastewater was not as high as the growth shown in the peptone water medium. It was also indicated that the growth of this organism in the wastewater sample was not inhibitory as in the solution containing amoxycillin  $(10\mu g/ml)$ .

A study of the effect on the same organism was also carried out using the antibiotic diffusion technique. The inhibition zones created from a concentration series of an antimicrobial substance were studied with the inhibition zone produced by wastewater.



The study carried out using the cylinder plate test showed an inhibition zone for wastewater. For a wastewater sample taken from the wastewater treatment plant after cleaning Rifampicin processed machines, a clear zone was observed giving an effect equivalent to a concentration of 2.9 µg of amoxycillin per milliliter.

From the observed results, heavy impact on the wastewater treatment plant operation from discharges was not indicated. However the precautions are needed to be taken when contemplating expansion etc. as the present observations and conclusions apply only to current loading conditions. Close monitor of waste effluent would be necessary in future if expansion is sought by the company.

v

## Table of Contents

Acknowledgement	iii
Summary	iv
Table of Contents	vi
List of Figures	ix
List of Tables	х
List of Abbreviations	xi

Chapter I

4

4

5

۲

#### Introduction and an Overview of the Pharmaceutical Industry in Sri Lanka.

1.1. Introduction	1
1.2. The Pharmaceutical Industry in Sri Lanka	3
1.3. Scope of the project	5



### Chapter 2

#### Pharmaceuticals, Pharmaceutical Industry and its Wastewater Treatment

2.1 Introduction	6
2.2 Classification of antimicrobial drugs	6
2.2.1 The drugs of importance in this study	8
2.3 Liquid waste generated and wastewater treatment plants in	
Pharmaceutical Industry	10
2.3.1 Liquid waste generated from Pharmaceutical Industry	10
2.3.2 Wastewater treatment in Pharmaceutical Industry	11
2.4 Activated Sludge	14
2.5 Concepts of indicating the effect by antimicrobial substances	17
2.6 Observed effects on waste treatment plants due to antimicrobial	
substances in the pharmaceutical industry	19

Page

### Chapter 3

٨

4

### State Pharmaceuticals Manufacturing Corporation Production Facility and its Wastewater Treatment Plant

3.1 Introduction 2	
3.2 State Pharmaceuticals Manufacturing Corporation	21
3.2.1 The SPMC Production Facility	24
3.2.2 Process flow schematics	25
3.2.3 Water consumption at SPMC	· 28
3.2.4 Drain Layout	34
3.3 Wastewater Treatment Plant	36

### **Chapter 4**

### Study of General Characteristics of the Wastewater Treatment Plant

4.1 Introduction		43
4.2 Metho	odology	43
	BOD Determination iversity of Moratuwa, Sri Lanka.	48
4.2.2	COD Determination cronic Theses & Dissertations	49
4.2.3	TSS & TS Determination	49
	Viable Count of Bacteria	49
4.3 Calcu	lation and Results	52
4.3.1	Process Efficiency	52
	F/M (Food to Microorganism) Ratio	52

### Chapter 5

#### Antimicrobial Effect Study

5.1	Metho	dology	62
	5.1.1	Effect on BOD test	62
	5.1.2	Effect of wastewater on microorganism growth	62
	5.1.3	Inhibition effect of wastewater - Cylinder Plate Method	64
5.2	Result		67
	5.2.1	Effect on BOD test	67
	5.2.2	Effect of wastewater on microorganism growth	69
		Inhibition effect of wastewater - Cylinder Plate Method	73

#### Chapter 6

#### Discussion

### Chapter 7

-1

5

#### **Conclusions and Suggestions for Further Study**

7.1 Conclusions		83
7.2 Suggest	ions for Further Study	84
7.2.1	Capacity to handle production expansion	84
7.2.2	Waste minimization impacts on wastewater performance	84

#### References

#### Appendices

University of Moratuwa, Sri Lanka, Electronic Theses & Dissertations www.lib.mrt.ac.lk

A Drugs Produced at SPMC

**B** Samples Collected Days

- C1 General Characteristics of wastewater
- C2 Cleaning/washing in General Drugs Manufacturing Zone and Penicillin Zone
- C3 Variation of BOD with the variation in sample dilution Equalization Tank
- C4 Variation of BOD with the variation in sample dilution
  - Wastewater from General Drugs Production Zone
  - Wastewater from Penicillin Drugs Production Zone
- C5 Absorbance observed in the spectrophotometer
- C6 Cylinder Plate Test Clear Zone Diameters
- D General Standards for discharge of effluents into inland surface waters
- E Wastewater transfer in the Wastewater treatment plant and retention times in each tank.

# List of Figures

للله

4

**.**¥

۶

ĸ

i L

1.1	Pharmaceutical Products - Imports to Sri Lanka	4
2.1	Generalized Schematic of a bacterial cell	7
2.2	Structure of some penicillins	9
2.3	WWTP Block Diagram	12
2.4	WWTP Block Diagram - Pharmaceutical Manufacturer in North Chicago, USA	13
2.5	WWTP Block Diagram - Wastewater containing organic compounds	13
2.6	Activated Sludge Process	14
3.1	State Pharmaceutical Manufacturing Corporation	21
3.2	Location map of State Pharmaceutical Manufacturing Corporation	22
3.3	Detailed map of wastewater flow from SPMC into Bolgoda Lake	23
3.4	Production Zones at SPMC	25
3.5	Process flow Schematic - General Drugs	26
3.6	Process flow Schematic - Penicillin Drugs	27
3.7	Water utilization Flow diagram	38
3.8	Typical Process Equipment washed in Production Areas	30
3.9	Procedure for cleaning equipment at a change over	33
3.10	Drain Layout	35
3.11	Wastewater Treatment Plant (WWTP - SPMC)	37
3.12	Wastewater Treatment Plant - Flow Diagram	38
4.1(a)	Plan view and details of the WWTP	43
4.1(b)	Sampling Points of the WWTP	45
4.2	Variation in total solids	54
4.3	Variation in total suspended solids	54
4.4	Variation in pH	55
4.5	Variation in temperature	55
4.6	Variation in turbidity	56
4.7	Plate count of bacteria (Plate count agar)	57
4.8	Plate count of bacteria (Nutrient agar)	57
4.9	Variation of BOD	58
4.10	Variation of COD	58
4.11	F/M Ratio	61
4.12	BOD/COD Ratio in the equalization tank	61
5.1	BOD vs Dilution % - Samples from Equalization Tank	68
5.2	Samples inoculated with 0.05 ml of the <i>E. coli</i> culture	72
5.3	Samples inoculated with 0.10 ml of the <i>E. coli</i> culture	72
5.4	Samples inoculated with 0.15 ml of the <i>E. coli</i> culture	72
5.5	Clear Zone diameter vs Antibiotic concentration	73
5.6	Cylinder Plate Test : Graph	74
5.7	Cylinder Plate Test : Clear Zone Observation	75

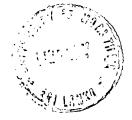
### List of Tables

1.1	Pharmaceutical Industry	1
1.2	Characteristics of wastewater from Primary Manufacturing	2
2.1	Classification of antimicrobial agents	6
2.2	Pharmaceutical process wastes	11
2.3	OUR for different wastes	18
3.1	Antimicrobial drugs produced at SPMC	25
3.2	Water consumption observations	32
3.3	Wastewater Treatment Plant design conditions (design data)	36
4.1	Analytical Technique	47
4.2	Wastewater Treatment Plant Efficiency - overall efficiency	59
4.3	Wastewater Treatment Plant Efficiency - activated sludge plant	59
4.4	F/M Ratio	60
4.5	BOD/COD Ratio in Equalization Tank	60
4.6	Dissolved Oxygen Content in Aeration Tank	60
5.1	Absorbance observed in the spectrophotometer	71
5.2	Cylinder Plate Test - Effect of wastewater	74



University of Moratuwa, Sri Lanka. Electronic Theses & Dissertations www.lib.mrt.ac.lk

х



4

5

.

-4

¥

### List of Abbreviations

BAT	Best Available Technology
BATNEEC	Best Available Technology Not Entailing Excessive Cost
BBT	Bacterial Bioluminescence Test
BOD	Biochemical Oxygen Demand
BP	British Pharmacopoeia
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EPA	Environmental Protection Agency
F/M	Food to Microorganism
GAC	Granular Activated Carbon
GD	General Drugs
GMP	Good Manufacturing Practices
MLSS	Mixed Liquor Suspended Solids
N/A	Not Applicable
NA	Nutrient Agar
NTU	Nephelometric Turbidity Units
OUR	Oxygen Uptake Rate
PCA	Plate Count Agar
R&D	Research and Development
SBL	Still Base Liquor
SIC	Standard Industrial Classification
SPC	State Pharmaceutical Corporation
SPMC	State Pharmaceutical Manufacturing Corporation
SS	Suspended Solids
TS	Total Solids
TSS	Total Suspended Solids
USP	United States Pharmacopoeia
UV	Ultra Violet
WHO	World Health Organization
WWTP	Wastewater Treatment Plant



4

4

لحد