Characterization of liquid effluent from a Pharmaceutical Production Facility.

Ms. Manisha Gunasekera, *Department of Chemical Engineering, University of Moratuwa.*
Dr. Indrika Abeygunawardana, *Department of Microbiology, University of Kelaniya.*
Dr. Ajith De Alwis, *Department of Chemical Engineering, University of Moratuwa.*
Mr. W.Pathirana, *State Pharmaceuticals Manufacturing Corporation, Ratmalana.*

Abstract

Pharmaceutical manufacturing industry is expected to generate effluents having a wide variety of anti microbial substances. The effluent has to meet the Central Environmental Authority's relevant discharge standards. The biological treatment processes should be capable of handling effluents, containing these inhibiting materials without being subjected to inhibitory effects.

A wastewater treatment plant of a Pharmaceutical formulation manufacturing company that produces solid dosage forms was analyzed. This wastewater treatment plant deals with both human waste and factory waste together. The manufacturing process deals with the formulation, filling and packaging of preparations for prescription products.

The wastewater was investigated for its general characteristics such as temperature, pH, turbidity, BOD, COD, TSS and TS. The average BOD_5 and COD removal efficiency of this wastewater treatment plant was 68 and 45% respectively. The TSS removal efficiency was 70%. Different liquid culture media including wastewater from the equalization tank were inoculated with an equal amount of the microorganism *E.coli* and were measured for absorbance in the spectrophotometer after 24 hrs, 48 hrs and 72 hrs. Presence of an anti microbial substance in the wastewater was also determined by determining the sensitivity to the microorganism *E.coli* using the cylinder-plate technique.

Introduction

There are eight major pharmaceutical manufacturers in Sri Lanka presently. The contribution from these local manufacturers is about 35% of the total requirement in this country (WHO Mission 1993). Rest of the pharmaceutical requirement is mainly met through imports. The two main steps in the pharmaceutical manufacturing industry are bulk manufacturing industry and formulation industry. All the pharmaceutical manufacturing industries in Sri Lanka fall under the latter category.

The pharmaceutical production facility of this study produces solid dosage forms of drugs. This facility which is equipped with the most modern machinery has an annual capacity of 550 million units of tablets or capsules and 60000 liters of dry syrup. The organization produces about 42 different drugs presently. The production area of this facility consists of two main sections where penicillin drugs and non-penicillin drugs are manufactured separately. Pipe borne water is used and is entirely supplied by the National Water Supply and Drainage Board. All effluents are collected and treated in a wastewater treatment plant before being discharged. This treated wastewater is then discharged to an open canal, which leads to the Bolgoda Lake.

Water Consumption

The incoming pipe born water is treated before it is used in the facility. The water consumption at this facility is about 1100 m^3 per month. As solid dosage formulation, filling and packing are the main activities, the process water usage is minimal (Table 1). For the preparation of starch as a binder and sugar and film coating blends, water is used in small quantities. However, in these production areas water is extensively used for subsequent cleaning and washing. Processing equipments are washed and cleaned when the manufacturing product is changed. Water is also used for the washing of accessories such as containers and equipment used for preparing paste and film coating materials. The retained process materials adhering on to the surfaces get removed with the wash water.

When considering characteristics of wastewater from a primary manufacturing of pharmaceutical drugs, the BOD_5 of the final effluent strengths in some wastes may be 600 mg/l and some pharmaceutical waste have BOD_5 in excess of 200000mg/l. (Environmental Technology Consultants 1993). In a dry dosage formulation pharmaceutical manufacturing facility, such high strength effluents are not expected to happen (EPA 1991). Since many non-contaminated water is also entering the wastewater treatment plant in this study low strength effluents could be expected.

The Wastewater Treatment Plant

The wastewater treatment plant of this facility consists of a wastewater collection tank. Sewage screened through a bar screen enters the collection tank and the wastewater from production areas enters this tank separately (fig.1). It collects wastewater and transfers it to the equalization tank (fig.2). This wastewater is screened through a fine screen before entering the equalization tank. The contents in the equalization tank are mixed continuously using air diffusers placed at the bottom of the tank. Wastewater is transferred from the Equalization tank to the aeration tank through a flow rate-controlling device. During this transfer wastewater flows through the aeration tank, sedimentation tank, sterilization (Chlorination) tank and to the outlet of the wastewater treatment plant under gravity flow. In aeration tanks aeration is done continuously throughout the day by air diffusers placed at the bottom of the tanks. Sludge collected at the bottom of the sedimentation tank is transferred to a sludge storage tank once a day and during this transfer part of the sludge is sent back to the aeration tank as the return sludge. The rate of return of the activated sludge in a typical activated sludge plant is usually between 50% to 100% times the (volumetric basis) inflow of the wastewater.

Materials and Methods

General Process Analysis

Wastewater samples collected from collection tank, equalization tank, two aeration tanks and from the outlet were analyzed for the parameters shown is table 2.

Samples taken from the equalization tank were analyzed for BOD for six different dilutions. (i.e. 3.33%, 5%, 6.67%, 8.33%, 10% and 13.33%). The purpose of this test was to ascertain the presence of any substance in wastewater, which can cause inhibition in the microbial activity of this test.

Anti microbial effects

Growth characteristics of microorganisms in different media vary depending on the amount of nutrients and anti microbial substances present while keeping other growth factors constant. The growth of the microorganism *E.coli* DMBUK3001 was observed by measuring absorbance (wave length 600 nm) by using different culture materials. Culture media were 5ml samples of 0.1% peptone water, 10 μg/ml amoxycillin solution (1ml of 50 μg/ml amoxycillin solution in 4ml of 0.1% peptone water), distilled water and filtered wastewater collected from equalization tank (filtered through a membrane filter nominal pour size 0.45 μm). The microorganisms *E.coli* grown in 0.5% peptone water was inoculated in these media by aliquots of 0.05 ml, 0.1 ml and 0.15 ml, and were incubated at 37^0C. The turbidity was measured at the time of incubation and after 24 hours, 48 hours and 72 hours. A control sample was also analyzed for each media.

The presence of anti microbial substances could be observed by microbial characteristics such as bioluminescence, oxygen uptake rate, inhibitory effects etc. The inhibition of the growth of a microorganism from a wastewater sample from equalization tank was tested using the cylinder plate technique, and a comparison was done with the sensitivity of Amoxycillin to *E.coli* DMBUK3001. Amoxycillin standard solutions were prepared in distilled water having concentration 500μg/ml, 100μg/ml, 50 μg/ml, 10 μg/ml and 1μg/ml. The organism *E.coli* in the 0.5% peptone water (70% transmittance, 600nm wavelength) was spread (0.08ml) on nutrient agar medium. Quantities 0.08ml from each of the amoxycillin standard solutions and wastewater were tested for inhibition zones. Wastewater was used after filtering through a membrane filter (nominal pore size 0.45μm) and sterilized distilled water was used as the blank solution.

Results and Discussion

General Effluent Characteristics

Wastewater from the collection tank, equalization tank, aeration tanks and from the outlet have temperature in the range 28^0C to 31^0C. The lowest recorded pH was 6.88 from the collection tank and the highest recorded pH value 8.46 was also from the collection tank. Therefore the pH variation was highest in the collection tank. Collection tank holds the wastewater discharged from the factory for approximately 40 minutes. Therefore the wastewater pH vary with the variation of the wastewater discharged at the time of collection. The pH values in the aeration tanks, where the biological treatment takes place, vary between 6.88 and 8.0. The general range of operation of an aeration system is between pH 6.5 and 8.5 (Hammer 1977).

The turbidity observed in the collection tank was within the range 12-48 (NTU) and that of equalization tank was 10-44 (NTU). The turbidity measured in the outlet samples varies in the range 4.5-16 (NTU). For an effluent from a wastewater treatment plant these values could be considered as low (very good quality with regard to this parameter) as even for drinking water, the WHO guidelines give a value of 5 NTU which is generally objectionable to consumers (WHO 1984).

BOD_5 in the collection tank vary between 14 mg/l to 62 mg/l. Of the total number of 12 samples six values were less than 30 mg/l which is the maximum tolerance limit of the general standards given for discharge of effluents into inland surface wasters by Central Environmental Authority. In the aeration tanks the maximum BOD_5 value observed was 29mg/l. The outlet wastewater has BOD_5 values 100% less than the maximum permissible BOD_5, 30 mg/l. The average BOD_5 of the influent in collection tank was 37 mg/l and that of the effluent of the wastewater treatment plant was 12 mg/l. Therefore the BOD_5 removal efficiency of this plant was 68%. The main wastewater treatment process of this wastewater treatment plant is a biological treatment process. In the aeration tank 1, 50% of the F/M ratios were above 0.5 and in the aeration tank 2 about 17% were above 0.5 (fig. 3). The maximum BOD_5 value observed in the aeration tanks was 29mg/l. Therefore, wastewater treated in this process has very low strength effluent.

The COD values in the wastewater of collection tank vary in the ranges 33mg/l to 257mg/l. The average COD value in this tank was 122mg/l. The COD value variation in the equalization tank was between 26mg/l to 161 mg/l. In the outlet wastewater COD values observed varied between 18mg/l to 239 mg/l. The average of these values was 67 mg/l. Therefore the COD removal efficiency was 45%. Although the COD removal efficiency was low the effluent discharged from this wastewater treatment plant complies with the maximum tolerance limit 250 mg/l of COD. When comparing the ratios BOD/COD, 92% of the wastewater in the equalization tank were less than 0.5 (Table 3).

The effluent discharged from the wastewater treatment plant has a maximum TSS of 26 mg/l and the maximum tolerance limit for this parameter is 50mg/l. Therefore this parameter also complies with the standards. Total suspended solids in the inlet to the wastewater treatment plant vary from 12 mg/l to 96 mg/l and the variation in the outlet was from 4 mg/l to 26mg/l. The TSS removal efficiency for these ranges was 70%.

The total viable count of the bacteria were in the ranges 11700 CFU/ml to $67x10^4$ CFU/ml in aeration tanks on Nutrient Agar medium and on Plate Count Agar it was observed in the ranges 12500 - $25x10^5$ CFU/ml.

BOD_5 variation in various sample dilutions

The variations in the BOD_5 value for the six dilutions of five samples taken from the equalization tank are shown in fig.4. In two of the five days a decrease in the BOD value was observed with the decreasing of dilutions. In lower dilutions high concentration of the inhibition substance is present, consequently the inhibition of biological activity is greater. In samples 1 and 2 a decrease in BOD_5 from 64 mg/l to 44 mg/l and 69 mg/l to 45 mg/l respectively were observed with the decrease in dilution. The average dissolved oxygen in aeration tanks was 6.6 mg/l. This shows that there was adequate aeration for the biological treatment process. The dissolved oxygen level was quit high and this has the tendency to reduce toxic effects.

Inhibitory effect on E.coli

The absorbance variation observed for sample 1 (0.05 ml aliquots of *E.coli*), Sample 2 (0.10ml aliquots of *E.coli*) and Sample 3 (0.15 aliquots of *E.coli*) are shown in fig. 5. During the first 24 hours, highest increase in the absorbence was observed in the culture grown in 0.1% peptone water. During the next 24 hours to 48 hours the increase in absorbance was less. Since there were no inhibitance for the microorganism's growth and the presence of enough nutrients for the microorganism growth, high absorbance values were observed. The next highest increase in absorbance was seen in the wastewater from the equalization tank. Therefore this organism *E.coli* does not grow in wastewater in the same rate as it is in the 0.1% peptone water. In distilled water, during the first 24 hours absorbance of the samples increase but not as much as the increase in wastewater. An inhibitory growth was shown in the 10μg/ml amoxycillin solution.

Determination of presence of an anti-microbial substance in the wastewater

The figure 6 shows the variation in the clear zone diameter with the amoxycillin concentration in a semi-log scale. From the best fit curve the equivalent amoxycillin concentration to the wastewater sample collected from the equalization tank was given as 2.9 μg/ml. Therefore this wastewater contains an anti microbial substance sensitive to *E.coli* micro-organism equivalent to the sensitivity of 2.9μg/ml amoxycillin to the same organism.

Conclusion

The characteristics of the wastewater in this facility show extremely low organic contents. Large quantities of water not contaminated with any contaminants enter and dilute the wastewater stream. The decrease in BOD values with the lowering of dilution shows that the wastewater samples contain substance or substances, which inhibit the biological activity. The effective zone of the wastewater is equivalent to a one where amoxycillin concentration is 2.9μg/ml. Since this factory is planing to meet some foreign demand, an increase in the production capacity is expected in the future. Therefore a vigilant approach to detect any anti microbial substance entering the wastewater treatment plant should be adopted in managing this plant. Anti microbial substances entering the wastewater treatment plant could either kill microorganisms or inhibit their growth and result in low treatment efficiencies. If it is possible to have a detection for anti microbial substance in wastewater streams entering this treatment plant, measures could be taken to reduce the anti microbial concentration to a level that would not affect the biological activity in the activated sludge treatment process.

References

Environmental Technology Consultants Ltd., (ETC) 1993, "Anaerobic Digestion of industrial wastewater, A survey of potential application in the United Kingdom Industry ETSU B 1294, pp52

EPA 1991, Guides to Pollution Prevention, The Pharmaceutical Industry

Hammer M.J. 1977, Water and wastewater Technology, pp 94

Standard Methods, 19th edition 1995, For Examination of water and wastewater, APHA AWWA WEF

WHO 1984, Guidelines for Drinking Water Quality, Volume 2

Table 1 Water Consumption of Different Processes

Water Consumption	Stage
High (>1000 l/day)	Bottle washing, Washing of work personnel uniforms, Oscillating/mixing, Cooling water for Dry Granulator, Fine Mill and Vacuum Pump in the capsule-filling machine.
Medium (1000 l/day to 100 l/day)	Blending and Kneading Machine, Canteen, Quality Control Laboratory, Toilets, Paste Preparation.
Low (<100 l/d)	Sugar coating pans washing General washing

Table 2 Method of analysis of parameters – General Characteristics

Parameter	**Method/Equipment**	**Reference**	**Comment**
Temperature	Thermometer	Standard	on site
PH	pH meter	Methods	measurement
Turbidity	Nephelometry	19^{th} Edition	
BOD_5^{20}	Titrimetry	1995	
COD	Open reflux, Titrimetry		
TSS	Gravimetry		
TS	Gravimetry		
Viable count of bacteria	Pour plate		

Table 3 BOD/COD ratio of the wastewater from Equalization Tank

Sampling Day	BOD/COD
1	0.03
2	0.26
3	0.20
4	0.03
5	0.17
6	0.24
7	0.40
8	0.63
9	0.41
10	0.19
11	0.36
12	0.50

Fig.1 Drain Layout

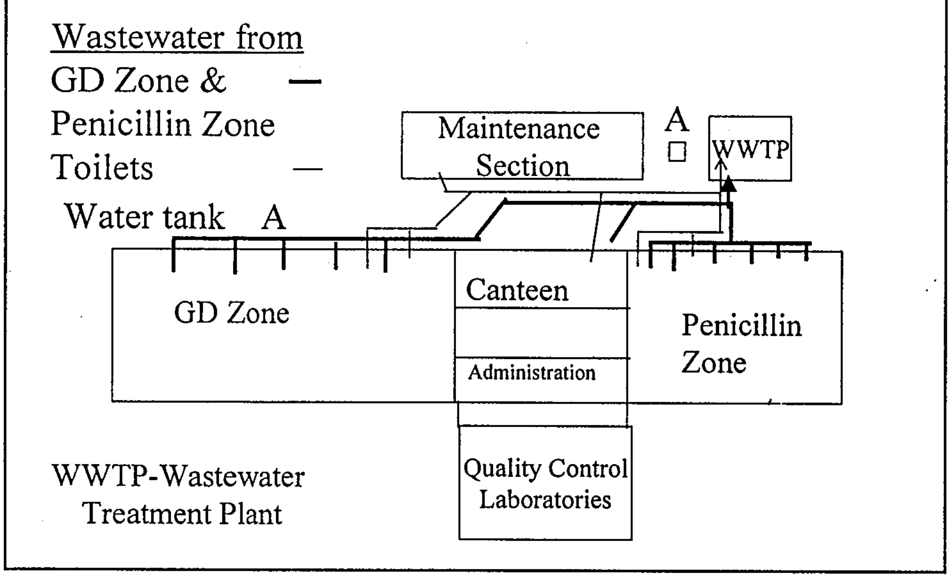
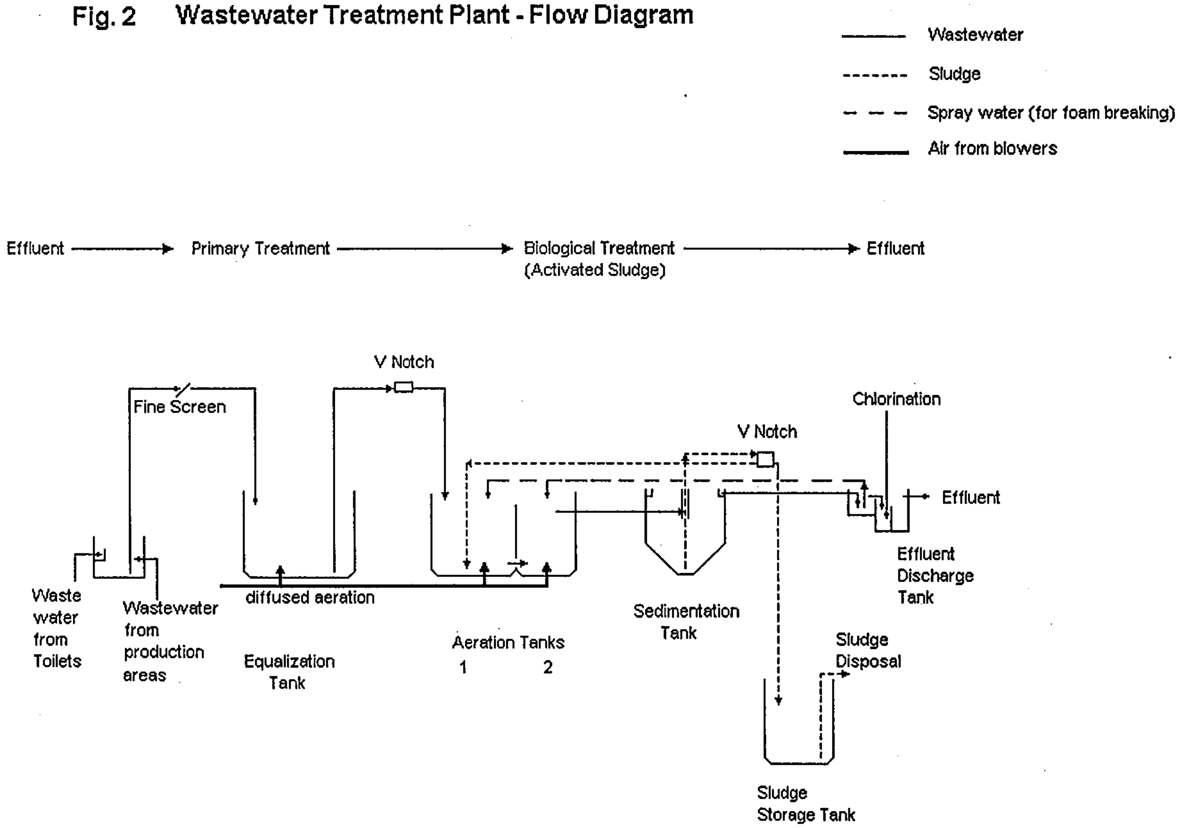


Fig. 2 Wastewater Treatment Plant - Flow Diagram



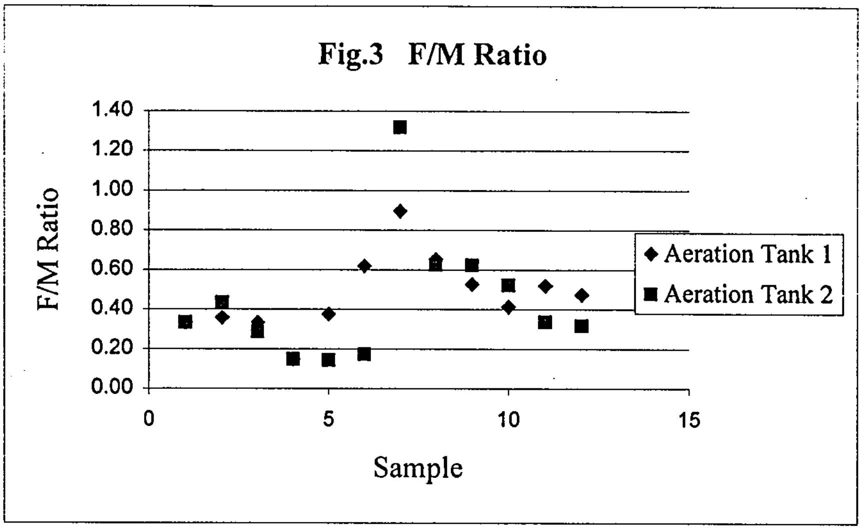


Fig. 4 BOD Vs DILUTION % - SAMPLES FROM EQUALIZATION TANK

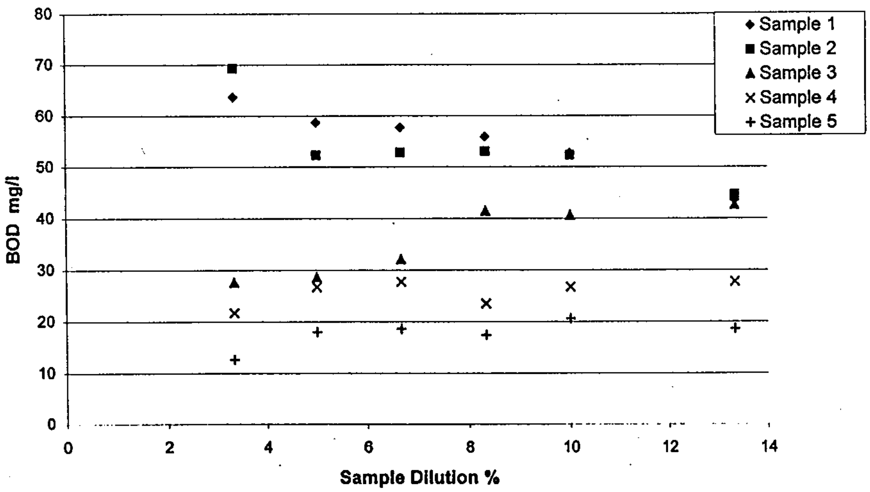
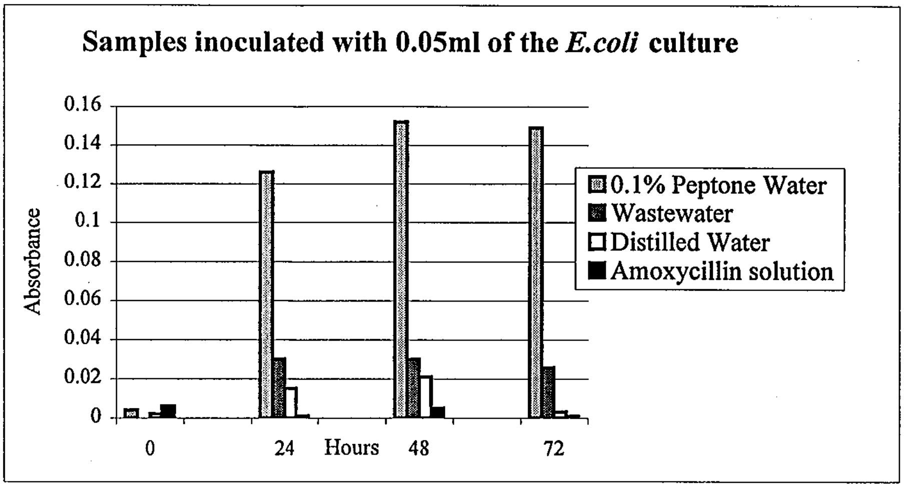
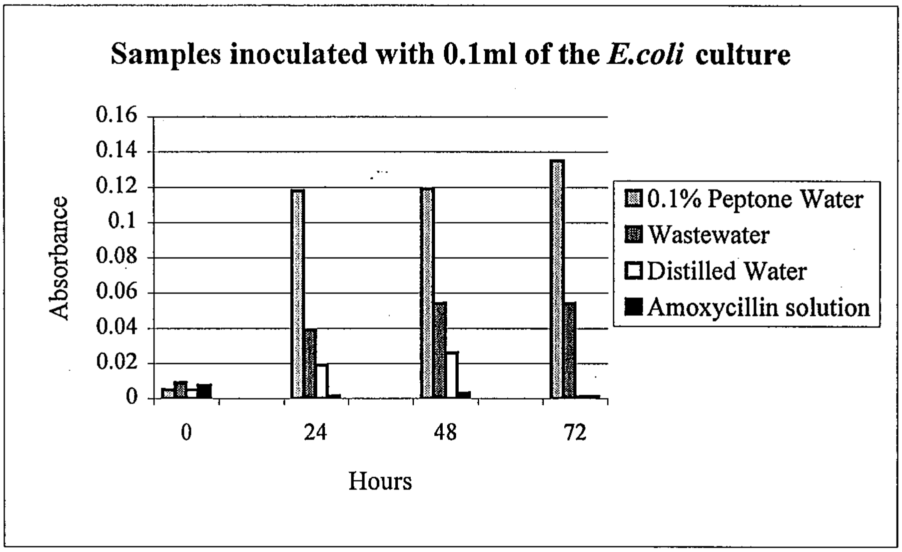


Fig. 5 Absorbance variation Observed





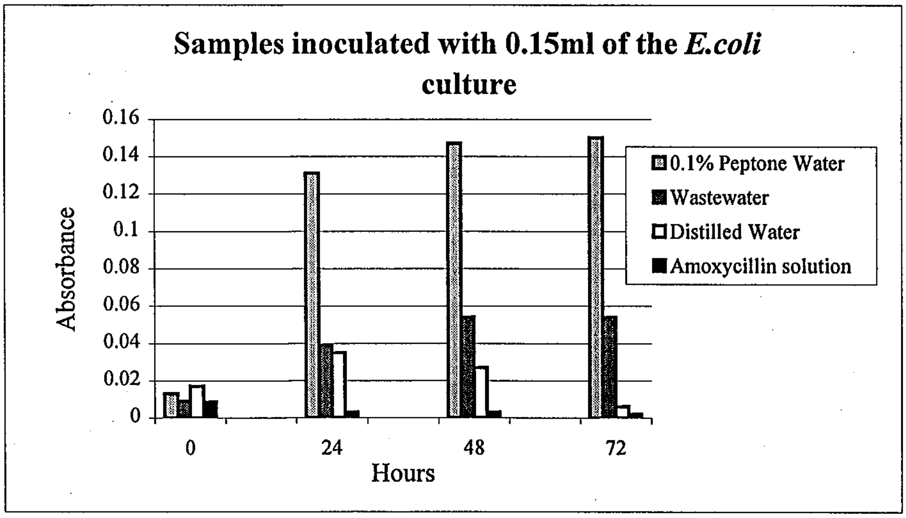
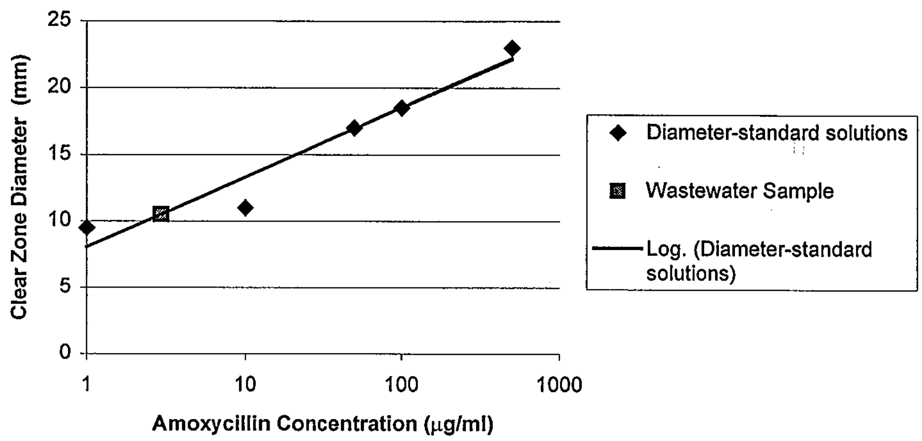


Fig. 6 Cylinder Plate Test



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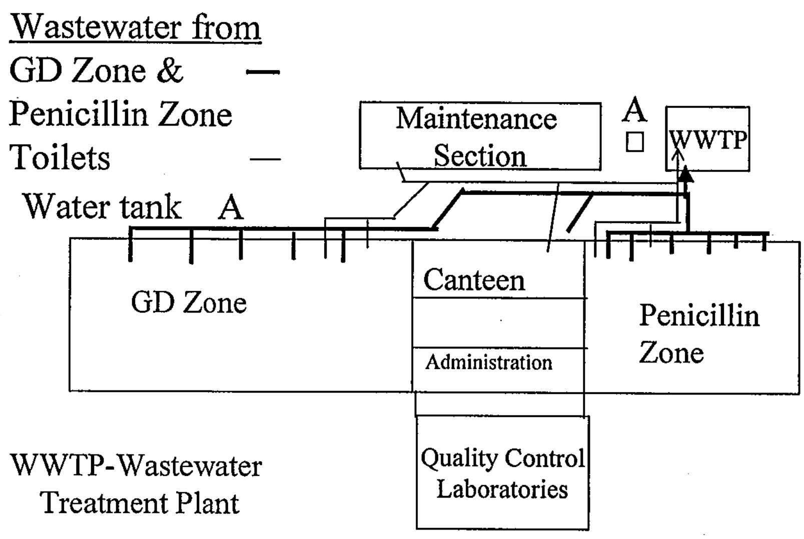

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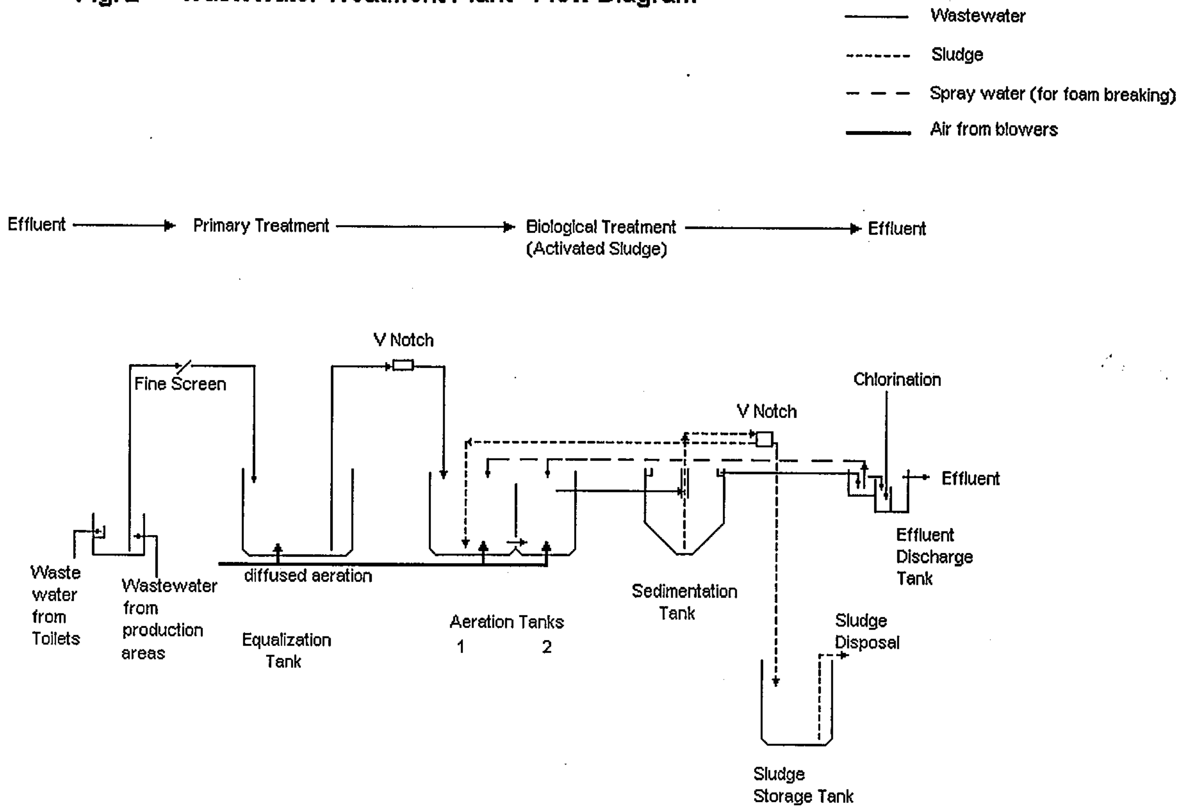

Fig.3 F/M Ratio

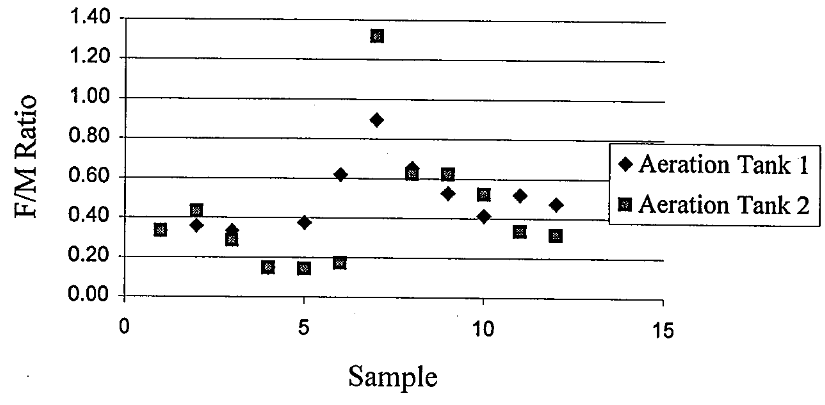

Fig. 4 BOD Vs DILUTION % - SAMPLES FROM EQUALIZATION TANK