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**BIOLOGICAL TREATMENT OF MILK AND SOYBEAN WASTEWATER  
WITH BIOPRODUCTS**

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Bachelor of Science in Civil Engineering

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July, 2007

Submitted in partial fulfillment of requirements for the degree

MASTER OF SCIENCE IN CIVIL ENGINEERING

at

CLEVELAND STATE UNIVERSITY

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**BIOLOGICAL TREATMENT OF MILK WASTEWATER AND SOY MILK  
WASTEWATER WITH BIOPRODUCTS**

**TIANZHU BI**

**ABSTRACT**

Dairy industries discharge larger amounts of wastewater as compared to other food industries. Wastewater contains high amount of total organic carbon materials and nutrients, such as fat, protein, and lactose.

Biological treatment is widely used to treat this kind of wastewater due to the fluctuation of amount and content of dairy wastewater. This study investigated removal of total organic carbon (TOC) from two types of dairy wastewater—milk and soy milk wastewater. The bioproducts used in experiments were baker's yeast, beer's yeast, live liquid microorganism (LLMO), Enforcer Overnite Toilet Care Liquid, and Enforcer Overnite Toilet Care Granular. The parameters included in this study were shaking time, concentration of wastewater, types of wastewater and bioproducts.

Overnite Toilet Care Granules and Baker's yeast were very effective to remove TOC from milk wastewater. But when Overnite Toilet Care Granules dissolved, more particles were produced and increased the amount of TOC. So Baker's yeast was more suitable to treat milk wastewater than the others. The best result is 57% of TOC removal and happened at 6 hours when concentration of TOC was 25 mg/l. G1 is the best bioproduct for TOC removal from soybean milk wastewater. 75.2% of TOC was removed by using G1. It was more than twice higher than TOC removal by using Baker's yeast and Overnite

Liquid Drain Care. Although the removal rate of using beer's yeast is almost the same as using Baker's yeast, Beer's yeast did not show steady results. Beer's yeast and Liquid Drain Care did not yield good results for treating both milk and wastewater. Because Beer's yeast and Liquid Drain Care contained unknown components and low concentrations of bacteria.

Key Words: Milk Wastewater, Soy Milk wastewater, Baker's yeast, Beer's yeast, LLMO, Enforcer Overnite Toilet Care Liquid, and Enforcer Overnite Toilet Care Granular

## TABLE OF CONTENTS

ACKNOWLEDGMENTS .....	iii
ABSTRACT .....	iv
Table of contents .....	vi
LIST of TABLES.....	ix
LIST OF FIGURES .....	xii
CHAPTER I	
INTRODUCTION .....	1
CHAPTER II	
OBJECTIVES.....	4
CHAPTER III	
LITERATURE REVIEW .....	5
3.1 Food Processing .....	5
3.2 Dairy wastewater.....	6
3.2.1 Description of Dairy Wastewater .....	6
3.2.2 Chemical Processes .....	7
3.3 Aerobic treatment of food industrial wastewater .....	8
3.4 Anaerobic treatment of food industrial wastewater .....	9
3.5 Aerobic and/or anaerobic method .....	11
3.6 Common Biological Method to Treat Dairy Wastewater .....	13
3.6.1 Activated sludge .....	13

3.6 .2 Trickling filter .....	15
3.6.2.1 Definition of Trickling filter.....	15
3.6.2.2 Purposes of Trickling Filter.....	15
3.6.2.3 Selection of Biofilm Media.....	16
3.6.3 Membrane bioreactor.....	16
3.6.4 Wetland .....	17

## CHAPTER IV

MATERIALS.....	20
4.1 Materials .....	20
4.1.1 Types of LLMO used in the experiment.....	20
4.1.2 Yeast.....	20
4.1.3 Brewer’s yeast .....	21
4.1.4 Baker’s yeast .....	22
4.2 Bioaugmentation.....	23
4.3 Wastewater.....	24
4.3.1 Milk Wastewater.....	24
4.3.2 Soybean Wastewater.....	24
4.4 Methods .....	24
4.4.1 Making milk wastewater.....	24
4.4.2 Making soybean milk wastewater .....	25
4.4.3 Procedure of measuring TOC.....	26

## CHAPTER V

RUN PROPOSAL .....	28
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CHAPTER VI	
RESULTS DISCUSSION .....	46
6.1 Results of Run 1 to Run 18.....	46
6.2 Comparison of all runs .....	53
CHAPTER VII	
CONCLUSION .....	55
7.1 Conclusions.....	55
7.2 Engineering Significance.....	56
7.3 Recommendations.....	56
REFERENCES.....	57
APPENDICES.....	65
APPENDIX A	
CALIBRATION OF SOYBEAN MILK AND MILK SOLUTION.....	66
APPENDIX B	
RESULTS OF TABLES.....	70
APPENDIX C	
RESULTS OF FIGURES .....	88

## LIST OF TABLES

Table I Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast .....	30
Table II Run #2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast .....	31
Table III Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast.....	32
Table IV Run #4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast .....	33
Table V Run #5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast.....	34
Table VI Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast.....	35
Table VII Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granule .....	36
Table VIII Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnite Toilet Care Granules .....	37
Table IX Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnite Toilet Care Granules .....	38
Table X Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care .....	39
Table XI Run #11 25, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care ...	40
Table XII Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care...	41
Table XIII Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1 .....	42
Table XIV Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast ..	43
Table XV Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care .....	44

Table XVI Run #16 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1 .....	45
Table XVII Calibration of Milk Solution.....	66
Table XVIII Calibration of Soybean Milk .....	68
Table XIX Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast Results	70
Table XX Run #2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast Results .....	71
Table XXI Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast Results .....	72
Table XXII Run #4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast .....	73
Table XXIII Run #5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast .....	74
Table XXIV Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Beer's Yeast.....	75
Table XXV Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granules .....	76
Table XXVI Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnite Toilet Care Granules .....	77
Table XXVII Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnite Toilet Care Granules .....	78
Table XXVIII Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care .....	79
Table XXIX Run #11 25, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care .....	80
Table XXX Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care	81
Table XXXI Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1 .....	82

Table XXXII Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast .....	83
Table XXXIII Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care.....	84
Table XXXIV Run #16 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1 .....	85
Table XXXV Comparison between Runs, Ranged by Type of Wastewater .....	86
Table XXXVI Comparison of All runs, Ranged by Type of Bioproduct.....	87

## LIST OF FIGURES

Figure 1 Calibration of Milk Solution .....	67
Figure 2 Calibration of Soybean Milk .....	69
Figure 3 Bar Chart of Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast Results.....	88
Figure 4 Line Chart of Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast Results.....	89
Figure 5 Bar Chart of Run#2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast Results.....	90
Figure 6 Line Chart of Run#2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast Results.....	91
Figure 7 Bar Chart of Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast Results.....	92
Figure 8 Line Chart of Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast	93
Figure 9 Bar Chart of Run #4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast Results .....	94
Figure 10 Line Chart of Run#4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast Results.....	95
Figure 11 Bar Chart of Run #5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast	

Results.....	96
Figure 12 Line Chart of Run#5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast Results.....	97
Figure 13 Bar Chart of Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast Results.....	98
Figure 14 Line Chart of Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast Results.....	99
Figure 15 Bar Chart of Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granules Results.....	100
Figure 16 Line Chart of Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granules Results.....	101
Figure 17 Bar Chart of Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnite Toilet Care Granules Results.....	102
Figure 18 Line Chart of Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnite Toilet Care Granules Results.....	103
Figure 19 Bar Chart of Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnite Toilet Care Granules Results.....	104
Figure 20 Line Chart of Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnite Toilet Care Granules Results.....	105
Figure 21 Bar Chart of Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care Results.....	106
Figure 22 Line Chart of Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care Results .....	107

Figure 23 Bar chart of Run #11, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care Results.....	108
Figure 24 Line Chart of Run #11 25, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care Results .....	109
Figure 25 Bar Chart of Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care Results .....	110
Figure 26 Line Chart of Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care Results .....	111
Figure 27 Bar Chart of Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1 Results .....	112
Figure 28 Line Chart of Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1 Results .....	113
Figure 29 Bar Chart of Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast Results .....	114
Figure 30 Line Chart of Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast Results .....	115
Figure 31 Bar Chart of Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care Results.....	116
Figure 32 Line Chart of Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care Results.....	117
Figure 33 Bar Chart of Run #16 25, 50, 100 and 150 mg/l Soybean Milk with 5.5 ml G1 Results.....	118
Figure 34 Line Chart of Run #16 25, 50, 100 and 150 mg/l Soybean Milk with 5.5 ml G1	

Results.....	119
Figure 35 %TOC Removal of Milk wastewater vs. Types of Bioproducts .....	120
Figure 36 %TOC Removal of Soybean Milk wastewater vs. Types of Bioproducts .....	121
Figure 37 Comparison of %TOC Removal between Milk and Soybean Milk by Same Types of Bioproducts.....	122
Figure 38 %TOC Removal vs. Types of Bioproducts .....	123
Figure 39 TOC Removal Rate vs. Types of Bioproducts .....	124



## **CHAPTER I**

### **INTRODUCTION**

The purpose of wastewater treatment is to remove pollutants that lower dissolved oxygen (DO) level. Because aquatic microorganisms will utilize oxygen-demanding materials as a food source and use oxygen for their metabolism, this leads to lower the concentration of oxygen in aquatic system and jeopardize higher life forms such as fish.

Compared to other food industries, dairy industries produce the largest volume of pollutant according to its water consumption (one liter of processed milk can produce 0.2-10L effluent)[1]. Dairy wastewater contains a large amount of lactose, fats, and proteins. This kind of wastewater which contains a high quantity of organic pollutants and nutrients needs to be treated before discharge. Several technologies can be used to treat dairy wastewater - biological treatment involving aerobic, anaerobic and aerobic/anaerobic systems, chemical treatment, land treatment and irrigation and membrane treatment technology. Among these methods, the biological treatment method is widely used to treat

dairy and dairy process wastewater [2]. Because fats, lactose and proteins are easily degraded by bacterial populations, the aerobic process is more preferred and common. The aerobic process is more effective compared to the anaerobic and anoxic method for removing organic pollutant with concentration range between 50-4000 mg/l.

On the other hand, soybeans are important source of vegetable oil and protein worldwide. Soybean products are the main ingredients in many meat and dairy substitutes. The main producers of soy are the United States, Brazil, Argentina, China and India. Historically, soybean has been used in China for 5,000 years as a food and a component of drugs. In China, Japan, and Korea the bean and products made from the bean are a popular part of the diet. Soy took on a very important role in the United States after World War I [3]. Common forms of soy include soy meal, soy flour, soy milk, tofu, textured vegetable protein, tempeh, soy lecithin and soybean oil.

Recently, the increasing application of soybeans and soybean-containing foods is lowering rates of cancer. One group of phytochemicals, the isoflavones, is found in soybeans and soybeans are for practical purpose the only source of isoflavones in the human diet [4]. Phytochemicals are non-nutritive plant chemicals and the plants which produce this kind of chemical can protect themselves from disease. It was demonstrated that phytochemicals can also help humans against disease. With the increasing amount of soy milk consumed, it is necessary to think about how the waste should be treated.

The treatment of soymilk and milk is very important due primarily because of the waste materials involved. The most common treatment methods include bio-treatment by bacteria, fungi or algae, composting, solidification, extraction, incineration, neutralisation, vitrification, and smelting. There are two types of biological wastewater treatment,

trickling or biological filter and activated sludge. Both types of treatment have two vessels. The first reactor contains microorganisms that remove organic organisms from wastewater. The second vessel called sedimentation tank, where the microorganism and treated wastewater get separated [5].

The purpose of this study is to investigate several parameters which affect the results of biological waste treatment. The parameters include the strength and type of wastewater, amount of shaking time, types and dosage of live liquid micro-organism (LLMO).

## **CHAPTER II**

### **OBJECTIVES**

The following objectives were considered in the study:

To evaluate the effect of concentration of soybean wastewater and milk wastewater on TOC removal.

To evaluate the effect of time on TOC removal

To evaluate the effect of soybean wastewater versus milk wastewater on TOC removal

To evaluate the effect of different bacteria types on TOC removal

To evaluate the effect of bacteria concentration on TOC removal

The experiments were conducted under laboratory scale conditions.

## **CHAPTER III**

### **LITERATURE REVIEW**

#### **3.1 Food Processing**

Water has a role in virtually every product produced in the food industry, and is used for various applications. Industrial processes often use water once and then discharge it back to the environment as effluent. However, its quality changes due to introduction of contaminants [6]. Therefore, food industry waste water discharged into aquatic systems is a main source of pollution.

Because food industries include a variety of foodstuffs, the industry's wastewater is organic in nature. High organic matter causes excessive loading of organic matter and nutrients into an aquatic system, which causes eutrophication. Eutrophication breaks the balance among water supply, fisheries and recreation. Ultimately, eutrophication

threatens public health [7].

Eutrophication generally promotes excessive plant growth and decay, favors certain weedy species over others, and may cause a severe reduction in water quality. In aquatic environments, enhanced growth of choking aquatic vegetation or phytoplankton (e.g. algal blooms) disrupts normal functioning of the ecosystem, causing a variety of problems such as a lack of oxygen in the water, an important component needed for fish and shellfish to survive. Water then becomes cloudy, colored with either a shade of green, yellow, brown, or red. As previously stated, human society is impacted as well--eutrophication decreases the resource value of rivers, lakes, and estuaries such that recreation, fishing, hunting, and aesthetic enjoyment are hindered. Health-related problems can occur where eutrophic conditions interfere with drinking water treatment [8].

## **3.2 Dairy wastewater**

### **3.2.1 Description of Dairy Wastewater**

Dairy wastewater contains a high concentration of nutrients, such as nitrite and phosphorus, two major compounds that cause algae bloom. Algae bloom leads not only to the unpleasant odor, but also excess sludge accumulation. Odor is caused by excess algae using up the dissolved oxygen and turning an aerobic condition into an anaerobic condition. Dead algae will settle at the bottom of the pond and also use dissolved oxygen to degrade. It is very costly to degrade and dispose of accumulated sludge. It will be very helpful to reduce the concentration of nutrient in wastewater to fix this problem.

Unlike other industries, parameters of food processing industries vary greatly. Those parameters include: BOD, pH, volume of wastewater and also concentration of pollutants.

In food processing industries, BOD can range from 100 ppm to 100,000 ppm, pH ranges between 3.5 and 11.0. The biggest difference between food industrial wastewater and other industries, or municipal wastewater, is the high concentration of organic pollutant [9].

### **3.2.2 Chemical Processes**

Most food industrial wastewater consists of simple sugars and starch. During organic degradation, organic matter is first hydrolyzed and fermented to produce organic acids and hydrogen gas, and then methanogens convert organic acids to methane gas. Biohydrogen gas is a product of food processing and domestic wastewaters [10].

The largest residual from food processing is liquid or solid waste. These processing residuals include cheese whey, tofu whey, process water and wastewater. Because food process residuals are used as animal feed, in land application and even have human uses, food processes residuals require treatment to adhere to federal regulations. The Activated Sludge Process is the most common treatment method used to remove BOD and nutrients such as nitrogen and phosphorus from food processing liquid residual. Filling and spreading are two major foods processing solid waste disposal methods. Moisture should be removed in order to reduce the land disposal cost [11].

Since dairy wastewater contain high concentrations of phosphorus and nitrogen, combining nitrification and denitrification with Enhanced Biological Phosphorus Removal is a more effective method than chemical precipitation. Because dairy wastewater characteristics vary throughout the industry, this treatment process requires more flexibility operation and online control [12].

### **3.3 Aerobic treatment of food industrial wastewater**

Aerobic treatment is a process where bacteria remove organic pollutants under aerobic condition. The microorganism converts organic materials into water and carbon dioxide. Trickling filter, activated sludge and rotating biological contactor are usually used in the aerobic process [13].

Author Wakelin and Author Forster conducted a study that treated grease-containing fast food restaurant wastewaters by aerobic treatment. A weir tank reactor with a rectangular polypropylene cistern, two chambers, a weir chamber and a main chamber was used in this study. The organic loading rate from the daily kitchen activities was 5kg m<sup>3</sup>/d. Two cultures, Microbial Culture (MC1), which is related to a strain number in a given place during a given time and activated sludge, have been identified as useful bacterial mixed together to remove fast food grease residues. Fats, oils and greases were reduced 84-96%. The combination of a mechanical mixing regime and the periodic removal of a portion of microbial solids from the weir tank reactor liquor contributed to the high performance of weir tank reactor [14].

Authors B. Malladi and S.C. Ingham investigated a thermophilic aerobic method to treat potato-processing wastewater. The potato processing wastewater was collected in a container and settled for 1 hour. Supernatant is decanted in a jacketed Wheaton jar. The initial BOD ranged from 620 to 1743 mg/l and suspended solids ranged from 0.31 to 0.49 mg/ml. Fermentation occurs at 55oC and aerated at 0.61 l/min. After 96 hours, 98% of BOD, 75% of total suspended solids, and an average of 96% of the starch are removed [15].

Authors Manyele et al. used aerobic microorganisms to provide secondary treatment



for beverage processing wastewater. Three-phase fluidised bed bioreactors were set up under optimum operating conditions—chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total suspended solids (TSS) , pH and dissolved oxygen concentration (DOC) were measured.

Initial pH of two samples of wastewater was adjusted to 9.0 and 11.5 respectively. After 1 day, the pH of treated bioreactor contents remained at 9.3; after 5 days, 95% of TSS was removed for both initial pH levels. Other like COD removal, the TKN and NH<sub>3</sub> removal are independent of the initial pH. In addition, COD dropped by 98% when at initial pH of 9.0 and by 50% when at initial pH of 11.5 [16].

Author Fang conducted a study of aerobic treatment of dairy wastewater. In this study, three stage aeration reactors and one settling tank with a detention time of 3.8, 8 and 1.82 hours respectively. Influent dairy wastewater is 230 L/hr with 1060 mg/l of BOD<sub>5</sub>, 630 mg/l of TOC, 109 mg/l of TKN, and 8.5 mg/l of NH<sub>4</sub>-N. With detention times of 19.8 hours, effluent reduced TOC by 93% , BOD<sub>5</sub> by 99%, TKN by 91% and 92% of NH<sub>4</sub>-N respectively. Even though the characteristics of dairy wastewater vary, the ratio of BOD<sub>5</sub>/TOC, NH<sub>4</sub>-N/TKN and VSS/TSS do not change significantly [17].

### **3.4 Anaerobic treatment of food industrial wastewater**

The difference between anaerobic and aerobic wastewater treatment is aeration. An example of an anaerobic wastewater treatment, known as anaerobic digestion, does not require air supply. This particular anaerobic process occurs in sealed tanks. First, microorganisms convert waste into organic acids, ammonia, hydrogen and carbon dioxide; second, methanogen convert the residual into methane and carbon dioxide [18].

Anaerobic treatment has been widely applied to treat food industrial wastewater,

because of its low capital expenditure requirement. Anaerobic treatment can treat high organic content wastewater and generate less sludge than aerobic treatment. Anaerobic treatment can also produce valuable biogas whose production rate is directly related to the consumption of organic load [19].

Methane gas as a non-polluting fuel can reduce the release of carbon dioxide. Biological gas can be produced from the fermentation of substrates, but the use of commercially produced food products is not economical. Food industrial wastewater is a promising source of production of biogas. It can not only reduce the waste treatment and disposal cost, but also can be used as heating source. Biohydrogen gas is production from food processing and domestic wastewaters. [10]

Potato processing industrial wastewater contains high concentration of biodegradable components like starch and proteins. High concentration of suspended solids, BOD and large quantities of potential foaming substances, such as proteins and fats contribute to the complex nature of potato processing wastewater. Small scale anaerobic digestion of potato processing wastewater and co-digestion with pig slurry and/or abattoir waster was investigated by Monou et al. co-digested with pig slurry to enhance anaerobic digestion. This combination achieved a volatile solid removal of 72%, an average biogas production of 35 ml, and a maximum methane content of 32% 22 days. Another combination, co-digested with abattoir wastewater, did not improve the anaerobic process because the poor buffering and low pH value [20].

Omil et al. had successfully used anaerobic filter reactor and a sequential batch reactor to treat dairy wastewater from a full scale plant. After two years observation, the effluent achieved the discharge standard and fat can be removed prior to the anaerobic

reactor. Fat had been degraded without sludge flotation. The sludge amount was about 2-3 kg VSS/d. According to their study, the influent COD in anaerobic filter was 5-6 kg/m<sup>3</sup>·d and effluent COD was reduced by 90%. After treatment by sequential batch, the effluent COD content was below 200 mg/l and total nitrogen below 10 mg N/l [21].

### **3.5 Aerobic and/or anaerobic method**

Author Matosic et al. investigated the treatment of soft drinks production wastewater by considering treatment efficiency between a membrane bioreactor (MBR) and conventional activated sludge. The cost of an MBR is higher than conventional processes, but it is more flexible to the variation of composition and fluctuation of wastewater. The investigated facility considered both bottling natural spring water and also soft drinks. When the production switches from water to soft drink, the content of wastewater varies greatly. The result shows activated sludge process has high COD and TOC content in its effluent which cannot be discharged. On the other side, MBR reduces over 90% of COD, BOD and TOC respectively. Significant membrane fouling was occurring during the first 10 days and it was slowing down after that. Using intensive chemical cleaning with hypochlorite, acid and alkaline solution can restore the original permeability of membrane [22].

Author Galambos et al. used reverse osmosis (RO) and nanofiltration (NF) membranes to treat two different types of wastewater from the food industry. According to the study, when COD was below 125 mg O<sub>2</sub>/l, the treated wastewater can be discharged into natural water, if discharge into sewer the limit is 800mgO<sub>2</sub>/l. The results showed that reverse osmosis membrane is more efficient than nanofiltration membrane. The treated effluent by RO can be released into natural waters or be recycled, but the effluent treated by

NF can be discharged into sewer only [23].

Authors Kadlec, Burgoon and Henderson designed a combination of surface flow wet lands, intermittent vertical flow wetlands, ponds and land application. This engineered natural system can effectively reduce potato processing wastewater COD by 75%, TSS by 69% and TKN by 46% and the treated effluent can be used as irrigation supply [24].

Authors Burgoon, Kadlec and Henderson considered the use of an integrated natural system to treat high strength potato processing water. The integrated natural system consisted of a free water surface, vertical flow wetlands, and a facultative storage lagoon. The constituents of the potato processing wastewater included a volumetric flow of 5300 m<sup>3</sup>/d, with an average COD concentration of 2800 mg/l, 150 mg/l TN and 350 mg/l TSS was treated annually. Based on two years operation and observation, COD removal in the summer and winter were 95% and 75% respectively. Ammonia removal was reduced from 85% in the summer to 30-50% in the winter. Addition of exogenous carbon can be reduced to 95% NO<sub>3</sub>-N [25].

Author Balannec et al. described the treatment of dairy processing wasters by membrane operation. They investigated the performances of eight different nanofiltration and reverse osmosis membrane by dead end filtration. After compared several NF and RO membranes, they found one single membrane operation is sufficient to milk constituents concentrated but one finishing step has to be added for the production of reusable water [26].

Author Vymazal suggested that constructed wetlands with horizontal sub-surface flow (HF CWs) can be used to treat industrial wastewater like food-processing wastewater. Several studies of using HF CWs to treat food-processing wastewater had

been mentioned in his paper. These cases included HF CW treating seafood processor wastewater, cheese-processing wastewater, seasonal food (tomato sauce, apple and grape juice, olive oil) processing wastewater and other types of food-processing wastewater. HF CWs system was also useful to treat winery and distillery wastewater which contain high content of organic and solids content, high acidity and large variation of seasonal flow rate [27].

Author Tchamango et al. applied electrocoagulation to treat dairy wastewater. A pair of aluminum electrodes was used to carry out the experiment. The results showed that 61% of COD, 89% of phosphorus, 81% of nitrogen, and 100% of turbidity were removed respectively. Chemical coagulant aluminum sulphate was used to perform chemical coagulation as comparative study of electrocoagulation. The results indicated that the removal rates of nitrogen and turbidity are almost same, but removal of phosphorus and COD are slightly higher by chemical coagulation. The treated effluent by electrocoagulation can be used as recycling water due to its low conductivity [28].

An entrapment technique can be used at small or land-limited food industry to treat the high organic concentration wastewater. By using microorganism from activated sludge and other mixed microorganism immobilized in cellulose triacetate, the entrapment technique can effectively reduce soluble COD. This method can be used to treat high strength organic wastewater and reduce the sludge production [29].

### **3.6 Common Biological Method to Treat Dairy Wastewater**

#### **3.6.1 Activated sludge**

Activated sludge is a process that uses air and microorganism to treat wastewater.

Activated sludge consists of different groups of microorganisms entrapped within a polymeric network. The floc of activated sludge is negatively charged and the multivalent cations are important for the stability of the structure. Among all cations,  $\text{Ca}^{+2}$  plays a key role for bridging polymeric networks and bacteria together. Filamentous microorganisms are important for the structure of activated sludge. Poor quantity of filamentous microorganisms contributes to low settle ability, poor dewater ability and compaction. And over quantity of that lead to slowly settling, poor compaction and bulking sludge [30].

Activated sludge process converts organic pollutant to carbon dioxide, water and new cells. The excess cells, called sludge, will be separated from treated water at settling tank, where 1 kg BOD removed will produce 0.5 kg dry excess sludge. The removal of excess sludge is efficient and low cost because this treatment takes 25% to 65% of total treatment cost. There are several methods have been used to reduce sludge—alkaline-thermal treatment, ozonation, chlorination, metabolic uncoupler, and dissolved oxygen [31].

Biological treatment, generally the activated sludge process, has become the main wastewater treatment method to treat municipal and industry wastewater all over the world[32]. Activated sludge is one of the common suspended growth reactors and most of them are continuous stirred tank reactor (CSTRs). The suspension of suspended growth is typically provided by an air pump which provides constant stirring of wastewater and microorganism. The mixing is sufficient and all constituents are uniform throughout the CSTR. This process developed around 1912-1914 [33], while the first plants appeared in the early 1960s and were mainly extended aeration plants or oxidation ditches. The most important advantages of using activated sludge were freedom from the fly and odor

problems associated with bacteria beds and a reduced requirement for land area. [34] Currently over 90% of the municipal wastewater treatment plants within the United States use it as the core part of treatment process ([31]). Overall, the activated sludge process is an efficient and economic way to treat organic wastewater not only SS and BOD but also nitrogen and phosphorus. It can provide high quality effluent.

### **3.6 .2 Trickling filter**

#### **3.6.2.1 Definition of Trickling filter**

Trickling filter consists of support media, usually rocks, gravel or plastic. Wastewater flows from the top to the bottom of trickling filter. Microorganisms attach to the surface of bed media and form aerobic layer and anaerobic layer. Aerobic condition is maintained by forced air flowing through the bed or natural convection of air [35].

Trickling filters remove organic pollutants by microbial films which attach to the surface of media in trickling filter. The biofilter which use microorganism to reduce organic develops on the top of media surface where oxygen and food is very sufficient. When wastewater from the setting tank trickles over the bed of media, the pollutant in the water will be removed by the biofilm. Finally, the biofilm became thick and heavy, it will fall from the media then the new process start over again [36].

#### **3.6.2.2 Purposes of Trickling Filter**

The primary purpose of a trickling is to remove organic pollutant. In addition, trickling filters can be used to remove nitrogen. Nitrification which oxidize ammonia to nitrite and then to nitrate by microorganism begins after organic removal since nitrifiers

need more time to grow and heterotrophic bacteria dominant the biofilm at first. Bacteria attach to the surface of support media and utilize the organic material as food. Bacteria generate an extrapolymeric matrix which allows them to firmly adhere to the surface [37].

There are many factors that determine a successful performance of a trickling filter. A major component known as carrier material determines the performance of biofilter. The materials of media include natural, inert, synthetic and mixtures of both. The media is not only used to support the biofilm, but also trap contaminants which can be degraded further by bacteria [38].

### **3.6.2.3 Selection of Biofilm Media**

According to Odd-Ivar Lekang, there are several factors affect the selection of biofilter media. These factors include void ratio, specific surface area, weight, homogeneous water flow, and economics. Void ratio is the ratio of void volume and volume of media. Specific area is defined as the total surface area of filter media per unit of mass of media. The media with light weight can be easily handled. Homogeneous water flow refers to the water flow that has the same or similar nature. The media has the reasonable price and cost effective [39].

### **3.6.3 Membrane bioreactor**

The membrane bioreactor (MBR) combines biological treatment with membrane filtration process. MBR is more effective than conventional biological treatment process to remove COD, BOD and SS. For example, suspended solid removal complete by filtration rather than gravity, MBR has independent solid retention time and hydraulic



retention time. According to the application, membrane bioreactor can be divided into three categories— filtration membrane, gas diffusion membrane and extractive membranes [40].

An MBR consists of two parts— biological suspended growth reactor and another part is membrane. Two common types, microfiltration and ultrafiltration are frequently used to treat wastewater. Membrane acts as separate unit to remove certain particles. In regards to treatment of wastewater, MBR and activated sludge operate in the similar way; however MBR does not need a clarifier. The advantages of MBR are that smaller plant size, completely suspended solids removal and high treatment efficient. But MBR is much more expensive to install, operate and maintain [41].

#### **3.6.4 Wetland**

Wetlands are lands saturated with water. It is a very important for a large spectrum of habitats, such as temporary shallow waterbodies, lake margins, large river floodplains, coastal beaches, coral reefs and beds of marine algae or seagrasses [42].

Wetlands use a high nutrient tolerance root system of reeds such as elephant to treat or renovate wastewater [43]. An alternative type of wetland beyond natural wetlands is constructed [44].

A wetland is one of the most effective ecosystems in the world. Plants play an important role in nutrients cycling. One of the most common plants, floating macrophytes are widely used for wastewater treatment. Nutrient-use efficiency (NUE) is considered to be an important plant factor which combines a variety of nutrient uptake and release process. NUE changes with types of plants and nutrient availability [46].

Compared with stabilization ponds and lagoons, wetlands can offer high degree of

process control and develop applicable design and cost criteria for a given and desired level of wastewater. Recently some studies document the possibility of using wetland to remove waterborne pollutants [47].

Wetlands are used to remove aquatic pollutants. Aquatic pollutants are removed by wetlands through physical, chemical and biological process, such as sedimentation, soil adsorption, and biological transformation. There are more than 500 wetlands used in the world to treat municipal and industrial wastewater [48].

Wetlands have different hydraulic retention times which range from 2 to 20 days. High hydraulic retention is applied when wetlands are used for BOD removal and nitrification by diffusion aeration. On the contrast, low hydraulic retention time is usually employed to get higher quality treated wastewater and other design objectives, such as denitrification and habitat enhancement [49].

Wetlands have been used to enhance wastewater discharge from municipal wastewater treatment. Some food industries also use wetland for the tertiary treatment for processing wastewater [50]. An alternative type of wetland beyond natural wetlands is constructed.

Constructed wetlands are artificial wetlands that follow the natural processes to treat wastewater. There are two types of constructed wetlands- surface flow and subsurface flow. Wastewater flows from the top of the soil is called surface flow; wastewater flows through porous medium is called subsurface flow [45]. As an engineered and managed 'natural system', constructed wetlands use less energy, more reliable and less cost than reactor tanks and basins. A wetland system can also be served as wild life habitat or nature centre. Author Hammer indicates that there are four factors in pollutant removal— wetland

microbial populations, wetland macrophytes, wetland substrates, and water column [51].

## **CHAPTER IV**

### **MATERIALS**

#### **4.1 Materials**

##### **4.1.1 Types of LLMO used in the experiment**

The most common LLMO product used for the experiment is G-1. G-1 is used for solubilizing grease and fat. G-1 can be used in sewage collection systems, wet wells, grease traps, drain lines and septic tank maintenance, and also can treat industrial waste with high grease/fat content [52].

##### **4.1.2 Yeast**

Yeasts are fungi. The yeast open species *Saccharomyces cerevisiae* has been used in baking and fermenting alcoholic beverages for thousands of years [53]. All yeasts need

carbon and nitrogen for maintenance and growth. Yeast can also metabolize inorganic nitrogen and organic nitrogen sources for growth [54].

Author Kaszycki et al. tested the probability of methylotrophic yeast to treat several different types of industrial wastewater. The results concluded that *Hansenula polymorpha* can effectively remove methanol and formaldehyde from real industrial wastewater samples. *Hansenula polymorpha* has high adaptation to the changeable and poor environmental condition [55].

Malandra et al studied the microbiology of a biological contactor to treat winery wastewater. The biofilm on a rotating biological contactor can reduce an average 43% of COD with 1 hour detention time. The authors isolated eight types of bacteria and seven types of yeast species from biofilm and evaluated them within wastewater. The results concluded that yeast isolation more effective than bacteria to remove COD. The results validated that the presence of yeast species contribute to the removal of pollutant during treatment process [56].

Author Yang et al set up a two-step biological system consisting of mixed yeast and activated sludge treat Monosodium glutamate processing wastewater. The yeast successfully removed over 80% COD of high strength wastewater and raised the pH from 2.5 to 5.0-6.0. The results indicated that the preceding activated sludge system can reduce 50-70% COD of yeast system effluent [57]. The two major types of yeast include brewer and baker.

#### **4.1.3 Brewer's yeast**

Brewer's yeast is also called brewing yeast. The classification of yeast according to brewers is top and bottom fermenting. Top fermenting yeasts come from foam at the top of

the wort during fermentation. Bottom fermenting yeasts refer to those produce larger-type beers and also ale-type beers. For both types, yeast is fully distributed through the beer when it is fermenting ([53]).

Saccharomyces yeasts are the most useful yeast in the brewing industries and its biomass is the second by-product from brewing industries. The function of yeast during fermentation of cooled wort process is to convert sugar to alcohol. Saccharomyces yeasts are characteristic for fast growing, ethanol effective production and good tolerance for environment stress. Author Ferreira et al. studied several potential usage of this kind of yeast, such as fish nutrition, microorganism food ingredients and biosorbent for toxic industrial wastewater. Both living and non-living biomass have the ability to remove heavy metal from aqueous solutions [58].

#### **4.1.4 Baker's yeast**

The function of yeast used in baking is to converts the fermentable sugars in the dough into carbon dioxide. The dough will expanded as carbon dioxide forms bubbles. During this process, we can get the soft and spongy texture of product ([53]).

Author Zhang et. al investigated the capacity of three baker's yeasts to absorb  $\text{Cu}^{2+}$ . There are three type of yeast— ethanol, caustic-pretreated and pristine baker's yeast. From the results, baker's yeast can be successfully used as biosorbent for  $\text{Cu}^{2+}$  removal. However, ethanol and caustic pretreatment baker's yeast are more effective than pristine baker's yeast, due to the functional groups on the surface of baker's yeast have been improved by pretreatment [59].

## 4.2 Bioaugmentation

Bioaugmentation is a process that indigenous, wide type or genetically engineered microorganisms are applied to the bioreactor or the polluted sites to enhance the performance of existing biological process. There are three criteria to determine whether the bacteria are suitable for bioaugmentation— assist complex microbial communities in degrading pollutant, completely grow when introduced into the system, and must not adversely affect the indigenous microbial communities [60].

The chemical content of wastewater from chemical industry varies greatly depending on particular technological process and also seasonal changes. The original activated sludge cannot adapt to this change, due to the prohibition of biocenosis which is the specific living habitat for all the interacting organisms.

Authors Kaszycki and Kolozek confirmed the applicability of using methylotrophic yeast for bioaugment activated sludge biocenoses. The result showed that original and yeast-augmented activated sludge have the similar performance to remove formaldehyde at low concentration but yeast-augmented activated sludge is more effective to removal formaldehyde at high concentrations [61].

Bioaugmentation is considered a promising and attractive method to improve wastewater treatment performance. Microorganisms play an important role in the removal of pollutant; therefore, it is important to select the proper microorganisms and adopt a suitable strategy. Some techniques widely used to assess the persistence of added bacterial and the effects on indigenous population, denaturing gel electrophoresis, analysis of the polymerase chain reaction-amplified ribosomal DNA fragments, and in-situ green fluorescent protein fluorescence detection [62].

## **4.3 Wastewater**

### **4.3.1 Milk Wastewater**

Wastewaters used in this experiment are milk wastewater and soybean milk wastewater. Milk wastewater is prepared by milk powder and tap water. The brand of milk powder is Our Family and bought from Walmart.

Milk contains about 4.9% carbohydrate and most of carbohydrate is lactose with trace amounts of monosaccharides and oligosaccharides. Lactose belongs to disaccharides and it is composed by glucose molecular and galactose molecular. Two sugar molecules must be broken before the sugar can be used for energy.

There are approximate 3.4% total fat in milk. Enzyme action, exposure to light and oxidation can degrade milk fat. Enzyme which can degrade milk fat comes from the native milk, airborne bacterial contamination and also bacteria added intentionally. Milk contains 3.3% protein [63].

### **4.3.2 Soybean Wastewater**

Soybean milk wastewater is prepared by soybean milk and tap water. The brand of soybean milk is MOGAMI and bought from local store named Park to Shop. Soybean milk has around 3.5% protein, 2% fat, 2.9% carbohydrate and 0.5% ash ([3]).

## **4.4 Methods**

### **4.4.1 Making milk wastewater**

1. Using balance to measure 411 mg milk powder.
2. Put powder into 2 L plastic beaker No.1.



3. Using 1000 ml round bottom flask to measure 1 L of distill water and pour into beaker No.1.

4. Put magnet into beaker No.1 and put it on the magnetic stirrer. After the milk powder totally dissolved into water, we get generating a 150 mg/l milk solution.

5. Using balance to measure 543 mg milk powder.

6. Repeat steps 2 to 4.

7. Using 1000 ml round bottom flask to measure 2 L of distill water and pour into beaker No.2.

8. Repeat step 4. After the milk powder totally dissolved, we get 100 mg/l milk solution.

9. Using 1000 ml round bottom flask to measure 1 L of 100 mg/l milk solution form beaker No.2 and pour into 2 L plastic beaker No.3.

10. Measure 1 L of distilled water and pour into beaker No.3 to get 50 mg/l milk solution.

11. Measure 1 L of 50 mg/l milk solution from beaker No.3 and pour into 2 L plastic beaker No.4.

12. Measure 1 L of distilled water and pour into beaker No.4 to get 25 mg/l milk solution.

#### **4.4.2 Making soybean milk wastewater**

1. Using 10 ml graduated flask to measure 7 ml of soybean milk.

2. Put 7ml of soybean milk into 2 L plastic beaker No.5.

3. Using 1000 ml round bottom flask to measure 1 L of distill water and pour into beaker No.5.

4. Put magnet into beaker No.1 and put it on the magnetic stirrer. After the milk powder totally dissolved into water, we get 150 mg/l soybean milk solution.
5. Using 10 ml graduated flask to measure 9.4 ml of soybean milk.
6. Put soybean into 2 L plastic beaker No.6.
7. Using 1000 ml round bottom flask to measure 1 L of distill water and pour into beaker No.6.
8. Repeat step 4. After the milk powder totally dissolved, we get 100 mg/l soybean milk solution.
9. Using 1000 ml round bottom flask to measure 1 L of 100 mg/l soybean milk solution form beaker No.2 and pour into 2 L plastic beaker No.7.
10. Measure 1 L of distilled water and pour into beaker No.7 to get 50 mg/l soybean milk solution.
11. Measure 1 L of 50 mg/l soybean milk solution from beaker No.7 and pour into 2 L plastic beaker No.8.
12. Measure 1 L of distilled water and pour into beaker No.8 to get 25 mg/l soybean milk solution.

#### **4.4.3 Procedure of measuring TOC**

1. Put 24 100ml bottles on the table and put tags which have been written the number of bottles and the hours on their caps respectively.
2. Pour 100 ml of 25mg/l milk solution into No.1-6 bottles, 50 mg/l of milk solution into No.7-12 bottles, 100 mg/l of milk solution into No.13 – 18 bottles and 150 mg/l of milk solution into No.19 – 24 bottles respectively.
3. Put the caps on and close tightly.

4. Leave the 0 hr bottles on the table and put all other 20 bottles on the shaker.

Record the time.

5. Do filtration for 0 hr bottles twice. Pour the filtrate into new bottles and put tags on their caps.

6. Put the step 5 bottles in the refrigerator.

7. At 2, 4, 6, 12, 24 hrs pick up the bottles from the shaker and repeat step 5, 6.

8. Put all the filtrate into TOC tubes respectively and then put the TOC tubes on TOC machine.

9. Use TOC machine to get the results.

## **CHAPTER V**

### **RUN PROPOSAL**

Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast

Run #2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast

Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast

Run #4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast

Run #5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast

Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast

Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granules

Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnite Toilet Care Granules

Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnite Toilet Care Granules

Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care

Run #11 25, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care

Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care

Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1

Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast

Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care

Run #16 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Baker's Yeast Dosage (ml)	Shaking Time(hrs)
1	milk	25	1	0
2	milk	25	1	2
3	milk	25	1	4
4	milk	25	1	6
5	milk	25	1	12
6	milk	25	1	24
7	milk	50	1	0
8	milk	50	1	2
9	milk	50	1	4
10	milk	50	1	6
11	milk	50	1	12
12	milk	50	1	24
13	milk	100	1	0
14	milk	100	1	2
15	milk	100	1	4
16	milk	100	1	6
17	milk	100	1	12
18	milk	100	1	24
19	milk	150	1	0
20	milk	150	1	2
21	milk	150	1	4
22	milk	150	1	6
23	milk	150	1	12
24	milk	150	1	24

Table I Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Baker's Yeast Dosage (ml)	Shaking Time(hrs)
1	milk	25	5.5	0
2	milk	25	5.5	2
3	milk	25	5.5	4
4	milk	25	5.5	6
5	milk	25	5.5	12
6	milk	25	5.5	24
7	milk	50	5.5	0
8	milk	50	5.5	2
9	milk	50	5.5	4
10	milk	50	5.5	6
11	milk	50	5.5	12
12	milk	50	5.5	24
13	milk	100	5.5	0
14	milk	100	5.5	2
15	milk	100	5.5	4
16	milk	100	5.5	6
17	milk	100	5.5	12
18	milk	100	5.5	24
19	milk	150	5.5	0
20	milk	150	5.5	2
21	milk	150	5.5	4
22	milk	150	5.5	6
23	milk	150	5.5	12
24	milk	150	5.5	24

Table II Run #2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Baker's Yeast Dosage (g)	Shaking Time(hrs)
1	milk	25	10	0
2	milk	25	10	2
3	milk	25	10	4
4	milk	25	10	6
5	milk	25	10	12
6	milk	25	10	24
7	milk	50	10	0
8	milk	50	10	2
9	milk	50	10	4
10	milk	50	10	6
11	milk	50	10	12
12	milk	50	10	24
13	milk	100	10	0
14	milk	100	10	2
15	milk	100	10	4
16	milk	100	10	6
17	milk	100	10	12
18	milk	100	1.5	24
19	milk	150	1.5	0
20	milk	150	1.5	2
21	milk	150	1.5	4
22	milk	150	1.5	6
23	milk	150	1.5	12
24	milk	150	1.5	24

Table III Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast



Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Beer's Yeast Dosage (ml)	Shaking Time(hrs)
1	milk	25	1	0
2	milk	25	1	2
3	milk	25	1	4
4	milk	25	1	6
5	milk	25	1	12
6	milk	25	1	24
7	milk	50	1	0
8	milk	50	1	2
9	milk	50	1	4
10	milk	50	1	6
11	milk	50	1	12
12	milk	50	1	24
13	milk	100	1	0
14	milk	100	1	2
15	milk	100	1	4
16	milk	100	1	6
17	milk	100	1	12
18	milk	100	1	24
19	milk	150	1	0
20	milk	150	1	2
21	milk	150	1	4
22	milk	150	1	6
23	milk	150	1	12
24	milk	150	1	24

Table IV Run #4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Beer's Yeast Dosage (ml)	Shaking Time(hrs)
1	milk	25	5.5	0
2	milk	25	5.5	2
3	milk	25	5.5	4
4	milk	25	5.5	6
5	milk	25	5.5	12
6	milk	25	5.5	24
7	milk	50	5.5	0
8	milk	50	5.5	2
9	milk	50	5.5	4
10	milk	50	5.5	6
11	milk	50	5.5	12
12	milk	50	5.5	24
13	milk	100	5.5	0
14	milk	100	5.5	2
15	milk	100	5.5	4
16	milk	100	5.5	6
17	milk	100	5.5	12
18	milk	100	5.5	24
19	milk	150	5.5	0
20	milk	150	5.5	2
21	milk	150	5.5	4
22	milk	150	5.5	6
23	milk	150	5.5	12
24	milk	150	5.5	24

Table V Run #5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Beer's Yeast Dosage (g)	Shaking Time(hrs)
1	milk	25	10	0
2	milk	25	10	2
3	milk	25	10	4
4	milk	25	10	6
5	milk	25	10	12
6	milk	25	10	24
7	milk	50	10	0
8	milk	50	10	2
9	milk	50	10	4
10	milk	50	10	6
11	milk	50	10	12
12	milk	50	10	24
13	milk	100	10	0
14	milk	100	10	2
15	milk	100	10	4
16	milk	100	10	6
17	milk	100	10	12
18	milk	100	1.5	24
19	milk	150	1.5	0
20	milk	150	1.5	2
21	milk	150	1.5	4
22	milk	150	1.5	6
23	milk	150	1.5	12
24	milk	150	1.5	24

Table VI Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Overnite Toilet Care Granules (g)	Shaking Time(hrs)
1	milk	25	0.5	0
2	milk	25	0.5	2
3	milk	25	0.5	4
4	milk	25	0.5	6
5	milk	25	0.5	12
6	milk	25	0.5	24
7	milk	50	0.5	0
8	milk	50	0.5	2
9	milk	50	0.5	4
10	milk	50	0.5	6
11	milk	50	0.5	12
12	milk	50	0.5	24
13	milk	100	0.5	0
14	milk	100	0.5	2
15	milk	100	0.5	4
16	milk	100	0.5	6
17	milk	100	0.5	12
18	milk	100	0.5	24
19	milk	150	0.5	0
20	milk	150	0.5	2
21	milk	150	0.5	4
22	milk	150	0.5	6
23	milk	150	0.5	12
24	milk	150	0.5	24

Table VII Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granule

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Overnite Toilet Care Granules (g)	Shaking Time(hrs)
1	milk	25	1	0
2	milk	25	1	2
3	milk	25	1	4
4	milk	25	1	6
5	milk	25	1	12
6	milk	25	1	24
7	milk	50	1	0
8	milk	50	1	2
9	milk	50	1	4
10	milk	50	1	6
11	milk	50	1	12
12	milk	50	1	24
13	milk	100	1	0
14	milk	100	1	2
15	milk	100	1	4
16	milk	100	1	6
17	milk	100	1	12
18	milk	100	1	24
19	milk	150	1	0
20	milk	150	1	2
21	milk	150	1	4
22	milk	150	1	6
23	milk	150	1	12
24	milk	150	1	24

Table VIII Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnite Toilet Care Granules

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Overnite Toilet Care Granules (g)	Shaking Time(hrs)
1	milk	25	1.5	0
2	milk	25	1.5	2
3	milk	25	1.5	4
4	milk	25	1.5	6
5	milk	25	1.5	12
6	milk	25	1.5	24
7	milk	50	1.5	0
8	milk	50	1.5	2
9	milk	50	1.5	4
10	milk	50	1.5	6
11	milk	50	1.5	12
12	milk	50	1.5	24
13	milk	100	1.5	0
14	milk	100	1.5	2
15	milk	100	1.5	4
16	milk	100	1.5	6
17	milk	100	1.5	12
18	milk	100	1.5	24
19	milk	150	1.5	0
20	milk	150	1.5	2
21	milk	150	1.5	4
22	milk	150	1.5	6
23	milk	150	1.5	12
24	milk	150	1.5	24

Table IX Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnite Toilet Care Granules

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Liquid Drain Care (ml)	Shaking Time(hrs)
1	milk	25	1	0
2	milk	25	1	2
3	milk	25	1	4
4	milk	25	1	6
5	milk	25	1	12
6	milk	25	1	24
7	milk	50	1	0
8	milk	50	1	2
9	milk	50	1	4
10	milk	50	1	6
11	milk	50	1	12
12	milk	50	1	24
13	milk	100	1	0
14	milk	100	1	2
15	milk	100	1	4
16	milk	100	1	6
17	milk	100	1	12
18	milk	100	1	24
19	milk	150	1	0
20	milk	150	1	2
21	milk	150	1	4
22	milk	150	1	6
23	milk	150	1	12
24	milk	150	1	24

Table X Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Liquid Drain Care (ml)	Shaking Time(hrs)
1	milk	25	5.5	0
2	milk	25	5.5	2
3	milk	25	5.5	4
4	milk	25	5.5	6
5	milk	25	5.5	12
6	milk	25	5.5	24
7	milk	50	5.5	0
8	milk	50	5.5	2
9	milk	50	5.5	4
10	milk	50	5.5	6
11	milk	50	5.5	12
12	milk	50	5.5	24
13	milk	100	5.5	0
14	milk	100	5.5	2
15	milk	100	5.5	4
16	milk	100	5.5	6
17	milk	100	5.5	12
18	milk	100	5.5	24
19	milk	150	5.5	0
20	milk	150	5.5	2
21	milk	150	5.5	4
22	milk	150	5.5	6
23	milk	150	5.5	12
24	milk	150	5.5	24

Table XI Run #11 25, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care



Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Liquid Drain Care (ml)	Shaking Time(hrs)
1	milk	25	10	0
2	milk	25	10	2
3	milk	25	10	4
4	milk	25	10	6
5	milk	25	10	12
6	milk	25	10	24
7	milk	50	10	0
8	milk	50	10	2
9	milk	50	10	4
10	milk	50	10	6
11	milk	50	10	12
12	milk	50	10	24
13	milk	100	10	0
14	milk	100	10	2
15	milk	100	10	4
16	milk	100	10	6
17	milk	100	10	12
18	milk	100	10	24
19	milk	150	10	0
20	milk	150	10	2
21	milk	150	10	4
22	milk	150	10	6
23	milk	150	10	12
24	milk	150	10	24

Table XII Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	G1 (ml)	Shaking Time(hrs)
1	milk	25	5.5	0
2	milk	25	5.5	2
3	milk	25	5.5	4
4	milk	25	5.5	6
5	milk	25	5.5	12
6	milk	25	5.5	24
7	milk	50	5.5	0
8	milk	50	5.5	2
9	milk	50	5.5	4
10	milk	50	5.5	6
11	milk	50	5.5	12
12	milk	50	5.5	24
13	milk	100	5.5	0
14	milk	100	5.5	2
15	milk	100	5.5	4
16	milk	100	5.5	6
17	milk	100	5.5	12
18	milk	100	5.5	24
19	milk	150	5.5	0
20	milk	150	5.5	2
21	milk	150	5.5	4
22	milk	150	5.5	6
23	milk	150	5.5	12
24	milk	150	5.5	24

Table XIII Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Baker's Yeast Dosage (ml)	Shaking Time(hrs)
1	soybean	25	5.5	0
2	soybean	25	5.5	2
3	soybean	25	5.5	4
4	soybean	25	5.5	6
5	soybean	25	5.5	12
6	soybean	25	5.5	24
7	soybean	50	5.5	0
8	soybean	50	5.5	2
9	soybean	50	5.5	4
10	soybean	50	5.5	6
11	soybean	50	5.5	12
12	soybean	50	5.5	24
13	soybean	100	5.5	0
14	soybean	100	5.5	2
15	soybean	100	5.5	4
16	soybean	100	5.5	6
17	soybean	100	5.5	12
18	soybean	100	5.5	24
19	soybean	150	5.5	0
20	soybean	150	5.5	2
21	soybean	150	5.5	4
22	soybean	150	5.5	6
23	soybean	150	5.5	12
24	soybean	150	5.5	24

Table XIV Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Liquid Drain Care (ml)	Shaking Time(hrs)
1	soybean	25	5.5	0
2	soybean	25	5.5	2
3	soybean	25	5.5	4
4	soybean	25	5.5	6
5	soybean	25	5.5	12
6	soybean	25	5.5	24
7	soybean	50	5.5	0
8	soybean	50	5.5	2
9	soybean	50	5.5	4
10	soybean	50	5.5	6
11	soybean	50	5.5	12
12	soybean	50	5.5	24
13	soybean	100	5.5	0
14	soybean	100	5.5	2
15	soybean	100	5.5	4
16	soybean	100	5.5	6
17	soybean	100	5.5	12
18	soybean	100	5.5	24
19	soybean	150	5.5	0
20	soybean	150	5.5	2
21	soybean	150	5.5	4
22	soybean	150	5.5	6
23	soybean	150	5.5	12
24	soybean	150	5.5	24

Table XV Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	G1 (ml)	Shaking Time(hrs)
1	soybean	25	5.5	0
2	soybean	25	5.5	2
3	soybean	25	5.5	4
4	soybean	25	5.5	6
5	soybean	25	5.5	12
6	soybean	25	5.5	24
7	soybean	50	5.5	0
8	soybean	50	5.5	2
9	soybean	50	5.5	4
10	soybean	50	5.5	6
11	soybean	50	5.5	12
12	soybean	50	5.5	24
13	soybean	100	5.5	0
14	soybean	100	5.5	2
15	soybean	100	5.5	4
16	soybean	100	5.5	6
17	soybean	100	5.5	12
18	soybean	100	5.5	24
19	soybean	150	5.5	0
20	soybean	150	5.5	2
21	soybean	150	5.5	4
22	soybean	150	5.5	6
23	soybean	150	5.5	12
24	soybean	150	5.5	24

Table XVI Run #16 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1

## **CHAPTER VI**

### **RESULTS DISCUSSION**

#### **6.1 Results of Run 1 to Run 18**

##### **Run 1**

Run 1 used 1 ml baker's yeast as bioproduct to treat 25, 50, 100, 150 mg/l milk wastewater.

The TOC removal rates at 12 hours for all concentrations are bigger than at 24 hours.

When milk concentrations are 25, 50, 100mg/l, the largest TOC removal occurred at 12 hours, where removal rates were 28%, 23% and 33% respectively. As concentration of TOC increases the TOC removal increases. The largest TOC concentration removal was 150 mg/l, at 6 hours, the largest removal at 39.33%.

## **Run 2**

The run used 5.5 ml baker's yeast to treat four different concentration of milk solution. The percent TOC removal was 59.17%, 35.01%, 11.78% and 42.94% for 25, 50, 100, 150 mg/l of milk solution respectively. When milk concentrations are 25, 50,100 mg/l, their highest removal rates all happened at 6hrs. But the removal rate was very low, when milk concentration is 100 mg/l. when milk concentration ranged from 25 to 100 mg/l, TOC removal rate has the tendency to decrease as the concentration increased. The higher percent TOC removal was 59% at 6 hours with a milk solution concentration of 25 mg/l. On the contrary, TOC removals with 100 mg/l milk are below 12%.

## **Run 3**

This run adds the highest dosage of bioproduct, 10 ml of baker's yeast, to treat four different concentration of milk solution. The results indicated that the majority of % TOC removals were above or approximate 40%. The TOC concentrations were 46.88% for 25 mg/l of milk, 73.16% for 50 mg/l of milk, 39.81% for 100 mg/l of milk and 47.36% for 150 mg/l of milk.

### Summary of Results for Run 1 – Run 3

The TOC removal rates decreased as the concentration of milk increased in Run 1 and Run 2 and TOC removal rates were all above 40% for four different concentrations of milk in Run 4. The highest removal rates for three runs were 39.33, 59.17 and 73.16% respectively. As the dosage of Baker's yeast increase, the %TOC removal increases concurrently. Baker's yeast uses organic carbon as food source. The food is sufficient enough for high content of baker's yeast to product. High amount of Baker's yeast will help to accelerate the reduction of organic carbon in the limited time.

#### **Run 4**

Four different concentrations of milk solution are treated by 1 ml of Beer's yeast. The best removal results for each concentration from low to high concentration were 11.72%, 3.03%, 2.48% and 25.87% respectively. Initial TOC played more important role in TOC removal rate if extended the shaking time it did not change the removal rate.

#### **Run 5**

This run was similar as run 4 but use 5.5 ml of Beer's yeast, but 25 mg/l of milk solution was treated with 5.5 ml of Beer's yeast. But when concentration of milk was 25 mg/l, TOC increased at first and then was completely removed. This data was invalid. For other samples, get 10.17%, 35.73% and 12.63% form low to high concentration of milk solution. However, this result does not produce any results.

#### **Run 6**

Run 6 used 10 ml of baker's yeast to reduce content of TOC from milk solution. TOC increased after adding 10ml of Beer's yeast into highest concentration of milk wastewater and TOC cannot be removed in 24 hrs. The results are unfavorable for TOC concentrations of 25 mg/l, 50 mg/l, and 150 mg/l. However, results at a TOC concentration at 100 mg/l produced a 52.76 % TOC removal at time of 24 hours.

#### **Run 4 – Run 6 Results Discussion**

The results of most samples are not favorable as the %TOC removal fluctuates. A possible reason may involve the condition of the beer yeast. The beer's yeast was highly concentrated and had been stored in the freezer for several weeks before the beginning of the runs. There are visible flocs on the top and bottom within the beer's yeast. The composition of those flocs is unknown and maybe the live cells, dead cells, nutrient and



other substrate. The concentration of beer's yeast is separate from the liquid. The beer yeast liquid contains high concentration of nutrients but may be degraded by yeasts. The unknown substrate had the possibility to increase TOC of milk wastewater.

### **Run7**

This was the first run using 0.5 g Overnite Toilet Care Granules to treat milk solution. All four concentration of milk solution had a 30% removal. During this run, TOC concentrations measured 74.64%, 30.09%, 39.04% and 29.86% from low to high concentration of milk solution. The % removal has the tendency to decrease as the concentration of milk solution increase.

### **Run 8**

Run 8 used a 1g of Overnite Toilet Care Granules to treat milk solution. The % TOC concentration removed 36.89% for 25 mg/l, 74.76% for 50 mg/l, 27.56% for 100 mg/l, and 43.96% for 150 mg/l of milk solution. When concentrations of milk were 50,100 and 150 mg/l, highest TOC removal rate happened at 24 hrs. Overall, % TOC removal is inversely proportional to the concentration of milk solution. From liner diagram, we can see that the TOC decreased at first and then increased. TOC reached highest amount at 6 hours and finally had been removed within 24 hours.

### **Run 9**

During Run 9 1.5 g Overnite Toilet Care Granules were added to 25 mg/l, 50 mg/l, 100 mg/l and 150 mg/l of milk solution respectively. The results indicated an increase in TOC concentration when 1.5 g Overnite Toilet Care Granules were added into the milk solution. The highest %TOC removal occurs for this run at 100mg/l with a concentration was 73.18%. But most of results were negative, that means time was a very important

factor for using highest dosage of this bioproduct to remove TOC.

#### Run 7 to Run 9 Results Discussion

According to Material Safety Data Sheet (MSDS) which is provided by the Enforcer website, Enforcer Overnite Toilet Care Granule contains 1-10% cellulases and enzyme protein. The mechanism of cellulases is to first break down cellulose into individual cellulose fibers, then hydrolyze them into smaller sugars, and finally break them into glucose [64].

According to Jean Piccard and Mary Rising this enzyme is water soluble. It still remains in the milk after the casein and the fat were precipitated by adding acid [65]. The content of enzyme increases as the concentration of milk increase.

Overall, there are three possibilities:

When one adds the same dosage of Enforcer Overnite Toilet Care Granule into 25mg/l, 50mg/l, 100mg/l and 150 mg/l of milk solution, the Cellulases break cellulose into individual cellulose fibers which increase the TOC content.

The high concentration of the milk solution contains more enzymes and the enzymes may pass through the filter into the filtrate.

#### Run 10 to Run 12

Runs 10 through 12, added 1 ml, 5.5 ml and 10 ml of Liquid Drain Care to remove TOC from 15, 50, 100 and 150 mg/l of milk solution. The results have no pattern. The content of TOC increases greatly as the addition of liquid Drain Care.

#### Run 10 to Run 12 Results Discussion

The composition of Enforcer Overnite Toilet Care Liquid includes 10-20% cellulase and enzyme protein.

There are also two possibilities to explain why the readings are so high without any patterns.

1. The Overnite Toilet Care Liquid contains substrate that cause bubbles in the samples. These bubbles may increase the reading of TOC from TOC analyzer. The readings are much higher than the actual numbers.

2. The cellulase and enzyme protein contents are higher in Enforcer Overnite Toilet Care Liquid than in Toilet Care Granular.

3. Enforcer Overnite Toilet Care Granules were particles. When particles dissolved into wastewater, it will contribute TOC negative removal rate.

### **Run 13**

This was the last run to treat milk solution. A volume of 5.5 ml of G1 was used in this run to treat 25mg/l, 50 mg/l, 100 mg/l and 150 mg/l of milk solution. When concentrations of TOC were 50, 100 and 150mg/l, the highest TOC removal rates all occurred at 4 hours. A Linear diagram showed the removal rates had the tendency to increase along the time. The % TOC removal increases as the concentration of milk solution decreases. The highest % TOC removal happens at a time of 24 hours with a concentration of 50 mg/l.

### **Run 14**

Run 14 used 5.5 ml of baker's yeast to treat four different concentration of soybean milk wastewater. The concentrations of soybean milk are 25, 50, 100, and 150 mg/l.

The addition of 5.5 ml baker's yeast did not affect the concentration of soybean at 0 hour. The high removal rate for all four concentration of milk occurred at 24 hours. The %TOC removal was 26.51% for 25 mg/l, 34.42% for 50 mg/l, 22.56% for 100 mg/l and 36.30% for 150 mg/l.

TOC increased during the fermentation and finally decreased at 24 hours. Soybean milk produced more bubbles than milk when TOC Analyzer injects N<sub>2</sub> into sample tube. The bubbles may cause a fluctuation in results. The soybean milk contains more sugar than milk. The suitable results at 24 hours may be caused by baker's yeast break down the bubble-causing substrate in the solution.

### **Run 15**

This run includes four concentrations of soybean solution, 25mg/l, 50 mg/l, 100 mg/l and 150 mg/l. A volume of 5.5 ml of liquid drain is used as bioproduct. The Liquid Drain Care raised the TOC to 3000 ppm, concentrations not suitable to be analyzed by TOC analyzer.

The cause of high reading of TOC is due to the aggregation of soy protein or the denaturation of samples. It is also the possibility that the particle accumulated in the TOC analyzer and are not completely washed away before running the samples.

### **Run 16**

Run 16 used a volume of 5.5 ml of G1 is used as bioproduct in this run to reduce TOC for four different concentration of soybean milk. The results have the same tendency to use 5.5 ml of G1 to treat milk solution. It was determined that as the %TOC removal decreases as the concentration of soybean milk increase. 5.5ml of G1 was not suitable to treat high concentration of soybean milk wastewater. Because there were ash and particles in soybean milk, once the particle broke down, it concentrated to increase the amount of TOC. The higher concentrations of milk solution had the larger amount of particles. There were not enough bacteria in 5.5 ml of G1 to remove high amount of TOC.

## 6.2 Comparison of all runs

From Table 35, the highest % TOC removal of all runs is 74.76% when use 1 ml of overnight Toilet Care Granules to treat 50 mg/l of milk wastewater. %TOC removal keeps constant between 73-74% when use different concentration of Overnite Toilet Care Granules to treat milk wastewater. Using 5.5 ml of baker's yeast or liquid drain care to treat 150 mg/l of soybean at 24 hrs can get the almost same results, around 36% TOC removal.

From Table 36, Baker's yeast has the same efficiency (36% and 39%) to treat 150 mg/l milk and soybean wastewater, but it takes longer time for soybean wastewater to have a higher get its highest pollutant removal. When using 5.5 ml of G1 to treat milk and soybean wastewater, the highest TOC removal happened at 4 hours.

From Figure 35, Baker's yeast and Overnite Toilet Care Granules were very effective bioproduct to reduce amount of TOC of milk wastewater and their highest removal rate were almost the same. G1 also can be used to treat milk wastewater. Figure 36 showed that G1 was the most suitable bioproduct to remove TOC from soybean milk wastewater. Using Overnite Liquid Drain Care and Baker's yeast got the same TOC removal rate. Figure37 compared the %TOC removal between milk and soybean milk when same types of bioproducts were used. The results showed Baker's yeast is more suitable to treat milk wastewater and the removal rate of milk wastewater was almost two times higher than the removal rate of soybean milk wastewater. The TOC removal rate of milk and soybean milk by using Overnite Liquid Drain Care and G1 were almost same. From Figure 38, it was clear that the highest %TOC removal rate of milk wastewater was almost the same as the highest %TOC removal rate of soybean milk wastewater. Figure 38 indicated that the best bioproducts to remove TOC from milk wastewater were Baker's

yeast and Overnite Toilet Care Granules. Bacteria in Overnite Liquid Drain Care effectively removed TOC from soybean milk wastewater. Figure 39 compared the TOC removal rate of all runs. The highest TOC removal rate happened by using Liquid drain care to treat soybean milk wastewater. When the highest TOC removal rate happened, the removal rates by using Baker's yeast to treat milk wastewater were very low. That indicated the highest %TOC removal and highest TOC removal rate did not happen at the same time.

## **CHAPTER VII**

### **CONCLUSION**

#### **7.1 Conclusions**

Based on the results presented above, the most effective way to remove TOC from milk solution is using Overnite Toilet Care Granules and Baker's yeast. But Baker's yeast is more preferred than Overnite Toilet Care Granules, because Overnite Toilet Care Granules greatly increases initial content of TOC. The best result, 59.17% of TOC removal, happened at 6 hours when concentration of TOC was 25 mg/l.

Baker's yeast is also very effective method to remove TOC from soybean milk, but G1 is the best bioproduct for TOC removal. 75.2% of TOC was removed by using G1. Although the removal rate of using beer's yeast is almost the same as using Baker's yeast, beer's yeast did not show steady results.

Beer's yeast does not yield good results for most of samples due to high nutrients content and low concentration of bacteria. Liquid Drain Care is not suitable for treating either milk or soybean milk wastewater.

## **7.2 Engineering Significance**

As mentioned above that dairy wastewater contains high concentration of biodegradable TOC. Dairy wastewater needs to be treated before discharging into municipal sewer network. This study confirmed that milk and soy milk wastewater contain high concentration of total organic carbon. Biological treatment can be a very effective and environmental friendly method to reduce TOC. This study provided a strong possibility of using Baker's yeast to treat both milk wastewater and soy milk wastewater.

It is encouraged that dairy processing industries should set up laboratory scale experiment to find the most effective bioproduct to treat their specific wastewater. Economic products should be used to remove TOC.

## **7.3 Recommendations**

1. The bioproducts should be in liquid form or totally dissolved in water. This ensures that the bioproduct added into the wastewater has the same amount of microorganism. The substrate in the solid form, like Enforcer Overnite Toilet Care Granular in this study, has to break down cellulose into individual cellulose fibers, then hydrolyzes cellulose fibers into smaller sugars, and finally break them into glucose. This leads to the temporarily increase of TOC in wastewater.

2. The bioproduct should not produce bubbles. The bubbles greatly affect the results of TOC analysis.



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## APPENDICES

## APPENDIX A

### CALIBRATION OF SOYBEAN MILK AND MILK SOLUTION

Concentration of milk solution (mg/l)	TOC (ppm)
1000	356.2
500	189.7
250	96.03
125	45.61
62.5	23.37
31.25	10.91

Table XVII Calibration of Milk Solution

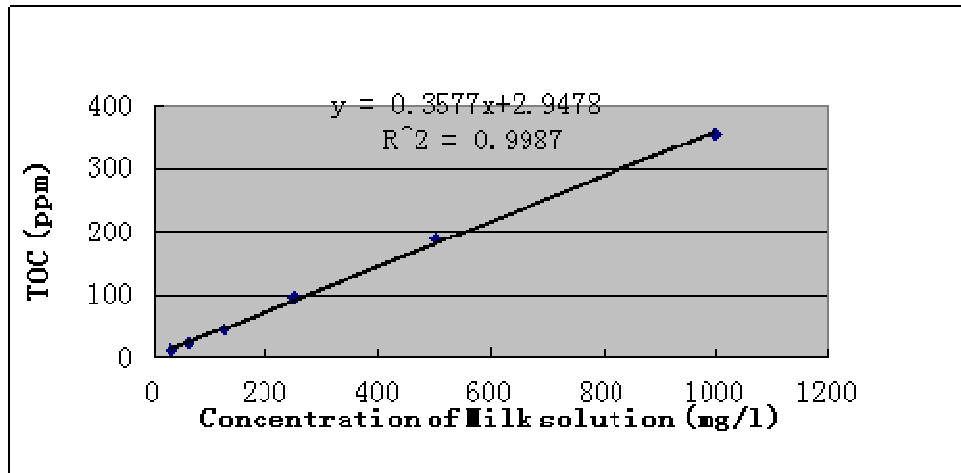


Figure 1 Calibration of Milk Solution

y	x
150	411.10
100	271.32
50	131.54
25	61.65

Soybean Concentration (ml of soybean/ l of water)	Run 1	Run 2	Run3	Average
0.75	29.77	30.8	32.33	30.97
1.5	61.62	64.37	61.6	62.53
3	121.1	134.6	124.4	126.70
6	251	374.1	268.3	259.65

Table XVIII Calibration of Soybean Milk

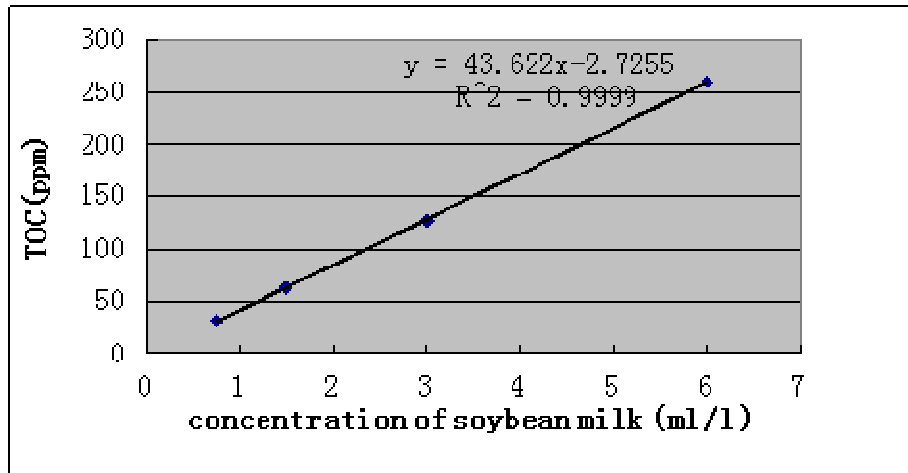


Figure 2 Calibration of Soybean Milk

y	x	2x
150	3.5	7.00
100	2.35	4.7
50	1.2	2.4

## APPENDIX B

### RESULTS OF TABLES

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Baker's Yeast Dosage (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	1	0	19.63	0
2	milk	25	1	2	23.09	-17.63
3	milk	25	1	4	20.98	-6.88
4	milk	25	1	6	16.29	17.01
5	milk	25	1	12	14.02	28.58
6	milk	25	1	24	18.69	4.79
7	milk	50	1	0	39.34	0
8	milk	50	1	2	40.04	-1.78
9	milk	50	1	4	33.29	15.38
10	milk	50	1	6	38.36	4.20
11	milk	50	1	12	30.32	22.93
12	milk	50	1	24	37.28	5.24
13	milk	100	1	0	85.93	0
14	milk	100	1	2	68.72	20.03
15	milk	100	1	4	76.90	10.51
16	milk	100	1	6	72.20	15.98
17	milk	100	1	12	57.92	32.60
18	milk	100	1	24	77.44	9.88
19	milk	150	1	0	139.70	0
20	milk	150	1	2	167.30	-19.76
21	milk	150	1	4	110.90	20.62
22	milk	150	1	6	84.75	39.33
23	milk	150	1	12	109.40	21.69
24	milk	150	1	24	123.60	11.52

Table XIX Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast Results

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Baker's Yeast Dosage (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	5.5	0	33.53	0
2	milk	25	5.5	2	20.88	37.73
3	milk	25	5.5	4	21.79	35.01
4	milk	25	5.5	6	13.69	59.17
5	milk	25	5.5	12	17.03	49.21
6	milk	25	5.5	24	21.75	35.13
7	milk	50	5.5	0	44.75	0
8	milk	50	5.5	2	41.62	6.99
9	milk	50	5.5	4	42.29	5.50
10	milk	50	5.5	6	27.05	35.01
11	milk	50	5.5	12	34.95	21.90
12	milk	50	5.5	24	29.92	33.14
13	milk	100	5.5	0	77.90	0
14	milk	100	5.5	2	81.39	-4.48
15	milk	100	5.5	4	70.58	9.40
16	milk	100	5.5	6	68.72	11.78
17	milk	100	5.5	12	69.60	10.65
18	milk	100	5.5	24	76.76	1.46
19	milk	150	5.5	0	137.40	0
20	milk	150	5.5	2	131.30	4.44
21	milk	150	5.5	4	111.70	18.70
22	milk	150	5.5	6	112.90	17.83
23	milk	150	5.5	12	78.40	42.94
24	milk	150	5.5	24	107.00	22.13

Table XX Run #2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast Results

Bottle No.	Waste Water	Concentration of Wastewater (mg/l)	Baker's Yeast Dosage (ml)	Shaking Time (hrs)	TOC (ppm)	%TOC Removal
1	milk	25	10	0	32.72	0
2	milk	25	10	2	20.44	37.53
3	milk	25	10	4	23.31	28.76
4	milk	25	10	6	26.96	17.60
5	milk	25	10	12	17.38	46.88
6	milk	25	10	24	38.68	-18.21
7	milk	50	10	0	62.19	0
8	milk	50	10	2	50.62	18.60
9	milk	50	10	4	43.06	30.76
10	milk	50	10	6	34.62	31.61
11	milk	50	10	12	46.27	25.60
12	milk	50	10	24	16.69	73.16
13	milk	100	10	0	95.03	0
14	milk	100	10	2	84.08	11.52
15	milk	100	10	4	63.30	33.39
16	milk	100	10	6	70.71	25.59
17	milk	100	10	12	57.20	39.81
18	milk	100	10	24	89.72	5.59
19	milk	150	10	0	165.50	0
20	milk	150	10	2	131.80	20.36
21	milk	150	10	4	142.10	14.14
22	milk	150	10	6	87.12	47.36
23	milk	150	10	12	108.20	34.62
24	milk	150	10	24	91.25	44.86

Table XXI Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast Results



Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Beer's Yeast Dosage (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	1	0	28.84	0
2	milk	25	1	2	30.30	-5.06
3	milk	25	1	4	25.46	11.72
4	milk	25	1	6	29.28	-1.53
5	milk	25	1	12	28.17	2.323
6	milk	25	1	24	32.61	-13.07
7	milk	50	1	0	62.28	0
8	milk	50	1	2	61.08	1.93
9	milk	50	1	4	70.15	-12.64
10	milk	50	1	6	64.47	-3.52
11	milk	50	1	12	60.39	3.03
12	milk	50	1	24	93.25	-49.73
13	milk	100	1	0	124.90	0
14	milk	100	1	2	129.00	-3.28
15	milk	100	1	4	125.90	-0.80
16	milk	100	1	6	121.80	2.48
17	milk	100	1	12	127.70	-2.24
18	milk	100	1	24	126.00	-0.88
19	milk	150	1	0	261.30	0
20	milk	150	1	2	201.40	22.92
21	milk	150	1	4	196.40	24.84
22	milk	150	1	6	200.90	23.12
23	milk	150	1	12	193.70	25.87
24	milk	150	1	24	204.00	21.93

Table XXII Run #4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Beer's Yeast Dosage (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	5.5	0	41.97	0
2	milk	25	5.5	2	46.17	-10.01
3	milk	25	5.5	4	45.15	-7.58
4	milk	25	5.5	6	0.00	100.00
5	milk	25	5.5	12	0.00	100.00
6	milk	25	5.5	24	0.00	100.00
7	milk	50	5.5	0	77.40	0
8	milk	50	5.5	2	70.2	9.30
9	milk	50	5.5	4	69.53	10.17
10	milk	50	5.5	6	73.97	4.43
11	milk	50	5.5	12	73.02	5.66
12	milk	50	5.5	24	107.30	-38.63
13	milk	100	5.5	0	211.60	0
14	milk	100	5.5	2	136.00	35.73
15	milk	100	5.5	4	149.50	29.35
16	milk	100	5.5	6	138.10	34.74
17	milk	100	5.5	12	141.80	32.98
18	milk	100	5.5	24	0	100
19	milk	150	5.5	0	219.40	0
20	milk	150	5.5	2	324.40	-47.86
21	milk	150	5.5	4	191.70	12.63
22	milk	150	5.5	6	216.10	1.50
23	milk	150	5.5	12	220.50	-0.50
24	milk	150	5.5	24	205.80	6.20

Table XXIII Run #5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Beer's Yeast Dosage (g)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	10	0	38.42	0
2	milk	25	10	2	38.95	-1.38
3	milk	25	10	4	37.73	1.80
4	milk	25	10	6	39.21	-2.06
5	milk	25	10	12	38.82	-1.04
6	milk	25	10	24	34.25	10.85
7	milk	50	10	0	69.79	0
8	milk	50	10	2	74.05	-6.10
9	milk	50	10	4	66.43	4.81
10	milk	50	10	6	108.4	-55.32
11	milk	50	10	12	76.33	-9.37
12	milk	50	10	24	68.30	2.13
13	milk	100	10	0	190.6	0
14	milk	100	10	2	126.6	33.58
15	milk	100	10	4	193.00	-1.26
16	milk	100	10	6	130.90	31.32
17	milk	100	10	12	135.70	28.80
18	milk	100	10	24	90.04	52.76
19	milk	150	10	0	193.30	0
20	milk	150	10	2	207.40	-7.29
21	milk	150	10	4	203.70	-5.38
22	milk	150	10	6	195.50	-1.14
23	milk	150	10	12	197.80	-2.33
24	milk	150	10	24	208.60	-7.92

Table XXIV Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Beer's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Overnite Toilet Care Granules (g)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	0.5	0	42.37	0
2	milk	25	0.5	2	51.15	-20.72
3	milk	25	0.5	4	62.89	-48.43
4	milk	25	0.5	6	10.75	74.63
5	milk	25	0.5	12	42.11	0.61
6	milk	25	0.5	24	57.49	-35.69
7	milk	50	0.5	0	133.60	0
8	milk	50	0.5	2	89.08	33.32
9	milk	50	0.5	4	138.60	-3.74
10	milk	50	0.5	6	107.80	19.31
11	milk	50	0.5	12	93.40	30.09
12	milk	50	0.5	24	138.80	-3.89
13	milk	100	0.5	0	168.30	0
14	milk	100	0.5	2	160.20	4.81
15	milk	100	0.5	4	146.40	13.01
16	milk	100	0.5	6	240.70	-43.02
17	milk	100	0.5	12	151.20	10.16
18	milk	100	0.5	24	102.60	39.04
19	milk	150	0.5	0	218.00	0
20	milk	150	0.5	2	202.70	7.02
21	milk	150	0.5	4	215.90	0.96
22	milk	150	0.5	6	182.90	16.10
23	milk	150	0.5	12	218.80	-0.37
24	milk	150	0.5	24	152.90	29.86

Table XXV Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granules

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Overnite Toilet Care Granules (g)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	1	0	99.80	0
2	milk	25	1	2	42.41	57.51
3	milk	25	1	4	65.88	33.99
4	milk	25	1	6	151.4	-51.70
5	milk	25	1	12	72.34	27.52
6	milk	25	1	24	62.98	36.89
7	milk	50	1	0	108.90	0
8	milk	50	1	2	71.87	34.00
9	milk	50	1	4	75.68	30.51
10	milk	50	1	6	97.61	10.37
11	milk	50	1	12	30.84	71.68
12	milk	50	1	24	27.49	74.76
13	milk	100	1	0	135.70	0
14	milk	100	1	2	132.50	2.36
15	milk	100	1	4	131.20	3.32
16	milk	100	1	6	149.80	-10.40
17	milk	100	1	12	182.30	-34.34
18	milk	100	1	24	98.30	27.56
19	milk	150	1	0	217.00	0
20	milk	150	1	2	181.50	16.36
21	milk	150	1	4	221.20	-1.94
22	milk	150	1	6	304.10	-40.14
23	milk	150	1	12	211.30	2.63
24	milk	150	1	24	121.60	43.96

Table XXVI Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnite Toilet Care Granules

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Overnite Toilet Care Granules (g)	Shaking Time(hrs )	TOC (ppm)	%TOC Removal
1	milk	25	1.5	0	185.90	0
2	milk	25	1.5	2	259.00	-39.32
3	milk	25	1.5	4	245.20	-31.90
4	milk	25	1.5	6	335.50	-80.47
5	milk	25	1.5	12	301.60	-62.24
6	milk	25	1.5	24	244.90	-31.74
7	milk	50	1.5	0	173.30	0
8	milk	50	1.5	2	201.90	-16.50
9	milk	50	1.5	4	171.90	0.81
10	milk	50	1.5	6	47.93	72.34
11	milk	50	1.5	12	149.40	13.79
12	milk	50	1.5	24	186.80	-7.79
13	milk	100	1.5	0	153.30	0
14	milk	100	1.5	2	57.82	62.28
15	milk	100	1.5	4	41.11	73.18
16	milk	100	1.5	6	195.50	-27.53
17	milk	100	1.5	12	165.30	-7.83
18	milk	100	1.5	24	233.30	-52.19
19	milk	150	1.5	0	108.20	0
20	milk	150	1.5	2	134.80	-24.58
21	milk	150	1.5	4	131.10	-21.16
22	milk	150	1.5	6	128.00	-18.30
23	milk	150	1.5	12	123.40	-14.05
24	milk	150	1.5	24	159.70	-47.60

Table XXVII Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnite Toilet Care Granules

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Liquid Drain Care (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	1	0	77.60	0
2	milk	25	1	2	85.53	-10.22
3	milk	25	1	4	42.72	44.95
4	milk	25	1	6	96.54	-24.41
5	milk	25	1	12	91.77	-18.26
6	milk	25	1	24	88.30	-13.79
7	milk	50	1	0	159.20	0
8	milk	50	1	2	108.20	-22.54
9	milk	50	1	4	133.10	16.39
10	milk	50	1	6	117.50	26.19
11	milk	50	1	12	190.80	-19.85
12	milk	50	1	24	104.60	34.30
13	milk	100	1	0	187.20	0
14	milk	100	1	2	248.00	-32.48
15	milk	100	1	4	197.00	-5.24
16	milk	100	1	6	263.40	-40.71
17	milk	100	1	12	185.60	0.85
18	milk	100	1	24	167.50	10.52
19	milk	150	1	0	245.70	0
20	milk	150	1	2	253.50	-3.17
21	milk	150	1	4	279.60	-13.80
22	milk	150	1	6	251.80	-2.48
23	milk	150	1	12	251.00	-2.16
24	milk	150	1	24	226.80	7.69

Table XXVIII Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Liquid Drain Care (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	5.5	0	280.40	0
2	milk	25	5.5	2	319.50	-13.94
3	milk	25	5.5	4	342.60	-22.18
4	milk	25	5.5	6	332.60	-18.62
5	milk	25	5.5	12	295.10	-5.24
6	milk	25	5.5	24	308.00	-9.84
7	milk	50	5.5	0	325.10	0
8	milk	50	5.5	2	341.10	-10.75
9	milk	50	5.5	4	328.50	-1.05
10	milk	50	5.5	6	334.60	-2.92
11	milk	50	5.5	12	350.20	-7.72
12	milk	50	5.5	24	320.30	1.48
13	milk	100	5.5	0	397.40	0
14	milk	100	5.5	2	417.90	-5.16
15	milk	100	5.5	4	386.80	2.67
16	milk	100	5.5	6	407.00	-2.42
17	milk	100	5.5	12	410.80	-3.37
18	milk	100	5.5	24	387.30	2.54
19	milk	150	5.5	0	520.40	0
20	milk	150	5.5	2	536.00	-3.00
21	milk	150	5.5	4	493.00	5.27
22	milk	150	5.5	6	356.00	31.59
23	milk	150	5.5	12	494.80	4.92
24	milk	150	5.5	24	418.00	19.68

Table XXIX Run #11 25, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care



Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Liquid Drain Care (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	10	0	513.60	0
2	milk	25	10	2	565.90	-10.18
3	milk	25	10	4	533.90	-3.95
4	milk	25	10	6	540.00	-5.14
5	milk	25	10	12	582.10	-13.34
6	milk	25	10	24	587.20	-14.33
7	milk	50	10	0	553.00	0
8	milk	50	10	2	595.00	-1.33
9	milk	50	10	4	624.40	-12.91
10	milk	50	10	6	648.00	-17.18
11	milk	50	10	12	639.90	-15.71
12	milk	50	10	24	687.00	-24.23
13	milk	100	10	0	714.70	0
14	milk	100	10	2	733.60	-2.64
15	milk	100	10	4	690.00	3.46
16	milk	100	10	6	683.00	4.44
17	milk	100	10	12	719.00	-0.60
18	milk	100	10	24	979.30	-37.02
19	milk	150	10	0	734.00	0
20	milk	150	10	2	750.60	-2.26
21	milk	150	10	4	926.50	-26.23
22	milk	150	10	6	775.50	-5.65
23	milk	150	10	12	829.90	-13.07
24	milk	150	10	24	856.50	-16.69

Table XXX Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	G1 (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	milk	25	5.5	0	41.06	0
2	milk	25	5.5	2	55.47	-35.09
3	milk	25	5.5	4	50.59	-23.21
4	milk	25	5.5	6	51.76	-26.06
5	milk	25	5.5	12	34.91	14.98
6	milk	25	5.5	24	53.01	-29.10
7	milk	50	5.5	0	118.60	0
8	milk	50	5.5	2	115.70	2.45
9	milk	50	5.5	4	41.85	64.71
10	milk	50	5.5	6	124.90	-5.3
11	milk	50	5.5	12	56.51	52.35
12	milk	50	5.5	24	54.62	53.95
13	milk	100	5.5	0	102.20	0
14	milk	100	5.5	2	145.40	-42.27
15	milk	100	5.5	4	60.41	40.89
16	milk	100	5.5	6	135.60	-32.68
17	milk	100	5.5	12	130.80	-27.98
18	milk	100	5.5	24	146.90	-43.74
19	milk	150	5.5	0	188.50	0
20	milk	150	5.5	2	175.00	7.16
21	milk	150	5.5	4	136.30	27.69
22	milk	150	5.5	6	219.20	-16.29
23	milk	150	5.5	12	217.20	-15.23
24	milk	150	5.5	24	184.50	2.12

Table XXXI Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Baker's Yeast Dosage (ml)	Shaking Time (hrs)	TOC (ppm)	%TOC Removal
1	soybean	25	5.5	0	22.56	0
2	soybean	25	5.5	2	24.96	-10.64
3	soybean	25	5.5	4	19.98	11.44
4	soybean	25	5.5	6	23.65	-4.83
5	soybean	25	5.5	12	21.85	3.15
6	soybean	25	5.5	24	16.58	26.51
7	soybean	50	5.5	0	45.29	0
8	soybean	50	5.5	2	47.99	-5.96
9	soybean	50	5.5	4	45.43	-0.31
10	soybean	50	5.5	6	69.78	-54.07
11	soybean	50	5.5	12	45.65	-0.79
12	soybean	50	5.5	24	29.70	34.42
13	soybean	100	5.5	0	87.55	0
14	soybean	100	5.5	2	88.69	-1.30
15	soybean	100	5.5	4	90.35	-3.20
16	soybean	100	5.5	6	125.60	-43.46
17	soybean	100	5.5	12	103.20	-17.88
18	soybean	100	5.5	24	67.80	22.56
19	soybean	150	5.5	0	139.20	0
20	soybean	150	5.5	2	137.70	1.08
21	soybean	150	5.5	4	142.60	-2.44
22	soybean	150	5.5	6	150.90	-8.41
23	soybean	150	5.5	12	123.60	11.21
24	soybean	150	5.5	24	88.67	36.30

Table XXXII Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	Liquid Drain Care (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	soybean	25	5.5	0	3338	0
2	soybean	25	5.5	2	3606	-8.03
3	soybean	25	5.5	4	3451	-3.39
4	soybean	25	5.5	6	3411	-2.19
5	soybean	25	5.5	12	3609	-8.12
6	soybean	25	5.5	24	3473	-4.04
7	soybean	50	5.5	0	2896	0
8	soybean	50	5.5	2	3214	-10.98
9	soybean	50	5.5	4	3488	-20.44
10	soybean	50	5.5	6	3911	-35.05
11	soybean	50	5.5	12	3440	-18.78
12	soybean	50	5.5	24	3501	-20.89
13	soybean	100	5.5	0	3232	0
14	soybean	100	5.5	2	2866	11.32
15	soybean	100	5.5	4	3399	-5.17
16	soybean	100	5.5	6	2531	21.69
17	soybean	100	5.5	12	3082	4.64
18	soybean	100	5.5	24	2711	16.12
19	soybean	150	5.5	0	3331	0
20	soybean	150	5.5	2	2723	18.25
21	soybean	150	5.5	4	3285	1.38
22	soybean	150	5.5	6	2783	16.45
23	soybean	150	5.5	12	2478	25.61
24	soybean	150	5.5	24	2144	35.63

Table XXXIII Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care

Bottle No.	Waste Water	Wastewater Concentration (mg/l)	G1 (ml)	Shaking Time(hrs)	TOC (ppm)	%TOC Removal
1	soybean	25	5.5	0	23.43	0
2	soybean	25	5.5	2	26.45	-12.89
3	soybean	25	5.5	4	5.81	75.20
4	soybean	25	5.5	6	23.42	0.04
5	soybean	25	5.5	12	21.06	10.12
6	soybean	25	5.5	24	21.82	6.87
7	soybean	50	5.5	0	43.38	0
8	soybean	50	5.5	2	48.27	-11.27
9	soybean	50	5.5	4	44.91	-3.53
10	soybean	50	5.5	6	41.63	4.03
11	soybean	50	5.5	12	41.08	5.30
12	soybean	50	5.5	24	30.44	29.83
13	soybean	100	5.5	0	85.95	0
14	soybean	100	5.5	2	103.4	-20.30
15	soybean	100	5.5	4	99.39	-15.64
16	soybean	100	5.5	6	97.20	-13.09
17	soybean	100	5.5	12	122.90	-42.99
18	soybean	100	5.5	24	78.80	8.32
19	soybean	150	5.5	0	155.50	0
20	soybean	150	5.5	2	165.00	-6.11
21	soybean	150	5.5	4	157.80	-1.48
22	soybean	150	5.5	6	151.40	2.64
23	soybean	150	5.5	12	141.70	8.87
24	soybean	150	5.5	24	117.80	24.24

Table XXXIV Run #16 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1

Run No.	Wastewater	Conc. (mg/l)	Type of Bioproduct	Dosage of Bioproduct (ml or g)	Shaking Time (hrs)	%TOC removal
1	milk	150	Baker's yeast	1	6	39.33
2	milk	25	Baker's yeast	5.5	6	59.17
3	milk	50	Baker's yeast	10	24	73.16
4	milk	150	Beer's yeast	1	12	25.87
5	milk	100	Beer's yeast	5.5	2	35.73
6	milk	100	Beer's yeast	10	24	52.76
7	milk	25	Overnite Toilet Care Granules	0.5	6	74.63
8	milk	50	Overnite Toilet Care Granules	1	24	74.76
9	milk	100	Overnite Toilet Care Granules	1.5	4	73.18
10	milk	25	Liquid Drain Care	1	4	44.95
11	milk	150	Liquid Drain Care	5.5	6	31.59
12	milk	100	Liquid Drain Care	10	6	4.44
13	milk	50	G1	5.5	4	64.71
14	soybean	150	Baker's yeast	5.5	24	36.30
15	soybean	150	Liquid Drain Care	5.5	24	35.63
16	soybean	25	G1	5.5	4	75.2

Table XXXV Comparison between Runs, Ranged by Type of Wastewater

Run No.	Wastewater	Conc. (mg/l)	Type of Bioproduct	Dosage of Bioproduct (ml or g)	Shaking Time (hrs)	%TOC removal
1	milk	150	Baker's yeast	1	6	39.33
2	milk	25	Baker's yeast	5.5	6	59.17
3	milk	50	Baker's yeast	10	24	73.16
14	soybean	150	Baker's yeast	5.5	24	36.30
4	milk	150	Beer's yeast	1	12	25.87
5	milk	25	Beer's yeast	5.5	6,12,24	100
6	milk	100	Beer's yeast	10	24	52.76
7	milk	25	Overnite Toilet Care Granules	0.5	6	74.63
8	milk	50	Overnite Toilet Care Granules	1	24	74.76
9	milk	100	Overnite Toilet Care Granules	1.5	4	73.18
10	milk	25	Liquid Drain Care	1	4	44.95
11	milk	150	Liquid Drain Care	5.5	6	31.59
12	milk	100	Liquid Drain Care	10	6	4.44
15	soybean	150	Liquid Drain Care	5.5	24	35.63
13	milk	50	G1	5.5	4	64.71
16	soybean	25	G1	5.5	4	75.2

Table XXXVI Comparison of All runs, Ranged by Type of Bioproduct

## APPENDIX C

### RESULTS OF FIGURES

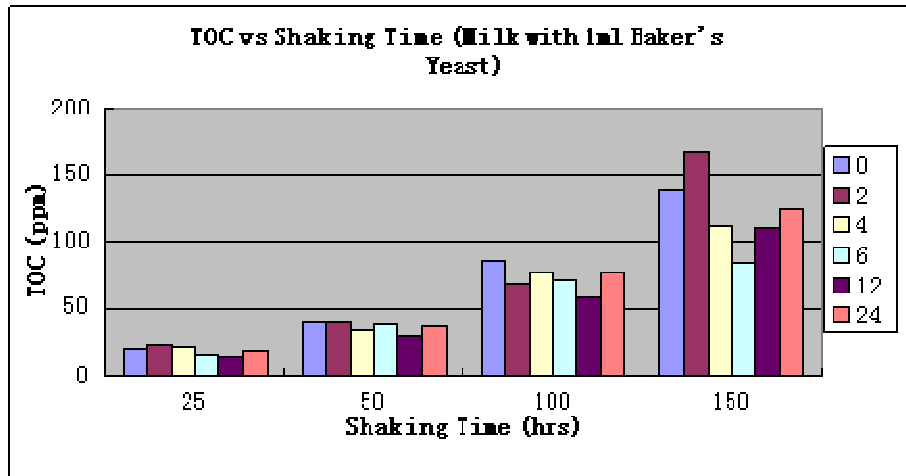


Figure 3 Bar Chart of Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast Results



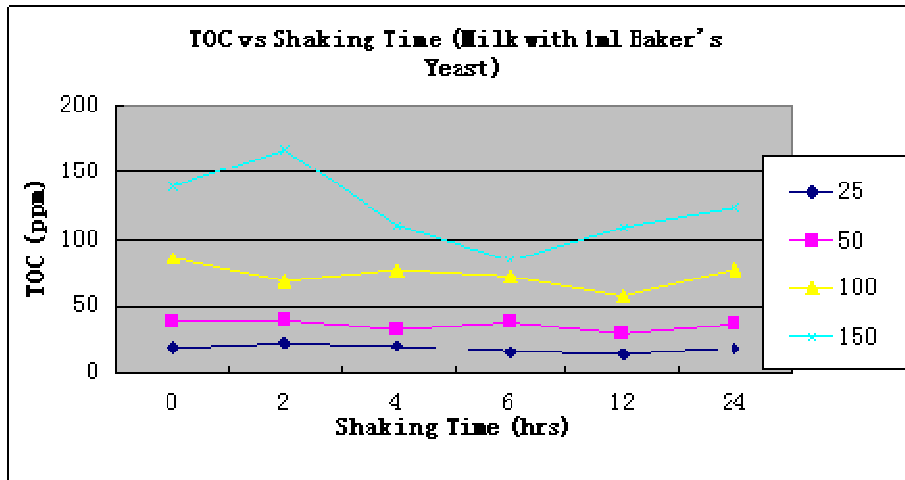


Figure 4 Line Chart of Run #1 25, 50, 100 and 150 mg/l Milk with 1 ml Baker's Yeast Results

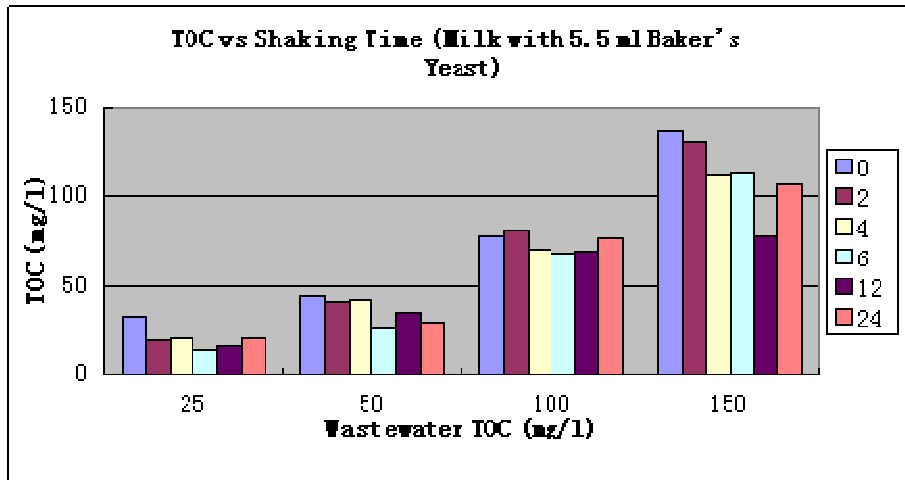


Figure 5 Bar Chart of Run#2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast Results

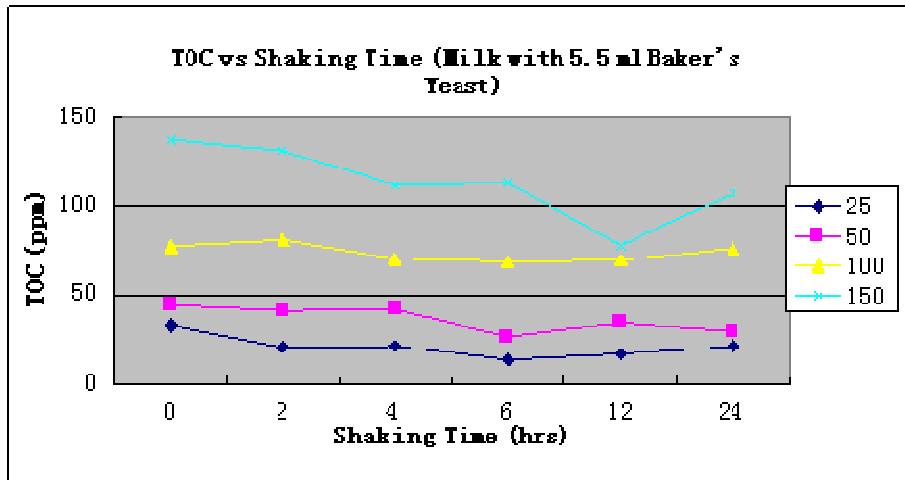


Figure 6 Line Chart of Run#2 25, 50, 100 and 150 mg/l Milk with 5.5 ml Baker's Yeast Results

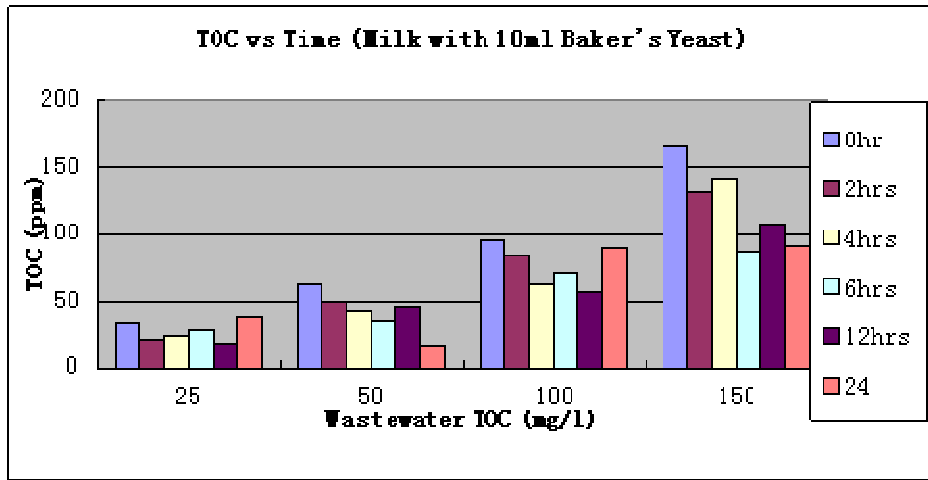


Figure 7 Bar Chart of Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast Results

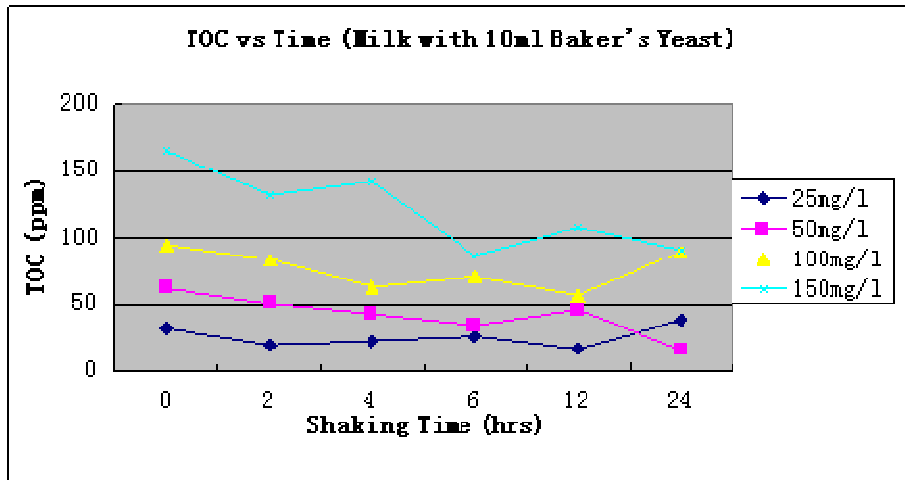


Figure 8 Line Chart of Run #3 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast

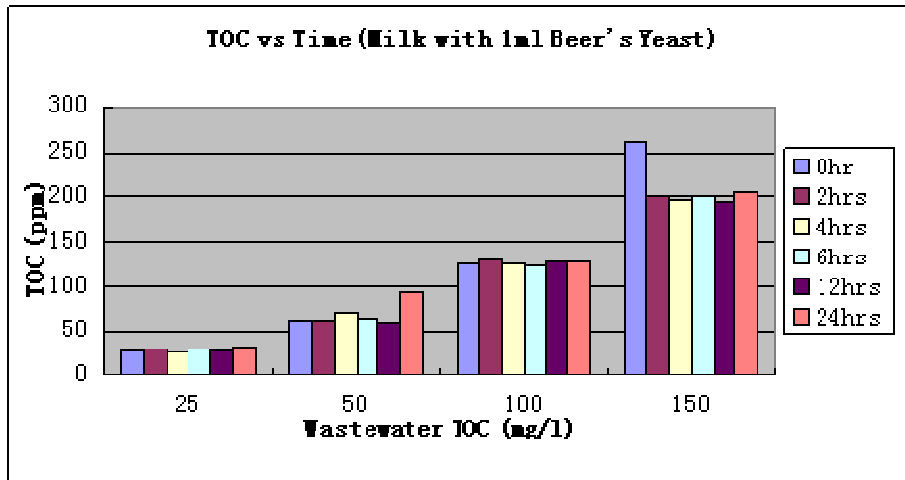


Figure 9 Bar Chart of Run #4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast Results

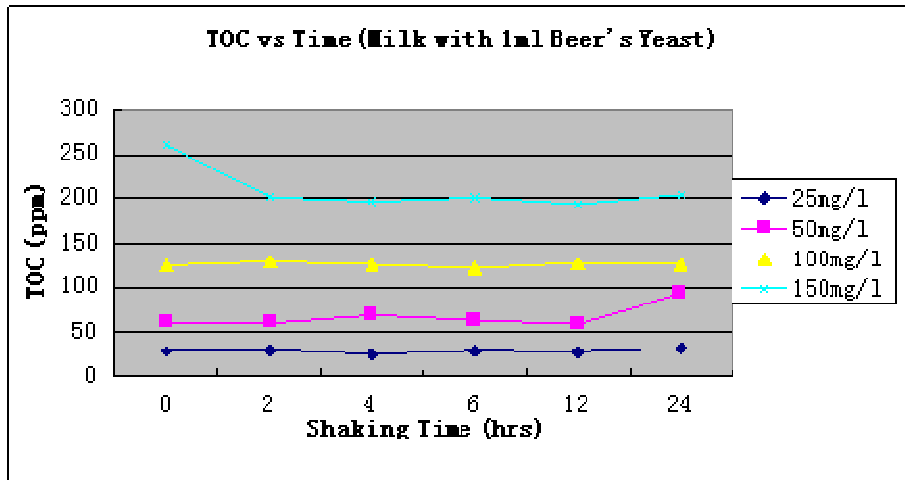


Figure 10 Line Chart of Run#4 25, 50, 100 and 150 mg/l Milk with 1 ml Beer's Yeast Results

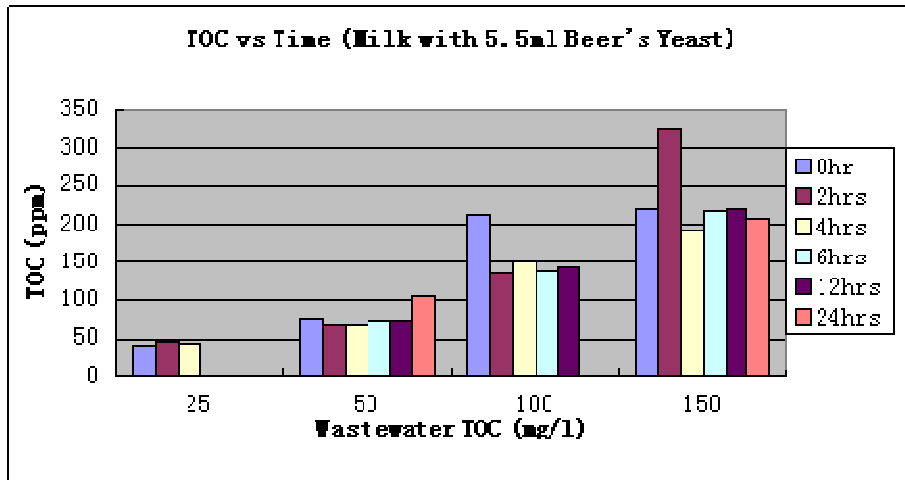


Figure 11 Bar Chart of Run #5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast Results



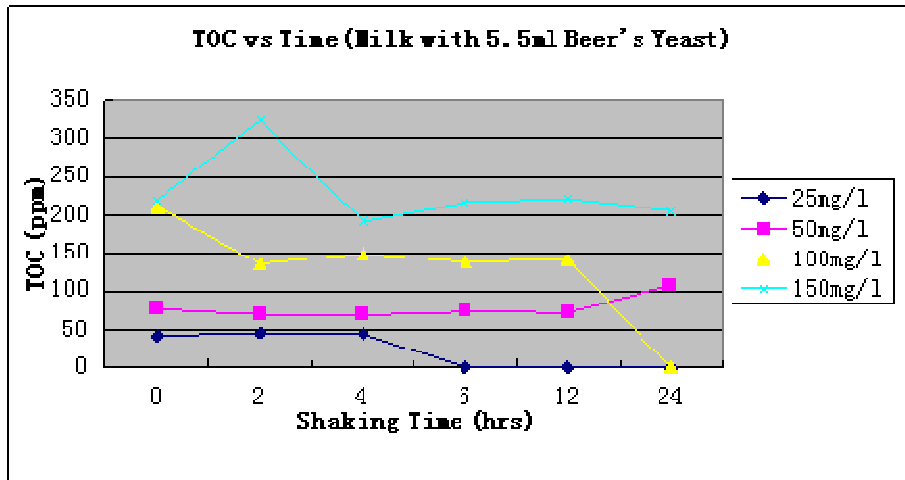


Figure 12 Line Chart of Run#5 25, 50, 100 and 150 mg/l Milk with 5.5 ml Beer's Yeast Results

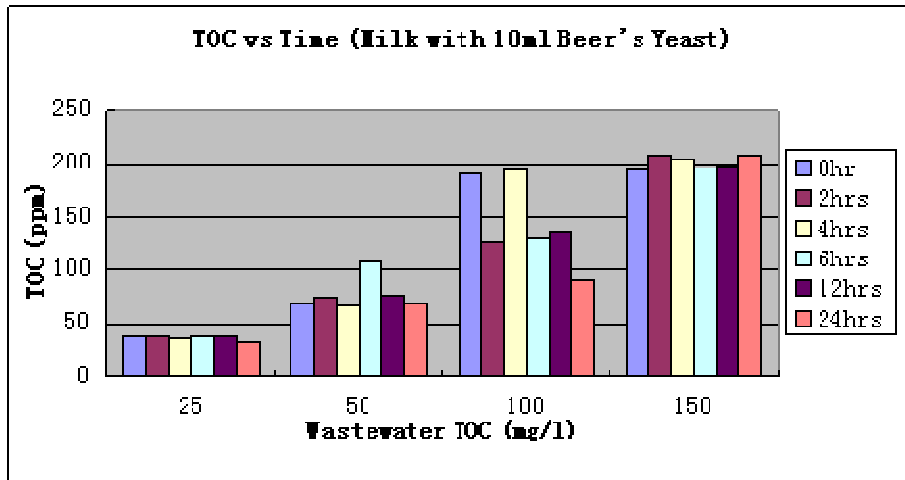


Figure 13 Bar Chart of Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast Results

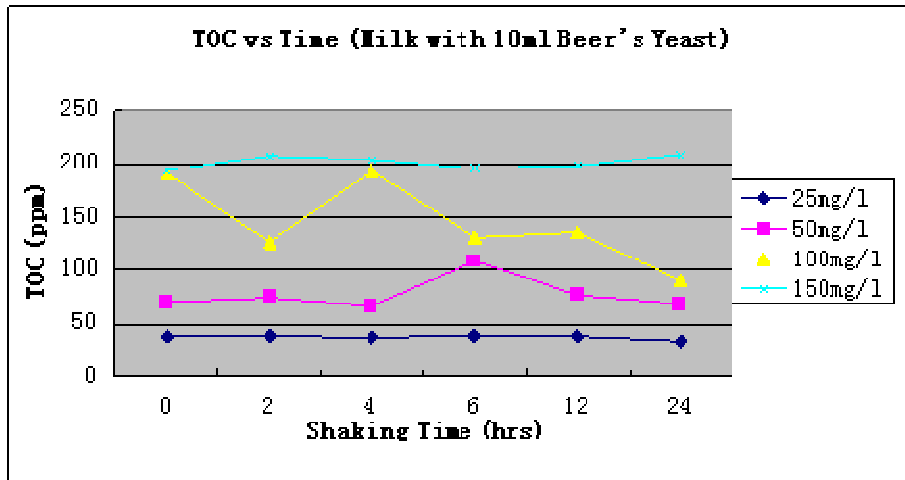


Figure 14 Line Chart of Run #6 25, 50, 100 and 150 mg/l Milk with 10 ml Baker's Yeast Results

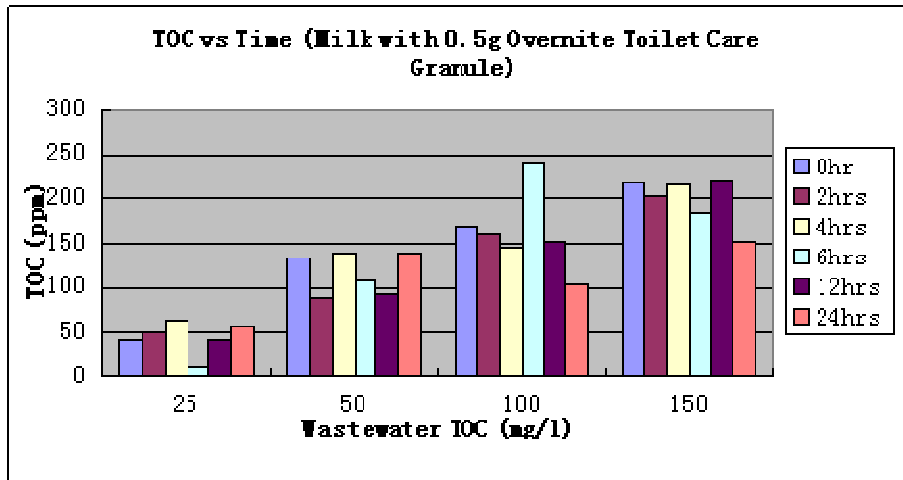


Figure 15 Bar Chart of Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granules Results

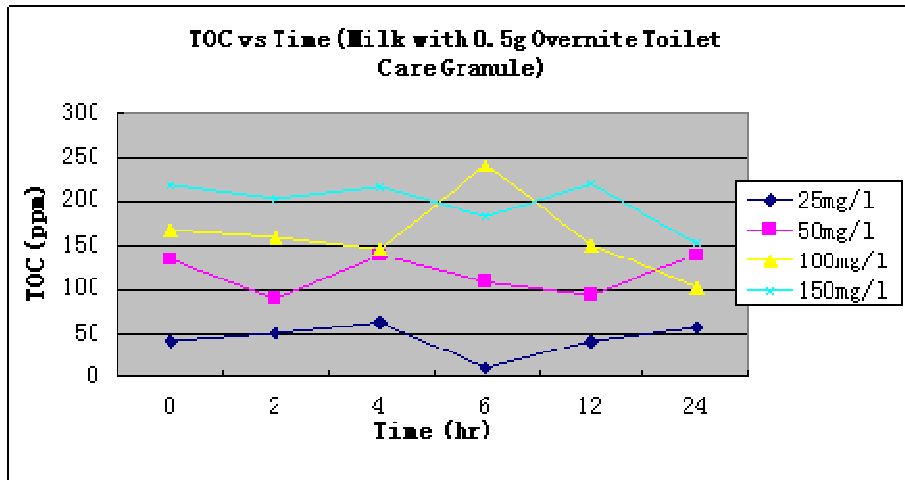


Figure 16 Line Chart of Run #7 25, 50, 100 and 150 mg/l Milk with 0.5 g Overnite Toilet Care Granules Results

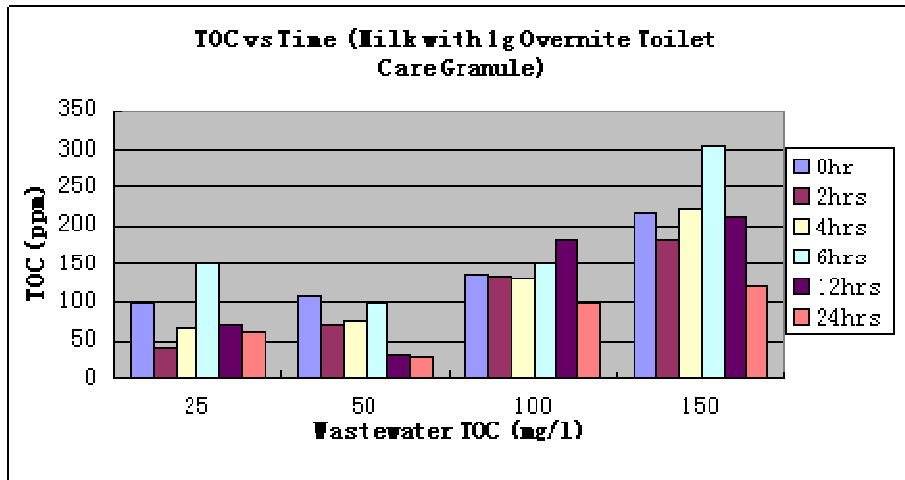


Figure 17 Bar Chart of Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnight Toilet Care Granules Results

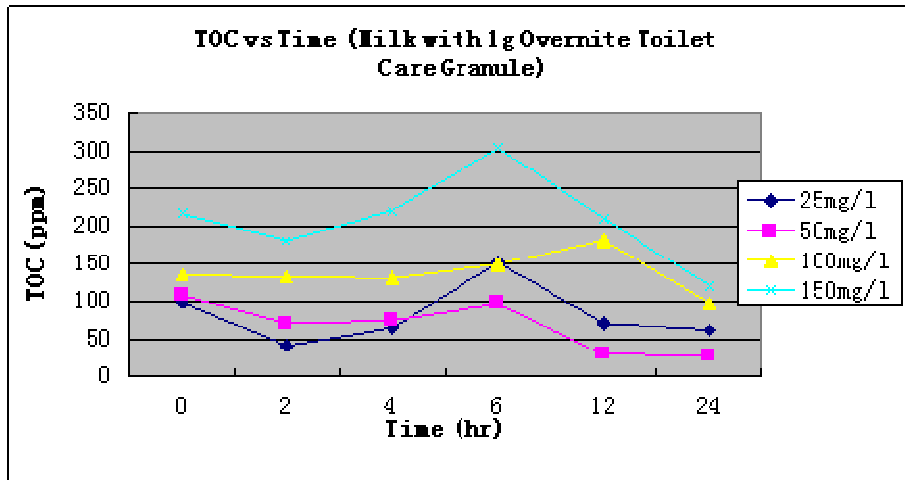


Figure 18 Line Chart of Run #8 25, 50, 100 and 150 mg/l Milk with 1 g Overnight Toilet Care Granules Results

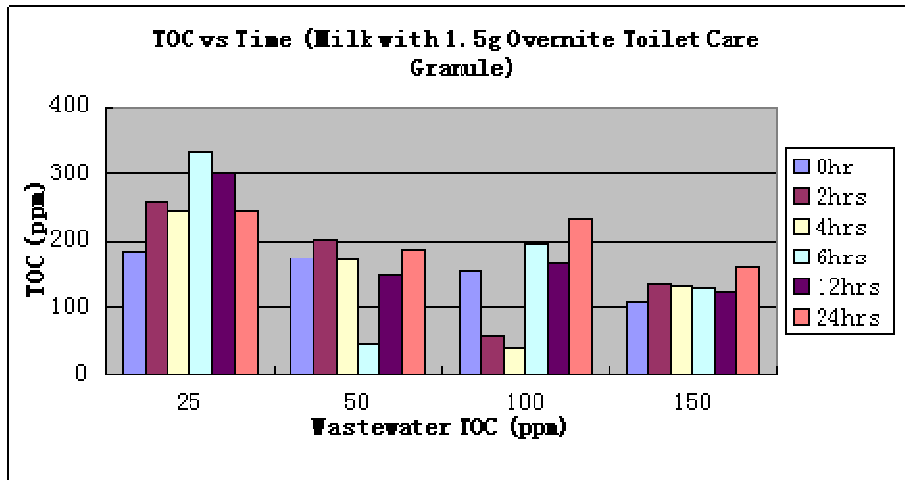


Figure 19 Bar Chart of Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnight Toilet Care Granules Results



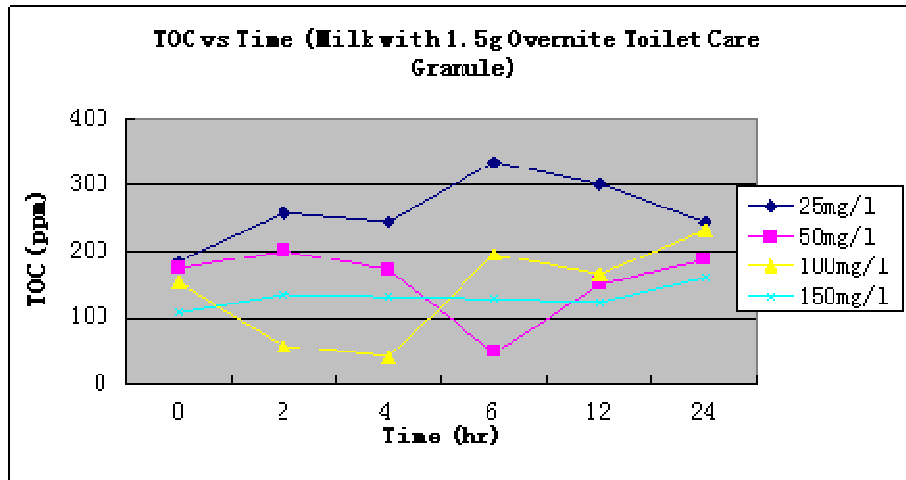


Figure 20 Line Chart of Run #9 25, 50, 100 and 150 mg/l Milk with 1.5 g Overnight Toilet Care Granules Results

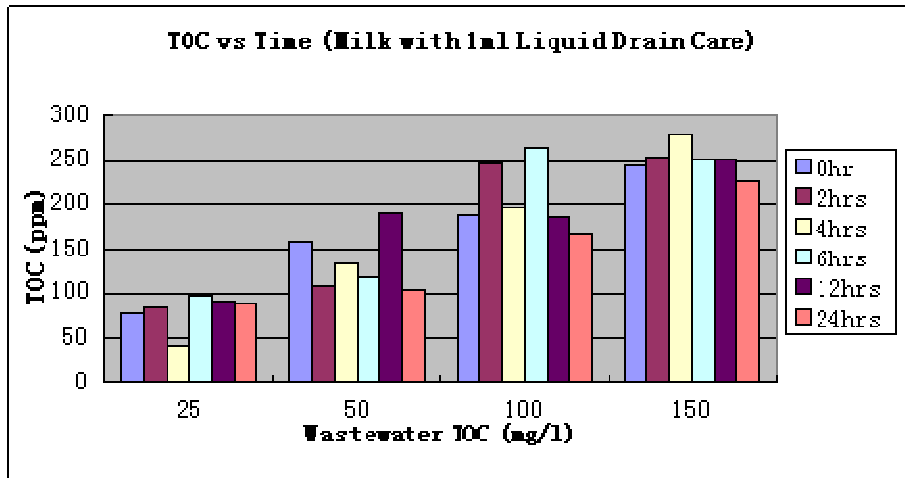


Figure 21 Bar Chart of Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care Results

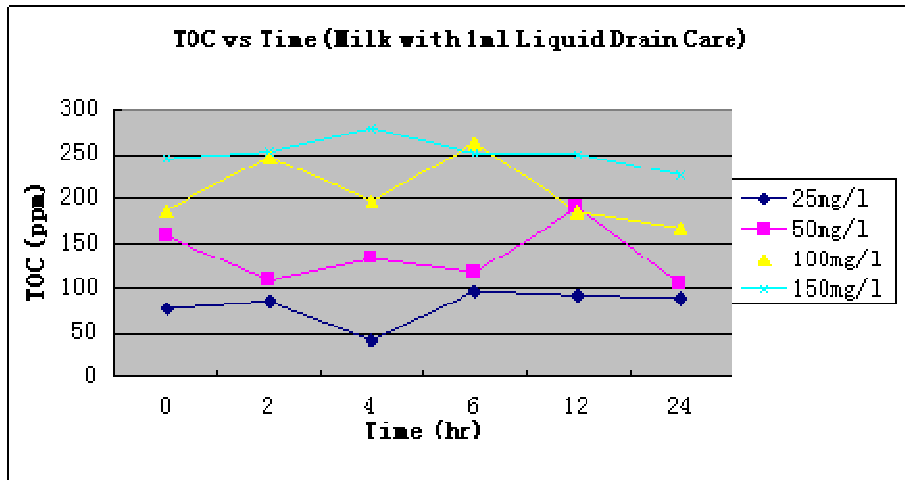


Figure 22 Line Chart of Run #10 25, 50, 100 and 150 mg/l Milk with 1 ml Liquid Drain Care Results

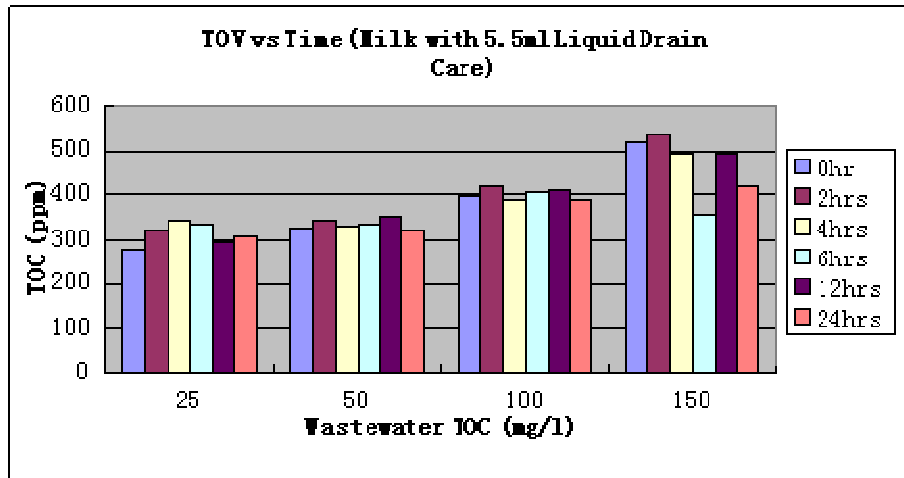


Figure 23 Bar chart of Run #11, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care Results

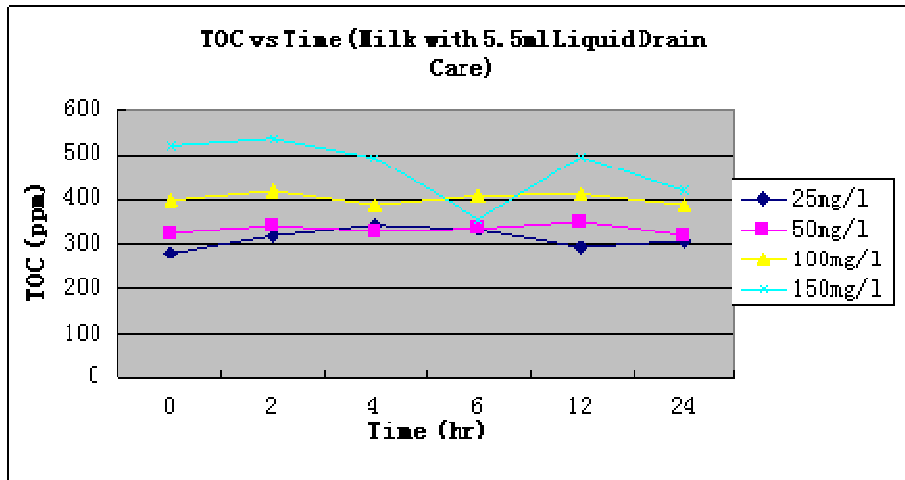


Figure 24 Line Chart of Run #11 25, 50, 100 and 150 mg/l Milk with 5.5 ml Liquid Drain Care Results

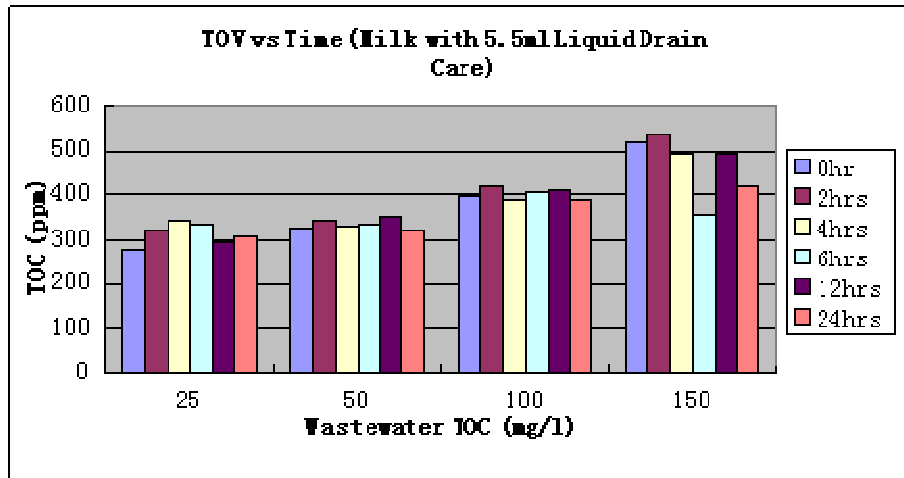


Figure 25 Bar Chart of Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care Results

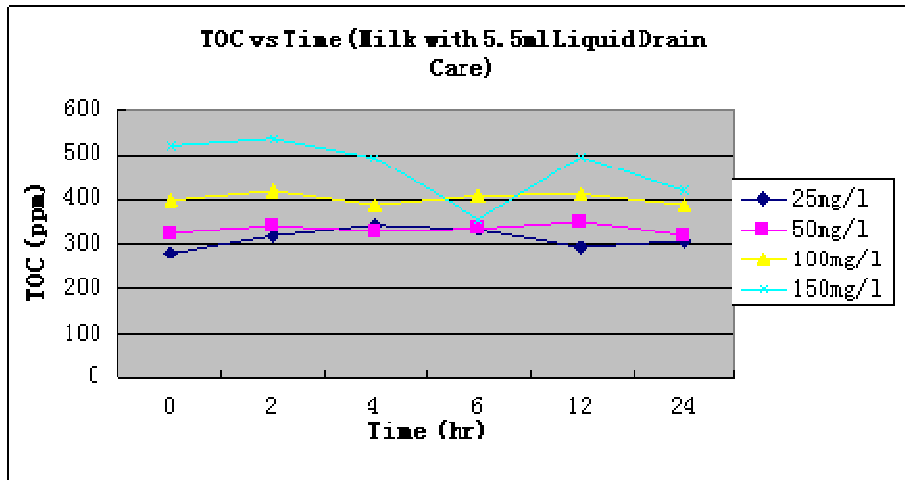


Figure 26 Line Chart of Run #12 25, 50, 100 and 150 mg/l Milk with 10 ml Liquid Drain Care Results

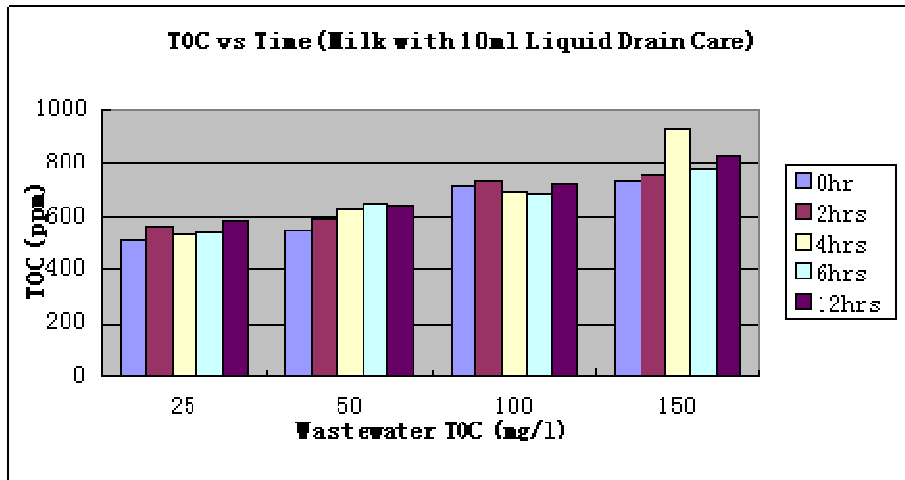


Figure 27 Bar Chart of Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1 Results



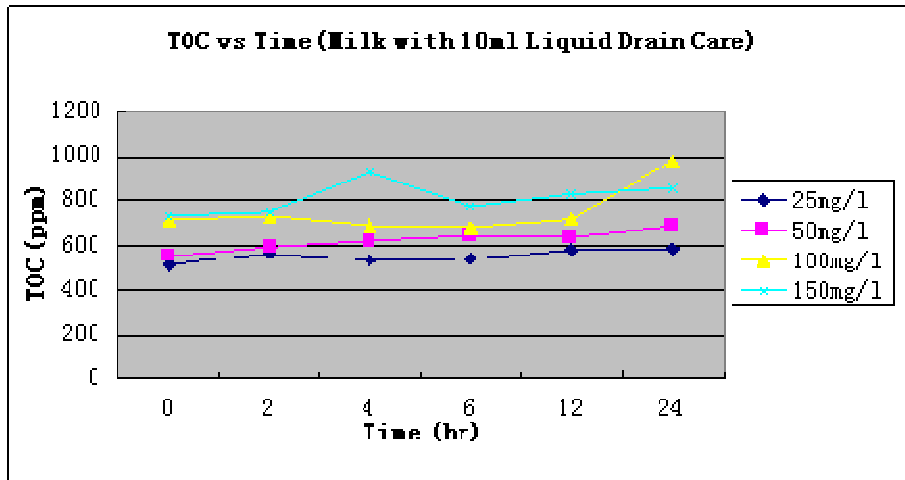


Figure 28 Line Chart of Run #13 25, 50, 100 and 150 mg/l Milk with 5.5 ml G1 Results

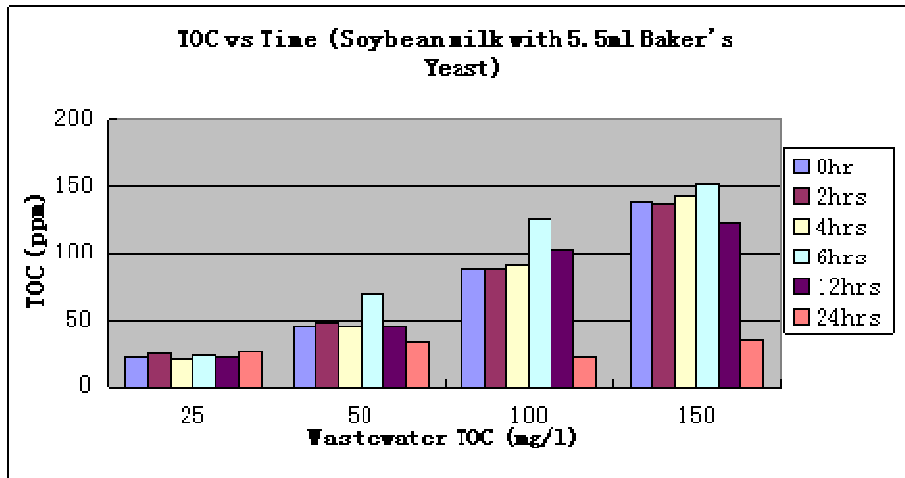


Figure 29 Bar Chart of Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast Results

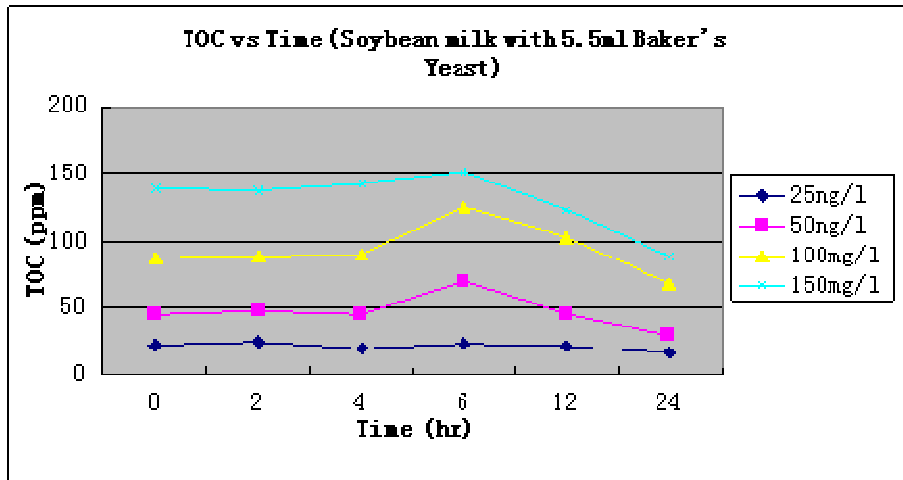


Figure 30 Line Chart of Run #14 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Baker's Yeast Results

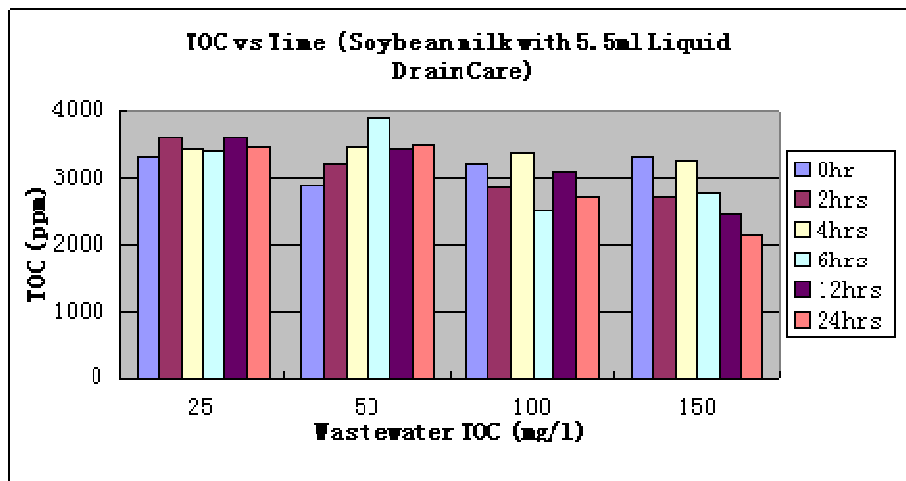


Figure 31 Bar Chart of Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care Results

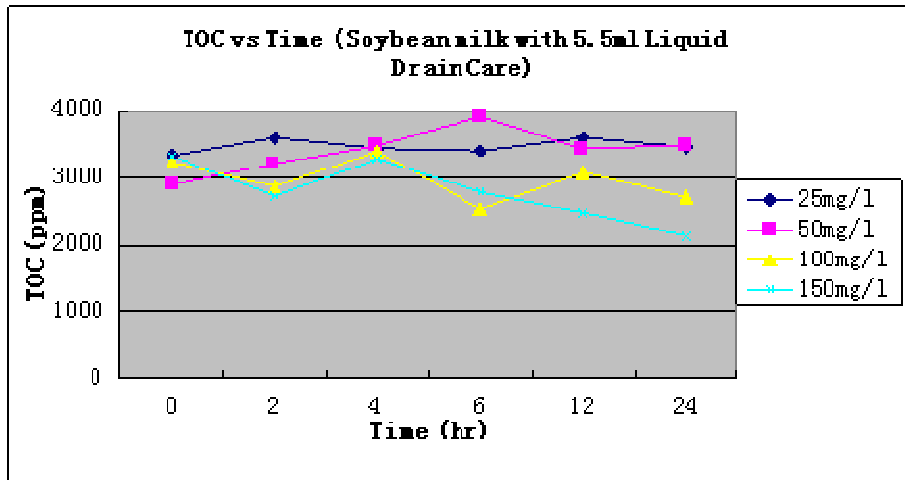


Figure 32 Line Chart of Run #15 25, 50, 100 and 150 mg/l Soybean with 5.5 ml Liquid Drain Care Results

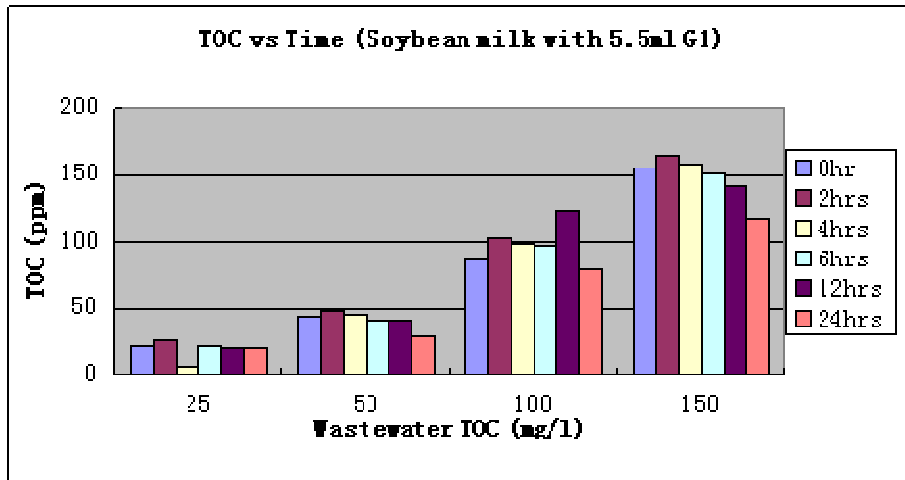


Figure 33 Bar Chart of Run #16 25, 50, 100 and 150 mg/l Soybean Milk with 5.5 ml G1 Results

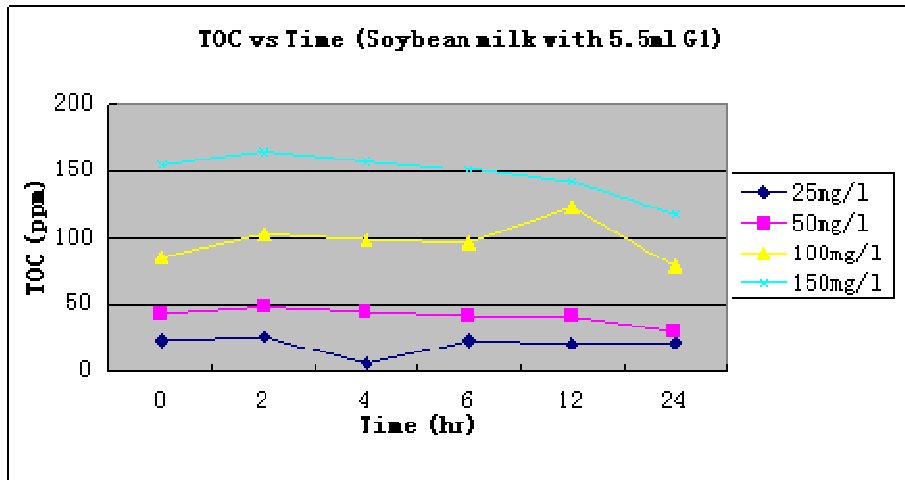


Figure 34 Line Chart of Run #16 25, 50, 100 and 150 mg/l Soybean Milk with 5.5 ml G1 Results

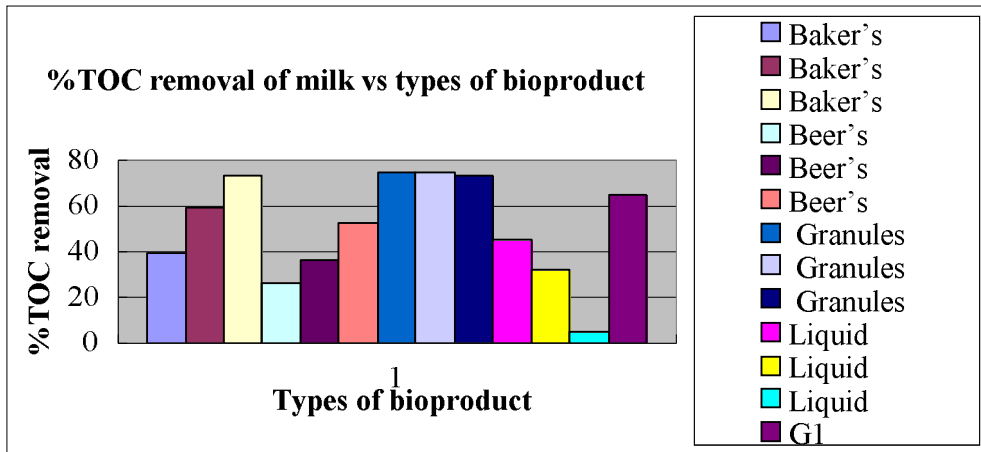


Figure 35 %TOC Removal of Milk wastewater vs. Types of Bioproducts



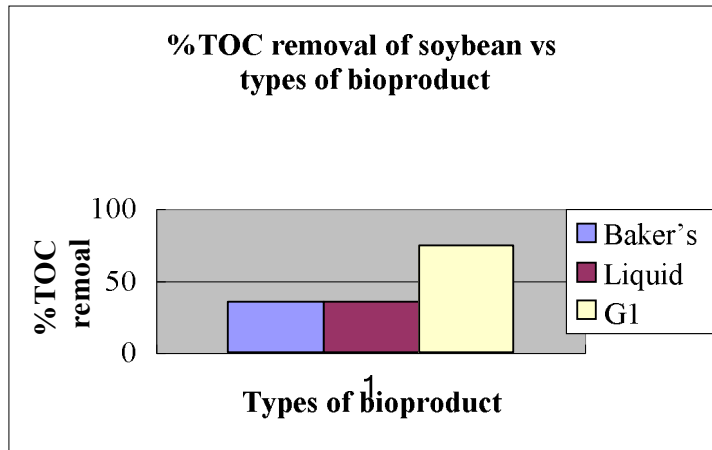


Figure 36 %TOC Removal of Soybean Milk wastewater vs. Types of Bioproducts

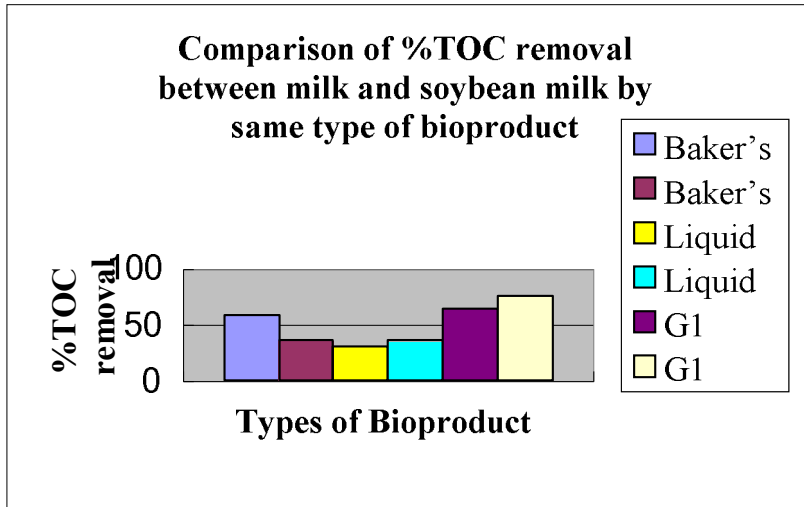


Figure 37 Comparison of %TOC Removal between Milk and Soybean Milk by Same Types of Bioproducts

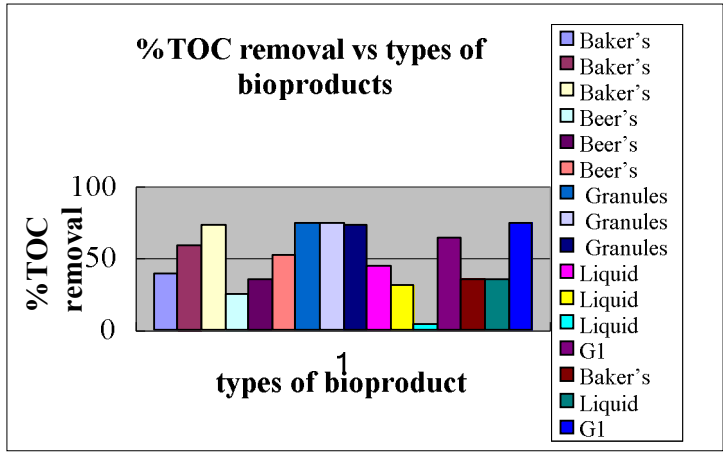


Figure 38 %TOC Removal vs. Types of Bioproducts

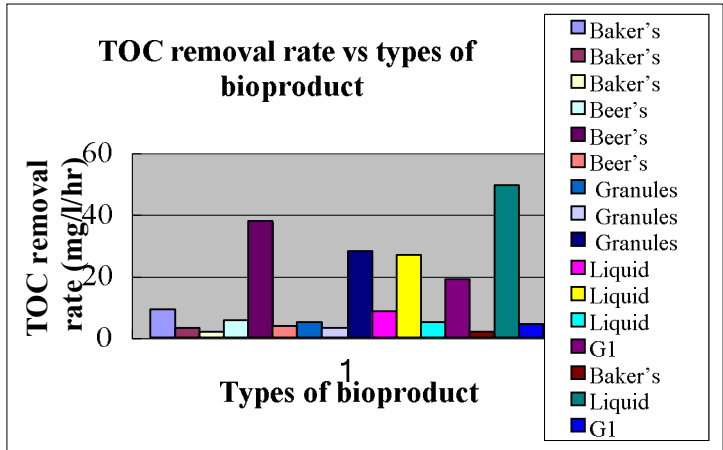


Figure 39 TOC Removal Rate vs. Types of Bioproducts

## VITA

The author, Ms. Tianzhu Bi, was born on September 7, 1983, in Dalian, China. She is the only child of parents Mr. Jianjun Bi and Mrs. Lianfang Han. Her father, Mr. Jianjun Bi, was a wastewater engineer and worked as a manager in the technology department of the Dalian Wastewater Treatment Plant. Her mother retired in 2002 and took care of the family since then. Ms. Bi graduated from the Dalian 36th Advanced High School in June 2003 and attended the Shenyang Jianzhu University, Shenyang, China, in August, 2003. Her under-graduate major was in civil and environmental engineering. Ms. Bi received her B.S degree in July. She came to Cleveland State University (CSU) in August 2008 to continue her graduate study and is expected to receive her MS Civil Engineering degree from CSU in December 2010. Mr. Wei Meng and Ms. Tianzhu Bi were married on December 16, 2009 and Mr. Wei Meng is also expected to receive his Ph.D. in Chemistry from Cleveland State University in December 2010.

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