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SCHOOL OF CHEMICAL AND BIO ENGINEERING

**Determination of Biokinetic Coefficients of
Distillery Wastewater Using Activated Sludge
Process**

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Determination of Biokinetic Coefficients of Distillery Wastewater Using Activated Sludge Process

A Thesis

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the requirements for Degree of Masters of Science
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Declaration

I the under signed, declare that this thesis is my original work and all the sources of materials used for this thesis have been duly acknowledged.

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Acknowledgment

I would like to praise my Lord, for he gave me patience, courage and motivation to undergo this research and complete it.

I am very grateful to Ato. Teshome Werku, my advisor who was very encouraging and helpful. You were understanding and patient with me and have helped me to be able to accomplish this.

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NOMENCLATURE

ANOVA	Analysis of variance
COD	Chemical oxygen demand
BOD	Biological Oxygen demand
BOD ₅	5 day Biological Oxygen demand
MLVSS	Mixed liquor volatile suspended solid
D.O.	Dissolved oxygen
SS	Suspended solid
TS	Total solid
VS	Volatile solid
TN	Total nitrogen
TP	Total phosphorus
S_o	Influent substrate concentration
S_e	Effluent substrate concentration
X or $X_{V,a}$	MLVSS in the reactor
HRT (θ_c)	Hydraulic retention time
Q	Flow rate
V	Reactor volume
k_1, k_{-1}, k_2, k_{-2}	Reaction rate constants
k	Maximum substrate utilization rate
k_s	Half velocity constant / Michaelis –Menten constants
Y	Cell yield coefficient
k_d	Decay coefficient
U	Substrate utilization rate
r_g	Net biomass production rate
μ	Specific biomass growth rate

P	Product after reaction
[E]	Enzyme concentration
[S]	Substrate concentration
[E-S]	Enzyme substrate complex

Abstract

Distilleries are among the existing industry which produces high effluent volume characterized with high temperature, low PH, very high COD and BOD, which is potentially dangerous to the environment if it is discharged without effective treatment. Therefore, this study is conducted to determine kinetic data which is crucial for effective treatment of this high volume distillery effluent using activated sludge process.

In this thesis work samples for the laboratory experiment were taken from Metehara sugar factory, with the following characteristic pH 3.8 – 4.5, COD 92,000 mg/L, BOD 45,410 mg/L, TS 49,950 mg/L, temperature 89 – 98 °C , Nitrogen 1150 mg/L and phosphorous 4100mg/L. In the determination of the kinetic coefficients, Michaelis-Menten relation for continuous process was used. Chemicals which are used in this experiment are ATH to avoid nitrification, KOH to absorb CO₂ and Na (OH) to maintain pH.

During the study, performance of a laboratory-scale biological treatment unit for the distillery waste was evaluated and the kinetic parameters for the activated sludge process were determined. A laboratory-scale treatment unit comprising aerobic digester and final clarifier was used for this purpose. The treatment unit was operated continuously for 270 days by varying the hydraulic retention times from 2 to 9 days. The biological oxygen demand (BOD) of the influent and effluent and the mixed liquor volatile suspended solids of the aeration tank were determined at various detention times to generate data for the kinetic coefficients.

The kinetic coefficients k (maximum substrate utilization rate), K_s (half velocity constant), Y (cell yield coefficient), and K_d (decay coefficient) were found to be 4.46 per day, 534 mg/L, 0.714, and 0.038 per day, respectively, based on BOD and MLVSS in the reactor. These coefficients used for the design of reactor volume and sludge handling facility for activated sludge process.

1. Introduction

Know a day's population growth, industrialization, urbanization, lack of awareness and life standard improvements are becoming the main threats to our environment. Many rivers of the world receive heavy flux due to industrial effluents. The wastewater consisting of substances varying from simple nutrient to highly toxic hazardous chemicals, which when used for irrigation caused both beneficial and damaging effects to various crops including vegetables [1].

Different industries have crucial effect for water body pollution; among those industries distillery industry is the one. Distilleries are the major agro-based industries, which utilizes molasses as raw material for the production of rectified spirit. In addition to rectified spirit, distilleries also produce ethanol, which can be mixed with diesel and used as biofuel and helps in reducing import of crude oil thereby saving foreign exchange [1].

In industrial scale production of fermented liquors had been started at small scale level in different industries like Mollamaru, Balezaf and National Alcohol and Liquor Factory and now more than ten factories are available in the country. Besides, two of the existing sugar factories Metahara and Finchaa were equipped with ethanol plant to utilize their byproduct molasses to produce ethanol [1].

The distillery waste effluent emanates two waste streams named: spent lees and vinasse. The spent lees is moderately polluted stream. Vinasse is a concentrated effluent that is highly acidic and brown in color. The spent lees causes eutrophication for the water bodies and the vinasse makes the soil acidic which is inhabitable for vegetation and other forms of life.

The dark colored nature of molasses in spent lees and vinasse blocks out the sunlight on rivers and streams, interfering with normal processes of photosynthesis. This reduces the oxygenation of the water and leads to the death of the aquatic life. This process is known as eutrophication. In addition the high acidity level of vinasse ranging from 3.8-4.4 is not suitable for soil. So this too needs to be treated before disposal [2].

These wastes can be treated by biological treatment specifically using activated sludge process before disposing of to the environment. The activated sludge is a biological reactor that involves bringing together wastewater and a mixture of microorganisms under aerobic conditions. The process is a combination of natural breakdown of organic matter by biological metabolism and the separation of the solids and liquids by bio-flocculation and the natural force of gravity.

The design of an effective activated sludge reactor requires consideration of the metabolic reaction in the reactor. To understand the behavior of the metabolic reaction the kinetic coefficients of the metabolism have to be determined using experiments.

This research aims to determine the kinetic coefficients for an activated sludge reactor system design to treat molasses distillery waste (both spent lees and vinasse) from Metehara Sugar Factory using laboratory experiments.

1.1 Statement of the Problem

The Federal Environment Protection Authority of Ethiopia has established the National goals to restore and maintain the chemical, physical and biological integrity of the nation's environment. Therefore, in 1997, the Ethiopian EPA established and set its definition of effluent treatment levels to achieve its environmental goals. The major effluent parameters were defined: 5 day Biochemical Oxygen Demand (BOD₅), Total suspended solids (TSS), Temperature and pH. These are limited to 60 mg/L, 50 mg/L, 40°C, 6 – 9 pH units respectively [3].

Know a day's Ethiopia is building several cane based sugar factories. According to Ethiopian Sugar Corporation, they have planned to integrate ethanol distillery for the new and existing sugar factories. The new and existing distilleries will create environmental stress due their highly polluted wastes. These wastes need to be treated before disposal or require an efficient process to recycle some of the wastes. Let's see the practice at Metahara Sugar Factory.

The distillery at Metahara Sugar Factory produces about 350 tons/ day concentrated vinasse, 236tons/day process condensate and 87.4 tons/day spent lees are produced. Sludge handling plant has holding capacity of 245 tons daily. Some of vinasse is mixed with mud from washing of sugar cane before it is crushed for sugar production. This is made into compost and used for fertilizing for their sugar cane plantation of 10 hectare. The spent lees is treated in an aerobic biological treatment plant and recovered for use in the distillery. But the factory claims the aerobic biological treatment in use requires chemicals that are too expensive which is burdening the factory. Surplus vinasse is disposed at a lagoon at the factory which is totally ineffective. This condition contributes to pollution and it is against the country environmental regulation.

The vinasse at Metahara Sugar Factory is highly acidic and normal micro fauna cannot survive in it except a few fungi, which also grow slowly. The vinasse temperature is in the range of 95 – 105 °C in

Atmospheric distillation scheme and 60 – 70 °C in multi – pressure distillation scheme. Prolonged land irrigation using vinasse, if not sufficiently diluted, may cause soil sickness [4].

This acidity of vinasse in land irrigation with prolonged use is believed to bring soil sickness and as a result affect the plantation. The surplus vinasse disposed in the lagoon is brown in color and releases obnoxious smell due to putriciable organics such as Skatole, Indole and other sulfur compounds [4].

If such practice continues the environment will seriously stressed so we need another treatment mechanism for the distillery wastes. This research will provide biological kinetic data, which is the basic to determine the volume of aeration tank (reactor) for the waste treatment. Studying this kinetic data also provides information for sludge handling facility. It also helps as a controlling manual for the wastewater technicians, because it provides what is to be done if the efficiency of the treatment plant downgrades.

1.2 Objectives

General Objective

The general objective of the research is to determine the kinetic coefficients for a biological reactor to treat waste from molasses distilleries waste.

Specific Objectives

The specific objectives are the following:

- To asses and characterize the Metahara Sugar Factory ethanol distillery waste.
- To determine the kinetic coefficients for the biological reactor used to treat waste from molasses distillery such as maximum substrate utilization rate (k), half velocity constant (K_s), cell yield coefficient (Y) and decay coefficients (K_d).

1.3 Significance of the Study

This study will provide kinetic coefficient data for the biological wastewater treatments of new and existing molasses based ethanol producing industries in Ethiopia. The kinetic coefficients will provide data for design of an effective aerobic biological treatment facility using the activated sludge process. The activated sludge method will be expected to remove up to 95% of the biological wastes and is best when existed compared to other treatment options [6].

The biological kinetic constants that will be determined in this research can be directly used in the model equations¹ to obtain the complete biological kinetic model of treatment plant for distillery waste. This model can then be used to design an effective full-scale wastewater treatment plant for distillery effluent.

Moreover, the country is building a number of sugar factories, findings in this paper can be used as an input for future researches for these factories and food industries as well.

¹ The model equations are presented in the literature review of this report.

2. Literature Review

Metahara sugar factory is designed to produce about 42.5 tons/day of rectified spirit alcohol and 40 tons/day of ethanol power alcohol. For this purpose the plant consumes about 206 tons of molasses having brix, 571 tons/day processes water and 400 tons/day soft water. About 731 tons/day fermented wash with 7.62% alcohol content and 20 tons/day sludge will be produced from fermentation section and pumped to distillation section. In the distillation section about 585 tons/day spent wash and 85 tons/day spent lees with pH of 3.3 – 3.8 as an effluent and 42.5 tons/day rectified spirit. The rectified spirit is dehydrated in dehydration section and about 40 tons/day ethanol of 99.99% strength and 2.4 tons spent lees. The voluminous dark brown spent wash is concentrated in multiple effect evaporator and produced 350 tons/day concentrated vinasse and 236 tons/day process condensate [1].

2.1 Environmental Concern on Distillery Wastes

Ethanol manufacturing from molasses generates large volumes of high strength wastewater that is of serious environmental concern. The effluent is characterized by extremely high chemical oxygen demand (COD) (80,000-100,000 mg/L) and biochemical oxygen demand (BOD) (40,000-50,000 mg/L), apart from low pH, strong odor and dark brown color' [6].

Apart from high organic content, distillery wastewater also contains nutrients in the form of nitrogen (1660-4200 mg/L), phosphorus (225-3038 mg/L) and potassium (9600-17,475 mg/L) that can lead to eutrophication of water bodies. Further, its dark color hinders photosynthesis by blocking sunlight and is therefore deleterious to aquatic life [6].

The general characteristics of distillery effluent are given in the table 1. It contains high BOD, COD, potassium content and some salts but the effluent does not contain any toxic heavy metals as it is a waste from plant materials.

2.1.1 Distillery Effluent Treatment

Distilleries are producing higher qualities of effluents which are produced from primary column, rectifier and recovery column, spent wash concentrating falling film evaporators as well as washing of process equipment and leakages of pumps, lines and different equipments. Leftover which is removed from bottom of primary column is called spent wash. The proportion of spent wash is nearly 14 -15 times the total alcohol production. The disposal of large quantities of distillery effluent poses environmental problems, as it contains a considerable amount of organic material [7].

Table 1 General characteristics of distillery effluent [1]

S/N	Characteristics	Continuous process	Batch process
1	Quantity of spent wash	6-9 liters/liter production of alcohol	15 liters/liter production of alcohol
2	Temperature of spent wash	60 – 100 ⁰ c	60 – 100 ⁰ c
3	Oder	Jiggery	Jiggery
4	Color	Dark brown	Dark brown
5	pH	4.3 – 4.6	4.3 – 4.6
6	BOD, mg/L	90000 – 95000	30000 – 40000
7	COD, mg/L	190000 – 200000	85000 – 95000
8	Total solid, mg/L	270000 – 280000	80000 – 90000
9	Potassium(k), mg/L	18000 – 20000	8000 – 10000
10	Chlorides, mg/L	13000 - 15000	5000 – 6000
11	Sulphates, mg/L	15000 – 18000	2000 – 5000
12	Phosphates as PO ₄ , mg/L	1000 – 1500	800 – 1200
13	Calcium (Ca), mg/L	2600 – 2700	500 – 600
14	Nitrogen (KTN), mg/L	200 – 2500	1000 – 1200
15	Sodium (Na), mg/L	300 – 500	200 – 500

The high organic content of ethanol spent wash makes anaerobic treatment attractive in comparison to direct aerobic treatment. Therefore, biomethanation is the primary step is often followed by two stage aerobic treatment before discharge into a water body or on land for irrigation. The post-anaerobic treatment stage effluent still has high organic loading and is dark brown in color, hence it is generally followed by a secondary, aerobic treatment. Solar drying of biomethanated spent wash is one option but the large land area requirement limits this practice. The other treatment option that has been demonstrated for biomethanated distillery effluent is activated sludge process [10].

The most common post-biomethanation step is the activated sludge process wherein research efforts are targeted at improvements in the reactor configuration and performance. For instance, aerobic sequencing batch reactor (SBR) is reported to be a promising solution for the treatment of effluents originating from small wineries. [11]. The treatment system consisted of a primary settling tank, an intermediate retention trough, two storage tanks and an aerobic treatment tank. A start up period of 7 day was given to the aerobic reactor and the system resulted in 93% COD and 97.5% BOD removal [12].

The activated sludge process and its variations utilize mixed cultures. To enhance the efficiency of aerobic systems, several workers have focused on treatment by pure cultures. Further, aerobic treatment has also been examined as a precursor to anaerobic treatment. In studies on both beet spentwash and

molasses, aerobic pre-treatment of beet spentwash with *Penicillium decumbens* resulted in about 74% reduction in phenolics content and 40% reduction in color. [6]. Anaerobic digestion without aerobic pre-treatment resulted in a sharp drop in COD removal efficiencies with decreasing HRT. The organic matter removal was marginally higher for beet molasses previously fermented with *P. decumbens*. The anaerobic reaction followed first-order kinetics and the rate constant decreased on increasing the organic loading with untreated molasses; however, it remained almost constant with pre-treated molasses. *Geotrichum candidum* is another species that resulted in partial elimination of phenolic inhibitors such as gentisic acid, gallic acid, quercetin, p-coumaric acid, etc., thereby enhancing the effectiveness of anaerobic process [14].

2.3 Activated sludge process

2.3.1 History

The activated sludge treatment process was developed in England during the early 1900's. In 1914, H.W. Clarke at the Lawrence Experimental Station, Massachusetts, studied sewage purification through aeration in the presence of microorganisms. Dr. G.S. Fowler (Consulting Chemist, Rivers Committee of Manchester Corporation) during a visit to the United States observed some of the Lawrence experiments and suggested to Edward Arden and William Lockett (Davyhulme Sewage Works, Manchester Corporation) that they carry out similar experiments. Arden and Lockett achieved high purification levels through the use of an aeration process, which incorporated the recovery of flocculent solids and their recycle to the aeration stage. Thus, the activated sludge method of wastewater treatment was born [15].

Many people feel that the activated sludge process cannot be controlled and will not perform reliably. Assuming that the plant is adequately designed, properly maintained and operated, the activated sludge process can and does produce an excellent effluent [15].

2.3.2 Microorganisms

Microorganisms may be classified according to the source of their energy and carbon requirements. Chemolithotrophs oxidize inorganic substances for their energy needs, whereas, chemoorganotrophs oxidize organic substances for their energy. Heterotrophs use organic substances as a carbon source for making cell materials, whereas, autotrophs use carbon dioxide as the source of carbon. Most of the microorganisms in activated sludge are chemoorganotrophic and heterotrophs [16].

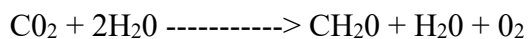
The principal microorganisms involved in the breakdown of organic matter in wastewater are single-celled bacteria. Other microorganisms of importance in biological treatment are: fungi, algae, protozoa, rotifers and nematodes. The predominant species are determined by the characteristics of the influent, the environmental conditions, process design and mode of operation [16].

Bacteria are small (0.5 - 1.0 microns by 1.0 - 5.0 microns), single-celled protista. They grow in many shapes: round, rod, spiral, comma or budding. They are aerobic, anoxic or anaerobic. The majority of the bacteria in activated sludge are facultative, that is, they can live in either aerobic or anoxic conditions. The bulk of the bacteria in activated sludge prefer the pH to be between 6.5 and 9.0. Bacteria adsorb to soluble and particulate wastewater solids and produce enzymes that break down those solids into nutrient forms that can be absorbed into the cell. Floc-forming bacteria produce compact flocs which settle well. Filamentous bacteria grow in either an open or bridging floc structure. It is important for a strong floc to have some filaments growing through it to act as a backbone. Excessive growth of filaments is known as filamentous bulking [16].

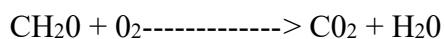
Fungi are multicellular, non-photosynthetic, heterotrophic protista. They are strict aerobes and must have free dissolved oxygen. They predominate at low nitrogen levels and/or low oxygen levels and grow well at pH values under 6.0 [16].

Algae are unicellular or multicellular autotrophic, photosynthetic protista. They are more important in lagoons than in activated sludge treatment plants. However, an understanding of the biochemical reactions for photosynthesis and respiration can be beneficial [16].

Photosynthesis:



Respiration:



Protozoa are motile single cell protists. They are several hundred microns in size, tend to be strict aerobes and are more sensitive to toxic materials. There are many types of protozoa: amoebae, flagellates, free-swimming ciliates, and stalked ciliates, each working in its own niche in the biological scheme [16].

Amoebae are single cells of protoplasm that move slowly in search of food by pushing protoplasm into areas within the cell membrane called pseudopodia, or false feet. The testate amoebae are usually associated with nitrified conditions where little unionized ammonia exists [16].

Flagellated protozoa are very small and propel themselves using a whip-like appendage called flagella. Since they move quickly, their energy requirements are much higher than amoebae or bacteria. Flagellates predominate when bacteria are dispersed and upon recovery from a toxic spill [16].

Free-swimming ciliates are much larger and move around using tiny hair-like structures called cilia. Bulk liquid free-swimmers are found in poorer effluent conditions and in activated sludge systems that yield turbid effluents. Crawlers are found in medium aged activated sludge and are usually associated with better effluent quality. The stalked ciliates are very energy efficient and are found in high numbers in effluents of very good quality. Rotifers are much larger multi-cellular animals which are generally strict aerobes. Nematodes and annelids (bristle worms) are multi-cellular worms. Nematodes occur in higher sludge age systems. Bristle worms (and water bears) occur in nitrifying systems [16].

2.3.3 Fundamentals of Activated Sludge Process

The activated sludge process simply involves bringing together wastewater and a mixture of microorganisms under aerobic conditions. The process is a combination of the natural breakdown of organic matter by biological metabolism and the separation of the solids and liquids by bio-flocculation and the natural force of gravity. This process therefore, serves two purposes: Reducing organic matter in wastewater by using a complex biological community in the presence of oxygen and converting the organic matter to new cell mass, carbon dioxide and energy; and producing solids capable of bio-flocculating and settling out in the clarifier to produce an effluent low in Biochemical Oxygen Demand (BOD) and Total Suspended Solids (TSS) [16].

During the transfer step (see Figure 1), soluble organic matter is absorbed through the cell wall and into the cell where it is converted. Insoluble matter is adsorbed onto the cell wall and broken down and then absorbed through the cell wall [16].

Before cell respiration and synthesis reactions can take place, the organic material (soluble or non-settle able particles) must be taken inside the bacterium. This proceeds in the following manner. First, the external food source comes into contact with the bacterial cell capsular layer (slime layer). The cell capsular layer provides elementary cell protection and serves as a depository for both food and waste materials. Next, the food source reaches the cell wall. The cell wall has been likened to the steel girders

of a building. It provides the cell with its basic shape and as a building's steel framework has openings in it as does the cell wall. These openings allow food to "pass" through the cell's semi-permeable membrane [16].

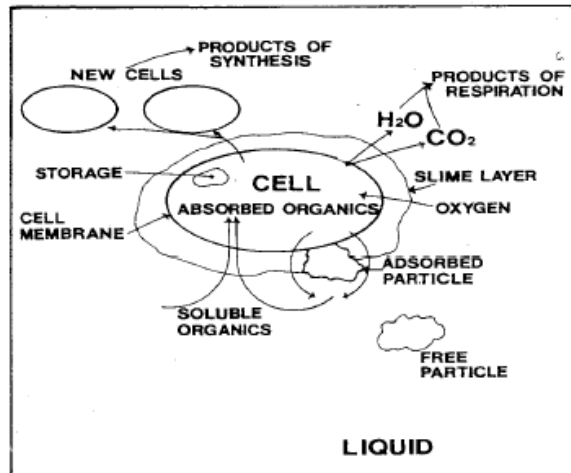


Fig. 1 Biological metabolism [16]

During these process two fundamental things occurs: The food can pass through this membrane to the interior of the cell for utilization without any action by the cell to obtain it (passive transport); or the food can be carried across the semi-permeable membrane (active transport). In this system the cell produces an enzyme (permease) that passes through the membrane and attaches to the food. This allows a food that may otherwise by unable to cross through the semi-permeable membrane to be utilized. The enzyme acts as a catalyst and is not changed in the transfer of food. Once the food is in the interior of the cell the enzyme becomes detached and is able to go back for more food. The permeases produced by cells are specific to certain substrates. Consequently, if the food cannot by utilized by one cell, it passes from cell to cell until one utilizes it or it passes out the effluent. This is why a biological system must be acclimated and why a varied group of microorganisms is required to breakdown a complex mixture of organic matter such as wastewater [16].

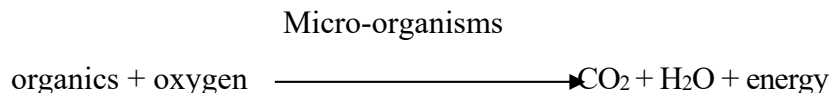
The conversion step is the second step towards the formation of activated sludge. The conversion step includes oxidation and synthesis. These two reactions make up the metabolic process. Metabolism is a life process involving a series of reactions in which some molecules are broken down and others are being formed. Metabolism can be divided into two parts: anabolism, or reactions involving the synthesis of compounds, and catabolism, or reactions involving the breakdown of compounds. Essential protein molecules which catalyze biochemical reactions are called enzymes. Some enzymes are within

the cell (endocellular) and some are secreted to the outside (exocellular). For a cell to grow and reproduce it requires a source of energy and carbon for the synthesis of new cells. If an organism derives its cell carbon from carbon dioxide it is called autotrophic. If it uses organic carbon it is called heterotrophic. Respiration is the process of deriving usable energy from high energy molecules. Bacteria capture and store energy in the chemical bonds of "energetic" compounds such as adenosine triphosphate (ATP). ATP is built up in special structures within the cells called mitochondria [16].

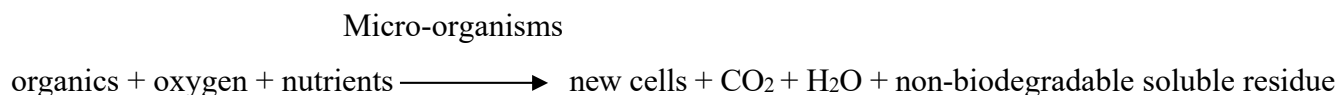
The reactions which take place during respiration are called oxidation-reduction. This involves the transfer of one or more electrons between two atoms. The first step involves the loss of an electron and is called an oxidation reaction while the second step involves the gain of an electron and is called a reduction reaction [16].

The biodegradation of organic matter found in wastewater by microorganisms has been viewed as a three-phase process with a portion of the removed organic matter being oxidized to supply energy and a portion being synthesized to new cells together with a subsequent oxidation of the new cells. These reactions can be illustrated by the following equations:

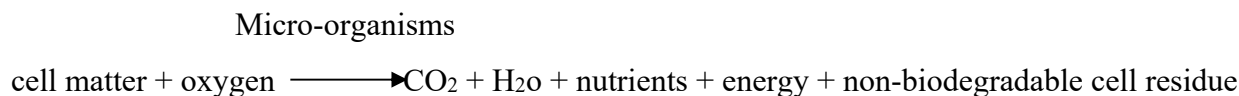
Oxidation:



Synthesis:



Endogenous Respiration:



In order to better understand the activated sludge process, which normally runs in a continuous flow mode, it is beneficial to first look at the process in a batch operation. This is done by taking a container of biologically degradable wastewater and aerating it with an air stone to provide sufficient oxygen and mixing energy. Measuring the number of microorganisms at constant time intervals, and plotting these numbers versus time, we get what is known as the growth curve. The growth curve has five distinct phases [16].

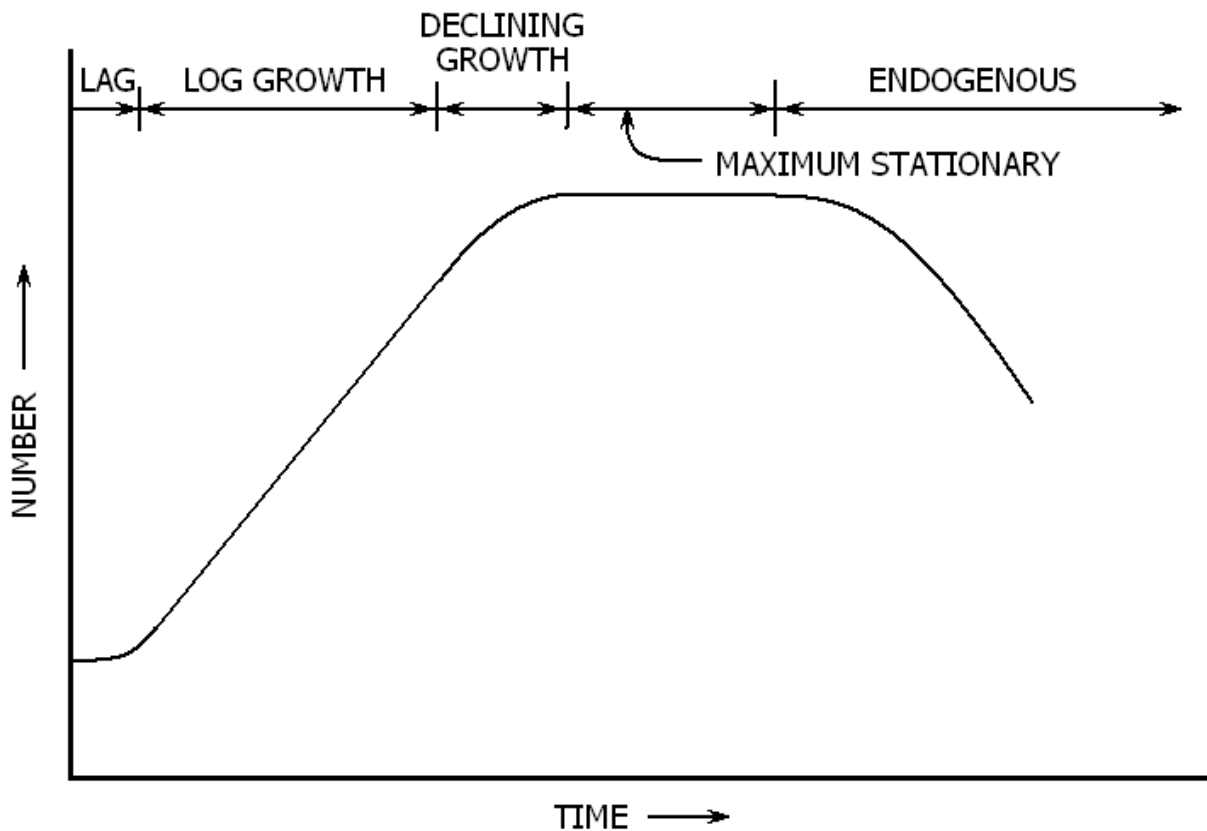


Fig.2. Microorganism's Growth Phase [16]

The five distinct phases are:

1. Adaptation (Lag) Phase - This portion of the curve represents the time required for the organisms to acclimate themselves to the organic material present in the wastewater. The number of bacteria is not increasing; however, a shift in the population of the different species of bacteria in the wastewater is occurring so that the bacteria that can best utilize these organic materials become predominate [16].
2. Log Growth Phase - Once the bacteria have "adapted", only the number of organisms present limit the rate of growth. Because bacterial cells reproduce by binary fission (i.e., cell division - one cell divides and becomes two, these two divide and become four, then eight, sixteen ...), this is known as logarithmic growth. Food is not a limiting factor for growth in this phase, that is, for each cell formed enough food is present to allow it to grow and divide [16].
3. Declining Growth Phase - In this phase food becomes a limiting factor to the growth of the bacterial cell mass because not every bacterium that is formed has the food required to grow [16].

BOD of the wastewater decreases with time as the organic matter is oxidized. A plateau is eventually reached corresponding to the amount of non-biodegradable matter (s_n) [17].

Concentration of MLVSS increases at first (from time 0 to time t_1) during the period when a substantial concentration of substrate (relatively high BOD) is present to provide abundant food to sustain growth of microorganisms. This growth corresponds to the synthesis of new microorganism cells, indicated in Fig. 3 as “synthesis phase.” After time t_1 when substrate concentration is considerably depleted, there is not enough food left to sustain growth of microorganisms. At this time microorganisms start consuming their “fellow microorganisms” as food. As this proceeds, concentration of MLVSS drops when the rate of destruction of microorganism’s cells exceeds that of synthesis of new cells. This corresponds to the “endogenous respiration phase.” The maximum on the MLVSS curve corresponds to time t_1 , when these two rates are exactly equal. Distance ΔX the net reduction of MLVSS concentration corresponds to change from t_1 to t_2 [17].

There are two fundamental differences between continuous or batch reactors, contrary to batch reactors BOD of the waste water in continuous reactor operating at steady state conditions remains constant (s_e). This corresponds to generally low substrate concentration, since the biological reactor is usually designed for removing most of the influent BOD. MLVSS in the continuous reactor operating at steady state condition is kept constant at a selected value ($x_{v,a}$). Maintenance of the MLVSS is obtained by providing the calculated amount of concentrated sludge. The kinetic data obtained is portrayed by the Michaelis-Menten Relationship where the formulation is shown below [17].

Two important corollaries of Michaelis-Menten Relationship are postulated below, the second one being utilized for the design of the continuous biological reactor.

1. At high substrate concentrations, BOD removal follows zero-order kinetics. This means that the rate of removal is essentially constant, independent of substrate concentration. This situation is found in early stages of the batch reactor operation, when substrate concentration is still very high. This corresponds to the section of the BOD curve Fig. 3 from time zero to approximately time t' . In this region, the tangent to the BOD curve, which equals the rate of substrate removal, coincides essentially with the curve itself (constant slope) [17].
2. BOD removal at low substrate concentrations (corresponding to BOD values below 500mg/liter) follows first-order kinetics. This means that the rate of removal is proportional to

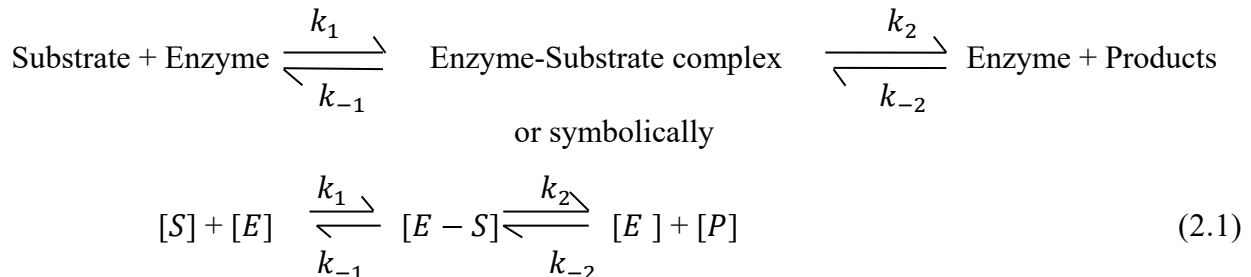
remaining substrate concentration. This corresponds to the section of the BOD curve beyond time t' . Slope of the BOD curve (which equals rate of substrate removal) decreases with time as the BOD value is lowered. A plot of these slopes vs. corresponding BOD values yields a straight line relationship [17].

2.3.5 The Derivation of Michaelis-Menten Relationship

Formulation of the Michaelis-Menten relationship is based on studies of pure cultures. However, it is used in determining kinetics of substrate degradation by a heterogeneous population of microorganisms, which is the case for the activated sludge process [17].

Degradation of wastes by microorganisms is accomplished through a complex series of chemical reactions. These reactions are catalyzed by organic-catalysts (enzymes) present in the microorganisms. Enzymes are large molecular weight compounds. Usually enzymes are quite specific in their functions as catalysts, i.e., a given enzyme ordinarily catalyzes a specific chemical reaction. Bacteria contain a great variety of enzymes, each one being responsible for a minor step in the complex process of biological metabolism [17].

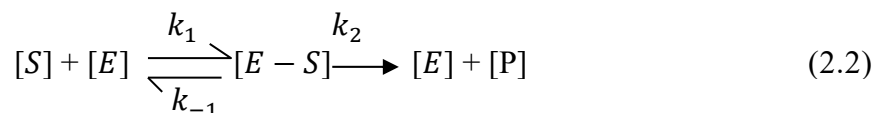
The action of enzymes is represented by the following chemical equation:



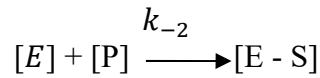
Where k 's stand for the reaction rate constants

As indicated by Eq. (2.1), substrate and enzyme unite to form an enzyme substrate complex. This is followed by the breaking down of this complex, resulting in the end products. The enzyme remains unchanged and is ready to reenter the reaction, acting therefore as a catalyst [17].

The rate of substrate removal is obtained from Eq. (2.1) by making the assumption that the breaking down of the enzyme substrate complex is irreversible. Then Eq. (2.1) is rewritten as



This assumption is essentially correct if measurements are taken shortly after introduction of substrate, which means that very little product has been allowed to form. Under these circumstances, since the rate of the inverse reaction is



and since $[P] \approx 0$, it may be assumed that the breaking down of the enzyme substrate complex is irreversible. Therefore Eq. (2.1) is rewritten as shown [Eq. (2.2)]. The rate of reaction measured under these conditions is that occurring immediately on contact of substrate and microorganism, and is referred to as the initial rate of reaction. To develop kinetic data it is necessary to measure a series of such initial rates, corresponding to different concentrations of substrate shortly after the substrate samples are brought into contact with the microorganism [17].

Substrate removal rate is denoted here by U . For a batch reactor, it corresponds to the slope of the BOD curve in Fig. 3 at any specified time t , corresponding to a concentration (s) of substrate. A specific substrate removal rate per mg/liter of MLVSS is utilized, i.e. [Eq. (2.3)].

$$U = - \left(\frac{1}{x_{v,a}} \right) \left(\frac{ds}{dt} \right) \tag{2.3}$$

(Minus sign is necessary since $\frac{ds}{dt} < 0$ and $U > 0$)

For the continuous reactor, the operating substrate concentrations (s) are considerably below 500 mg/liter (BOD_5), first-order kinetics is assumed in the formulation. Consider the continuous reactor operation under steady state and complete mixing conditions. This situation is illustrated in [fig. 4](#)

Assuming that rate of substrate removal $\frac{ds}{dt}$ follows first-order kinetics,

$$\frac{ds}{dt} = Ks \tag{2.4}$$

It is customary to express substrate removal rate per mg/liter of MLVSS present in the reactor.

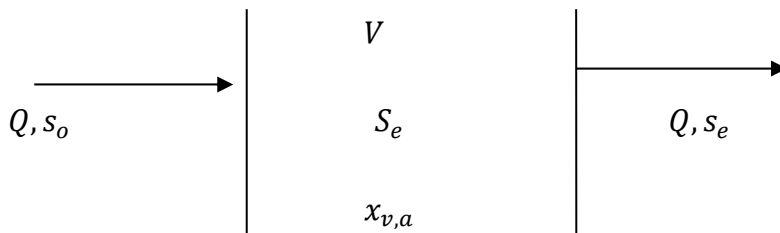


Fig. 4 Simplified diagram for continuous reactor [17]

Let $x_{v,a}$ be the MLVSS concentration. Equation (2.4) is then rewritten as

$$(1/x_{v,a})(ds/dt) = -Ks \quad (2.5)$$

The relationship between K and k is

$$K = kx_{v,a} \quad (2.6)$$

From Eq. (2.5)

$$\frac{ds}{dt} = -kx_{v,a}s \quad (2.7)$$

K is the substrate removal rate constant. When time t equal to residence time in the continuous reactor, concentration s corresponds to s_n , and Eq. (2.7) becomes

$$\frac{ds}{dt}(\text{cont. reactor}) = -Kx_{v,a}s_e \quad (2.8)$$

The following material balance for substrate is written for the reactor in Fig. 4

$$\begin{array}{l} \text{Change of substrate} \\ \text{in reactor} \end{array} = \begin{array}{l} \text{increase due to} \\ \text{influent flow} \end{array} - \begin{array}{l} \text{decrease due to} \\ \text{effluent flow} \end{array} - \begin{array}{l} \text{decrease due to} \\ \text{reaction} \end{array} \quad (2.9)$$

Under steady state conditions:

- Change of substrate in reactor = 0 (2.10)

- Increase due to influent flow = QS_o (2.11)

- Decrease due to effluent flow = QS_e (2.12)

- Decrease due to reaction = $-kx_{v,a}s_e V$ (2.13)

According to Eq. (2.7), the decrease in the amount of substrate due to the reaction is $-Kx_{v,a}s$ [minus sign already included in Eq. (2.9)]. Before substituting in Eq. (2.9) this value is multiplied by the reactor volume (V), since $kx_{v,a}s_e$ and represents per unit volume.

Substitution of the values given by eqs from (2.10) to (2.12) in Eq. (2.13) yields after manipulation

$$\frac{Q}{V} \left(\frac{s_o - s_e}{x_{v,a}} \right) = ks_e \quad (2.14)$$

However,

$$\theta_c = \frac{Q}{V} = \frac{mg}{mg/day} = \text{day} = \text{residence time in the reactor} \quad (2.15)$$

Consequently, Eq. (2.14) is

$$\left(\frac{s_o - s_e}{x_{v,a} \theta_c} \right) = ks_e \quad (2.16)$$

The term $\left(\frac{s_o - s_e}{x_{v,a} \theta_c} \right)$ which also appears in other formulations is the substrate removal rate (U). It corresponds to rate of removal of substrate in the continuous reactor per mg/liter of MLVSS present. Its units are:

$$\left(\frac{s_o - s_e}{x_{v,a} \theta_c} \right) = \frac{\frac{\text{mg}}{\text{liter}} \text{ of BOD removed}}{\left(\frac{\text{mg}}{\text{liter}} \text{ of MLVSS} \right) (\text{day})}$$

Equation (2.16) indicates that the substrate removal rate is proportional to substrate concentration S_e (first-order kinetics). Substrate removal rate constant k (day^{-1}) is determined according to Eq. (2.16) from a plot of $\frac{s_o - s_e}{x_{v,a} \theta_c}$ vs. s_e [17].

Data plotted in fig. 5 yield a straight line passing through the origin, assuming applicability of the mathematical model in Eq. (2.16). The left-hand member $\frac{s_o - s_e}{x_{v,a} \theta_c}$, vanishes as t approaches infinity (infinite residence time). Consequently, term s_e in the right-hand member approaches zero since $k \neq 0$. This corresponds to complete removal of substrate, which is not always the case since some substrates cannot be completely degraded by the aerobic biological process, even at infinite residence time. In these cases, the straight line cuts the abscissa at a value of $s_e > 0$ corresponding to the concentration of non biodegradable matter [17].

When non biodegradable matter is present, Eq.(2.16) is modified to the next equation.

$$\frac{s_o - s_e}{x_{v,a} \theta_c} \quad (2.17)$$

For the continuous reactor, as shown in the above formulation it corresponds to [in finite rather than differential form]

$$\frac{s_o - s_e}{x_{v,a} \theta_c}$$

where θ_c is the residence time in the continuous reactor. The substrate removal rate is equal to the rate of formation of product P, and is given by Eq. (2.18).

$$U = k_2[E - S] \quad (2.18)$$

Similarly, the rate of formation of the enzyme-substrate complex (E-S) is

$$\text{Rate of formation of } (E - S) = k_1[S][E] \quad (2.19)$$

The rate of conversion of enzyme-substrate complex to E and S is [Eq. (2.20)]

$$\text{Rate of conversion of } (E - S) = k_{-1}[E - S] \quad (2.20)$$

Therefore, the net change of concentration of enzyme-substrate complex is

$$\frac{d(E-S)}{dt} = \underset{\text{formation}}{k_1 [E][S]} - \underset{\text{destruction}}{k_{-1}[E - S]} - \underset{\text{destruction}}{k_2[E - S]} \quad (2.21)$$

Let the total concentration of enzyme in the reacting system be denoted as E . This includes not only free enzyme (E) but also enzyme in combined form as enzyme substrate complex ($E-S$), i.e., [Eqs. (2.22) and (2.23)]

$$[E_T] = [E] + [E - S] \quad (2.22)$$

$$\therefore [E] = [E_T] - [E - S] \quad (2.23)$$

Substituting $[E]$ in Eq. (2.21) by its value given in Eq. (2.23) yields

$$\frac{d[E-S]}{dt} = k_1(E_1 - [E - S])[S] - k_{-1}[E - S] - k_2[E - S] \quad (2.24)$$

At steady state conditions it is usually assumed that concentration of intermediate complexes (enzyme-substrate complex in this case) remains unchanged. This assumption is called the steady state approximation. Therefore

$$\frac{d[E-S]}{dt} = 0 \quad (2.25)$$

and Eq. (2.24) becomes Eq. (2.26)

$$k_1(E_1 - [E - S])[S] - k_{-1}[E - S] - k_2[E - S] = 0 \quad (2.26)$$

Solving for $[E - S]$,

$$[E - S] = \frac{[E][S]}{[S] + (k_{-1} + k_2)/k_1} \quad (2.27)$$

Term $(k_{-1} + k_2)/k_1$ is called the Michaelis- Menten constant and is designated as K_s .

$$K_s = (k_{-1} + k_2) / k_1 \quad (2.28)$$

Then, Eq. (2.27) is rewritten as Eq. (2.29).

$$[E - S] = \frac{[E][S]}{[S] + K_s} \quad (2.29)$$

Substituting this value in Eq. (2.18), the following expression for the substrate removal rate U is obtained:

$$U = k_2 \frac{[E][S]}{[S] + K_s} \quad (\text{Michaelis-Menten relationship}) \quad (2.30)$$

i) Corollaries of Michaelis-Menten Relationship

The two corollaries stated in are obtained from Eq. (2.30).

Corollary 1: High substrate concentrations

At high substrate concentrations,

$$[S] \gg K_s \quad (2.31)$$

Neglecting K_s in the denominator of Eq. (2.30) as compared to $[S]$ and simplifying,

$$U = k_2 [E] = k \quad (2.32)$$

Equation (2.32) indicates that at high substrate concentrations, removal of substrate takes place at a maximum rate (k) independent of concentration. It is assumed that at these high substrate concentrations all active sites of the enzymes are saturated with substrate, and so reaction proceeds as fast as possible independent of substrate concentration (zero-order reaction). This corresponds to the section of the BOD curve in Fig. 2.1 from time zero up to time t' , where the tangent to the BOD curve essentially coincides with the curve itself (constant slope) [17].

From Eq. (2.32) Eq. (2.30) is rewritten as

$$U = k \frac{[S]}{[S] + K_s} \quad (2.33)$$

Corollary 2: Low substrate concentrations

At low substrate Concentrations,

$$[S] \ll K_s \quad (2.34)$$

Neglecting $[S]$ in the denominator of Eq. (2.33) as compared to K_s ,

$$U = k[S] / K_s \quad (2.35)$$

Since k and K_s , are both constant for a specific waste, Eq. (2.35) is rewritten as

$$U = K[S] \quad (2.36)$$

where, $K = k/K_s$ (2.37)

Equation (2.36) indicates that at low substrate concentrations, substrate removal follows first-order kinetics. In Fig. 2.1 this corresponds to the section of the curve from a value of the abscissa $S = 0$ up to a value S'' . In this section, the curve is essentially replaced by a straight line passing through the origin (with slope = K). This situation corresponds to that encountered in continuous biological reactors operating at steady state conditions [17].

ii) Significance of Michaelis-Menten constant K_s

From Eq. (2.33) it is shown that K_s is equal to the substrate concentration when substrate removal rate V equals *half* the maximum, i.e., when $U_{MAX}/2$. This is shown by letting $U = U_{MAX}/2$ in Eq. (2.33) and solving for $[S]$. The final result is

$$[S] = K_s \quad (\text{for } U = k/2)$$

This is indicated in Fig. 4. [17]

iii) Determination of k : the Lineweaver Burk Plot

The value of k estimated from Fig. 6 is inaccurate since it is an asymptotic value. A better way of determining k is as follows. Take the inverse of Eq. (2.33),

$$1/U = (K_s/k) (1/[S]) + (1/k) \quad (2.38)$$

Based on Eq. (2.38) a linear plot of $1/U$ vs. $1/[S]$ is constructed, from which the characteristic constants k and K_s are determined from the slope and intercept of the straight line. This graph, which is shown in Fig. 7, is known as the Line weaver -Burk plot, as indicated the intercept at the abscissa corresponds to $(-1 /K_s)$, since for $1/U = 0$ one obtains $1/[S] = -1 /K_s$, from Eq. (2.38) [17].

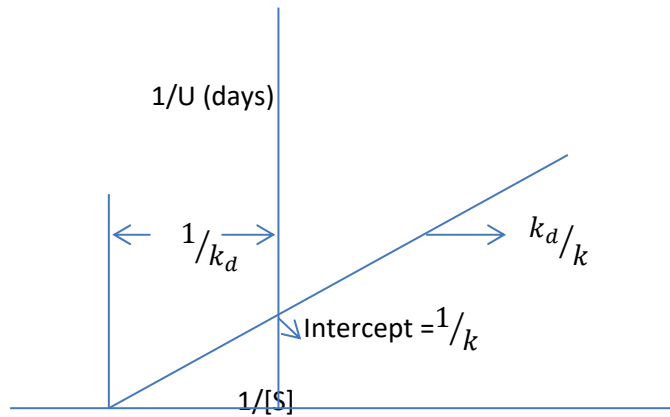


Fig. 5. Lineweaver –Burk Plot [17]

iv) Michaelis-Menten Relationship when Non-biodegradable Matter is Present in the System

If the concentration of non biodegradable matter is indicated as $[S_n]$, it is accounted for by substituting the value of $[S]$ by $([S] - [S_n])$ in Eq. (2.33). A similar substitution in the above formulation for continuous reactor led to Eq. (2.17) from Eq. (2.16) [17].

Therefore, modified Eq. (2) is

$$U = k \frac{[S] - [S_n]}{K_s + [S] - [S_n]} \quad (2.39)$$

The two corollaries shown above which are derived from Eq. (2.30) and also obtained from Eq. (2.38). Similarly, Fig. 5 is repeated when non-biodegradable matter is present (Fig. 5).

From Eq. (2.39), it is shown that

$$K_s = [S] - [S_n]$$

When $U = k/2$

The Lineweaver-Burk equation when non biodegradable matter is present is written by replacing [S] in Eq. (2.38) by ([S] - [S_n]). The corresponding Lineweaver-Burk plot follows directly from the equation thus obtained [17].

v) Michaelis-Menten Relationship in Terms of Specific Growth Rate of Sludge

The Michaelis-Menten relationship [Eq. (2.3)] is written as a function of the specific substrate removal rate. An equivalent form is written as a function of the specific growth rate of sludge, defined as:

$$\mu = (1/x_{v,a})(dx/dt) \quad (2.40)$$

When the substrate is being used at its maximum, the bacteria are also growing at their maximum rate. The maximum specific growth rate is those related the maximum specific substrate utilization rate as follow,

$$\mu_{\max} = kY \quad (2.50)$$

And

$$k = \frac{\mu_{\max}}{Y} \quad (2.51)$$

Where, μ_{\max} = maximum specific bacteria growth rate, g new cell/g cell. d

k = maximum specific substrate utilization rate, g/g.d

$$Y = \frac{\text{g of biomass produced}}{\text{g of substrate utilized (consumed)}}, \text{ true yield coefficient g/g}$$

$$\text{From } U = k \frac{[S]}{[S] + K_s} \text{ then } U = \frac{\mu_{\max}[S]}{Y[[S] + K_s]}.$$

The biomass rate is proportional to substrate utilization rate by the synthesis yield coefficient, and biomass decay is proportional to biomass present. Thus, the following relationship with the rate of growth and the rate of substrate utilization is applicable in both batch and continuous culture system.

$$r_g = -YU - k_d X \quad (2.52)$$

$$r_g = -Y \frac{kXS}{k_s+S} - k_d X \quad (2.53)$$

Where, r_g = net biomass production rate, g VSS/m³ .d

k_d = endogenous coefficient, g VSS/ g VSS. d

If we divide Eq.(2.53) by X

$$\frac{r_g}{X} = -Y \frac{kS}{k_s+S} - k_d, \text{ where } \mu = \frac{r_g}{X} \quad (2.54)$$

Where μ is specific biomass growth rate, gVSS/ g VSS. d

3. Materials and Methods

In this chapter materials and methods approached to generate the necessary data will be discussed in detail.

3.1 Experimental Framework

This research is conducted to develop kinetic relation for distillery waste for effective design of biological treatment using activated sludge process. The studies of all the experiments were planned according to this framework.

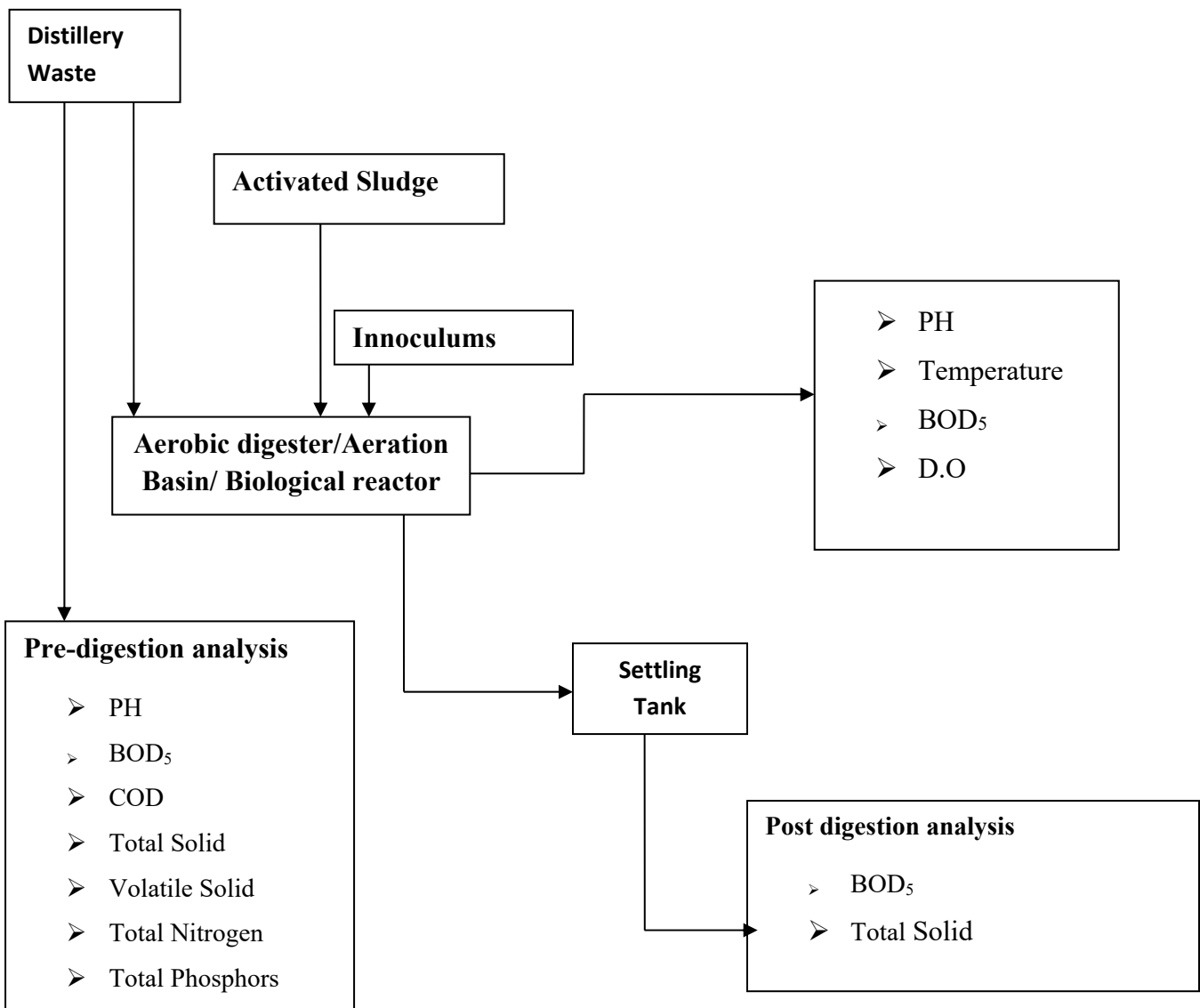


Fig. 6 Experimental Framework of the study

3.2 Materials

During conducting the experiment laboratory-scale Aerobic Digester (W11, Armfield), two 40 liters plastic jar for collecting samples from Metehara sugar factory ethanol plant stillage, Glass bottle, filter papers (whattman, 110mm), crucibles, Digital Balance (sartorius cp 3245), 78-1 Magnetic Stirrer Hot pelt, BOD incubator (WTW TS 606/4-i), Oven (Mammart, Germany), pH Meter (JENWAY 3505), Desiccator, Digital D.O meter, COD reactor (HANNA hi 839800), Vacuum pump (KNF LABPORT), Beaker, Graduating cylinders and Micropipette, was used to determine BOD, COD, SS, and kinetic coefficients based on standard methods [18].

Chemicals which are used in this experiment are:

- ATH to avoid nitrification
- KOH to absorb CO₂
- Na (OH) to maintain pH.

3.3 Methods

i. Sample collection and preparation

An ethanol distillery wastewater sample was collected from Metahara ethanol plant. The factory is located in the eastern part of Ethiopia around 250 km from Addis.

The Stillage Sample is discharging at 99 ° c which is collected and stored below 4°C in order to control biological activities until digestion. The sample was collected in the rainy season to consider the effect.

Before the start of the work, the reactor was seeded with 0.5 liters sludge taken from local toilet sewage for three days. The influent was subjected to settling in the effluent bottle. Due to lack of mechanical return sludge facility, the settled sludge was daily removed from the final clarifier in a beaker. The nitrogen (N) and phosphorus (P) requirements are based on the BOD of the wastewater, where a BOD: N: P of 100: 5: 1 is considered adequate.

ii. Study Variables

- Determining kinetic coefficients of the distillery waste for different hydraulic retention time.
- Characterization of the waste before and after digestion: COD, BOD₅, D.O, Temperature, pH, TS, VS, TN, TP.

iii. Experimental Design

The experiments were designed to determine the effect of hydraulic retention time which is taken as experimental factor by blocking the effect of other factors. Seven levels of this factor are considered (i.e. 2, 3, 4, 5, 7, 9 and 12 days). Each of the above levels has five replicate, having a hypothesis of the response variables, influent BOD₅, effluent BOD₅ and mixed liquor suspended solid (MLVSS). The single factor (with blocking) ANOVA was performed using Design expert® (V.6.0.8) trial version. The number of run needed to undergo this experiment is 35.

iv. Sample Analysis

Physical, chemical and biological characteristics (pH, MLVSS, BOD₅, COD, P and N) of distillery waste before and after digestion were analyzed at different institution. All analytical determinations were performed according to “Standard Methods” [18].

The parameters such as MLVSS, COD, BOD₅, Temperature, DO and pH were analyzed at Environmental laboratory of Chemical and Bio Engineering Department. The analysis for Phosphorus and Nitrogen were done at laboratory of Leather Institute of Ethiopia.

v. Statistical Analysis

After the determination of the amount BOD₅ before and after digestion, total and volatile solids in the aeration tank multiple comparisons with EXCEL are performed.

vi. Experimental Setup

The wastewater to the reactor is fed using a 5 liter influent plastic bottle. A completely mixed continuous flow reactor without recycle is used in this study. A peristaltic pump is used to regulate the flow to achieve a particular hydraulic retention time. The capacity of the aeration tank is 5 liter. A final clarifier followed the aeration tank with 3 liter capacity.

The laboratory-scale reactor was operated for about 270 days; by varying the hydraulic retention time of 2-12 days, the corresponding flow rates are in Table 2.

Table 2 Detention times and flow rate of influent

Hydraulic retention times (days)	Flow rate (1/day)
2	12.7
3	8.5
4	6.4
9	2.8
5	5.1
7	3.6
12	2.1

During the experiment flow, temperature, and pH values of the reactor were measured daily to ensure favorable environmental conditions in the reactor for biological treatment. Mixed liquor volatile suspended solids (MLVSS) in the reactor, BOD₅ of influent and effluent were measured thrice a week to determine kinetic coefficients. All the tests will be performed according to the procedures laid down in the "Standard Methods.

Table 3 Sampling schedule

Analysis	Frequency	Raw waste ^a	Mixed liquor ^b	Effluent ^c
BOD ₅ (mg/liter)	3/week	X	-	X
pH	Daily	X	X	X
Temperature(°C)	Daily	-	X	-
D.O(mg/liter)	Daily	-	X	-
MLVSS(mg/liter)	3/week	-	X	x

a - Sample withdrawn from raw waste container

b - Sample withdrawn from the reactor/ aeration tank

c - Sample withdrawn from the effluent bottle

3.4 Experimental Work and Procedures

i. Total Solids (TS)

Total solids are a measure of the suspended and dissolved solids in water. Matter suspended or dissolved in water or wastewater is considered as solids. A high amount of solids in water generally makes it not desirable for consumption. Solid analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with regulatory agency wastewater effluent limitations.

ii. Volatile Solids (VS)

Volatile solids are those solids lost on ignition (heating to 550°C). They are useful to approximate the

amount of organic matter present in the solid fraction of wastewater, activated sludge and industrial wastes.

iii. Measurement Procedure

A clean evaporating dish was dried to 105°C for 1 hour, cooled in desiccators and weighed immediately before use. A measured volume of well mixed sample was pipetted into a pre-dried and weighed evaporating dish. Then the dish was dried on drying oven at 105°C for 24 hours. Finally the dish was cooled in desiccators for balancing temperature and weighed immediately for total solid determination. The residue was ignited in a muffle furnace for 3 hours by method for volatile solid determination. The dish was transferred to desiccators for final cooling in a dry atmosphere and weighed immediately.

iv. Calculation

The amounts of TS and VS in the sample can be computed using the following equation respectively.

$$\text{mg total solids/L} = \frac{A-B}{\text{Sample volume, ml}} * 100$$

Where, A = weight of dried residue + dish, mg and

B = weight of dish, mg and,

$$\text{mg volatile solids/L} = \frac{x-y}{\text{Sample volume, ml}} * 100$$

Where, x = weight of dried residue dish + before ignition, mg

y = weight of residue + dish after ignition, mg

iv. Biological Oxygen Demand (BOD₅) Determination

Biochemical oxygen demand (BOD) is an analytical measure of how much oxygen is consumed in biological processes that degrade organic matter in wastewater by bacteria. It is therefore a measure of the concentration of organic matter in a waste that can be oxidized by bacteria ('bio- oxidized' or 'biodegraded'). Or BOD is the number of milligrams of O₂ required to carry out the oxidation of organic carbon in 1L of water. BOD is usually expressed on a 5 day, 20°C basis i.e. as the amount of oxygen consumed during oxidation of the wastewater for 5 days at 20°C.

BOD is widely used as an indicator of treatment efficiency, to determine the amount of organic pollution in surface water and also to determine the strength of wastewater by measuring how much dissolved oxygen is used by microorganisms during biochemical oxidation of any organic matter. High BOD₅ in effluent discharged to surface water can result in the depletion of dissolved oxygen in the aquatic environment, which can lead to a die-off of aquatic organisms and anaerobic conditions.

vi. Method

Lovibond BOD₅ system oxidirect was used to analyze the BOD₅ of the samples and their different mix ratios before and after digestion processes.

vii. Measurement Procedure

Initially sample preparation, estimation of measurement reagent and selection of volume for the sample were carried out. The sample volume was related to the expected BOD value. The oxidirect was designed to operate with ranges and samples volume i.e. up to 0-400 mg/L without any dilution; for 0-2000 mg/L expected value 56 ml sample volume with 3 drop ATH to avoid nitrification and 3-4 drop KOH; for 0-4000 mg/L expected value 21.2 ml sample volume with 1 drop ATH to avoid nitrification and 3-4 drop KOH; for greater than 4000 mg/L expected value 157 ml sample volume with 5 drop ATH to avoid nitrification and 3-4 drop KOH.

The necessary pre- treatment of the sample (setting pH value 6.5 to 7.5, filtering) was carried out. The optimum pH value for BOD₅ determination is between 6.5 and 7.5, but in the case of higher or lower, it was adjusted by HCl or H₂SO₄ and NaOH. Then the sample was measured precisely using appropriate over flow and poured into BOD bottle, magnetic stirring rod was also inserted. Five drop of nitrification inhibitor (ATH) was added into the sample bottle to inhibit nitrification. Four drop of KOH solution was also added into the seal gasket and the gasket was inserted in the neck of the bottle. Then the BOD sensors was screwed to the sample bottle and placed in the bottle rack. Finally measurement was started by incubate the sample in accordance with the instructions BOD₅ for 5 days at 20 °C.

a. Temperature

The temperature of the reactor remained in a range of 16-24 °C. All the processes of growth are dependent on chemical reactions, and the temperature influences the rate of these chemical reactions. Thus the rate of microbial growth as well as total amount of growth can be affected by temperature. The temperature will be assessed daily to create favorable environment for bacterial growth.

b. pH

The pH of the reactor during the study remained between 7.0 and 8.0 for most of the research period. Extremes of pH are fatal for most bacteria. The bacteria grow best when the pH is slightly on the acidic side. The optimum range for bacterial growth generally lies between 6.5 and 7.5 [13]. Activated sludge and aerated lagoons could be successfully operated when the pH was between 9 and 10.5 [11]. The pH is analyzed using Jenny pH meter in the laboratory and maintained using sodium hydroxide (Na (OH)).

c. Dissolved Oxygen

D.O. of the reactor remained between 3 mg/L and 4.2 mg/L for most of the study period. This value was ideal for the biological treatment systems working under aerobic conditions. The values of D.O. for the present study were above the minimum level of 2 mg/L which has been widely reported in the literature [20]. The level will be checked using digital D.O. meter from Adama Science and Technology University.

d. Kinetic Coefficients

Kinetic coefficients are usually determined through bench scale studies. For this purpose the bench scale completely mixed continuous flow reactor is going to be operated for several hydraulic retention times, i.e., 2, 3, 4, 5, 7, 9 and 12 days. At each hydraulic retention time the data will be collected at steady state conditions and mean values will be determined for S_0 (initial substrate concentration expressed as BOD), S_e (substrate concentration), and X (biomass concentration). For each detention time three readings of each S_0 , S_e , and X is going to be taken. The mean of these values is to be used for the calculation of the kinetic coefficients.

The kinetic coefficients are of great importance for appropriate design of the bioreactors which are used for the treatment of wastewater. The basic two equations which flows Michaelis-Menten model are used to mathematically describe the fundamental kinetics of the treatment that takes place as a result of microorganisms in biological treatment [20]:

$$\frac{dS}{dt} = \frac{kSX}{(K_s+S)} \quad (3.1)$$

$$\frac{dX}{dt} = Y \frac{dS}{dt} - K_d \quad (3.2)$$

Where X is mass of microorganisms, S is mass of organic matter used as food by the microorganisms (normally expressed as BOD), and Y is cell yield coefficient, the ratio of the mass of cells formed to the mass of substrate consumed. K_d represents the proportion of the total mass of microorganisms that self degrades (endogenous respiration) per unit time, k is maximum rate of substrate utilization per unit mass of microorganisms, and K_s is half velocity constant, substrate concentration at one half of the maximum growth rate, mass per unit volume.

Determination of k and K_s

The following linearized equation as described by Metcalf & Eddy [20] is used to find k and K_s :

$$\frac{X\theta_c}{S_o-S} = \frac{K_s}{k} \frac{1}{S} + \frac{1}{k} \quad (3.3)$$

Where θ_c is mean cell residence time and S_o is the initial substrate concentration expressed as BOD. By using the above equation, a graph is plotted with $\frac{1}{S}$ on the x-axis and $\frac{X\theta_c}{S_o-S}$ along the y-axis (Fig. 13). A linear regression line is fitted to the plotted data. The intercept on the y-axis and the slope of this line is used to find k and K_s .

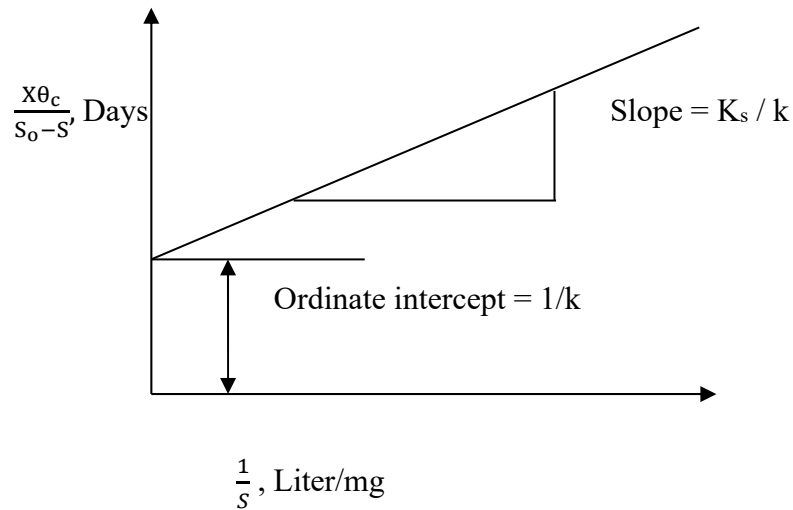


Fig. 7 Graphs of k_s and k basis on BOD₅

Determination of Y and K_d

The following linearized equation was used to find Y and K_d [17, 20].

$$\frac{1}{\theta_c} = \frac{S_o-S}{X\theta_c} Y - K_d \quad (3.4)$$

A graph is going to be plotted $\frac{1}{\theta_c}$ with *along* the y-axis and $\frac{S_o-S}{X\theta_c}$ along the x-axis. A linear regression line is fitted to the plotted data. The intercept on the y-axis and the slope of the line is going to be used to find K_d and Y .

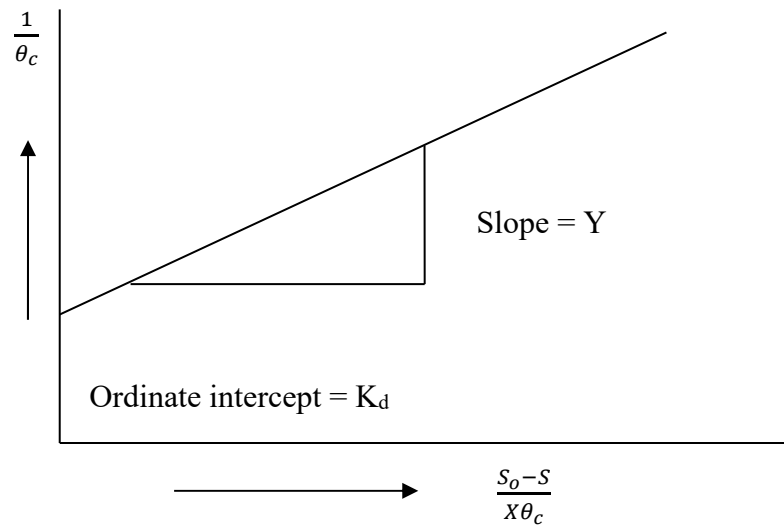


Fig. 8 Graphs of Y and K_d basis on BOD₅

4. Result and Discussion

4.1 Experimental Design

The resulting data, Table 4, were analyzed using Design expert® (V.6.0.8) software to determine the model adequacy of the experiment, where hydraulic retention time is the independent variable. The dependent variables used as response parameters were the Influent BOD (So), Effluent BOD (Se) and mixed liquor suspended solid (X). All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

Table 4 Experimental result using design expert (General factorial)

Std	Run	Block	Factor 1 A: Time (days)	Response 1 So (mg/L)	Response 2 Se (mg/L)	Response 3 X (mg/L)
26	1	Block 1	9	1200.7	27.2	644.1
16	2	Block 1	5	1259.76	39.4	712.6
1	3	Block 1	2	1235.34	102.3	720.9
31	4	Block 1	12	1309.1	21.9	523.6
6	5	Block 1	3	1360.6	82.3	750.2
21	6	Block 1	7	1278.7	32.4	631.3
11	7	Block 1	4	1470.7	53.2	856.2
17	8	Block 2	5	1297.89	45.1	757.4
12	9	Block 2	4	1398.45	43.9	827.3
22	10	Block 2	7	1160.9	35.3	659.4
2	11	Block 2	2	1200.5	84.3	750.5
27	12	Block 2	9	1150.8	20.9	656.01
32	13	Block 2	12	1136.7	23.2	559.2
7	14	Block 2	3	1298.7	59.4	815.6
3	15	Block 3	2	1250.09	87.3	779.2
18	16	Block 3	5	1176.87	30.4	791.2
13	17	Block 3	4	1299.09	38.5	835.2
23	18	Block 3	7	1067.6	26.1	678.1
33	19	Block 3	12	1095.2	19.6	565.3
8	20	Block 3	3	1265.7	71.4	830.1
28	21	Block 3	9	1020.5	26.1	638.2
24	22	Block 4	7	1264.9	27.2	663.1
14	23	Block 4	4	1267.09	49.3	830.5
19	24	Block 4	5	1298.9	33.6	735.2
29	25	Block 4	9	997.95	23.6	631.7
4	26	Block 4	2	1290.56	97.2	730.1
34	27	Block 4	12	1256.8	16.5	560.4
9	28	Block 4	3	1305.4	63.2	790.4
10	29	Block 5	3	1299.09	75.4	788
5	30	Block 5	2	1270.21	94.2	791.2

35	31	Block 5	12	1197.9	17.5	563.4
20	32	Block 5	5	1160.5	37.7	783.6
15	33	Block 5	4	1367.9	51.6	846.3
25	34	Block 5	7	1254.7	25.9	690.4
30	35	Block 5	9	1267.5	24.3	645.01

4.2 Statistical Analysis

Data were modeled by multiple regression analysis and the statistical significance of the terms was examined by analysis of variance for each responses. The statistical analysis of the data and three dimensional plotting were performed using Design Expert® Version 6.0.8. Software. The adequacy of regression model was checked by R^2 , Adj R^2 , Pred R^2 , Adeq Precision and F-test [18]. The significance of F-value was judged at 95% confidence level. The regression coefficients were then used to make statistical calculation to generate three-dimensional plots from the regression model. The degree of relationship between the variables was also checked by using correlation matrix of the factors and response variables.

4.2.1 Analysis of Variance

i. Analysis of Variance for Response 1 – Influent BOD (So)

A cubic polynomial regression model was assumed for predicting response. In order to determine whether or not the cubic model is significant, it was necessary to conduct analysis of variance (ANOVA), Table 5. The probability (*P-values*) values were used as a tool to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The smaller the *P-values* are, the bigger the significance of the corresponding coefficient.

Table 5 ANOVA for Influent BOD (So)

Source	Sum of Squares	dF	Mean Square	F value	p-value Prob >F
Block	66311.8	4	16577.95		
Model	1.47E+05	3	48941.38	10.27	0.0001*
A	1.12E+05	1	1.12E+05	23.56	< 0.0001
A ²	4438.99	1	4438.99	0.93	0.343
A ³	64405.26	1	64405.26	13.52	0.001
Residual	1.29E+05	27	4764.42		
Cor. Total	3.42E+05	34			
*Significant					

F- Value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to one and it is less likely that any of the factors have a significant effect on the response. It is calculated by Model Mean Square divided by Residual Mean Square. Here

the model F-value of 10.27 implies the model is significant. F-value of 8.53 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.

Probability values or "Prob > F" less than 0.0500 indicate model terms are significant. In this case A (residence time) and A³ (residence time)³ are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Coefficient of Variation (C.V.), the standard deviation expressed as a percentage of the mean; Predicted Residual Error Sum of Squares (PRESS), which is a measure of how the model fits each point in the design; the R-Squared, measure of the amount of variation around the mean explained by the model; Adj R-Squared that is a measure of the amount of variation around the mean explained by the model, Pred R-Squared, a measure of the amount of variation in new data explained by the model, and Adequate Precision, this is a signal to disturbance ratio due to random error, presented in the Table 6, below, which is used to decide whether the model can be used or not.

Table 6 Model adequacy measures for Influent BOD (So)

Std. Dev.	69.02	R-Squared	0.533
Mean	1240.95	Adj R-Squared	0.4811
C.V.	5.56	Pred R-Squared	0.2071
PRESS	2.18E+05	Adeq Precision	9.844

The "Pred R-Squared" of 0.2071 is not as close to the "Adj R-Squared" of 0.4811 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio of 9.844 indicates an adequate signal. Therefore this model can be used to navigate the design space.

The regression coefficients and the corresponding 95% CI (Confidence Interval) High and Low were presented in Table 7. 95% CI High and Low columns represent the range that the true coefficient should be found in 95% of the time. If this range spans 0 (one limit is positive and the other negative) then the coefficient of 0 could be true, indicating the factor has no effect.

From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of A (residence time), and the interaction terms of A³ had significant effect on the influent BOD (So).

Table 7 Regression coefficients and the corresponding 95% CI High and Low for Influent BOD (So)

Factor	Coefficient Estimate	95% CI Low	95% CI High
Intercept	1200.37	1160.95	1239.79
Block 1	61.18		
Block 2	-6.1		
Block 3	-73.09		
Block 4	-0.72		
Block 5	18.73		
A-time	-245.28	-348.96	-141.6
A ²	29.59	-33.31	92.48
A ³	216.4	95.63	337.16

ii. Analysis of Variance for Response 2 – Effluent BOD (Se)

A cubic polynomial regression model is suggested by the design program for this response. All statistical analysis including ANOVA test, post ANOVA statistics, normal plot of residuals etc. are done for the Effluent BOD (Se) data Table 8. All the tests indicated that the model is statistically acceptable.

Table 8 ANOVA for Effluent BOD (Se)

Source	Sum of squares	Df	Mean square	F value	p-value Prob>F
Block	300.78	4	75.19		
Model	21486.59	3	7162.2	257.07	< 0.0001*
A	216.52	1	216.52	7.77	0.0096
A ²	4414.04	1	4414.04	158.43	< 0.0001
A ³	947.51	1	947.51	34.01	< 0.0001
Residual	752.26	27	27.86		
Cor. Total	22539.63	34			
*Significant					

The ANOVA test indicates that the Model has F-value of 257.07 which implies that the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicating that model terms A (residence time), A² (residence time)² and A (residence time)³ are significant.

Table 9 Model adequacy measures for Effluent BOD (Se)

Std. Dev.	5.28	R-Squared	0.9662
Mean	45.93	Adj R-Squared	0.9624
C.V.	11.49	Pred R-Squared	0.9449
PRESS	1226.33	Adeq Precision	32.696

From Table 9 the "Pred R-Squared" of 0.9449 is in reasonable agreement with the "Adj R-Squared" of 0.9624. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio of 32.696 indicates an adequate signal. This model can be used to navigate the design space.

The regression coefficients and the corresponding 95 % CI High and Low were presented in Table 10 95 % CI High and Low columns represent the range that the true coefficient should be found in 95 % of the time. If this range spans 0 (one limit is positive and the other negative) then the coefficient of 0 could be true, indicating the factor has no effect.

Table 10 Regression coefficient and the corresponding 95% CI High and Low for Effluent BOD (Se)

Factor	Coefficient estimate	95% CI Low	95% CI High
Intercept	27.05	24.03	30.06
Block 1	5.32		
Block 2	-1.34		
Block 3	-3.15		
Block 4	-1.55		
Block 5	0.73		
A-time	-10.77	-18.7	-2.84
A ²	29.5	24.69	34.31
A ³	-26.25	-35.48	-17.01

From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of A (residence time) and the interaction terms of A² and A³ had significant effect on the Effluent BOD (Se).

iii. Analysis of Variance for Response 3 – Mixed liquor suspended solid (X)

A cubic polynomial regression model is suggested by the design program for this response. All statistical analysis including ANOVA test, post ANOVA statistics, normal plot of residuals etc. are done for the Mixed liquor suspended solid (X) data Table 11 All the tests indicated that the model is statistically acceptable.

Table 11 ANOVA for Mixed liquor suspended solid (X)

Source	Sum of square	df	Mean square	F value	p-value Prob>F
Block	7892.21	4	1973.05		
Model	2.69E+05	3	89704.49	76.72	< 0.0001*
A	93395.06	1	93395.06	79.88	< 0.0001
A ²	7114.54	1	7114.54	6.09	0.0203
A ³	19944.6	1	19944.6	17.06	0.0003
Residual	31567.9	27	1169.18		
Cor. Total	3.09E+05	34			
*significant					

The ANOVA test indicates that the Model has F-value of 76.72 which implies that the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms A (residence time), A (residence time)² and A (residence time)³ are significant.

Table 12 Model adequacy measures for mixed liquor suspended solid (X)

Std. Dev.	34.19	R-Squared	0.895
Mean	715.17	Adj R-Squared	0.8833
C.V.	4.78	Pred R-Squared	0.8314
PRESS	50707.32	Adeq Precision	16.838

From Table 12 the "Pred R-Squared" of 0.8314 is in reasonable agreement with the "Adj R-Squared" of 0.8833. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Here ratio of 16.838 indicates an adequate signal. This model can be used to navigate the design space.

The regression coefficients and the corresponding 95% CI High and Low were presented in Table 13. 95% CI High and Low columns represent the range that the true coefficient should be found in 95 % of the time. If this range spans 0 (one limit is positive and the other negative) then the coefficient of 0 could be true, indicating the factor has no effect.

Table 13 Regression coefficient and the corresponding 95% CI High and Low for Mixed liquor suspended solid (X)

Factor	Coefficient estimate	95% CI Low	95% CI High
Intercept	700.71	681.19	720.24
Block 1	-23.9		
Block 2	2.75		
Block 3	15.87		

Block 4	-9.25		
Block 5	14.53		
A-time	-223.72	-275.08	-172.36
A ²	-37.46	-68.61	-6.3
A ³	120.42	60.6	180.25

From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of A (residence time) and the interaction terms of A² and A³ had significant effect on the Mixed liquor suspended solid (X).

iv. Diagnostic Test

All diagnostic plots are also tested for each response for adequacy of the models (normal plot of residuals, residuals vs. predicted value, residuals vs. factor, box-cox plot, studentized residuals, leverage, etc.).

a. Diagnostic Test for Response 1 - Influent BOD (So)

The actual versus predicted values using model equation, Eq. (4.1) are tabulated in Table 14

Table 14 Actual vs. model predicted Influent BOD (So)

Standard order	Actual value	Predicted value	Residual	Student residual	Run order
1	1235.34	1320.01	-84.67	-1.447	3
2	1200.5	1252.73	-52.23	-0.892	11
3	1250.09	1185.75	64.34	1.099	15
4	1290.56	1258.11	32.45	0.554	26
5	1270.21	1277.57	-7.36	-0.126	30
6	1360.6	1365.91	-5.31	-0.085	5
7	1298.7	1298.63	0.071	0.001	14
8	1265.7	1231.64	34.06	0.543	20
9	1305.4	1304.01	1.39	0.022	28
10	1299.09	1323.47	-24.38	-0.389	29
11	1470.7	1372.62	98.08	1.577	7
12	1398.45	1305.34	93.11	1.497	9
13	1299.09	1238.36	60.73	0.976	17
14	1267.09	1310.72	-43.63	-0.701	23
15	1367.9	1330.18	37.72	0.606	33
16	1259.76	1350.54	-90.78	-1.465	2
17	1297.89	1283.26	14.63	0.236	8
18	1176.87	1216.28	-39.41	-0.636	16
19	1298.9	1288.64	10.26	0.166	24
20	1160.5	1308.1	-147.6	-2.382	32
21	1278.7	1261.55	17.15	0.276	6

22	1160.9	1194.27	-33.37	-0.538	10
23	1067.6	1127.28	-59.68	-0.962	18
24	1264.9	1199.65	65.25	1.052	22
25	1254.7	1219.1	35.6	0.574	34
26	1200.7	1182.02	18.68	0.314	1
27	1150.8	1114.74	36.06	0.606	12
28	1020.5	1047.75	-27.25	-0.458	21
29	997.95	1120.12	-122.17	-2.054	25
30	1267.5	1139.57	127.93	2.151	35
31	1309.1	1262.25	46.85	0.819	4
32	1136.7	1194.97	-58.27	-1.018	13
33	1095.2	1127.99	-32.79	-0.573	19
34	1256.8	1200.35	56.45	0.986	27
35	1197.9	1219.81	-21.91	-0.383	31

To see how well the model satisfies the assumptions of the ANOVA, the plots of residuals for Influent BOD (So) (Table 14) were analyzed.

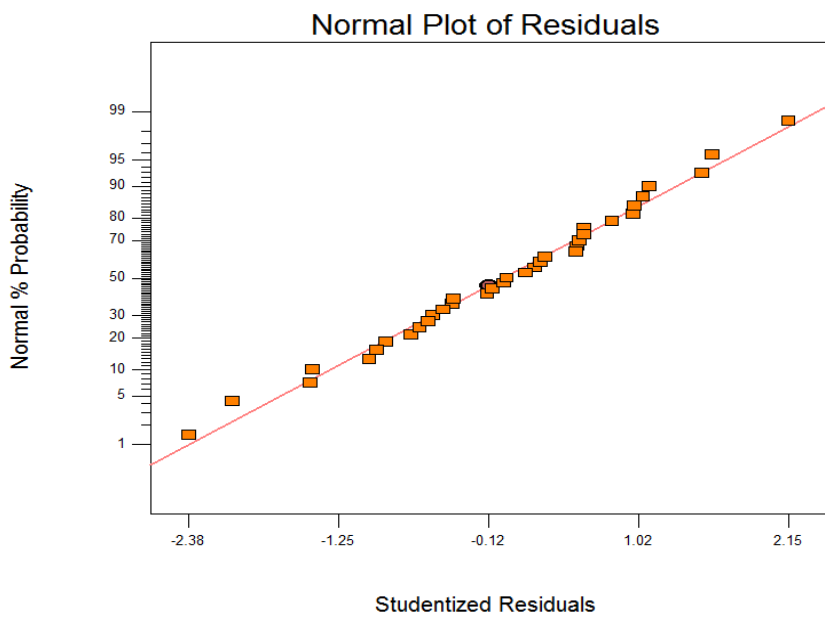


Figure 10 Normal plot of residuals for Influent BOD (So)

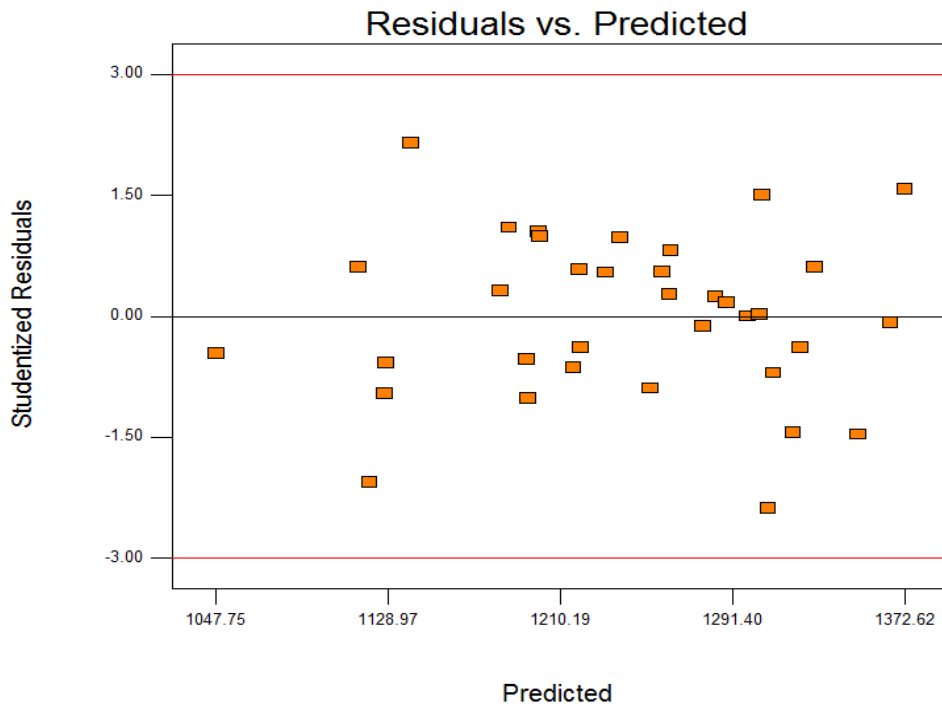


Figure 11 Plot of residuals vs. model predicted values for Influent BOD (So)

The normal probability plot, (Fig. 10), indicates the residuals following a normal distribution, in which case the points follow a straight line assuring the model satisfies ANOVA assumptions. A plot of the residuals versus the ascending predicted response values (Fig. 11), tests the assumption of constant variance. The plot shows random scatter (constant range of residuals across the graph) which is welcome deserving no need for a transformation to minimize personal error.

b. Diagnostic Test for Response 2 - Effluent BOD (Se)

The actual versus predicted values using model equation, Eq. (4.3) are tabulated in Table 15.

Table 15 Actual vs. model predicted Effluent BOD (Se)

Standard order	Actual value	Predicted value	Residual	Student residual	Run order
1	102.3	98.89	3.41	0.762	3
2	84.3	92.23	-7.93	-1.772	11
3	87.3	90.42	-3.12	-0.696	15
4	97.2	92.02	5.18	1.158	26
5	94.2	94.3	-0.1	-0.023	30
6	82.3	73.3	9	1.877	5
7	59.4	66.65	-7.25	-1.512	14
8	71.4	64.83	6.57	1.37	20
9	63.2	66.43	-3.23	-0.674	28
10	75.4	68.72	6.68	1.394	29

11	53.2	55.12	-1.92	-0.404	7
12	43.9	48.46	-4.56	-0.959	9
13	38.5	46.65	-8.15	-1.713	17
14	49.3	48.25	1.05	0.221	23
15	51.6	50.53	1.07	0.224	33
16	39.4	43.07	-3.67	-0.775	2
17	45.1	36.42	8.68	1.832	8
18	30.4	34.6	-4.2	-0.887	16
19	33.6	36.2	-2.6	-0.549	24
20	37.7	38.49	-0.79	-0.166	32
21	32.4	32.37	0.035	0.007	6
22	35.3	25.71	9.59	2.021	10
23	26.1	23.89	2.21	0.465	18
24	27.2	25.49	1.71	0.36	22
25	25.9	27.78	-1.88	-0.396	34
26	27.2	31.1	-3.9	-0.857	1
27	20.9	24.44	-3.54	-0.778	12
28	26.1	22.63	3.47	0.764	21
29	23.6	24.23	-0.63	-0.138	25
30	24.3	26.51	-2.21	-0.486	35
31	21.9	24.85	-2.95	-0.674	4
32	23.2	18.19	5.01	1.144	13
33	19.6	16.38	3.22	0.736	19
34	16.5	17.98	-1.48	-0.338	27
35	17.5	20.26	-2.76	-0.632	31

To see how well the model satisfies the assumptions of the analysis of variance (ANOVA), the plots of residuals for Effluent BOD (Se) (Table 15) were analyzed.

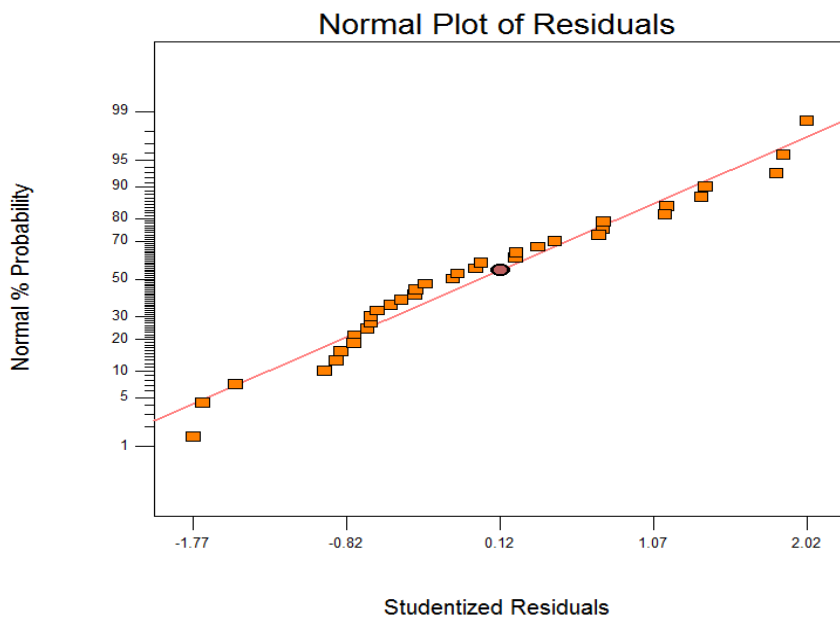


Figure 12 Normal plot of residuals for Effluent BOD (Se)

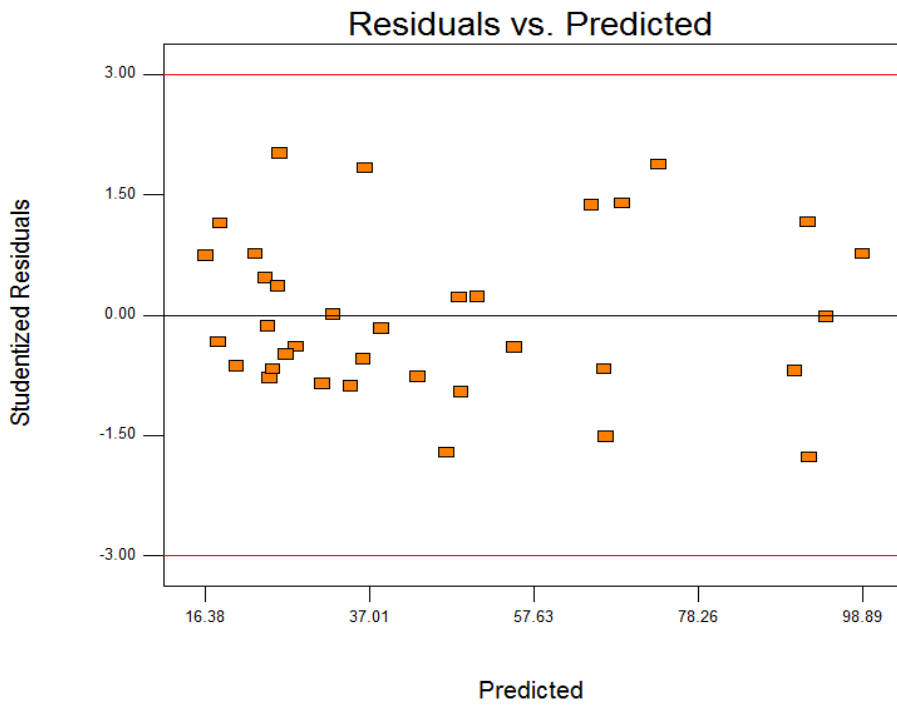


Figure 13 Plot of residuals vs. model predicted values for Effluent BOD (Se)

The normal probability plot, (Fig. 12), indicates the residuals following a normal distribution, in which case the points follow a straight line assuring the model satisfies ANOVA assumptions. A plot of the residuals versus the ascending predicted response values (Fig. 13), tests the assumption of constant

variance. The plot shows random scatter (constant range of residuals across the graph) which is welcome deserving no need for a transformation to minimize personal error.

c. Diagnostic Test for Response 3 - Mixed liquor suspended solid (X)

The actual versus predicted values using model equation, Eq. (4.5) are tabulated in Table 16.

Table 16 Actual vs. model predicted mixed liquor volatile suspended solid (X)

Standard order	Actual value	Predicted value	Residual	Student residual	Run order
1	720.9	742.66	-21.76	-0.75	3
2	750.5	769.3	-18.8	-0.648	11
3	779.2	782.43	-3.23	-0.111	15
4	730.1	757.3	-27.2	-0.938	26
5	791.2	781.09	10.11	0.349	30
6	750.2	770.17	-19.97	-0.643	5
7	815.6	796.81	18.79	0.605	14
8	830.1	809.94	20.16	0.649	20
9	790.4	784.81	5.59	0.18	28
10	788	808.6	-20.6	-0.663	29
11	856.2	771.55	84.65	2.747	7
12	827.3	798.2	29.1	0.945	9
13	835.2	811.33	23.87	0.775	17
14	830.5	786.2	44.3	1.438	23
15	846.3	809.98	36.32	1.179	33
16	712.6	752.6	-40	-1.303	2
17	757.4	779.25	-21.85	-0.712	8
18	791.2	792.38	-1.18	-0.038	16
19	735.2	767.25	-32.05	-1.044	24
20	783.6	791.03	-7.43	-0.242	32
21	631.3	676.82	-45.52	-1.481	6
22	659.4	703.46	-44.06	-1.433	10
23	678.1	716.59	-38.49	-1.252	18
24	663.1	691.46	-28.36	-0.923	22
25	690.4	715.25	-24.85	-0.808	34
26	644.1	589.04	55.06	1.869	1
27	656.01	615.69	40.32	1.369	12
28	638.2	628.81	9.39	0.319	21
29	631.7	603.68	28.02	0.951	25
30	645.01	627.47	17.54	0.595	35
31	523.6	536.06	-12.46	-0.439	4
32	559.2	562.7	-3.5	-0.124	13
33	565.3	575.83	-10.53	-0.371	19
34	560.4	550.7	9.7	0.342	27
35	563.4	574.49	-11.09	-0.391	31

To see how well the model satisfies the assumptions of the ANOVA, the plots of residuals for mixed liquor suspended solid (X) (Table 16) were analyzed.

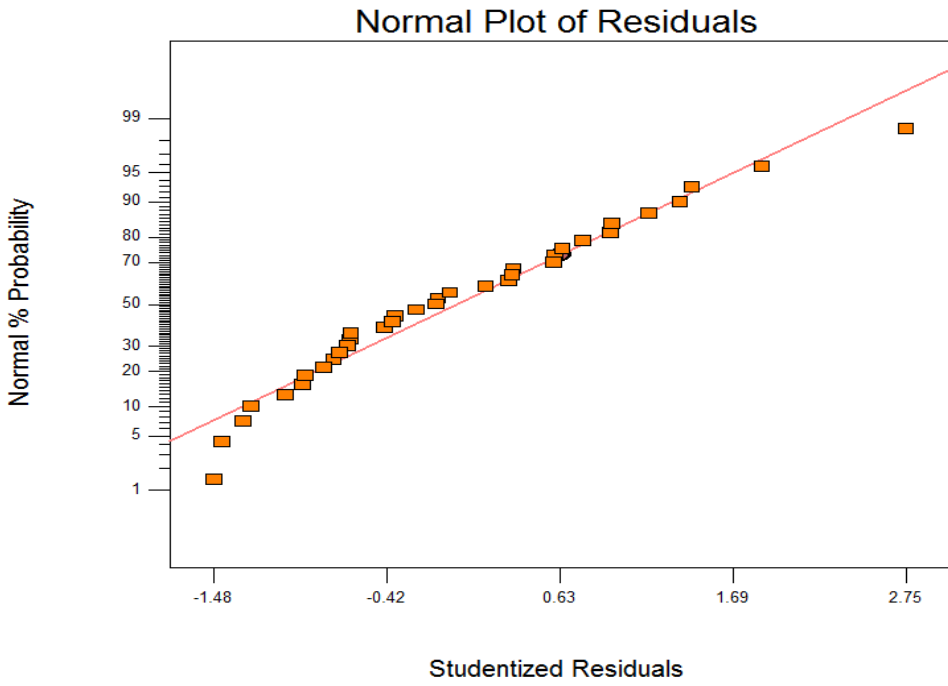


Figure 14 Normal plot of residuals for mixed liquor suspended solid (X)

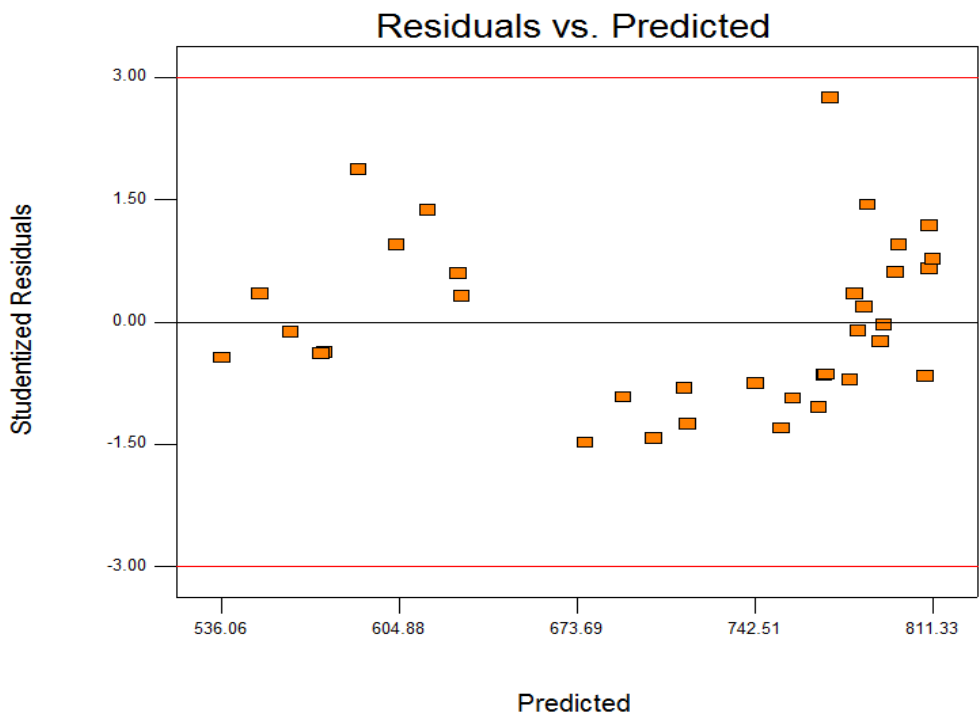


Figure 15 Plot of residuals vs. model predicted values for mixed liquor suspended solid (X)

The normal probability plot, (Fig. 14), indicates the residuals following a normal distribution, in which case the points follow a straight line assuring the model satisfies ANOVA assumptions. A plot of the residuals versus the ascending predicted response values (Fig. 15), tests the assumption of constant variance. The plot shows random scatter (constant range of residuals across the graph) which is welcome deserving no need for a transformation to minimize personal error.

4.2.3 Kinetic Determination

Before going to the detail of the laboratory work of aerobic digestion for kinetic data generation, the distillery waste was properly analyzed and characterized. Characterization of the waste is important parameter to know exactly what composition does the waste have. The Metahara sugar factory distillery waste characterized for different parameters are stated below.

Table 17 Characteristics of Metahara Distillery Waste

No.	Parameter	Metahara distillery waste
1	pH	4.0 – 4.5
2	COD	92,000 mg/L
3	BOD	45,410 mg/L
4	TS	49,950 mg/L
5	Temperature	89 – 98 °c
6	Nitrogen	1150 mg/L
7	Phosphorous	4100 mg/L

The reactor during this study was monitored on a daily basis for four parameters, i.e., temperature, pH, D.O., and mixed liquor volatile suspended solids (MLVSS). The first three parameters have a profound effect on biological growth and efficiency of biological treatment system [19] and the fourth parameter, i.e., MLVSS was used to determine the kinetic coefficients.

i. Temperature

The temperature of the reactor remained in a range of 17-25 °C. All the processes of growth are dependent on chemical reactions, and the temperature influences the rate of these chemical reactions. Thus the rate of microbial growth as well as total amount of growth can be affected by temperature.

ii. pH

The pH of the reactor during the study remained between 7.0 and 8.0 for most of the research period. Extremes of pH are fatal for most bacteria. The bacteria grow best when the pH is slightly on the acidic side. The optimum range for bacterial growth generally lies between 6.5 and 7.5 [20]. Activated sludge and aerated lagoons could be successfully operated when the pH was between 9 and 10.5 [19].

iii. Dissolved oxygen

DO of the reactor remained between 3 mg/L and 4.2 mg/L for most of the study period. This value was ideal for the biological treatment systems working under aerobic conditions. The values of DO for the present study were above the minimum level of 2 mg/L which has been widely reported in the literature [19].

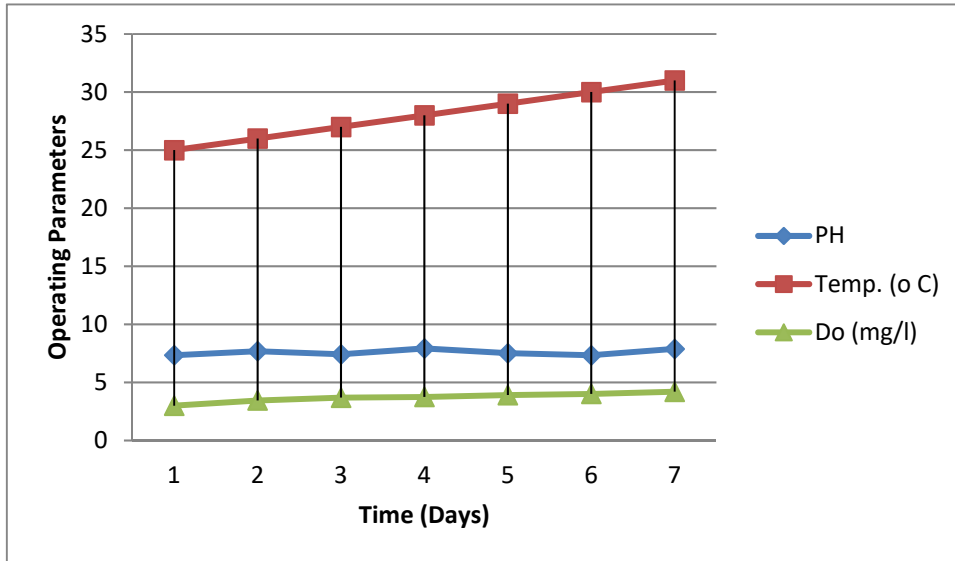


Fig. 16 operating parameters of the reactor

iv. Kinetic coefficients

Kinetic coefficients are usually determined through bench scale studies. For this purpose the bench scale completely mixed continuous flow reactor was operated for several hydraulic retention times, i.e., 2, 3, 4, 5, 7, 9 and 12 days. At each hydraulic retention time the data were collected at steady state conditions and mean values were determined for S_o (initial substrate concentration expressed as BOD), S_e (effluent substrate concentration), and X (biomass concentration). For each detention time three readings of each S_o , S_e , and X were taken, see Table 18. The mean of these values was used for the calculation of the kinetic coefficients.

Table 18 .Mean Values of data for kinetic coefficients

θ_c (days)	S_o (Influent) (mg/L of BOD)	S_e (effluent) (mg/L of BOD)	X (MLVSS) (mg/L of VSS)
2	1249.34	93.06	754.38
3	1305.898	70.34	794.86
4	1360.646	47.3	839.1
5	1238.784	37.24	756
7	1238.784	29.38	664.46
9	1238.784	24.42	643.004
12	1238.784	19.74	554.38

The kinetic coefficients are of great importance for appropriate design of the bioreactors which are used for the treatment of wastewater. The basic two equations are used to mathematically describe the fundamental kinetics of the treatment that takes place as a result of microorganisms in biological treatment [19].

$$\frac{ds}{dt} = \frac{kSX}{K_d + S}$$

$$\frac{dx}{dt} = Y \frac{ds}{dt} - k_d X$$

Where, X is mass of microorganisms, S is mass of organic matter used as food by the microorganisms (normally expressed as BOD), and Y is cell yield coefficient, the ratio of the mass of cells formed to the mass of substrate consumed., K_d represents the proportion of the total mass of microorganisms that self-degrades (endogenous respiration) per unit time, k is maximum rate of substrate utilization per unit mass of microorganisms, and K_s is half velocity constant, substrate concentration at one half of the maximum growth rate, mass per unit volume.

The following linearized equation as described by Metcalf & Eddy [38, 13] was used to find k and K_s :

$$\frac{X\theta_c}{S_o - S} = \frac{K_s}{k} \frac{1}{S} + \frac{1}{k}$$

Where θ_c is mean cell residence time and S_o is initial substrate concentration expressed as BOD.

By using the above equation, a graph was plotted with $\frac{1}{S}$ on the x-axis and $\frac{X\theta_c}{S_o - S}$ along the y-axis. A linear regression line was fitted to the plotted data. The intercept on the y-axis and the slope of this line were used to find k and K_s . The equation of the fitted line is also shown on the graph in Fig.17 below.

The following linearized equation is used to find Y and K_d [37, 14].

$$\frac{1}{\theta_c} = \frac{S_o - S}{X\theta_c} Y - K_d$$

A graph is plotted $\frac{1}{\theta_c}$ with along the y-axis and $\frac{S_o - S}{X\theta_c}$ along the x-axis. A linear regression line is fitted to the plotted data. The intercept on the y-axis and the slope is used to find the coefficients K_d and Y respectively. The equation of the fitted line is also shown in the Fig. 18 below.

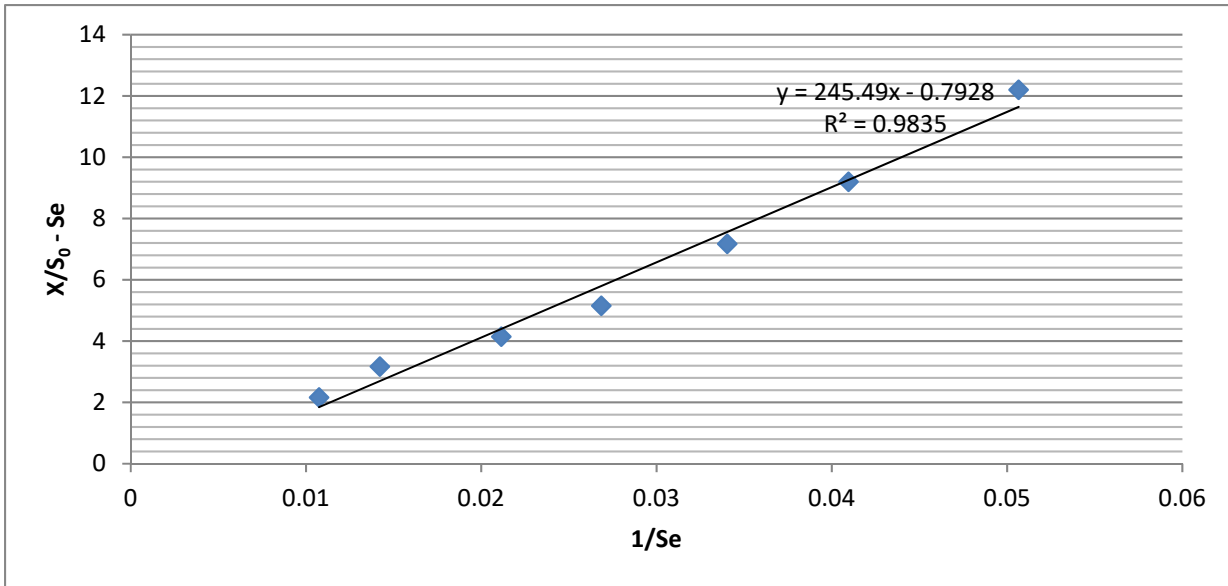


Fig. 17 Graph for k and K_s basis on BOD

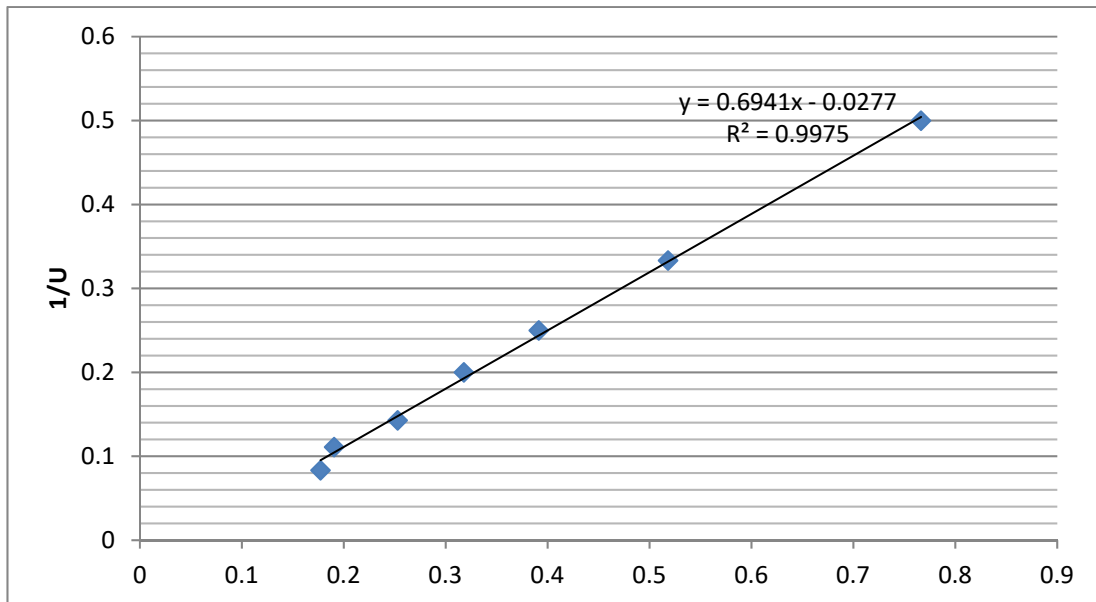


Fig. 18 Graph for Y and K_d basis on BOD

Results found from the experiment for the kinetic coefficients are in table 19.

Table 19 Results of kinetic coefficients from experiment

Kinetic Coefficients	Results
K	1.261352 per day
K_s	309.6493 mg/L of BOD
Y	0.6941 mg VSS/mg BOD
K_d	0.0277 per day

The best efforts were made to find the values of these coefficients for molasses based distillery industry waste. These data were compared with domestic waste, fish and cheese manufacturing waste in Table 20.

Table 20 Kinetic Coefficients for Various Wastewaters

Reference	k(per day)	K_s (mg/L)	Y(mgVSS/mg BOD)	K_d (per day)	Wastewater type
Metcalf&Eddy	5	60	0.6	0.10	Municipal
Haydar and Aziz	3.125	488	0.64	0.03	Tannery industry
Demirel et al	9.3	482.5	0.20	0.25	Dairy(anaerobic)
Bertola et al	0.09	0.006	0.45	0.024	Potato industry
Guptaand	0.216	56		0.068	Fertilizer industry

i. Maximum rate of substrate utilization (k)

k is the maximum rate of substrate utilization per unit mass of microorganisms. The value of k in this study came out to be 1.26 per day based on BOD, The value of k for domestic wastewater is 5 g COD per (g VSS) per day and the value of k for cheese processing wastewater is 9.3 per day, showing that the maximum rate of substrate utilization is less in the case of distillery-industry wastewater as compared to domestic and cheese processing wastewaters. The possible deviation may be due to the difference in the composition of the two wastewaters. As a matter of fact, the k value affects the volume of the reactor. The greater the value of k the smaller will be the size of the reactor [19].

ii. Half velocity constant (K_s)

K_s is the half velocity constant and is numerically equal to the substrate concentration. It is the maximum value at saturation concentration of growth limiting substrate. The value of K_s for this study came out to be 309.65mg/L sBOD. The range of K_s for domestic wastewater lies between 25 and 100 mg/1 BOD [13], for tannery wastewater K_s is 488 mg/L, and for cheesing processing it is 482.5 mg/L. A large value for K_s shows that the maximum specific yield of bacteria occurs at high substrate

concentration. Although K_s is one of the coefficients that are normally determined, yet it has no direct application in the process design. The only significance of K_s is more of a theoretical nature and gives an idea about change in specific growth rate of bacteria with a change in the concentration of growth limiting substrate [19].

iii. Biomass yield (Y)

Y represents the biomass yield, i.e., how biomass is produced against substrate utilized. The value of Y recorded for this study came out to be 0.69 mg VSS/mg BOD. The range of Y is 0.4-0.8 mg VSS/mg BOD for domestic wastewater and for cheese processing wastewater its value is 0.20 mg VSS/mg COD. [20] The significance of Y in process design is that it gives an estimate of the sludge produced as a result of wastewater treatment. The greater the value of Y, the greater will be the amount of sludge, and the size of sludge handling facility. Preliminary cost estimates for sludge handling can be found out once the size is known [19].

iv. Endogenous decay coefficient (K_d)

K_d is the microbial decay coefficient and represents the biomass lost to endogenous respiration per unit of biomass per unit time and has the dimensions per time. For this study the value of K_d came out to be 0.028 per day on the basis of BOD. The decay coefficient for domestic wastewater fluctuates in the range of 0.06-0.1 g VSS per (g VSS) per day and for cheese processing wastewater it is 0.25 per day. The lower value showed a lower bacterial decay rate in the case of distillery industry wastewater. The significance of K_d in process design is used when evaluating net sludge production in a treatment facility. Higher values of K_d reduce the net production of sludge. Although the effects are minimal, yet it can be used to fine tune the size of sludge handling facilities resulting in some economic benefits in the cost reduction [19].

Conclusion

The development of the bio-kinetic model for treatment of distillery wastewater is the first step towards design of a full-scale wastewater treatment plant. The development of such biokinetic model requires determination of the key biokinetic constants for the distillery wastewater. The raw data obtained from experimental runs with different residence time on a bench scale model was used to determine these biokinetic coefficients. In published research such biokinetic constants have been reported, both on the basis of BOD₅ and COD. In this work biokinetic constants have been determined using BOD₅. These biokinetic constants can now be directly used in the equations presented earlier in this paper, to obtain the complete biokinetic model for treatment of distillery wastewater. This model can then be used to design a full-scale wastewater treatment plant for distillery effluent.

The kinetic coefficients k (maximum substrate utilization rate), k_s (half velocity constant), Y (cell yield coefficient) and K_d (decay coefficient) were found to be 1.26 per day, 309.64 mg/L, 0.69 and 0.028 per day, respectively. The determination of these coefficients may be helpful in (1) understanding the kinetics of substrate utilization (2) sludge production and (3) design of biological treatment facilities based on activated sludge process for molasses distillery wastewater. Thus these coefficients have both academic value and practical significance.

Recommendation

The aim this work was to develop biokinetic model for Metehara distillery waste by varying the residence time but further research may be carried out using other combinations of the experimental parameters like MLVSS concentration, different size and volume of the aerated reactor and other designs of the settling tank.

The laboratory experimental work took more than three months because of the limitations on the reactor and its accessories. However, the duration can be lower to its minimum by using many reactors in series. Also helps the experimental data to be more reliable than doing it on single reactor.

Different researcher recommends, taking different samples will be more helpful in order to get reliable data. But I was not able to do that due to limited resource, budget, time and the place is far from Addis. During sample collection it is important to consider the high (winter) season to consider the effect of rain on the influent, this will be help full to have effective design of the biological waste water treatment plant.

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Appendices

Appendix 1. Biochemical Oxygen Demanded (BOD)

The stillage sample was related to the expected BOD value. The Oxidizer was designed to operate with the following ranges and sample volumes, allowing BOD measurement up to 0 – 4000 mg/l, without and dilution.

Range BOD mg/l	Sample volume in ml	Dosage ATH
0-40	428	10 drops
0-80	360	10 drops
0-200	244	5 drops
0-400	157	5 drops
0-800	94	3 drops
0-2000	56	3 drops
0-4000	21.7	1 drops

Procedures

1. The PH value of the stillage was measured and adjusted to be between 6.5- 7.5.
2. The stillage sample was mixed and homogenized well and allowed to settle for a short time.
3. The stillage volume was measured precisely; using the appropriate overflow measurement flask and placed into the sample bottle. The stillage sample was ensured in the bottle that contained a representative portion of any solids in suspensions. Each sample was measured three times for precision.
4. To protect consumption of oxygen by nitrifying bacteria, nitrification inhibitor ATH was added.
5. Clean magnetic stirrer rod was added to each bottle and 3-4 drops of 45% potassium hydroxide solution was added to the seal gasket to absorb the carbon dioxide then the seal gasket was inserted in the neck of the bottle.
6. Before measurement was begun the prepared sample was brought to the desired temperature that is BOD₅, at 20⁰c. This was achieved by placing the sample in a thermostatically controlled cabinet, while stirring the sample contiguously with the inductive stirring system.
7. The BOD sensors were placed in the sample bottles and tighten carefully. This was extremely important, as the system must be completely air tight. Then the BOD sample bottle was placed, with the sensor screwed in position, into the bottle rack. This was done in the thermostatically controlled cabinet itself.
8. The measurement process was started,

- A prepared bottle was placed in a vacant position of the rack and Switched on the unit and pressured the corresponding head key to activate this position.
 - The start key was pressed and the key was started the measurement for this bottle. When the “start” key was pressed, the unit was changed to “Start Mode “and was displayed the last selected range used for this head, with the sample volume required.
9. The sample was incubated in accordance with the instruction BOD₅ for 1 to 5 days at 20 °C.

Equipment and reagents

1. Incubation bottles
2. Incubators
3. KOH to absorb CO₂
4. ATH to avoid nitrification
5. BOD bottles
6. BOD sensor
7. BOD bottle rack
8. Measuring cylinder

Appendix 2 Determination Chemical Oxygen Demand (COD)

1. A homogeneous stillage sample containing settleable solids was homogenized with blender.
2. The Hanna Reactor was preheated 150 °C form 30min to 40 min.
3. The cap was removed from two Reagent vials.
4. Add exactly 2.0mL of sample was added to one vial, and 2.0mL of deionized water was added to the other vial (blank sample) while the vials was kipped at 45-degree angle.
5. The vials was inserted into the reactor and heated them for 2 hours at 150 °C
6. At the end of the digestion period the reactor was switched off and waited for twenty minutes to allow the vials to cool to about 120°C.
7. Each vial was inverted several times while stilled, warmed, then placed them in the test tube rack
8. The vials in the tube rack were leaved and cooled to room temperature. Will not shacked or inverted any more.

The program number was selected with corresponding to Oxygen Demand, Chemical LR (COD) on the secondary LCD by pressing program and placed the blank vial into the holder and pushed it and complete down Zero was and "SIP" was blinked on the display and waited for few seconds and displayed was shown "-0.0-". At this point the meter was zeroed and ready for

measurement. The blank vial was removed and placed into the holder and pushed it completely down Read direct was placed and "SIP" was blinked during measurement .The instrument was displayed the concentration in mg/L of oxygen demand on the liquid Crystal was measured.

Appendix 3. Total solids analysis of stillage.

The determination of the total solids contents, that is the sample residue left in the crucible after evaporation of the Stillage samples using oven both from National alcohol and Addis Ababa university Chemical engineering laboratory at 105°C. The Total Solid Content (%) is the amount of sample residues remaining following heating the Stillage samples was analyzed.

Test Principle:

A well-mixed sample of stillage is evaporated in a weighed dish and dried to a constant weight in a convection oven for overnight at a temperature of 105°C; the weighed samples are placed in the oven during the above specified time and dried until a constant mass is obtainable.

Equipment and Apparatus used:

- Porcelain evaporating crucibles
- Analytical balance
- Drying oven
- Desiccators
- Micropipettes
- Magnetic Stirrer
- Metal Tongs

Appendix 4. Determination of Volatile Solids Content (VS %) of Stillage Samples.

Analysis of volatile solids in a sample has important application in that it gives that it gives rough estimation of the amount of organic matter present in the distillery stillage. A weighted distillery stillage sample solids was ignited using Muffle furnace at a temperature of 550°C ($\pm 10^\circ\text{C}$) for one hour and the total Volatile Solids was determined.

Analysis of Volatile solids in a sample has important application in that it gives rough estimation of the amount of organic matter present in the wastes and effluents, solid fraction of wastewater and activated sludge.

1. After determining the final weight in total solids analysis, the respective cooled oven dried Stillage samples and crucible was placed in a muffle furnace and ignited at 550°C at a ±10°C for exactly 1hr.
2. Following the ignition duration for complete combustion process to take place, the oven ignition switch was switched off and allowed to cool down for 10 minutes.
3. The cooled muffle furnace dried samples was then placed and kept in desiccators for an hour. Immediately after sample was allowed to desiccate, samples were weighed together with the crucible in the sensitive analytical measuring balance.

Calculation

A = weight of dried residue + dish, mg, and

B = weight of dish, mg.

A = weight of dried residue + dish before ignition, mg

B = weight of residue + dish after ignition, mg.

Calculation

$$\text{mg total solid} / L = \frac{(A - B)}{\text{Sample volume, ml}} \times 100$$

A = weight of dried residue + dish, mg, and

B = weight of dish, mg.

$$\text{mg volatile solid} / L = \frac{(A - B)}{\text{Sample volume, ml}} \times 100$$

A = weight of dried residue + dish before ignition, mg

B = weight of residue + dish after ignition, mg.

Appendix 5 Determination of Phosphorus

Method

1. Hach method 8190: Molybdovanadate with acid per-sulfate digestion.
2. Adaptation of the standard method for examination of water and waste water 20th ed. 4500-P C, vanadomolybdophosphoric acid method. A persulfate digestion converts organic and condensed inorganic forms of phosphates to orthophosphate then the reaction between orthophosphate and the reagent cause a yellow tint in the sample.

Required reagent

1. Potassium per-sulfate powder pillows
2. 1.54N NaOH solution
3. Molybdovanadate reagent
4. Deionized water

Materials used

1. Hach reactor
2. Hach spectrophotometer model DR / 2010
3. Measuring cylinder
4. Pipette

Measurement procedure

1. Reagent blank correction.
 1. This method needs a reagent blank correction.
 2. A single vial may be used more than once.
 3. The blank vial is stable up to one day (room temperature).
 4. For most accurate measurement, run a blank for each set of measurements and always use the same lot of reagents for blank and sample.
 5. Choose a homogeneous sample.
 6. Preheat the Hach reactor to 150 °C.
 7. Remove the cap from two reagent vials.
 8. Add exactly 5ml of sample to one vial (sample vial).
 9. Add exactly 5 ml of deionized water to the other (blank vial).
 10. Replace the cap tightly and mix by inverting each vial a couple of times.
 11. Add the content of one potassium per-sulfate reagent powder pillows for phosphorus analysis to each vials.
 12. Replace the cap tightly and shake gently the vials until all the powder is completely dissolved
 13. Insert the vials into the reactor and heat them for 30 minute at 150 0C
 14. At the end of digestion period switch of the reactor and place the vials carefully in the test tube rack and allow cooling to room temperature.
 15. Select the program number corresponding to total phosphorus on the secondary LCD by pressing program increase or decrease symbol.
 16. Remove the cap from the vials and add exactly 2ml of sodium hydroxide solution (1.54 N) to each vial while keeping the vial at 45 degree.
 17. Replace the cap tightly and mix by inverting the vial a couple of time.
 18. Remove the cap from the vial and add exactly 0.5 ml of molybdovanadate reagent to each vial while keeping the vial at 45 degree.
 19. Replace the cap tightly and mix by inverting the vial a couple of time.

20. Place the blank vial into the holder and push it completely down.
21. Press timer and the display show the countdown prior to the measurement, alternatively, wait for 7 minute and pres zero in both cases 'sip' will blink on the display.
22. The display will show '-0.0-'now the meter is zeroed and ready for measurement.
23. Remove the blank vial.
24. Place the sample vial into the holder and push it completely down.
25. Press read direct and 'sip' will blink during measurement.
26. Instrument directly displays concentration in mg/l of total phosphorus on the liquid crystal display.
27. To convert the reading to mg/L of P₂O₅, multiply by a factor of 0.748.
28. To convert the reading to mg/L of phosphorus concentration, multiply by a factor of 0.326.

Appendix 6 Determination of Nitrogen

Method

1. Hach method 10072: TNT per-sulfate digestion

- Chromotropic acid method, a persulfate digestion converts all forms of nitrogen to nitrate. Then the reaction between nitrate and the reagents causes a yellow tint in the sample

Required reagent

- Reagent vial-total nitrogen hydroxide reagent
- Total nitrogen per-sulfate powder pillows
- Total nitrogen reagent A powder pillows
- Total nitrogen reagent B powder pillows
- Deionized water

Materials used

- Hach reactor
- Hach spectrophotometer model DR / 2010
- Measuring cylinder
- Pipette
- Measurement procedure
- Reagent blank correction
- This method needs a reagent blank correction

- A single vial may be used more than once
- The blank vial is stable up to one week (room temperature)
- For most accurate measurement, run a blank for each set of measurements and always use the same lot of reagents for blank and sample
- Choose a homogeneous sample
- Preheat the Hach reactor to 105 °C
- Remove the cap from two digestion vials
- Add the content of one packet of total nitrogen per-sulfate reagent powder pillows
- Add exactly 0.5 ml of sample to one vial (sample vial)
- Add exactly 0.5 ml of deionized water to the other (blank vial)
- Replace the cap tightly and shake vigorously the vials for about 30 seconds until all the powder is completely dissolved
- Insert the vials into the reactor and heat them for 30 minute at 105 °C
- At the end of digestion period switch of the reactor and place the vial in test tube rack after digestion to cool at room temperature
- Select the program number corresponding to total nitrogen on the secondary LCD by pressing program increase or decrease symbol
- Remove the cap from the vials and add the content of one packet of total nitrogen reagent powder pillow to each vial. Replace the cap tightly and shake gently the vials for 15 seconds
- Wait for 3 minute without shaking the vials to allow the reaction to complete
- Remove the cap from the vials and add the content of one packet of total nitrogen reagent B powder pillows to each vial. Replace the cap tightly and shake gently the vials for 15 seconds
- Wait for 2 minute without shaking to allow the reaction to complete
- Remove the cap from two other reagent vials
- Add exactly 2 ml of digested sample from the digested sample vial to one reagent vial (sample vial), and 2 ml of digested blank to other reagent vial (blank vial) while keeping the vial at 45 degree angle
- Replace the cap tightly and invert the vials 10 times
- Place the blank vial into the holder and push it completely down
- Press timer and the display show the countdown prior to the measurement, alternatively, wait for 5 minute and pres zero in both case 'sip' will blink on the display
- The display will show '-0.0-'now the meter is zeroed and ready for measurement

- Remove the blank vial
- Place the sample vial into the holder and push it completely down
- Press read direct and 'sip' will blink during measurement
- Instrument directly displays concentration in mg/l of total nitrogen on the liquid crystal display
- To convert the reading to NH₃, multiply by 1.22
- To convert the reading to NO₃, multiply by 4.43
- Interference
 - Bromide above 240 mg/L
 - Chloride above 3000 mg/L

Appendix 7. EPA Emission Limits

1. List of Gazata Notifications on environmental Issues

Sr. No.	Gazata No. / Circular No.	Date of Issue	Contents
1	295/2002	31-10-2002	Establishment of Environmental Protection Authority.
2	299/2002	03-12-2002	Environmental Impact Assessment Proclamation
3	300/ 2002	03-12-2002	Environmental Pollution Control Proclamation
4	--	Aug. 2003	Guidelines of Ambient Environment Standards for Ethiopia.
5	US/ ETH/ 99/068/ETHIOPIA	Sept. 2003	Standards for Industrial Pollution Control in Ethiopia.
6	--	Nov. 2003	Environmental Impact Assessment Procedural Guidelines Series- 1

2. Malting brewing distilling the production of wines and other alcoholic liquors.

Table 1 Emission limit values discharged to water

Constituent Group or Parameter	Emission Limit Value (mg/ l)
Temperature	40 ⁰ C
pH	6 – 9 pH units
BOD₅ at 20⁰ C	> 90% Removal or 60 mg/ l
COD	> 90% Removal or 250 mg/ l
Suspended Solids	50
Total Ammonia (as N)	20
Total Nitrogen (as N)	> 80% Removal or 40 mg/ l
Total Phosphorus (as P)	> 80% Removal or 5 mg/ l
Oils, Fats, and Grease	15
Mineral Oil (Interceptor)	20

Table 2 Emission limit values for emission to air

Substance	Concentration (mg/Nm ³)
Total Particulates (at a mass flow of 0.5 kg/h or above)	100
Hydrogen chloride (as HCl) (at a mass flow of 0.3 kg/h or more)	30

3. Manufacture of sugar.

Table 3 Emission limit values discharged to water

Constituent Group or Parameter	Emission Limit Value (mg/ l)
Temperature	40 ⁰ C
pH	6 – 9 pH units
BOD ₅ at 20 ⁰ C	> 90% Removal or 60 mg/ l
COD	> 90% Removal or 250 mg/ l
Suspended Solids	50
Total Ammonia (as N)	15
Total Nitrogen (as N)	> 80% Removal or 40 mg/ l
Total Phosphorus (as P)	> 80% Removal or 5 mg/ l
Oils, Fats, and Grease	15
Mineral Oil (Interceptor)	20

Table 4 Emissions limit values for emission to air.

Substance	Concentration (mg/Nm³)
Total Particulates (at a mass flow of 0.5 kg/h or above)	100
Hydrogen chloride (as HCl) (at a mass flow of 0.3 kg/h or more)	30

4. Standards which shall be applied to all effluents which shall be applied to land

Tables 5 Emission standards for industrial effluents for land application

Constituent Group or Parameter	Emission Limit Value (mg/ l)
Basic Parameters	
pH	5.5-9 pH units.
Biochemical Oxygen Demand (BOD) ₅	500
Total Dissolved Solids (TDS)	2100
Fats, Oils and Grease	30
Metals	
Arsenic (as As)	0.25
Barium (as Ba)	10
Borom (as B)	5
Cadmium (as Cd)	1
Chromium (as total Cr)	2
Chromium (as hexavalent Cr)	0.5
Cobalt (as Co)	1
Copper (as Cu)	2
Cyanide (as Cn)	0.5
Lead (as Pb)	0.5
Manganese (as Mn)	5
Mercury (as Hg)	0.001
Nickel (as Ni)	3
Selenium (as Se)	1
Silver (as Ag)	1
Tin (as Sn)	5
Zinc (as Zn)	5
Total Heavy Metals (Combined)	15
Inorganic Chemicals	
Chloride (as Cl)	1000
Fluoride (as F)	20
Sulphate (SO ₄)	1000

