CHARACTERISATION OF AEROBIC BIOTREATMENT OF MEAT PLANT EFFLUENT

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by

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ABSTRACT

This study investigated the bio-kinetics of a meat-processing wastewater in an activated sludge system. The main pollutant loading of the wastewater under investigation was characterized as 1350 mgCOD/L, 70 mgNH_4-N/L, and 127 mgTKN/L in average.

The respirometric method and aerobic batch methods were used to evaluate the biodegradability and the kinetics of carbon removal and nitrification of meat-processing wastewater in an activated sludge system.

The readily biodegradable COD accounts for 15~17 % of the COD in meat-processing wastewater, while the inert portion, including soluble and particulate, accounts for another 10 % of the COD. Approximately, 1/3 of the meat-processing wastewater composition is in soluble/fine colloidal form and the remaining 2/3 is in particulate form.

For heterotrophos growing on soluble meat-processing wastewater, the determined values of kinetic constants for carbon removal were 0.63 mgcellCOD/mgCOD for the observed COD based yield coefficient (Y_H), 0.40 mgVSS/mgCOD for the observed mass (VSS) based yield coefficient (Y_o), 1.4 for COD/VSS ratio, 3.3 day^{-1} for the maximum specific growth rate ($\mu_{H,MAX}$), and 10 mgCOD/L for the half-saturation constant (K_s). The death-regeneration decay coefficient of heterotrophos (b_H) was 0.38 ~ 0.49 d^{-1}. For heterotrophos growing on unfiltered meat-processing wastewater, the relationship between S_o/X_o and the observed corresponding specific growth rate (μ, d^{-1}) was found to

fit a Monod type function. The maximum specific growth rate of heterotrophos in unfiltered meat-processing wastewater was determined as 9 d^{-1}, while the half-saturation constant was found to be 22.

In regard of nitrification, the maximum specific growth rate of autotrophos in soluble meat-processing wastewater was 0.56 ~ 0.71 d^{-1}.

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Chapter 1 Introduction

1.1 The New Zealand Meat Industry

Over three hundred thousand tonnes of New Zealand beef and veal are exported to eighty-five countries around the world annually. This market including the Pacific Region, European Region, North America, the Caribbean, Middle East, South East Asia, Japan and Korea, brings important contributions to the national income of New Zealand. [2] The inspected cattle slaughtering, including adult cattle, calves and vealers, was 2.5 millions carcasses in average (was 3.06 million and 2.86 million for the years 1993 and 1994 respectively). [1, 2]

The New Zealand meat industry is under increasing pressure from environmental authorities due to the influence of the resource conservation movement reflected in regulations made by local authorities.

1.2 Environmental Concerns of the Meat Industry

Environmental concerns that may be related to the meat industry include the eutrophication and depletion of dissolved oxygen in aquatic systems, ammonia toxicity, contaminated drinking waster (due to the nitrification products, nitrate and nitrite), odour release (H_2S, NH_3 and fecal material), and waste disposal. To meet domestic regulations as well as global trade guidelines and standards, such as Environmental Management and Auditing System, EMAS, ISO 09000 and ISO14000, the industry management needs to act and address these issues now to achieve compliance with both forms of regulations.

1.3 Treatment of Meat-processing Wastewater

High level of organic nitrogen (protein), fat and large amount of organic matters characterize the quality of primarily treated meat-processing effluent. With respect to pollution parameters, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total suspended solid (TSS) and fat are

all (sedimentation) and dissolved air flotation (DAF) are widely used primary treatment techniques in the New Zealand Meat industry. Secondary treatment techniques include physiochemical treatment systems (PCTs, such as pH adjustment and/or addition of precipitants) and biological treatment systems (such as anaerobic/aerobic lagoons or tanks, irrigation, and artificial wetlands) are also commonly used in the New Zealand meat industry. [1,3,4,5] Trickling filters and sequential batch reactors have also been employed in the recent decade. [6,7] In general, only about 50% of pollutants (COD and TKN) are removed by means of PCT techniques, and about 50% to 90% of COD and TKN is removed by the biological treatment systems. [3,4,5,6,7] Amongst these techniques, the activated sludge treatment system is considered to be the best option for simultaneous removal of carbonaceous and nitrogenous oxygen demand. [14,15,17,19]

1.4 Design and Modelling of Activated Sludge Treatment Systems for the Meat Industry

Although the New Zealand meat industry has significant experience in biological treatment of the meat-processing wastewater, there is little literature on the kinetics of the biological treatment of meat-processing wastewater. On the other hand, engineers often find limitations of time and budget hinders their exploration of the potential solutions for design by testing a lab-scale reactor or a pilot plant. Hence, they frequently turn to mathematical models to define the best feasible design. Appropriate parameters and constants are essential to the reliability of their modelling. Consequently, the information of substrate (meat-processing wastewater) components and process kinetic constants are crucial to a successful design.

1.5 Major Assumptions

This study is based on an assumption that the activated sludge models and their major theories associated with domestic sewage can be applied to meat-processing wastewater, including the following:

(1) The organic matter in a meat-processing wastewater can be subdivided into a number of categories: non-biodegradable organic matter, which includes inert soluble organic matter (S_i) and inert suspended organic matter (X_i), and biodegradable matter, which includes readily biodegradable materials and

slowly biodegradable materials (X_s); Amongst these, soluble inert organic matter contributes to the effluent organic concentration, while particulate inert organic matter becomes a part of the (volatile) suspended solids in the activated sludge system, and neither of them are involved in any conversion process; Readily biodegradable materials are mainly soluble matter, while slowly biodegradable materials are mainly particulate matter;

(2) The growth of activated sludge in GF/C filtered (soluble) meat-processing wastewater follows Monod's equation: [45]

$$\frac{1}{X}\frac{dX}{dt} = \mu = \mu_{MAX}\frac{S}{K_s + S} \qquad (2\text{-}13)$$

where

μ = specific growth rate (day^{-1}),

μ_{MAX} = maximum specific growth rate (day^{-1}),

X = microorganism concentration (mg/L),

S = growth-limiting substrate concentration,

K_s = half-saturation constant (mg/L);

(3) The growth of activated sludge in the unfiltered meat-processing wastewater follows a modified Monod type equation (equation 4-1) – a modified Strenstrom's equation, [51] which later was related to the rate expressions for hydrolysis of slowly biodegradable substrate in the Task Group Model No.1: [19]

$$\mu = \frac{\mu_{H,MAX,overall} \bullet (S_o / X_o)}{K_{S_o/X_o} + (S_o / X_o)} \qquad (4-1)$$

where

μ = specific growth rate of activated sludge growing on unfiltered wastewater

$\mu_{H,MAX,overall}$ = maximum specific growth rate of activated sludge growing on unfiltered wastewater

S_o = initial COD concentration of unfiltered wastewater

X_o = initial concentration of biomass, COD based

K_{S_o/X_o} = half-saturation constant for utilizing unfiltered wastewater

(4) Decay of activated sludge in meat-processing wastewater is a death-

regeneration process: The death of biomass results in the release of slowly biodegradable substrate (X_s) that can be recycled back to soluble substrate used for cell growth. [28]

1.6 Objectives

In order to meet the challenge of discharging effluents which comply with the strengthened environmental standards as cost-effectively as possible, information of biological treatment kinetics are crucially required.

To assist the optimum design of activated sludge systems for meat-processing wastewater, it was decided this thesis to provide information as follows:

(1) The characteristics and biodegradation of meat-processing wastewater; and
(2) The kinetic constants of carbon removal and nitrification for meat-processing wastewater in an activated sludge system.

Chapter 2 Literature review

2.1 Characteristics and Treatments of Meat-processing Wastewater in New Zealand

The New Zealand meat industry processes approximately 2.5 millions cattle slaughtered each year. [2] A typical meat-processing plant in New Zealand produces up to 10,000 m^3/day of wastewater with a pollution load equivalent to a city of 60-100,000 inhabitants. [1,8] High oxygen demand (due to organic matter such as fat and protein) and high levels of nutrient concentrations (nitrogen, phosphorous, sulfur, etc.) are the two major pollutants in meat-processing wastewater, the discharge of which can result in oxygen depletion, nuisance slicks, color, turbidity, eutrophication, and toxicity in the aquatic system. This chapter reviews the sources and types of meat-processing wastewater, representative characteristics of meat-processing wastewater, waste reduction/reuse techniques, options for wastewater management /treatment in the industry, and the general principles and operational parameters for pertinent present treatment options.

2.1.1 Characteristics of meat-processing wastewater

2.1.1.1 Sources and types of wastes

Sources of meat-processing wastewater include stockyards, slaughtering and boning operations, rendering processes, blood collection and processing, gut processing, and skin and pelt processing (fellmongering). [3,9,10,11,12]

Wastes generated from these processes include fecal material and urine voided while holding stock, material produced by cutting and emptying the paunches of ruminants, losses of blood from blood processing, fat and residual tissue, and chemicals used in fellmongery operations, and losses of stick-water from rendering process. [3,9,10,11,12] Many of these materials can be recovered or removed through conducting waste minimization practices to avoid the entry of contaminants into the waste stream. [13]

2.1.1.2 Characters of meat-processing wastewater

The materials contributing to meat-processing wastewater have been quantified or characterized in the literatures. [3,9,10,11,12] Overall, high levels of organic nitrogen (protein) and large amounts of separable material [1,3] characterize meat-processing wastewater. In terms of pollution parameters, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total suspended solids (TSS), volatile solids (VS), and fat are important.

Some inorganic constituents, such as brines, detergents, disinfectants and pesticides, strong alkalis and acids, may also be introduced to meat-processing wastewater if best management practices (BMPs) / waste minimization practices have not been carried out appropriately. [13]

Sampling for wastewater characterization was normally taken after primary treatment (settling or screening) due to the large amounts of separable material in the raw wastewater. [1,3] Table 2-1 shows a typical organic loading of meat-processing wastewater.

The overall oxygen demand of slaughterhouse wastewater can be attributed to two major groups of compounds, fat and grease, and blood. [1]

Table 2-1 Representative characteristics of primarily treated meat-processing plant effluents [reproduced from [3]]

Pollutant	Range*	Representative Value*
5-D biochemical oxygen demand (BOD_5)	700 - 1,800	1,000
Chemical oxygen demand (COD)	1,000 - 3,000	2,000
Total Kjeldahl nitrogen (TKN)	70 – 180	110
Ammonia nitrogen (NH_3-N)	5 – 50	20
Total suspended solids (TSS)	200 - 1,200	500
Fat	100 – 900	400
Total phosphorus	5 – 20	12

* All units are g/m^3

2.1.1.3 Significance of meat-processing wastewater

Meat-processing wastewater is usually associated with the following issues due to its relatively high loads of carbonaceous and nitrogenous organics:

1. Eutrophication and depletion of dissolved oxygen in aquatic system

Ammonia nitrogen acts as a primary nutrient that may stimulate phytoplankton and plant growth. [14] Nitrate is also a major nutrient which in excessive amounts may induce prolific growth. [14] Phosphorus is another typical nutrient found in meat wastewater. Algae blooms tend to occur if the concentration of inorganic nitrogen (ammonia nitrogen, nitrite, and nitrate) and phosphorus exceed respective values of 0.3 mg/L and 0.01 mg/L. [15] When receiving water becomes enriched (or over-fertilized) with nutrients and energy, either by natural processes or human input, the domination of excessive plant and /or algae growth may occur. The decay and decomposition of these plant and phytoplankon result in odor and sediment oxygen demand, which tend to cause depletion of dissolved oxygen.

The aerobic bio-degradation of high levels of organic nitrogen (protein), fat, and large amounts of separable material in aquatic system will also consume a

significant amount of dissolved oxygen.

Depletion of dissolved oxygen affect the respiration of fish, benthic aquatic animals, and attached plant growth. This suffocation of aquatic aerobosis results in both economic loses of fishery industry and losses of bio-diversity in aquatic ecosystem.

2. Ammonia toxicity

Sediments of suspended solids from meat-processing wastewater are mostly degradable constituents. The degradation and decay of these sediments in anaerobic conditions produce toxic products, such as H_2S, NH_3, which affect fish and shellfish. Free ammonia (NH_3) has shown acutely toxic to fish at TL_{50} (Tolerance Limit) values ranging from 0.083 to 4.6mg/L. [16]

3. Public health concern

The nitrification products, nitrate and nitrite, from oxidation of nitrogenous organics in meat-processing wastewater, cause a public health concern, primarily related to methmoglobinemia syndrome (in its lay term, blue baby) and carcinogensis. [17]

Under specific conditions in the stomach and saliva nitrate can be reduced to nitrite. Nitrite can further oxidize in the hemoglobin molecular, from the ferrous to the ferric state, the resulting methemoglobin is incapable of exchanging oxygen, and anoxia or death may occur. Drinking water guidelines and standards recommend that the concentration of nitrate in drinking water should not exceed 10 mg/L. [17]

Nitrites can react with amines and amides to form nitrosamines and nitrosamides. Carcinogenesis (gastric cancer, in particular) has been found associated with the ingestion of N-nitroso compound. [17]

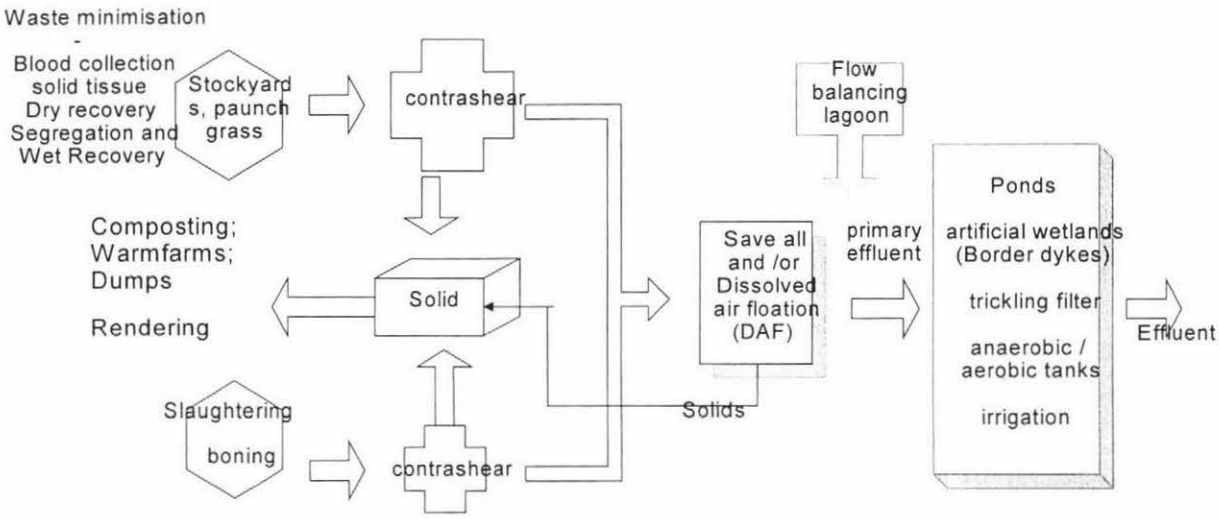
4. Pollution of groundwater

Nitrogen loading in groundwater may be increased due to the infiltration of nitrogenous surfacewater. Increased N-load affects potable use of groundwater. (17)

2.1.2 Treatment technologies of meat-processing wastewater in New Zealand

The meat-processing industry in New Zealand has employed both primary and secondary wastewater treatment techniques. Figure 2-1 shows a typical treatment of the waste stream of meat-processing plants.

Figure 2-1 Typical wastestream treatment of meat processing plants



2.1.2.1 Primary Treatment

Wastewater pretreatment in the New Zealand meat-processing industry consists of using screens sedimentation or both screens sedimentation and flotation. (1,3) The major functions of primarily treated wastewater treatment in the meat industry include the removal of large particles, such as manure and paunch contents, removal of higher density solids, such as bones, pieces of meat, etc., and reducing the levels of total fat and suspended solids. (1,3,13)

Pretreatment units usually contain screens, contrashear, sedimentation tanks, and flotation tanks. (1,3) Solids recovered from pretreatment units can be subject to

rendering or composting for recovery, or landfill for final disposal.

2.1.2.2 Secondary Treatment

Pretreated meat-processing wastewater usually goes through a flow-balancing lagoon before secondary treatment.

2.1.2.2.1 Physicochemical treatment (PCT)

Much of the COD in meat-processing wastewater is in a colloidal or soluble form and this material is not recovered by simple physical treatment. This colloidal and soluble fraction contains a considerable quantity of protein which is recognized as a potentially valuable resource.

Cooper & Russell (1991) [3] compiled and abstracted the results of researches employing pH adjusting and/or addition of specific precipitants for precipitating proteins from wastewater. An advantage of PCT is that the solids recovered can be used as animal feed supplements. On the other hand, high levels of metal content in the precipitated solids reduce their commercial value. The increased amount of sludge produced by chemical precipitation and the chemical are disadvantages of PCT. [3]

Treatments of meat-processing wastewater by various PCT techniques could reduce 57 % ~ 74% of total COD, 39%~65% of soluble COD, 30%~63% of TKN, and 78%~92% of fat. No significant ammonia-nitrogen removal has been observed through the PCT technique used in the meat industry. [3] However, at high pH, with large quantities of air, NH_3 can be stripped from solution. The release of odours, the costs of lime, and acid for pH adjustment reduce its applicability.

2.1.2.2.2 Biological treatment

Biological wastewater treatment is a process in which the microorganisms, provided with nutrients, convert the colloidal and soluble organic matters into various gases and cell tissue. [15] The microorganisms involved in biological treatment are mainly bacteria, some fungi, algae, protozoa and little metazoa. The prevalence of each microbial category in a biological wastewater treatment system varies with types of bioprocess being adopted. [18]

The two major constituents in meat-processing wastewater, fat and protein, are both essential to microbial growth. They provide sources of energy, carbon for synthesis of new cellular material, and inorganic elements such as nitrogen, phosphorus, sulfur, potassium and calcium as nutrients for microbial growth. Therefore, biological treatment has been commonly used in meat-processing industry. [1,3,13] Biological treatment is also often considered the most cost-effective option for waste stabilization where applicable.

Lagoon based biological treatment systems, irrigation, and artificial wetlands are commonly used in the New Zealand meat industry. Some aerobic systems are mostly space oriented and operated at continuous flow, suspended or attached growth conditions.

Cooper *et al* (1979) [1] and Cooper & Russell (1991) [3] summarized the achievements of the application of biological wastewater treatment systems in the industry. The removals of BOD from anaerobic lagoons treating meat-processing wastewater ranged between 65% and 95%. [3] An anaerobic lagoon (with a hydraulic retention time (HRT) of 11days and at a temperature of 20-25°C) obtained removal of total COD, soluble COD, TSS, Organic-N, and fat by 74%, 68%, 66%, 52%, 79% respectively. [4] Aerated lagoons were found able to remove 50% of total COD and 70-80% of fat. [5] Removals of 54% BOD_5 and 97% ammonia were reported in a sequential batch reactors treating meat wastewater. [6,7]

2.2 Biological Treatment Processes for Carbon Removal and Nitrification

The microbial processes that have been applied to wastewater treatment for carbon and nitrogen removal are briefly reviewed.

2.2.1 Microbial processes of carbon removal

2.2.1.1 Anaerobic systems and anaerobic biological process

Anaerobic treatment systems such as lagoons/ponds are commonly used for treating meat-processing wastewater in New Zealand. Other high rate anaerobic systems, such

as contact reactors and up-flow sludge blanket reactors may be used to treat meat-processing wastewater. [3.13]

The microbial conversion of carbonaceous organic matter in the anaerobic treatment process can be divided into three steps:

1. Enzyme-mediated transformation (hydrolysis), where higher-molecular-mass compounds are converted into compounds suitable for use as a sources of energy and cell carbon;
2. Acidogenesis, where selected groups of bacteria convert the compounds resulting from the first step into lower-molecular-mass intermediate compounds; and
3. Methanogenesis, where specific group of bacteria convert the intermediate compounds into simpler end products, mainly methane and carbon dioxide. [13,15]

Figure 2-2 summarizes the typical microbial process in anaerobic systems. [13,15] The reactor must be void of dissolved oxygen and free from inhibitory materials such as heavy metals to establish and maintain the dynamic equilibrium between nonmethanogenic and methanogenic bacteria in the anaerobic system.

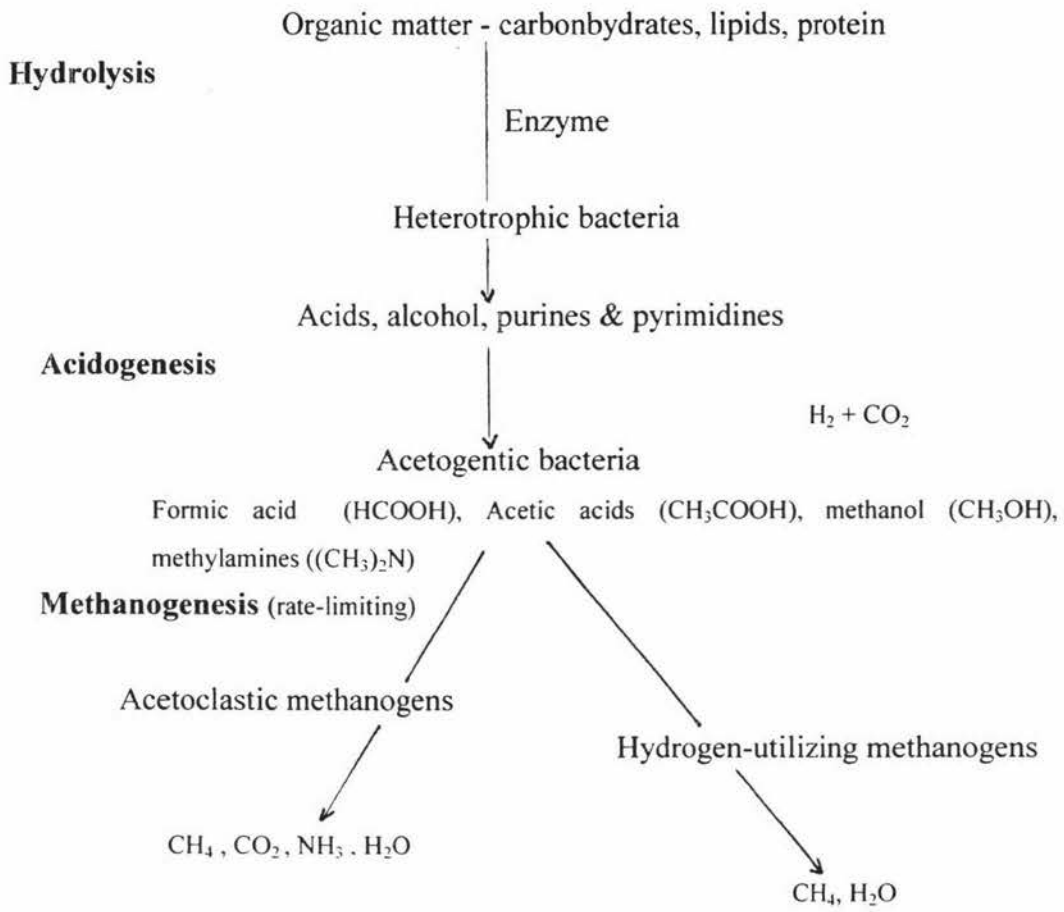


Figure 2-2 Microbial process in anaerobic systems [13,15]

The pH range of the aqueous environment in anaerobic reactors is optimum between 6.6 - 7.6. Sufficient alkalinity needs to be present to ensure the pH does not decrease below 6.2 as the methane bacteria cannot function below this point. Nutrients such as nitrogen and phosphorus need to be sufficient to ensure proper growth of the microbial populations. [15]

Advantages of anaerobic treatment: [3,13]

1. Low energy input requirement;
2. Most of the carbonaceous organic constituents in the wastewater can be converted to methane in the absence of oxygen;
3. Low production of excess sludge, low sludge to dispose of, and therefore low nutrient requirements;

4. High organic loading rates can be applied;
5. Acclimated sludge can be stored for long periods without deterioration.

Disadvantages of anaerobic treatment: [3,13,15,16,17]

1. Due to slow growth rates in anaerobic treatment systems, it requires a relatively larger reactor for long sludge retention time (SRT) in the digester to stabilize the waste;
2. Meat-processing wastewater from anaerobic treatment systems usually requires further treatment before being discharged. This is because the organic forms of nitrogen and sulfur are converted to ammonia and hydrogen sulfide. Both chemicals can be toxic to aquatic organisms; [16, 17]
3. Complete removal of nitrogen can not be achieved in anaerobic digester;
4. The anaerobic process is sensitive to factors such as finely dispersed colloidal material, excessive oil and grease, excessive fibrous material which adversely affect sludge settle-ability; anaerobic organisms are sensitive to inhibitants such as heavy metals, hydroxychloride, anionic detergents, high cation concentration including Na and Ca;
5. The causes of instability of the anaerobic processes have not yet been fully understood and the process has a reputation for failing and requiring longer startup time after failure.

2.2.1.2 Aerobic treatment and aerobic biological process

Aerated lagoons/ponds and activated sludge tanks have been widely used as aerobic systems to treat meat-processing wastewater. [5,34,35,36,37] The Sequential Batch Reactor (SBR), a time oriented periodic/unsteady state system, has also been used for meat-processing wastewater. [6,7]

Activated-sludge process (including SBRs), and aerated lagoons, have been classified as suspended-growth biological processes. [15] In this type of processes, microorganisms, which are responsible for the conversion of the organic matter or other constituents in the wastewater to gases and cell tissue, are maintained in suspension within the liquid.

In aerobic processes, the microbial culture carries out the bio-degradation of organic constituents. The bio-degradation process includes reactions of oxidation and synthesis and microbial decay, as explained below: [15, 14]

Oxidation and synthesis:

$$\underset{\text{(organic matter)}}{COHNS} + O_2 + \text{nutrients} \xrightarrow{\text{Bacteria}} \underset{\text{(new bacterial cells)}}{CO2 + NH_3 + C_5H_7NO_2} + \underset{\text{portion of influent organics)}}{\text{(SMP + nondegradable}} \quad \text{Soluble microbial products} \qquad (2\text{-}1)$$

Oxygen equivalence of decayed biomass:

$$\underset{\text{(New bacterial cells)}}{C_5H_7NO_2} + 5O_2 \xrightarrow{\text{Bacteria}} 5CO_2 + 2H_2O + NH_3 + \text{energy} + N + P + \text{SMPs} + \text{'bug bones'} \qquad (2\text{-}2)$$

The aerobic environment is created by means of mechanically mixing diffused oxygen (or air) with the liquor within the reactor. A completely mixed regime is usually maintained. After a specified period of hydraulic retention time (HRT), the bio-floc is conveyed to the secondary settling tank, where the biomass is separated by gravity from the treated wastewater. When sludge is recycled, a portion of the settled biomass returns to the aerobic reactor, so that one can maintain the desired concentration of biomass in the aerobic biological reactor. Another proportion is wasted to control the design sludge retention time (SRT). [15]

The aerated-lagoon process is similar to the activated-sludge process, except that settling usually happens within the aeration basin and no recycle is provided. Biomass concentrations in lagoon systems are usually much less than those for activated sludge treatment and a much longer hydraulic retention time (HRT) is required. The effluent quality is generally not as good as in the activated sludge process. [15]

Advantage of aerobic processes: [1.3.13.6.7.38]

1. Aerobic processes provide the oxygen required for microbial nitrification by which ammonia is converted first to nitrite and then to nitrate;
2. Provides a high quality effluent in terms of carbonaceous BOD and nitrogenous BOD;
3. Simplistic, low capital costs;

Disadvantages of aerobic processes [15. 39. 38. 40]

1. Aerobic waste-treatment processes are generally energy-intensive processes. Energy is required for the maintenance of adequate concentration of dissolved oxygen and for mechanical mixing;
2. Aerobic processes involve high sludge yields, high nutrient requirement, odor, and insect nuisance;
3. System performance is likely to be impaired by flow and load transients;
4. When dealing with strong wastes, system tends to be ineffective;
5. Remove little (20~25%) nitrogen.[38.40]

2.2.2 Microbial processes of nitrification

Microbial nitrogen removal can take place with alternating periods of aerobic and anoxic conditions. Organic nitrogen is firstly deaminated to ammonia nitrogen by enzymatic reactions. When sufficient oxygen is supplied, most of the ammonia nitrogen is then oxidized by nitrifying microorganisms (Nitrosomonas and Nitrobactors) to nitrite and nitrate, with a small portion of it being assimilated into microbial cells. When conditions are anoxic, denitrifiers convert oxidized nitrogen to nitrogen gas.

2.2.2.1 Microbial conversion of organic nitrogen to ammonia

The conversion of organic nitrogen to ammonia includes the following processes:

1. Proteinaceous materials (i.e. urea, muscle, blood) from waste streams are hydrolyzed to amino acids in the presence of proteolytic enzymes. [14] Some amino acids are used as building blocks in synthesis of cellular macromolecules, the others are bio-degraded to free ammonia nitrogen (NH_3-N) and ionic ammonia

nitrogen (NH_4^+-N);

2. D*eamination* is the predominant reaction for amino acids, resulting in the release of ammonia to the solution. [14] In the pH ranges of normal biological wastewater treatment processes, most nitrogenous organic compounds in incoming waste stream can be readily deaminated. [14]

3. Ammonia nitrogen, measured as NH_3-N and NH_4^+-N, mg/L, becomes the major dissolved nitrogenous constituent in meat-processing wastewater. It can be used in synthesis and removed by excess sludge (*assimilation*), or oxidized to nitrite-nitrogen (NO_2^--N, mg/L) and nitrate-nitrogen (NO_3^--N, mg/L), and removed via the denitrification process. [15. 14]

The percentage of free ammonia in total ammonia concentration is important in terms of toxicity and mass balance considerations. The equilibrium for reaction of $NH_3 + H_2O \Leftrightarrow NH_4^+ + OH^-$ shows that free ammonia increases with increasing pH and temperature. At pH values commonly encountered in most biological treatment processes, ionic ammonia normally predominates. [14]

2.2.2.2 Reactions involved in microbial nitrification

Nitrification

$$2NH_4^+ + 3O_2 \xrightarrow{\textit{Nitrosomonas}} 2NO_2 + 4H^+ + 2H_2O + \text{energy} \qquad (2\text{-}3)$$

$$2NO_2^- + O_2 \xrightarrow{\textit{Nitrobacteria}} 2NO_3^- + \text{energy} \qquad (2\text{-}4)$$

Therefore

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O \qquad (2\text{-}5)$$

$$\Delta G = -270 \text{ KJ/mole}$$

Winogradsky, 1890 [14 Chap.6] found that nitrification is associated with the metabolism of a group of chemoautotrophic bacteria obligate in their reliance upon inorganic nitrogen compounds for energy. *Nitrosomonas* species were observed to derive their

energy from the oxidation of ammonia to NO_2^-. *Nitrobactor* species were found to depend on NO_2^- oxidation as an energy source.

Heterotrophic nitrification also exists, [14 Chap.6] but these bacteria only oxidize ammonia nitrogen to nitrite, and nitrite is then consumed through assimilation. [14 Chap. 6] This microbial process is not considered significant in conventional biological wastewater treatment processes.

Nitrification by autotrophic bacteria is an aerobic process requiring inorganic carbon for cell synthesis.

2.2.2.3 Regulating nitrogen discharges

As illustrated earlier in this chapter, the impacts of nitrogen, particularly free ammonia, to the receiving aquatic environment include the impairment of dissolved oxygen balance, [17, 14] toxic effect on aquatic life, [14] biostimulation of plant and algal growth in surface water, [14] and public health threats. [17] Many nations have already set regulatory limits for nitrogen discharges to receiving water. Details of nitrogen discharge limits of several nations can be found in the references. [14, 17, 16]

2.2.3 Oxygen requirement in an activated sludge system for carbon removal

The oxygen requirement in an activated sludge system in regard of carbon removal can be described by the following equations:

For biomass growth:

Soluble organics + A' O_2 + N + P + cells → A (new cells) + CO_2 + H_2O + SMP (soluble microbe product) + ΔH (2-1-1)

For biomass decay:

B cells + B' O_2 → CO_2 + H_2O + N + P + non-degradable particulate cellular residue + SMPs (2-2-1)

where

A = mg of biomass produced per mg of COD removal, i.e. true growth yield coefficient, $Yo_{t\ (vss\ based)}$.

A'= mg O_2 utilized per mg of COD oxidized to end products (CO_2 and H_2O)

SMPs ≈ 2~20% of non-viable cellmass, including SRP and soluble biodegradable microbial decay product.

In the above equations, the biomass growth (+A) occurs at the expense of soluble substrate (-1); oxygen is comsumed in the metabolic process [-(1-A)] = A'. One can expect that 1.42($A_{(vss\ based)}$) + A' ≈ 1 (assuming COD of 1 Kg cellmass = 1.42kg; and because SMP only accounts for about 2~20% of non-viable cellmass, oxygen required for degradation of SMPs can be neglected).

The above equations show that only a fraction (A') of carbon substrate (measured as COD) provides electrons for energy use in metabolic activities, and becomes oxidized to CO_2, and an equivalent amount of oxygen is required (i.e. A') in the activated sludge system as electron acceptor. Most of the remaining fraction (A= 1-A') of carbon substrate is incorporated into cellmass. For domestic sewage, A' (i.e. Yo_t) was found to be 0.45 and 0.49 (mgVSS/mgCOD). [25, 26] When "death regeneration" decay (assume decay rate b_H = 0.54 day^{-1}) is taken into account, and 80 % of biomass cell is assumed to be biodegradable, a fraction of $0.45 \times 1.42 \times 0.54 \times 0.8 = 0.345$ (d^{-1}) substrate is released back to the system, leaving $0.45 \times 1.42 \times 0.54 \times 0.2 = 0.07$ (d^{-1}) as non-biodegradable residues in the system. When "endogenous respiration" decay (b'_H = 0.24 day^{-1}) is taken into account, a fraction of $0.45 \times 1.42 \times 0.24 \times 0.2 = 0.031$ (d^{-1}) becomes non-biodegradable residue in the system. This shows that the activated sludge system does not require 100% of the carbon substrate equivalent O_2 (measured as COD substrate) into the system. However, oxygen requirement has been considered to be the most essential factor in an activated sludge system. The measurement of oxygen consumption provides information such as the fractions of influent COD, [32,92] the maximum specific growth rate of heterotrophos, [32] coefficient for endogenous respiration b'_H, [92] concentration of heterotrophic biomass, [85,92] and hydrolysis constant K_h and the fraction of slowly biodegradable matters in the influent COD. [92]

2.2.3.1 Oxygen uptake and influent COD fractions in activated sludge systems

Ekama *et al.*, (1986) [32] conducted an aerobic batch test employing the oxygen uptake data for determining the readily biodegradable COD fraction in an activated sludge system. In their aerobic batch tests, a pre-selected volume of wastewater (V_{ww}) of

known total COD strength (S_{ti}) was mixed with a pre-selected volume of mixed liquor (V_{ml}) of known MLVSS (X_v) in batch reactors to create conditions with specified initial substrate over biomass (S_o/X_o) ratios. The oxygen uptake rate (OUR) was measured immediately after the mixing approximately every 5 to 10 minutes for about 4 to 5 hours. The observed oxygen uptake rate (OUR) from the start of the test remained high for a period of 1-3 hours depending on the readily biodegradable COD fraction, whereafter the OUR decreased fairly rapidly and leveled off at a second plateau level. Ekama *et al.* defined the initial high OUR being a consequence of the utilization of the readily biodegradable COD from the wastewater and that derived from hydrolysis of the particulate biodegradable COD.

In accordance with Ekama *et al.*, the constant OUR over the high plateau was due to the heterotrophs growing at the maximum level. The reason for specifying a appropriate initial S_o/X_o in these batch tests was because that at very high S_o/X_o ratios, the $X_s/X_{B,H}$ ratio (which defines the hydrolysis rate of slowly biodegradable COD) also was very high, so that hydrolysis rate operated at its maximum rate. The increased S_o/X_o made the step change from the first to the second OUR plateau much smaller and unclear. In an aerobic batch test, they used an initial S_o/X_o ratio of 0.41 (mgCOD/mgCOD) to obtain the OUR corresponding to the utilization of readily biodegradable COD.

The readily biodegradable COD fraction was related to the oxygen utilized in the consumption of this COD and is proportional to the area between the initial high OUR plot and a horizontal line projected to the vertical axis at the level of the second OUR plateau.

The calculation of readily biodegradable COD was as follows:

$$S_{si} = \{1/(1-Y_H)\} \bullet \Delta O \bullet (V_{ml} + V_{ww})/V_{ww} \qquad \text{(mgCOD/L)} \quad (2\text{-}6)$$

where

V_{ml} = volume of mixed liquor (at concentration of X_v mgVSS/L) (ml)

V_{ww} = volume of wastewater (ml)

S_{si} = influent biodegradable COD concentration of wastewater sample (mg COD/L)

ΔO = mass of oxygen utilized in S_{si} consumption per litre batch mixture (mg O/L), i.e. the area mentioned in the above paragraph

Y_H = yield coefficient, mg COD based biomass / mg COD substrate utilized

$1/(1-Y_H)$ = mg COD consumed per mg O utilized = 3 for $Y_H = 0.67$

or as suggested by Kappeler (1992): [92]

$$S_{si} = \left[\int r_{O_2,Total} - \int r_{O_2,Baseline-respiration}\right]/(1-Y_H) \qquad \text{(mg COD/L)} \qquad (2\text{-}7)$$

where

r_{O_2} = oxygen consumption at time t

Of the initial 530 mg COD /L of unsettled municipal wastewater added into a Ekama's aerobic batch test, 116 mg/L COD (i.e. a fraction of 22%) was defined to be readily biodegradable. In a Kappeker's batch-test estimation (with a S_o/X_o ratio, mgCOD/mgVSS, of 1 to 2), a fraction of 11 % of readily biodegradable COD was found for a 250 mg/L settled Switzerland domestic sewage. [92]

2.2.3.2 Oxygen uptake and the maximum specific growth rate of heterotrophs in activated sludge systems

Using the above OUR measurement, Ekama *et al.* (1986) [32] also determined the maximum specific growth rate of heterotrophs in an activated sludge system. The constant OUR over the high plateau was due to the heterotrophs growing at the maximum level. The maximum specific growth of the heterotrophs ($\mu_{H,MAX}$) was proportional to the vertical height of the initial high OUR (provided nitrification being inhibited) and was calculated as follows:

$$\mu_{H,MAX} = K_{ms} Y_{vssbased} \qquad (2\text{-}8)$$

where

K_{ms} = maximum readily biodegradable substrate utilization rate (mgCOD/mgAVSS/day)

$Y_{vssbased}$ = the yield coefficient of heterotrophos (=0.45 mgVSS/mgCOD)

The K_{ms} in equation (2-8) was calculated as below:

$$K_{ms} = \{1/(1-Y_H)\} \bullet OUR_i \bullet 24 \bullet (V_{ww} + V_{ml})/(f_{av} X_v V_{ml}) \qquad (2\text{-}9)$$

where

OUR_i = the initial high OUR in mgO/L/h

24 = number hours per day

X_v = MLVSS concentration of the mixed liquor added to the batch test (mgVSS/L)

f_{av} = the active fraction of the MLVSS

The f_{av} was calculated to be 0.44 by Ekama *et al.* [32] Details for calculating f_{av} fraction can be found in publications of Marais and Ekama (1976) and WRC (1984). [25, 180] The $\mu_{H,MAX}$ was determined to be 3.6/d in an aerobic batch test conducted by Ekama *et al.* (1986). [32]

2.2.4 Oxygen requirement in an activated sludge system for nitrification

Theoretically, the oxygen required for nitrification is 4.57gO_2 per g ammonium-nitrogen without consideration of biomass synthesis. In practice, the stoichiometry of nitrification is usually based on the observation that 4.33g O_2 are consumed for each gram of nitrate nitrogen formed. [19, 14] Consequently, an oxygen equivalent of biomass generation in nitrification condition, Y_N, g cell COD / g N, may be estimated as 4.57 - 4.33 = 0.24 gO_2/gN or $\frac{(4.57 - Y_N)}{Y_N} = \frac{(4.57 - 0.24)}{0.24} = 18.042\, {gO_2}/{gcellCOD}$. The value of $Y_N = 0.24\, {gO_2}/{gN}$ is currently accepted as the overall autotrophic yield characterizing nitrification. [19]

Given a suitable sludge retention time (SRT), nitrification can take place in an activated sludge system. An oxygen requirement of over 4-mg of oxygen per milligram of ammonia nitrogen oxidized to nitrate nitrogen shows the importance of including nitrification of oxygen demand in meat processing effluent.

2.2.5 Summary

The activated sludge process provides a means of preventing the oxygen depletion in waters caused by discharges of primary meat-processing wastewater, which contains high amounts of carbonaceous and nitrogenous oxygen demand.

2.3 Substrate Removal and Microbial Growth in an Activated Sludge System

2.3.1 Kinetics of bacterial growth in an activated sludge system

In 1950s, Eckenfelder and Weston (1956) [42] and Heukelekian *et al.* (1951) [43] described the rate of change of bacterial mass. The work combined the bacterial growth and decay as: [44]

$$\frac{dX}{dt} = -a\frac{dS}{dt} - bX \qquad (2\text{-}10)$$

where dX/dt = rate of change of bacterial mass (mg/L/day)

dS/dt = rate of change of substrate concentration (mg/L/day)

(Based on an assumption that the substrate concentration, S, representing a single nutrient, limits the rate of bacterial activity)

b = bacterial decay rate (day^{-1})

X = concentration of total biomass (mg/L)

a = yield constant (mg biomass /mg substrate)

If microbial growth occurs in a completely mixed batch culture, to which substrate is added only once at time zero, the mass of viable biomass with time can be approximated from integrating the above equation, by neglecting the biomass decay term, as follows: [44]

$$X = X_0 + a(S_0 - S) \qquad (2\text{-}11)$$

Where X_0 and S_0 are the initial viable biomass and substrate concentration respectively, X and S are biomass and substrate concentration at the end of some time period, t, and a is the yield coefficient.

Stratton and McCarty (1967) developed a general equation describing the decrease in substrate, as a function of time for microbial growth in a completely mixed batch culture: [44]

$$-\frac{1}{k}\{[\frac{-K_s}{X_0+aS_0}-\frac{1}{a}]\log_e[X_0+aS_0-aS]+[\frac{K_s}{X_0+aS_0}]\log_e[\frac{SX_0}{S_0}]+[\frac{1}{a}]\log_e X_0\}=t \qquad (2-12)$$

where

k = substrate utilization rate constant, mg substrate/ day per mg of biomass

K_s = half-saturation constant (mg/L)

X_0 = concentration of biomass at time zero (mg/L)

a = yield constant (mg biomass / mg substrate)

S_0 = concentration of substrate at time zero (mg/L)

t = time (days)

Equation 2-12 is the result of integration of Monod`s equation (equation 2-13). Provided that the appropriate parameters are known, this equation can be used to predict the decrease in substrate concentration. For nitrification, X_0 and S_0 in equation 2-12 are the concentration of nitrifying biomass and ammonia nitrogen substrate at time zero and X and S are these values at a period of time, t.

In summary, reliable kinetic constants such decay rate, rate of change of bacterial mass, rate of change of substrate concentration (or substrate utilization rate), yield coefficient, half-saturation constant are required for modelling the biomass growth in an activated sludge system.

2.3.2 Modeling substrate removal in an activated sludge system

Monod (1942) observed substrate removal in pure culture and described the rate of cell growth through the following expression, [45] which relates the specific organism growth rate to the concentration of substrate surrounding the organism:

$$\frac{1}{X}\frac{dX}{dt}=\mu=\mu_{MAAX}\frac{S}{K_s+S} \qquad (2\text{-}13)$$

where

μ = specific growth rate (day^{-1})

μ_{MAX} = maximum specific growth rate (day^{-1})

X = microorganism concentration (mg/L)

S = growth-limiting substrate concentration

K_s = half-saturation constant (mg/L)

Since Monod's equation (equation 2-13), there have been many suggestions with respect to the function of substrate removal. Garrett & Sawyer (1952) suggested that soluble BOD removed from sewage was a discontinuous function. [46] Eckenfelder (1966) suggested that soluble substrate (BOD_5) removal was a linear function. [47] Eckhoff and Jenkins (1967) suggested that soluble COD removal was a pseudo-first-order expression. [48] Tischler and Eckenfelder (1969) [49] presented a zero-order / non-linear scenario for removals of substrates such as glucose, phenol, and aniline. Stratton and McCarty (1967) [44] described the rate of substrate utilization by a non-linear function, based on an assumption that the substrate concentration (S) represents a single nutrient and its concentration limits the rate of bacterial activity, as follows:

$$dS/dt = -kXS/(K_s + S) \qquad (2\text{-}14)$$

where S = substrate concentration (mg/L)

t = time (days)

k = substrate utilization constant, mg/day per mg of biomass

K_s = half-saturation constant (mg/L)

X = total biomass (mg/L)

This is just a simple modification of the Monod equation, as elaborated in the following:

$$\mu = \frac{dX}{dt}\frac{1}{X} = \frac{\mu_{MAX} S}{K_s + S} \qquad (2\text{-}15)$$

$$\frac{dS}{dt} \bullet \frac{dX}{dS} = \frac{\mu_{MAX} SX}{K_s + S} \qquad (2\text{-}16)$$

$$\text{let } \frac{dX}{dS} = y \text{ , then } \frac{dS}{dt} = \frac{(\frac{\mu_{MAX}}{y})SX}{K_s + S} \qquad (2\text{-}17)$$

$$\text{define } k = \frac{\mu_{MAX}}{y}, \qquad (2\text{-}18)$$

$$-\frac{dS}{dt} = \frac{kSX}{K_s + S} \qquad (2\text{-}14)$$

2.4 Multi-Component Activated Sludge Models and Microbial Residues in an Activated Sludge System - A Conceptual Basis of Activated Sludge in this Study

2.4.1 Development of multi-component activated sludge models

Until 1970, Lawrence and McCarty [59] adopted the Monod equation and developed an early activated sludge model using biological solids retention time θ_c as an independent parameter in biological treatment process design and control. Andrews and Busby (1973) [54] proposed a function of adsorption and storage for substrate onto organism, i.e. substrate disappearing from the liquid phase. With this function, the rate of disappearance of substrate from the liquid phase is related to substrate concentration in the liquid and the maximum mass of substrate that can be stored onto the organism.

In 1976, Marais and Ekama (1976) [25] added two components, endogenous residue due to endogenous respiration and the accumulation of inert volatile solids due to the presence of this material in the influent, into above models. Marais and Ekama's model was developed for a steady state (under constant load and flow conditions) single completely mixed activated sludge reactor with domestic wastewater as influent. In their model, oxygen consumption was related to growth and endogenous respiration. Since then, the oxygen utilization rate has been recognized as a very sensitive parameter in terms of mass balance and system performance. Significant advances of this model include:

(1) the separation of active and inert fractions in wastewater;

(2) the expression of biological reactions in terms of active mass;

(3) the ability to determine the oxygen utilization rate directly from the biological reactions; and

(4) applying the adsorption and storage mechanism (Andrews and Busby (1973) [54]) in substrate removal process modelling.

In 1977, Ekama and Marais [26, 27] described the dynamic behavior of the completely mixed activated sludge process using a mathematical model. Their model was able to describe the response of single and multiple reactor process configurations under steady and cyclic loading conditions.

Dold *et al.* (1980) [28] developed a general activated sludge model that embedded several hypotheses for the activated sludge process:

(1) Bi-substrate hypothesis – Domestic wastewater contains two substrates (a rapidly biodegradable soluble and a slowly biodegradable particulate substrate). The biomass growth on rapidly biodegradable soluble substrate follows Monod (1942) equation; [45] while the biomass growth on the latter is related to the rate of extracellular enzymatic breakdown of large complex molecules to simple ones before transference through the cell walls;

(2) Extracellular growth-limiting reactions are modeled by active site theory;

(3) Decay is a death-regeneration process but not endogenous respiration process. The difference between these two processes will be explained later in this chapter.

Haandel, *et al.* (1981) [29] modified the model developed by Dold *et al.* (1980) [28] and extended it to include the behavior of the denitrification process in single sludge systems.

Ekama and Marais (1986) [30] applied the general activated sludge model developed by Dold *et al.* (1980) [28] and the extended modified model of Haandel, *et al.* (1981) [29] to an anoxic-aerobic activated sludge process.

Based upon the above modelling knowledge and experience, The IAWPRC Task Group (1987) [19] concluded a multi-component mathematical activated sludge model

(Activated Sludge Model No.1) that incorporated carbon oxidation, nitrification and denitrification. Many important concepts regarding activated sludge system were adopted in this model, such as:

(1) It is the balance between concentrations of electron donors (carbonaceous and nitrogenous organic substrate) and acceptors concentration (oxygen or nitrate) that mostly affects activated sludge concentration;

(2) By selecting appropriate process stoichiometry and estimating the electron acceptor requirements, the activated sludge process rate can be expressed, together with reliable kinetic constants, the concentration of activated sludge can be predicted;

(3) Switching functions can work effectively to turn process rate equations on and off as environmental conditions changed;

(4) The organic matter (carbonaceous and nitrogenous matter) in a wastewater may be subdivided into multi-components, such as non-biodegradable organic matter, which includes inert soluble organic matter (S_i) and inert suspended organic matter (X_i), and biodegradable organic matter, which includes readily biodegradable material (S_s) and slowly biodegradable material (X_s); Amongst these, soluble inert organic matter contributes to the effluent COD, while particulate inert organic matter becomes a part of the volatile suspended solids in the activated sludge system, and neither of them are involved in any conversion process;

(5) The volatile solids concentration in the activated sludge system is the sum of five particulate terms: slowly biodegradable substrate (X_s), active heterotrophic biomass ($X_{B,H}$), active autotrophic biomass ($X_{B,A}$), particulate residual arising from biomass decay (X_p), and particulate inert organic matter (X_i);

(6) Decay is assumed to result in the release of slowly biodegradable substrate (X_s) which is recycled back to soluble substrate used for cell growth (Dold *et al.* (1980)) [28] – so called "death – regeneration" decay model

Certain assumptions regarding substrate removal were made in the model of The Task Group (1987): [19]

- The Monod expression is suitable for the removal of readily biodegradable

substrate;

- A three-step mechanism of hydrolysis, adsorption and synthesis can be suitable for describing the utilization of slowly biodegradable substrate;
- The rate-limiting step was identified as hydrolysis and defined by expression:

$$\frac{dX_s}{dt} = -k_h \frac{X_s / x_a}{K_x + X_s / X_a} X_a \qquad (2\text{-}19)$$

where X_s = slowly biodegradable substrate concentration

X_a = active biomass concentration

k_h = maximum specific hydrolysis rate

K_x = half-saturation coefficient for hydrolysis

Activated Sludge Model No.2 (IAWPRC Task Group, Henze *et al.* (1994)) [31] was later presented to include additional biological processes to deal with biological phosphorus removal. However, phosphorus removal is not included in this study.

According to Görgün *et al.* (1995), [33] meat-processing wastewater is likely to contain high percentage of slowly biodegradable substrate. Understanding the activated sludge behavior associated with slowly biodegradable substrate can be helpful for meat-processing wastewater treatment in the activated sludge system.

The above multi-component models are well structured to enclose all types of substrate fractions, including the readily biodegradable and slowly biodegradable substrate. Therefore, the Activated Sludge Model No.1 is a conceptual basis of this study regarding substrate characterization and the activated sludge process evaluation.

The development of modeling the substrate utilization and active mass growth with respect to slowly biodegradable substrate can be found in literature. [27,50,51,28,19,52,53, 54,55,56]

By making critical corrections on the previous developed model of Busby, [56] which described the formation of active biomass from substrate stored by active biomass,

Strenstrom (1975) [51] proposed that the growth rate regarding stored biodegradable substrate to be described by the following equation:

$$r_{X_a} = R_{X_a}\left(\frac{f_s}{K_{fs} + f_s}\right)X_a \qquad (2\text{-}20)$$

where

r_{X_a} = active mass production rate (mg /L/T) for stored (i.e. slowly biodegradable) substrate

R_{X_a} = maximum specific growth rate (T^{-1}) regarding slowly biodegradable substrate

f_s = fraction of sludge mass which can be stored, stands for X_s / X_a

K_{fs} = half-saturation coefficient for stored substrate

The above expression of Strenstrom (1975) [51] later led to the rate expression for hydrolysis of slowly biodegradable substrate in the Task Group Model No. 1: [19]

$$r_{hydrolysis} = k_h \frac{X_s / X_{B,H}}{K_x + \left(X_s / X_{B,H}\right)}\left[\left(\frac{S_o}{K_{O,H} + S_o}\right) + \eta_h\left(\frac{K_{O,H}}{K_{O,H} + S_o}\right)\left(\frac{S_{NO}}{K_{NO} + S_{NO}}\right)\right]X_{B,H} \qquad (2\text{-}21)$$

where

$r_{hydrolysis}$ = biomass production rate for hydrolysis of slowly biodegradable substrate

k_h = hydrolysis constant

K_x = half-saturation coefficient

$X_{B,H}$ = active heterotrophic biomass (mg COD/L)

$K_{O,H}$ = half-saturation coefficient for oxygen (mg COD/L)

K_{NO} = half-saturation coefficient for nitrate and nitrite (mg COD/L)

S_O = oxygen (mg/L)

S_{NO} = nitrate and nitrite (mg/L)

η_h = correction factor for anoxic hydrolysis

The $X_{B,H}$ in equation 2-21 is corresponding to the X_a in equation 2-20, the $X_s/X_{B,H}$ in equation 2-21 corresponds to the f_s in equation 2-20, the K_x in equation 2-21 corresponds to the K_{fs} in equation 2-20, and the term of k_h $[(\frac{S_o}{K_{O,H}+S_o}+\eta_h\left(\frac{K_{O,H}}{K_{O,H}+S_o}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)]$ in equation 2-21 is corresponding to the R_{X_a} in equation 2-20.

Therefore, it is rational to assume that activated sludge growing on unfiltered meat-processing wastewater, which was found to have a high COD content with a predominant slowly biodegradable fraction, follows a expression which is similar to the above expressions regarding the utilization of slowly biodegradable substrate. [51, 19] To justify this assumption, the author will examine if the microbial growth on unfiltered meat-processing wastewater in an activated sludge system will follow a modified Strenstrom's growth rate expression.

2.4.2 Decay in an activated sludge system

Before Marais and Ekama (1976) [25] carried out an extensive investigation into aerobic digestion of activated sludge, the traditional concept of decay of biomass was "endogenous respiration." This concept described the decay of biomass by the equation:

$$dX_H/dt = -b'_H \bullet X_H . \qquad (2\text{-}22)$$

Where

X_H = concentration of heterotrophic biomass (mg/L)

b'_H = traditional decay rate of heterotrophic biomass (day^{-1})

t = time (days)

In "endogenous respiration" concept, a fraction of the biomass is unbiodegradable and remains as endogenous residue, i.e. $dX_{ex}/dt = f_{ex}b'_H X_H$, where X_{ex} is concentration

of endogenous residue and f_{ex} is the proportion of this unbiodegradable residue. The net mass loss of biomass was directly equated to oxygen consumption for endogenous respiration as following:

$$dO_2 / dt = -(1 - f_{ex}) b'_H X_H \qquad (2\text{-}23)$$

Dold *et al.* (1980) [28] proposed a new concept of biomass decay-regeneration. The most important point in this new concept is that except for the nonbiodegradable residue, the rest of the dead biomass will be released back into the liquid and become a portion of slowly biodegradable substrate in the system, and later be synthesized to new biomass through microbial respiration process. The processes include decay of biomass, release of nonbiodegradable residue and slowly biodegradable substrate, synthesis of new biomass, and oxygen consumption, i.e.

For decay of biomass:

$$dX_H / dt = -b_H X_H \qquad (2\text{-}24)$$

where

b_H = decay rate of heterotrophic biomass in death regeneration model

For release of slowly biodegradable substrate:

$$dX_{s,fromdeadX_H} / dt = (1 - f_{px}) b_H X_H \qquad (2\text{-}25)$$

where

f_{px} = fraction of nonbiodegradable residue in death regeneration model

X_s = concentration of slowly biodegradable substrate

For synthesis of new biomass:

$$dX_{H,new} / dt = Y_H \bullet (1 - f_{px}) b_H X_H \qquad (2\text{-}26)$$

where

Y_H = yield coefficient of heterotrophic biomass

For oxygen consumption:

$$dO_2 / dt = -(1 - Y_H)(1 - f_{px}) b_H X_H \qquad (2\text{-}27)$$

Under steady state conditions, the "endogenous respiration" and "death regeneration" decay models should yield the same residue and consume the equal amount of oxygen, which means:

Equating the residue of the tow models:

$$f_{ex} b'_H X_H = f_{px} b_H X_H \qquad (2\text{-}28)$$

and

Equating the oxygen consumption of the two models: (Equation 2-23 and 2-27)

$$-(1 - f_{ex}) b'_H X_H = -(1 - Y_H)(1 - f_{px}) b_H X_H \qquad (2\text{-}29)$$

The decay rate (b_H) and the fraction of nonbiodegradable residue (f_{px}) in the "death regeneration" decay model can be calculated from the following equations by knowing the decay rate (b'_H) and the proportion of unbiodegradable residue (f_{ex}) in the "endogenous respiration" decay model:

$$b'_H / (1 - Y_H (1 - f_{px})) = b_H \qquad (2\text{-}30)$$

and

$$[(1 - Y_H)/(1 - Y_H \bullet f_{ex})] f_{ex} = f_{px} \qquad (2\text{-}31)$$

The above evolution was detailed by Dold *et al.* (1980). [28]

Marais *et al.* (1976) [25] concluded a f_{ex} value of 0.2 and a b'_H value of 0.24 day^{-1} (based on traditional decay theory). On the other hand, Task Group Model No.1 [19] suggested f_{px} value to be 0.08 and b_H value to be 0.62 day^{-1} (death-regeneration decay theory). The discrepancy in the values of the similar rate parameters is due to the adoption of different decay models, i.e. the f_{ex} and b'_H are for the endogenous respiration model, while the f_{px} and b_H are for the death-regeneration model. The significance of f_{ex}, the proportion of unbiodegradable residue (X_{ex}, in terms of the "endogenous respiration" decay), and f_{px}, the fraction of nonbiodegradable residue

(X_{px}, in terms of the "death regeneration" decay), will be reviewed in the following section.

The concepts of the two decay models are illustrated in Figure 2-3

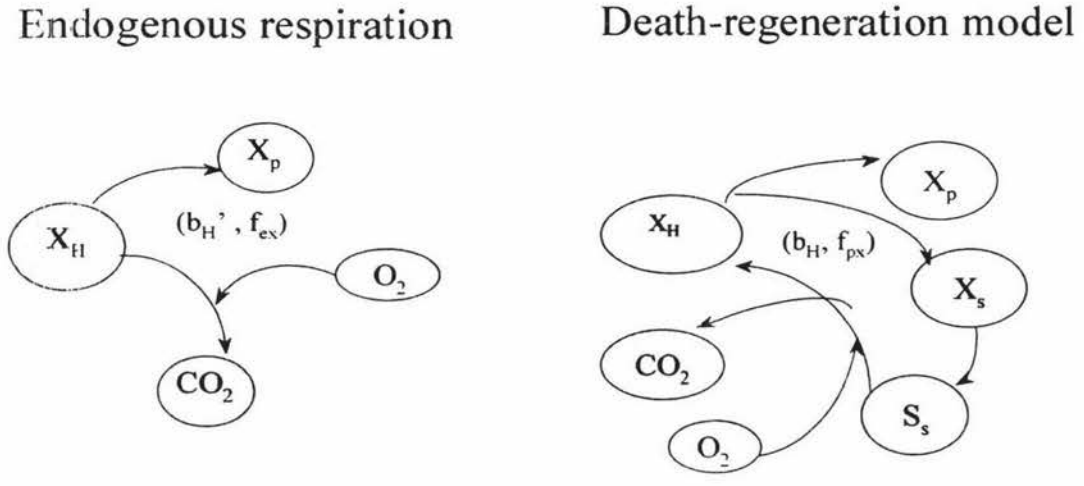


Figure 2-3 The concepts of activated sludge decay models, drawn from the contents of Dold *et al.* [28]

2.4.3 Microbial products in an activated sludge system

2.4.3.1 Particulate microbial residual, X_{px} or X_{ex}

Washington and Symons (1962) [60] indicated that VSS measurements contained a fraction that is inert to microbial activity. Mckinney (1962) [24] agreed and defined this fraction as "particulate residual organic matter originating in bacterial cells and released to the solution during the course of endogenous metabolism." He suggested that this inert fraction (f_{ex}) was 0.2~0.25. McCarty and Brodersen (1962) [61] noted that a certain percentage of synthesized mass is not endogenously respired but builds up in the reactor. Weddle and Jenkins (1971) [62] added that "particulate microbial residual" is a viability parameter that varied with the sludge age of the system. Busby (1973) [56] used the following expression to account for this microbial inert residue:

$$r_{XI} = Y_{xp} R_{XI} X_a \qquad (2\text{-}32)$$

where

r_{XI} = inert residue production rate (mg VSS/L/day)

Y_{xp} = yield coefficient, mass of volatile inert residue produced per unit active mass destroyed

R_{XI} = specific microbial decay rate (day^{-1})

X_a = concentration of active biomass (mg VSS/L)

The Y_{xp} in equation 2-32 has the similar meaning of f_{ex} in Mckinney's [24] definition and the f_{px} definition in the Task Group Model No.1. [19] Knowing the value of Y_{xp} (or f_{ex}, f_{px}) and R_{xi} (or b'_H, b_H), the designer can use this equation to anticipate the amount of inert residue produced from the activated-sludge decay process.

2.4.3.2 Soluble microbial residual products, S_p, or SRPs

Grady and Williams (1975) [63] suggested that soluble COD in the effluent appears not to be a fraction of the influent substrate, but could be organic matter released through microbial activities. Daigger & Grady (1977) [64] reviewed the information related to organic generation by microbial activities and noted that specific substrate tests coupled with COD measurements allow the determination of the amount of residual organic products. Chudoba (1985) [65] noticed that a portion of this soluble organic matter is refractory or at least very slowly biodegradable.

Orhon *et al.* (1989) [66] supported that a significant portion of the soluble effluent COD is non-biodegradable and described the SRPs formation to be from the hydrolysis of the decayed biomass. That is:

The active biomass X_a loses its viability and is converted into X_{px} (inert particulate residue) and X_s (slowly biodegradable particulate organic matter). The X_s is then hydrolyzed into S_s (readily biodegradable organic matter). In hydrolysis process, only a fraction of the particulate matter is converted into readily degradable substrate, the remaining becomes soluble and forms SRPs at the rate as follows:

$$\frac{dS_p}{dt} = \beta \bullet f_{cv} \bullet k_h \frac{X_s / X_B}{K_X + X_s / X_B} X_B \qquad (2\text{-}33)$$

where

β = stoichiometric coefficient, or Y_{sp}

f_{cv} = conversion factor (mg COD/mg VSS)

k_h = hydrolysis rate constants

K_X = half-saturation constant for growth on slowly biodegradable organic matter

Orhon *et al.* (1989) [66] found the level of residual COD to be directly associated with microbial decay. They indicated that around 10% (= β or Y_{sp}) of the hydrolyzed non-viable organic matter was the source of SPRs.

Artan *et al.* 1990 [67] considered that Task Group Model is inadequate in establishing a relationship between influent and effluent COD, and introduced the soluble residual products (SRPs) in a modified model.

In summary, it has been recognized that the presence of SPRs is important in modeling and the system performance evaluation of the activated sludge process.

2.4.4 Summary

The above multi-component models, particularly the Activated Sludge Model No.1, [19] including its adopted concepts and assumptions of activated sludge, and the concept of microbial residual production developed in the literature form the conceptual basis of the activated sludge in this study.

2.5 Reactor Configuration in Activated Sludge Systems

There are a number of parameters found to be associated with the performance of activated sludge that should be considered when configure an activated sludge system.

2.5.1 Reactor hydraulics

Mckinney (1962) [24] identified "mean hydraulic detention time" as one of the components involved in substrate removal mechanism, and found that the increase of "mean hydraulic detention time" will reduce the effluent BOD_5:

$$S = \frac{S_1}{1 + k_{mc} t} \qquad (2\text{-}34)$$

where S = effluent BOD_5

S_1 = influent substrate concentration

k_{mc} = rate constant from substrate removal

t = mean hydraulic detention time

In this equation, the substrate concentration was considered as zero-order with regard to biomass concentration.

By experimental observations, Eckenfelder (1966) [47] added biomass concentration into the above expression showing that the higher concentration of biomass will also reduce the effluent BOD_5:

$$S = \frac{S_1}{1 + k_{ec} t X} \qquad (2\text{-}35)$$

where k_{ec} = rate constant

X = MLVSS concentration representing biomass

Both of the above two equations were empirical models.

Pearson (1966) [57] also integrated hydraulic detention time into an expression of specific substrate removal rate, as following:

$$q = \frac{S_1 - S}{\theta_h X} \qquad (2\text{-}36)$$

where $q = {}^{mgBOD_5 orCOD}/_{mgVSS \bullet day}$, specific substrate utilization rate

S_1 = influent substrate concentration ($mgBOD_5$ or COD/L)

S = effluent substrate concentration ($mgBOD_5$ or COD/L)

θ_h = V/Q (day)

X = MLVSS concentration (mg/L)

In this equation, biomass concentration in the reactor and hydraulic retention time (HRT) both affect the rate of substrate removal in an activated sludge system. In conditions without inhibitors or other limitations, the longer HRT and/or higher X, the higher substrate removal rates. However, substrate becomes limited with increasing HRT. Metcalf & Eddy [15] suggested a HRT of 3-5 hour for complete-mixed activated sludge.

2.5.2 Loading factor

Ruchhoft and Smith (1939) [14 Chap.1] first defined a loading parameter (kgBOD/kgSS.day) which is based on the activated sludge. Heukelekian *et al.* (1953) [14, Chap. 1] also suggested (influent mgBOD)/(mgSS) to be a loading parameter. Both low organic loading rates and high organic loading rates have been found related to filamentous bulking. [115-118] Chiesa and Irvine (1985) [118] concluded that bulking could occur at the same treatment plant at different times throughout the year unless a strong selective pressure that favors floc formers over filaments is imposed. A proper balance of feast and famine conditions was found to be a key factor for controlling settleability of activated sludge. [118]

To evaluate the kinetics associated with commonly encountered nonfilamentous bacteria, monitoring the loading rate in activated sludge is important. For batch studies, the S_0 / X_0 ratio can be an indicator of loading factor. Chudoba et al. [69] found that at the $S_o/X_o = 8$ with glucose and $S_o/X_o = 8.5$ with alanine the synchronized division took place, as indicated by a break point on COD removal line. For continuous complete-mixed activated sludge systems, a value of F/M ratio = 0.2~0.6 (kgBOD5/kgMLVSS/d) was suggested by Metcalf & Eddy. [15]

Kappeler (1992) [92] suggested batch tests to be carried out with a S_o/X_o ratio

(mgCOD/mgVSS) of approximately 1 to 20 for estimating the maximum specific growth rate of heterotrophos $\mu_{H,MAX}$ for an activated sludge fed with settled domestic sewage.

Batch tests carried out with an appropriate range of S_o/X_o can be important for evaluating kinetics such as Y_H and $\mu_{H,MAX}$. Chudoba *et al.* [69] described the effects of S_o/X_o on the value of yield coefficient, as presented in later section of "Bio-kinetic constants for carbon removal in an activated sludge system - Y_H and S_o/X_o."

2.5.3 Sludge age

Grould (1949) [14 Chap.1] first introduced the concept of sludge age. It was defined as mgSS/mgSS added per day. Garrett & Sawyer (1952) and Pearson (1966) later presented an expression describing the net bacterial growth rate: [46, 57]

$$\frac{1}{\theta_x} = Yq - K_d \qquad (2\text{-}37)$$

where $\frac{1}{\theta_x}$ = the net specific growth rate (day^{-1})

θ_x = the maximum solids residence time, or sludge age (day)

Y = the yield coefficient (mgVSS/mg COD)

K_d = unit solids decay rate (day^{-1})

q = specific substrate utilization rate, $mgCOD)/mgVSS \bullet day$

The above expression relates substrate removal with growth and decay. In other words, $\frac{1}{\theta_x}$ represents the net growth of biomass in an activated sludge system.

Jenkins & Garrison (1968) and Lawrence & McCarty (1970) later adopted the concept of sludge age as one of the key parameters for modeling and designing the activated sludge model. [58, 59] A sludge age of 5-15 days is preferable for complete mixed activated-sludge processes. [15] To ensure the desired sludge age, returned activated-sludge with a flowrate of 50 – 100 percent of the wastewater flowrate is normally required for large plants and up to 150 percent of the wastewater flowrate for small plants. [15]

2.5.4 Reactor configuration and nitrogen removal

Nitrogen removal in primary treatment is merely the settling of particulate matter. Conventional activated sludge systems are designed mainly to function as a microbial assimilation process, and such a system, it is unlikely to achieve good nitrogen removal. Therefore, nitrification/denitrification processes must be included in process design to achieve effective biological conversion and removal of nitrogen. [14]

2.6 Bio-kinetic Constants for Carbon Removal in an Activated Sludge System

2.6.1 Yield coefficient (Y_o or Y_H) and S_o/X_o

The growth yield coefficient, Y_H, mg biomass COD/mg substrate COD, stands for the fraction of the COD metabolized and converted to new cell mass, i.e. the fraction "A" in equation 2-4. Payne (1970) [68] provided evidence showing that this yield coefficient is constant with respect to the COD utilized. Chudoba *et al.* (1992) [69] suggested that the observed "yield" decreases with increasing S_o/X_o ratio, and explained the phenomenon by a theory that the quantity of substrate oxidized per unit of biomass synthesized can be greater for a growth response than for a storage response. This exploration was based on certain theories and findings referenced by Chudoba *et al.* (1992), [69] as summarized below:

(1) Microbial growth reflects the increase in biomass with the increase in cell number (i.e. multiplication) or the increase in storage or accumulation (without the increase in cell number);

(2) Microbial storage or accumulation involves only the transport of substrates, a small portion of oxidation, and the synthesis of the storage polymers;

(3) When microbial growth was supplied with limited amount of carbon and energy source (i.e. at low S_o/X_o ratio), storage process was usually observed, and only negligible cell multiplication took place. The increases in weight of cellmass (mostly due to storage) were not at the expenses of high substrate removal. Consequently, a higher yield would be occurred;

(4) When a low amount of biomass was supplied with a higher quantity of substrate, increasing rates of substrate removal and biomass growth were observed. The cell multiplication was found to be a high-energy demanding process, especially for the formation of proteins, thus, the yield coefficients would be expected to be small.

2.6.2 Carbon removal kinetic constants and temperature dependence

Values of kinetic constants for conditions at $20^{\circ}C$ and their temperature dependence for a steady state carbonaceous degradation activated sludge model have been suggested by Marais and Ekama (1976) as following: [25]

Yield coefficient (Y_H, mgVSS/mgCOD) = 0.45, and is temperature independent;

Heterotrophic organism endogenous respiration rate (b'_H, day^{-1}) = 0.24;

Temperature dependency equation for endogenous respiration rate:

$$b'_{HT} = b'_{H20}\,\theta^{(T-20)},\ \theta = 1.029$$

Endogenous residue fraction (f, mgVSS/mgVSS) = 0.2

COD/VSS ratio (f_{cv}, mgCOD/mgVSS) = 1.42

Ekama and Marais (1977) [26] suggested kinetic constants for use in activated sludge processes at conditions of 20°C as follows:

Yield coefficient (Y_0, mgVSS/mgCOD) = 0.49

Endogenous respiration rate (b'_H, day^{-1}) = 0.24

Death regeneration decay rate (b_H, day^{-1}) = 0.54

Fraction of inert particulate influent (f_{Xi}) = 0.09

Fraction of inert soluble COD in the influent (f_{si}) = 0.05

Fraction of cellmass which is unbiodegradable in terms of endogenous respiration decay concept (f'_{px}) = 0.20

Fraction of cell mass which is unbiodegradable in terms of death regeneration decay concept (f_{px}) = 0.09

Half-saturation coefficient for substrate entering into storage (K_{sl}, mgCOD/L) =150.0

Half-saturation coefficient for COD released from storage for cell synthesis (K_s, mgCOD/L) = 100.0

Dold *et al.* (1980) [28] and Haandel *et al.* (1981) [29] used Y_h(mgVSS/mgCOD) = 0.45, p (COD:VSS ratio, mgCOD/mgVSS) =1.48, f' (the fraction of the active mass X_a that

disappears is unbiodegradable and remains as endogenous residue in terms of endogenous respiration decay concept) = 0.2, b_H (traditional decay rate, day^{-1}) = 0.24 K_s (half-saturation constant for growth on soluble COD, mgCOD/L) =20, and K_{ms} (the maximum specific soluble substrate utilization constant, mgCOD/mgVSS/d) =8 in their model for the activated sludge process as values for 20°C. By adopting the death regeneration decay concept (see equations 2-24~ 2-27), they also produced the values for kinetics such as b_H =0.62 d^{-1} and f (the fraction of the active biomass X_a that disappears is unbiodegradable and remains as endogenous residue in terms of death regeneration decay concept) = 0.08.

To the above values for kinetic constants, Warner *et al.* (1986) [30] added K_{msa} (the maximum specific readily biodegradable COD utilization rate for active biomass) = 5.6 mgCOD/mg activeVSS/day, $\mu_{H,MAX}$ (the maximum specific growth rate, day^{-1}) = $K_{msa}Y_h$ = 5.6*0.45 = 2.5day^{-1}, and K_{ss} (half-saturation coefficient for readily biodegradable COD utilization) = 5 mg COD/L for use in application of the general kinetic model to anoxic – aerobic digestion of activated sludge.

The Task Group Model No. 1 [19] recommends the following values including Y_H = 0.67 mg COD/ mg COD, f_p=0.08, $\mu_{H,MAX}$ = 6 day^{-1}, b_H = 0.62 day^{-1}, and K_{ss} = 20.0 mgCOD/L for carbonaceous organic biodegradation at $20^{\circ}C$ for modelling the activated sludge.

2.7 Bio-kinetic Constants for Nitrification Process

2.7.1 Yield constants and microbial synthesis

McCarty (1964) [70] suggested the theoretical magnitudes of nitrifying biomass yield constants, Y_{NH3-N} and Y_{NO_2-N}, to be 0.29 mg *VSS*/ mg NH_3-*N* for ammonia oxidation and 0.084 mg *VSS*/mg NO_2-*N* for nitrite oxidation when based on fresh culture, i.e. microorganism decay is negligible. Dold *et al.* (1980) [28] used Y_N = 0.10 mg VSS/mg N for modelling nitrification in the activated sludge process. Task Group No.1 [19]

suggested Y_N = 0.24 mg cell COD formed per mg N degraded (at 20°C). Assuming a bacterial cell composition of 10.9 % nitrogen, the quantity of nitrogen used for cellular synthesis by the nitrifiers represents approximately only 4% of the total nitrogen oxidized. [44,70] Nitrogen utilization due to microbial synthesis could therefore be neglected for integrating the net change in rate of nitrite production. [44]

2.7.2 Substrate utilization constant and half-saturation constant in nitrification

In nitrification, the substrate utilization constant, k, mg substrate/day/mg biomass, and half-saturation constant, K_{NH}, mg substrate/L, are functions of environmental conditions (i.e. pH, temperature, and DO), composition of wastewater, and strain of nitrifying microorganism. The concentration of viable nitrifying organisms is affected by the immediate history of the wastewater under study. For example, activated sludge from highly nitrifying plant contains more viable nitrifying organisms, while from a low nitrification system there is only small amount of nitrifying organisms. [44] Dold *et al.* (1980) [28] used K_{NH}=1.00 mg/L for modelling nitrification in the activated sludge process. This small value indicates that in a nitrification activated sludge system, when substrate (ammonia-nitrogen) is available, the growth of nitrifying organisms reaches the maximum growth rate very shortly, i.e. $\mu_N \approx \mu_{N,MAX}$.

2.7.3 Alkalinity consumption in nitrification process

Since in a nitrification process pH tends to decrease due to proton release, alkalinity is required to maintain the range of pH.

According to the stoichiometric equation,

$$NH_4^+ + 1.856O_2 + 1.98HCO_3^- \rightarrow 0.021C_5H_7NO_2 + 0.98NO_3^- + 1.876CO_2 + 2.92H_2O \quad (2\text{-}38)$$

nitrification will consume 1 mole HCO_3^- per mole of $NH_3 - N$ incorporated into cells, and 2 moles HCO_3^- per mole $NH_4^+ - N$ oxidized. Therefore, alkalinity consumed will be 7.07g $CaCO_3 / gNH_4^+ - N$. [14 Chap.6]

2.7.4 Sludge retention time of nitrification

The growth rate of nitrifying bacteria is slower than that of heterotrophic microorganism. It is important to have sufficient sludge retention time (SRT) to sustain a specific amount of nitrifying biomass. A sludge retention time greater than that needed for heterotrophic microorganisms is typically necessary. An equation for $SRT_{nitrification}$ calculation is:

$$SRT_{nitrification} = \frac{1}{(\mu_{N,MAX} - b_N)} \qquad (2\text{-}39)$$

where $SRT_{nitrification}$ = nitrification system sludge retention time (days)

$\mu_{N,MAX}$ = maximum specific growth rate of nitrifying biomass (day^{-1})

b_N = decay rate of nitrifying (mgNitrifyingVSS/mgNitrifying VSS/day)

Dold *et al.* (1980) [28] used $\mu_{NMAX20} = 0.62$ day^{-1} and $b_{N20} = 0.04$ day^{-1} for modelling nitrification of unsettled wastewater in the activated sludge process. The rate kinetics are affected by environmental conditions in the system as detailed in the following section.

2.7.5 Environmental factors associated with nitrification

Environmental factors such as temperature, pH, and the concentration of dissolved oxygen (DO) affect nitrification growth kinetics. It has been accepted that a Monod-type function can be used for calibrating these environmental factors on the growth rate of nitrifying organisms. [19]

The equation $\mu_N = \mu_{N,MAX} \frac{NH_3 - N}{K_{NH_3-N} + NH_3 - N} \bullet \frac{DO}{K_{DO} + DO}$ (2-40) can be used for DO correction,

where

μ_N = the specific growth rate of nitrifying organisms (day^{-1})

$\mu_{N,MAX}$ = the maximum specific growth rate of nitrifying organisms (day^{-1})

K_{NH_3-N} = half-saturation constant for ammonia-nitrogen ($mgNH_3$-N/L)

K_{DO} = half-saturation constant for dissolved oxygen (mgO_2/L).

For temperature correction, an Arrhenius temperature dependency coefficient, $\theta = 1.123$, has been suggested for pH value between 7.2 and 8.0. [90, 28] Temperature correction can be added into equation 2-40 and forms the following expression:

$$\mu'_{N,T} = \mu_{N,MAX} \frac{NH_3 - N}{K_{NH_3-N} + NH_3 - N} \bullet \frac{DO}{K_{DO} + DO} \bullet \theta^{(T-20)} \qquad (2\text{-}41)$$

where T = operating temperature (°C)

$\mu_{N,T}$ = growth rate under the stated conditions of temperature and dissolved oxygen (day^{-1})

Bickers (1996) [114] developed an average value of θ to be 1.083 with a 95% confidence interval for the temperature dependency coefficient of nitrification for meat processing wastewater.

The growth rate of nitrifying organisms, $\mu'_{N,T}$, is also influenced by pH. When pH is between 7.2 and 9.0, another correction factor of $[1 - 0.833(7.2 - pH)]$ can be added into above equations, as presented by McCarty & Eddy: [15 p.702]

$$\mu'_{N,T,pH} = \mu_{N,MAX} \frac{NH_3 - N}{K_{NH_3-N} + NH_3 - N} \bullet \frac{DO}{K_{DO} + DO} \bullet \theta^{(T-\theta)}[1 - 0.833(7.2 - pH)] \qquad (2\text{-}42)$$

where $\mu'_{N,T,pH}$ = growth rate under the stated conditions of temperature, DO, and pH (day^{-1})

$\mu_{N,MAX}$ = maximum growth specific growth rate of nitrifying organisms (day^{-1})

T = operating temperature (°C)

DO = dissolved oxygen (mgO_2/L)

K_{DO} = dissolved-oxygen half-saturation constant, usually taken as 1.3

pH = operating pH

θ = Arrhenius temperature dependency coefficient

Other form of pH correction coefficient has also been reported, such as θ_{pH} = 2.350. [28] In practice, Eckenfelder [71] suggested to apply a safety factor of 1.5 to 2.5 to provide sufficient sludge resident time so that nitrification can effectively occur.

2.7.6 Evaluation of nitrification kinetics

2.7.6.1 Measuring the rates of nitrification

It has been observed that the conversion of ammonia to nitrate follows a zero order reaction and the maximum nitrification rate can be estimated from the slope of the plot of the nitrate formation during the experimental periods. [91] Respirometric methods have also been used to characterize heterotrophic and nitrifying biomass in activated sludge samples. [92, 93, 94, 95, 96] Measuring the pH is another alternative in monitoring biological nitrogen removal processes. [96] This is because nitrification as well as denitrification influences the pH of the mixed liquor by the destruction and formation of alkalinity respectively. [17] Gernaey *et al.* (1998) [96] reviewed research on the pH profile of nitrification and denitrification in activated sludge systems, and conducted titration experiments for estimation of *Nitrosomonas* kinetic parameters.

2.7.6.2 Evaluation of the maximum specific growth rate of nitrifying microorganisms

The growth rate of nitrification microorganisms (mixed nitrifying biomass, including both *Nitrosomonas* and *Nitrobactor*) has been reported in the range of 0.34 to 0.65 day-1 by monitoring the removal of ammonia nitrogen. [19] A value of 0.86 day^{-1} for the maximum specific growth rate for nitrification biomass was used by the task group in modelling the activated sludge for domestic sewage at 20°C and pH = 7. [19] A value of 0.60 day^{-1} for the maximum specific growth rate of nitrifying biomass at 20°C (μ_{nm20}) was used by Warner et al. (1986). [30] By monitoring the production of oxidized nitrogen, Sözen and Orhon (1996) [99] assessed the net specific growth rate of nitrifying microorganisms for meat-processing wastewater to be 0.57 and 0.63 day^{-1} at $20^{\circ}C$.

The equations describing growth of nitrifying microorganism in an aerobic batch reactor are as following: [19, 100, 99]

$$\frac{dS_{NO}}{dt} = \frac{\mu_{N,MAX}}{Y_N} \frac{S_{NH}}{K_{NH} + S_{NH}} X_N \qquad (2\text{-}43)$$

where

S_{NO} = concentration of oxidized nitrogen

$\mu_{N,MAX}$ = the maximum specific growth rate of nitrifying biomass

Y_N = yield coefficient of nitrifying biomass

S_{NH} = concentration of ammonia nitrogen

K_{NH} = half-saturation constant for ammonia nitrogen removal

X_N = concentration of nitrifying biomass

When S_{NH} is high enough not to be rate limiting, equation 2-43 becomes:

$$\frac{dS_{NO}}{dt} \approx \frac{\mu_{N,MAX}}{Y_N} X_N \qquad (2\text{-}44)$$

and the growth rate of nitrifying biomass becomes:

$$\frac{dX_N}{dt} = (\mu_{N,MAX} - b_N) X_N \qquad (2\text{-}45)$$

where b_N = the decay of nitrifying biomass

equation 2-45 on integration becomes:

$$X_N = X_{N0} e^{(\mu_{N,MAX} - b_N)t} \qquad (2\text{-}46)$$

where X_{N0} = initial concentration of nitrifying biomass

t = time

substituting into equation 2-43 gives:

$$\frac{dS_{NO}}{dt} = \frac{\mu_{N,MAX}}{Y_N} X_{N0} e^{(\mu_{N,MAX} - b_N)t} \qquad (2\text{-}47)$$

Integration of equation 2-47:

$$S_{NO} - S_{NO0} = \frac{\mu_{N,MAX} X_{N0}}{Y_N(\mu_{N,MAX} - b_N)} e^{(\mu_{N,MAX} - b_N)t} - \frac{\mu_{N,MAX} X_{N0}}{Y_N(\mu_{N,MAX} - b_N)} \qquad (2\text{-}48)$$

where S_{NO0} is the initial concentration of oxidized nitrogen.

Antoniou *et al.* (1990) [100] setup an experiment with sufficiently low values of S_{NO0} and X_{N0}, so that the related terms in equation 2-48 may be neglected, and

equation 2-48 becomes:

$$\ln S_{NO} = \ln\left(\frac{1}{Y_N}\frac{\mu_{N,MAX}}{(\mu_{N,MAX} - b_N)} X_{N0}\right) + (\mu_{N,MAX} - b_N)t \qquad (2\text{-}49)$$

In equation 2-49, oxidized nitrogen concentration is linear with respect to time; the slope of this equation is the net growth rate of nitrifying biomass.

Without neglecting any term in equation 2-48, Sözen *et al.* (1996) [99] used a curve fitting approach to evaluate the maximum specific growth rate of nitrifying biomass. In their approach, they defined $k = \frac{Y_N(\mu_{N,MAX} - b_N)}{\mu_{N,MAX} X_{N0}}$ and $a = \mu_{N,MAX} - b_N$, and the equation 2-48 for oxidized nitrogen became:

$$S_{NO} = S_{NO0} + \frac{1}{k}\left[e^{at} - 1\right] \qquad (2\text{-}50)$$

Let $y = at$, the equation 2–49 was revised to be:

$$y = \ln\left[k(S_{NOE} - S_{NO0}) + 1\right] \qquad (2\text{-}51)$$

They employed a curve fitting technique to define the optimum values of parameters k and a, and found the values of "a" (i.e. $\mu_{N,MAX} - b_N$, the maximum specific growth rate of nitrifying biomass) for activated sludge in meat-processing effluent to be in the range of 0.56 and 0.63 d^{-1}.

2.7.6.3 Estimating ammonia nitrogen utilization constant (k_{NH}), half-saturation constant (K_{NH}), and the rate of oxygen consumption in nitrification

Stratton and McCarty (1967) [44] monitored the change in concentration of substrate nitrogen (C and C_0) measured at various time intervals (t), and developed the equation 2-12 for estimating nitrification kinetic constants:

$$-\frac{1}{k_{NH}}\left\{\left[\frac{-K_{NH}}{M_0 + aC_0} - \frac{1}{a}\right]\log_e\left[M_0 + aC_0 - aC\right] + \left[\frac{K_{NH}}{M_0 + aC_0}\right]\log_e\left[\frac{CM_0}{C_0}\right] + \left[\frac{1}{a}\right]\log_e M_0\right\} = t \qquad (2\text{-}12)$$

where

k = substrate utilization constant (mg/day/mg VSS)

K_{NH} = half-saturation constant (mg/L)

M_0 = biomass concentration at time zero (mg/L)

a = yield coefficient (mg VSS / mg COD)

C_0 = substrate concentration at time zero (mg/L)

C = substrate concentration at time t (mg/L)

t = time (days)

With the observed data of change in substrate nitrogen concentration, the above equation, and a best least squares fitting method, Stratton and McCarty (1967) [44] determined the nitrification rate constants k_{NH}, K_{NH}, and the initial concentration of nitrifying bacteria (M_0).

The mass of viable nitrifying biomass M at any time t can then be estimated. The course of nitrification and the rate of oxygen consumption due to biological nitrification can also be predicted. The details of the modeling equations can be found in the publication of Stratton and McCarty (1967). [44]

2.7.6.4 The fraction of nitrifying biomass

There are other approaches to estimate the concentration of nitrifying biomass.

Copp and Murphy (1995) [97] made reference to techniques of determining the mass of nitrifying organisms in an activated sludge sample, and an in situ nitrifier mass estimation technique (MET, using dominant culture of nitrifying organisms) was developed to estimate the nitrifier population within an activated sludge sample.

Using another calculation equation, Harremoës and Sinkjær (1995) [91] estimated the percentage of nitrifying biomass in the MLVSS to be 1.5% ~2.0% in activated sludge samples of a wastewater treatment plant in Ontario, Canada. They also suggested that by correcting the rates for the fraction of nitrifying biomass, the differences among the rates calculated per gVSS in plants could be eliminated. For example, in plant where

the fraction of nitrifying biomass is smaller, the nitrification rate such as removal of ammonia nitrogen based on total biomass in the system, $mgNH_3$-N/mgVSS/d, may show a smaller value than that estimated from another plant where the fraction of nitrifying biomass is higher. If the nitrification rate was calculated on the base of nitrifying biomass, but not the total biomass, the rates between the two plants could be in fact the same.

Cautiousness must be taken when apply nitrification rate, $mgNO_x$formed/mgVSS/d or $mgNH_3$-Nremoved/mgVSS/d, to evaluate the kinetics of nitrifying biomass, especially when the sludge is from different sources (cultivation or biotreatment systems).

2.7.6.5 Yield coefficient, Y_N, the maximum specific growth rate, $\mu_{N,MAX}$, decay rate, b_N, and half-saturation constant utilizing ammonia-nitrogen, K_{NH}

Applying Gee`s (1987) [101] analyses, Copp and Murphy (1995) [97] determined the yield $Y_N = 0.13 gVSS gN^{-1}$, the decay rate b_N=0.17d^{-1}, the maximum specific growth rate $\mu_{N,MAX} = 0.55 d^{-1}$, and the half-saturation constant $K_{NH} = 3.0 mgNL^{-1}$ for mixed nitrifiers. Of the resulting values from their evaluation, except for decay rate, all the other kinetic values fell within the ranges that they have seen in the literature:

Y_N = 0.05 ~0.21gVSS/gN

b_N = 0.004 ~ 0.119 d^{-1}

$\mu_{N,MAX}$ =0.25 ~1.23 d^{-1}

K_{NH} = 0.06 ~5.6 mg N/L

The decay rate of nitrifying biomass b_H = 0.17 day^{-1} determined by Copp and Murphy (1995) [97] (by measuring the OUR of a suspension void of ammonia or nitrite) is significantly different from the default values in previously developed activated sludge models. [19, 28] Therefore, they suggested that erroneous predictions of nitrifier populations in activated sludge may occur if use the Activated Sludge Model No.1 default value. As to temperature dependency, the decay rates determined at different temperatures in Copps analysis were found not identical. Nevertheless, with findings

such as (1) the overlapped confidence intervals and (2) the inclusion of overall death rate values within all of the confidence intervals, Copps *et al.* [97] suggested that temperature does not affect the decay rate of nitrifying biomass.

Studying high strength nitrogenous wastewater, Gupta and Sharma (1996) [41] determined the yield coefficient to be 0.15 $gVSS_N/gN$ and the decay coefficient to be $0.07d^{-1}$ for nitrifying biomass.

Henze (1988) [98] determined the yield coefficient of nitrifying biomass to be 0.16 $gVSS_N/gN$ and the decay coefficient of the nitrifying biomass to be 0.12 d^{-1} at 20°C. In addition, Henze suggested a temperature-calibrating coefficient $\theta_{b,nit} = 0.088$ for decay rate calibration.

The above information shows:

(1) a variation of decay coefficients of nitrifying biomass among results from previous researches, e.g. Copp *et al.* [97]: 0.17 d^{-1}, Activated Sludge Model No.1[19]: $0.04d^{-1}$, Gupta and Sharma [41]: $0.07d^{-1}$, and Henze [98]: $0.12d^{-1}$, and

(2) the discrepancy of whether or not decay coefficient is temperature dependent imply an necessity of further investigation.

This suggests that decay coefficient of nitrifying biomass and its temperature dependency coefficient still need to be further investigated.

2.7.7 Experimental designs in the literature for evaluation of nitrification constants -Batch tests and other approaches

Batch tests have been widely employed in the literature for estimating the maximum nitrification rates. Methods employed in the literature for determining kinetics of nitrification are listed in Table 2-2.

Table 2-2 A list of methods for evaluating kinetics of nitrification

Published year	Authors	Types of experimental unit	Parameters
1969	Balakrishnan *et al.* [103]	Batch	NOx
1980	Hall & Murphy [104]	Batch	Ammonia-nitrogen, NOx, TKN
1983	Painter *et al.* [105]	Fill and draw	NOx, Ammonia-nitrogen
1984	Gupta [106]	Continuos	Ammonia-nitrogen, NOx
1990	Antoniou *et al.* [100]	Batch	NOx, Ammonia-nitrogen
1990	Hanaki *et al.* [107]	Continuous, Batch	NOx, Ammonia-nitrogen
1990	Hanaki *et al.* [108]	Continuous, Batch	Ammonia-nitrogen, NOx
1991	Givens *et al.* [109]	Batch	Ammonia-nitrogen, NOx
1992	Lesouef *et al.* [110]	Batch	Ammonia-nitrogen
1992	Kappeler *et al.* [92]	Batch	OUR
1993	Vanrolleghem & Verstraete [94]	Batch	OUR
1994	Sinkjar *et al.* [111]	Alternating mode for nitrification /denitrification	Ammonia-nitrogen
1994	Novák *et al.* [23]	Batch	OUR
1996	Sözen *et al.* [99]	Batch	NOx

The preference of conducting batch studies for estimating kinetics of nitrification can be seen from this table.

Chapter 3 Methods and materials

3.1 Source and Type of Wastewater

The wastewater under investigation was the primary effluent from a typical meat-processing plant that includes slaughtering and boning processes. The plant processes (slaughtering and boning) an average of 500 cattle per day, which is typical a medium-size in the New Zealand meat industry. The wastewater consisted of fecal material and urine from the stockyards, pieces of paunches, losses of blood, fat, and residual tissue from slaughtering and boning processes. There were no rendering and fellmongering processes.

3.2 Storage of Primarily Treated Meat-processing Wastewater

Primary wastewater was taken fortnightly from a conveying channel located between the primary sedimentation tank and the first pond of the secondary treatment system. The primary effluent was transported in 20-liter containers and stored in a 4°C refrigerator for evaluating its characteristics, biodegradability, and bio-kinetics for carbon removal and nitrification. A portion of the primary effluent was fed to a laboratory scale continuous activated sludge system (CSTR) in a constant temperature laboratory (20°C) to grow microorganisms which are able to remove carbon (i.e. heterotrophic biomass), and conduct nitrification (i.e. autotrophic biomass) to supply the biomass for all batch experiments.

3.3 Characterization of Primarily Treated Meat-processing Wastewater

3.3.1 Source of wastewater

The wastewater was taken from the 4°C refrigerator where the primarily treated meat-processing wastewater was stored. Before taking samples for analyses, the wastewater was thoroughly shaken to maintain its homogeneity.

3.3.1.1 Analyses of primarily treated meat-processing wastewater

The pollution load parameters such as chemical oxygen demand (both unfiltered and GF/C filtered), total Kjeldahl nitrogen (both unfiltered and GF/C filtered), ammonia nitrogen, total phosphorous, total suspended solid (TSS), and volatile suspended solid (VSS) were measured within 36 hours when fresh wastewater was withdrawn from the

meat-processing plant. Other parameters such as pH, BOD_5, oil and grease, and alkalinity were also measured occasionally.

With respect to carbonaceous constituents' study, it is known that TOC does not give information about the oxidation state of the organic compounds. On the other hand, BOD is only a rough estimation of biodegradation. It does not provide a correct indication of the electron donor potential of carbonaceous organic constituents. COD provides electron equivalence between organic substrate, biomass and the oxygen utilized. [14 Chap. 9. 19. 20] Ammonia and nitrate nitrogen can also be converted into an equivalent COD basis for calculating the utilization rate. Furthermore, the mass balance can be made in terms of COD. [19]

Measurement of COD (using the closed reflux, colorimetric method), TSS, VSS, TKN, NH_4^+-N, total phosphorus (using vanadomolybdophosphoric acid colorimetric method) and alkalinity (using titration method) were performed according to *Standard Methods* (APHA, 1995). [21]

Total suspended solids (TSS) and volatile suspended solids (VSS) were performed in duplicate, with the average of the two values taken as the result. The analyses of TSS and VSS were carried out using Whatman GFC filters. The filters were prepared by placing at 105(± 1)°C in a oven until needed. The 105(± 1)°C dried filters were cooled in a desiccator before being weighted for use. For VSS analyses, filters (with SS on them) were ignited in a muffle furnace at 600(± 25)°C for 20 minutes along with at least a blank filter (without SS on it), then cooled in a desiccator before being weighted. The lost weight of the blank filter was used to calibrate the lost weight of filters themselves so that the net weight of VSS can be calculated. TSS and VSS were calculated as follows:

$$TSS\ (mg/L) = [((P'_{105} + SS_{105}) - P'_{105.b})] * 1000/V \qquad (3\text{-}1)$$

$$VSS\ (mg/L) = [(P'_{105} + SS_{105}) - (P'_{600} + SS_{600}) - (P'_{105.b} - P'_{600.b})] * 1000/V \qquad (3\text{-}2)$$

where

P'_{105} = the weight of filter after being dried at 105(+/-1)°C

SS_{105} = the weight of SS after being dried at 105(+/-1)°C

$P'_{105,b}$ = the weight of blank filter after being dried at 105(+/-1)°C

P'_{600} = the weight of filter after being dried at 600(+/-25)°C

SS_{600} = = the weight of SS after being dried at 600(+/-25)°C

$P'_{600,b}$ = the weight of blank filter after being dried at 600(+/-25)°C

V = volume of sample, ml

COD measurements were performed in triplicate, while TKN and NH_4^+-N measurement were performed in duplicate when feasible. A BI-100 Electrolytic Respirometer was used to measure BOD_5 of the wastewater occasionally. The oil and grease analysis uses the "total grease" measurement method of New Zealand Meat Industry Research Institute. [22]

Whatman GF/C filters were used for defining soluble meat-processing wastewater. The selection of filter paper was evaluated through a filtration test as shown in Figure 3-1. Filtration by pore size of 2.5 μm and 0.45 μm filter paper were found not practical because of the slow speed. Therefore, GF/C filter paper (pore size of 1.2 μm) was used for filtration and to define soluble substrate for this study.

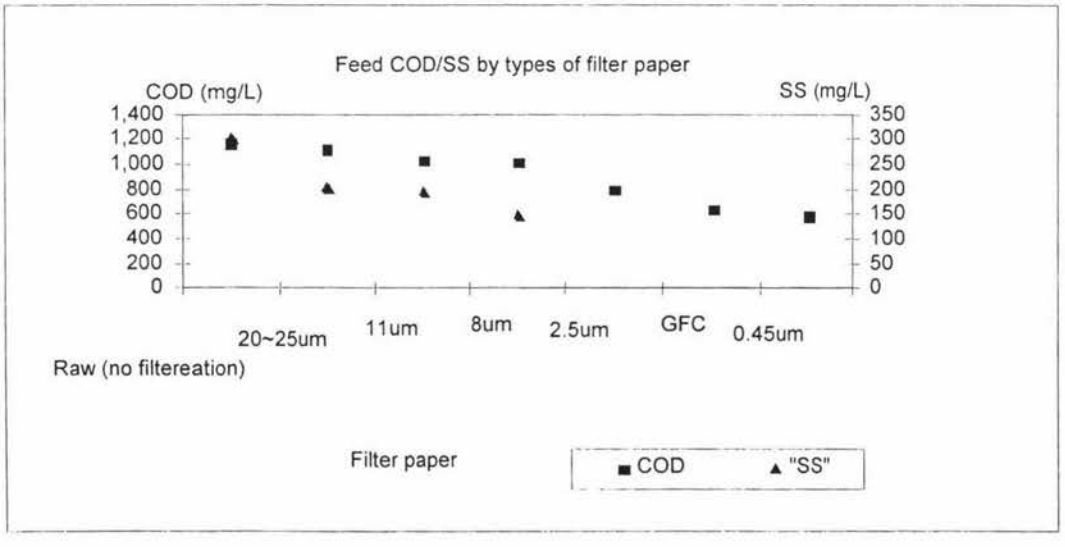


Figure 3-1 COD and SS by types of filter papers (assume GF/C filtrate is soluble substrate)

When large amount of filtered wastewater was needed for batch studies, centrifuge

was used before filters, the centrifuging was conducted for 15 minutes using RC5C Rotor at 20°C and 9000 RPM.

3.3.2 Evaluation of biodegradability for meat-processing wastewater

3.3.2.1 Parameters under investigation

From literature of the developed activated sludge models, accurate data on biodegradability of the target wastewater is essentially required to trigger a reliable model. To facilitate the development of activated sludge modeling for meat-processing wastewater, one objective of this study is to provide information on biodegradability of meat-processing wastewater. The parameters under discussion of biodegradability include: readily biodegradable COD (RBCOD), soluble biodegradable COD, inert suspended organic matter, inert soluble organic matter, coefficient for soluble microbial production, coefficient for particulate microbial production.

3.3.2.2 Batch methods

To evaluate the fraction of readily biodegradable COD (RBCOD), an aerobic batch test method used by Ekama *et al.* (1986) [32] (Respirametric method) is applied. (Appendix A – Data of Run25) On the other hand, a batch COD method presented by Orhon & Artan (1994) [14] was used for evaluating the fraction of soluble biodegradable COD. (Appendix B) The later was also used for evaluating the fraction of inert suspended organic matter, fraction of inert soluble organic matter, coefficient for soluble microbial production and coefficient for particulate microbial production. (Appendix B)

3.3.2.3 Source of biomass and wastewater

As mentioned earlier in this chapter, the source of biomass used for biodegradability batch experiments was cultivated in laboratory scale CSTR system. The biomass for biodegradability studies was prepared using the same preparation procedures for experiments evaluating the bio-kinetic constants of carbon removal. Both of the CSTR system and the biomass preparation procedures are presented in later sections of "The source of biomass" and "Biomass preparation". The wastewater used for biodegradability evaluation was the primarily treated meat-processing wastewater freshly taken from the processing plant.

3.3.3 Evaluation of bio-kinetic constants of carbon removal for meat-processing wastewater in an activated sludge system

3.3.3.1 Batch methods

A preference of conducting batch studies for estimating kinetic constants can be widely found in literature. [14,19,20,32,69,85,92] Batch tests using flasks, shakers, and respirometers were carried out to evaluate the bio-kinetic constants of carbon removal.

3.3.3.2 Temperature control

This study used a temperature constant room controlled at $20^{o}C \pm 2^{o}C$ to grow biomass and conduct the batch experiments. The obtained bio-kinetics can be used as referential values in future modelling and design tasks.

3.3.3.3 The source of biomass

3.3.3.3.1 Biomass growing system

A laboratory scale quasi-continuous activated system (CSTR), as shown in Figure 3-2, was established for biomass cultivation, which provided acclimated biomass for kinetic studies. The air was supplied to the reactor by a compressor. Dissolved oxygen concentrations (monitored by a YSI Model 57 Dissolved Oxygen Meter) were maintained at a level above 3 mg/L. The primarily treated meat-processing wastewater was fed into the reactor (A M1072-2300 2L Vessel, New Brunswick Scientific Co. Inc.) quasi-continuously to maintain a hydraulic retention time of 14 hours and a sludge retention time of 5~7 days. The culture was kept in darkness by wrapping the outside of the glass reactor vessel with aluminum foil. Figure 3-2 shows the configuration of the quasi-continuous system. The pH (monitored by a Type 5997-20 pH controller) of the system is maintained in the range of 6.5 to 8.5. $NaHCO_3$ was used to adjust the pH level when it dropped below 6.5.

The initial seed of the CSTR system was provided by a pilot scale SBR of Meat Industry Institute of the New Zealand in Halmilton in October 1997.

The wastewater (the feed of the system) stored in the 4°C refrigerator was manually stirred using a metal stick 2 to 3 times a day to avoid significant settling of suspended solids. The wastewater in the refrigerator was replaced by fresh one fortnightly.

3.3.3.3.2 Temperature and pH control of the CSTR system

The temperature of this biomass growing system was controlled at $20^{o}C \pm 2^{o}C$. The biomass grown in the system was used to obtain bio-kinetics at $20^{o}C \pm 2^{o}C$.

The addition of $NaHCO_3$ was employed to bring the pH value of the lab. scale CSTR system back to 7.5 when the pH value dropped to below 6.5.

3.3.3.3.3 Performance of the CSTR system

After a 4-month start-up period, the quasi-continuous system stabilized. Primarily treated wastewater, mixed liquor, returning sludge, and effluent samples were taken from the system twice a month for COD (both filtered –*F-S COD* and unfiltered – *F-COD*), TKN (both filtered – *F-S TKN* and unfiltered – *F-TKN*), NH_4^+-N as well as TSS, and VSS analysis.

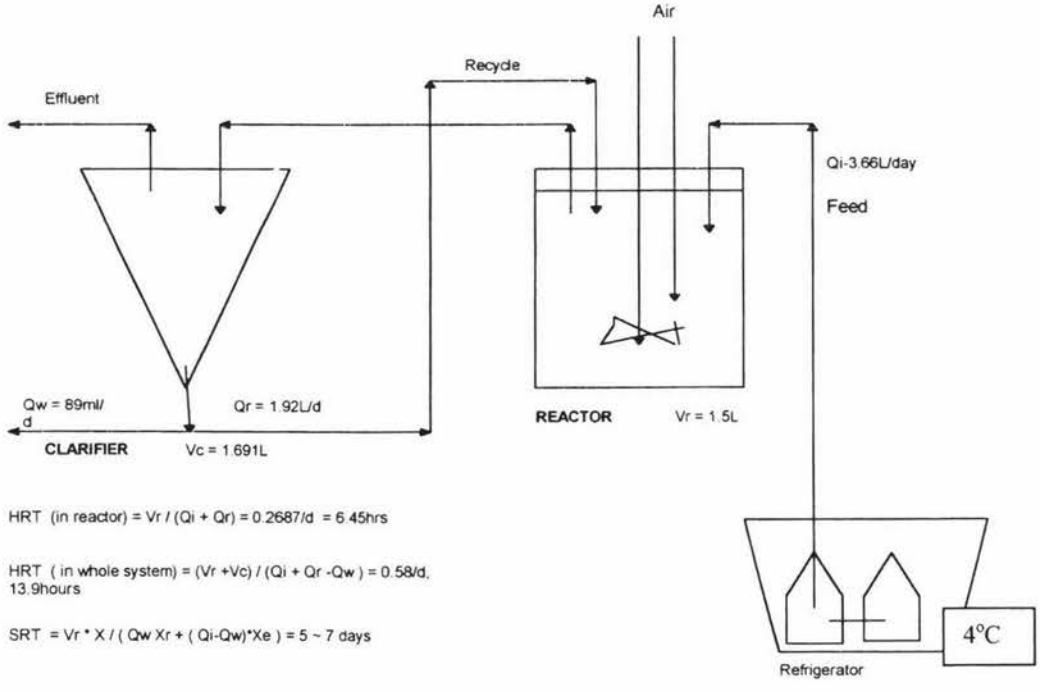


Figure 3-2 Biomass cultivation - lab. scale CSTR system

As seen in Figure 3-3~3-5, the MLSS and MLVSS in this laboratory scale CSTR system is in the range of 1500 mg/L to 4000 mg/L. The system demonstrated good capacities of COD removal and TKN removal. This suggested that the biomass

supplied from this cultivation system during the experimental period would include both heterotrophic and autotrophic biomass.

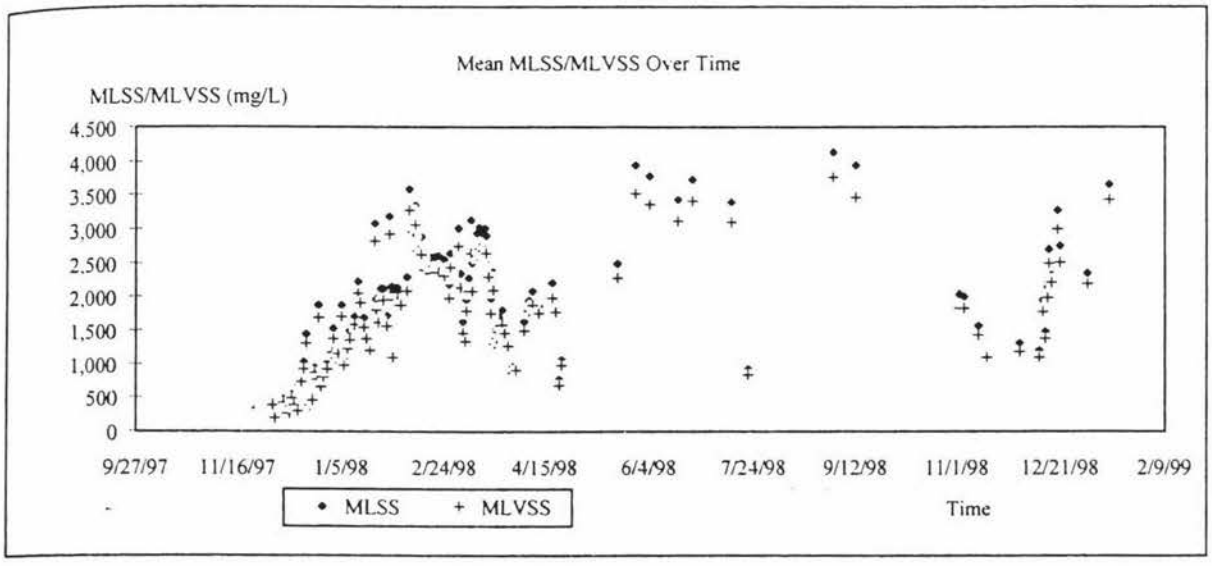


Figure 3-3 Biomass (MLSS & MLVSS) in lab. Scale CSTR system

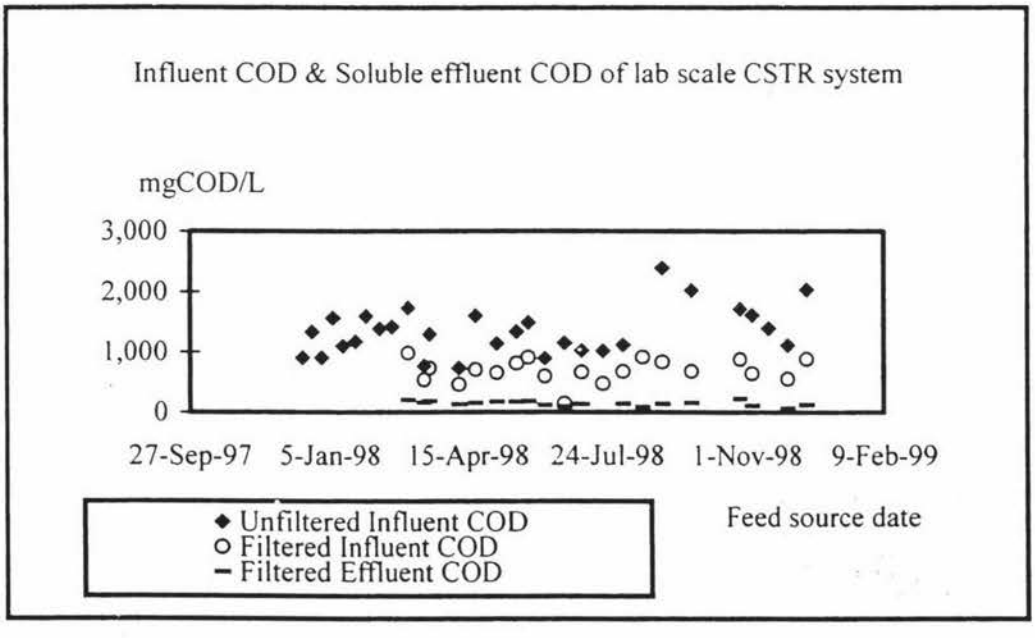


Figure 3-4 Carbon (COD) removal in lab. Scale CSTR system, 93~39% (Average 74%) carbon removal.

Unfiltered Influent COD, COD-F = COD of the unfiltered meat-processing wastewater fed into the CSTR system

Filtered Influent COD, COD –F-S = COD of the GF/C filtered meat-processing wastewater fed into the CSTR system

Filtered Effluent COD- E-S = COD of the GF/C filtered effluent of the CSTR system

The COD values presented in Figure 3-4 were the average of samples taken from each feed source day (i.e. the date the wastewater was taken from the meat plant). The overall 90 samples of filtered effluents taken during the year of 1998 have been within the range of 51.3 to 282.6 mgCOD/L with an average of 146.4 mgCOD/L, despite the variance and high strength of the system's feed COD concentrations. The system's COD removal capability was 74 % in average, which is higher than the performance of aerated lagoons, and some batch reactors. [5. 6.7]

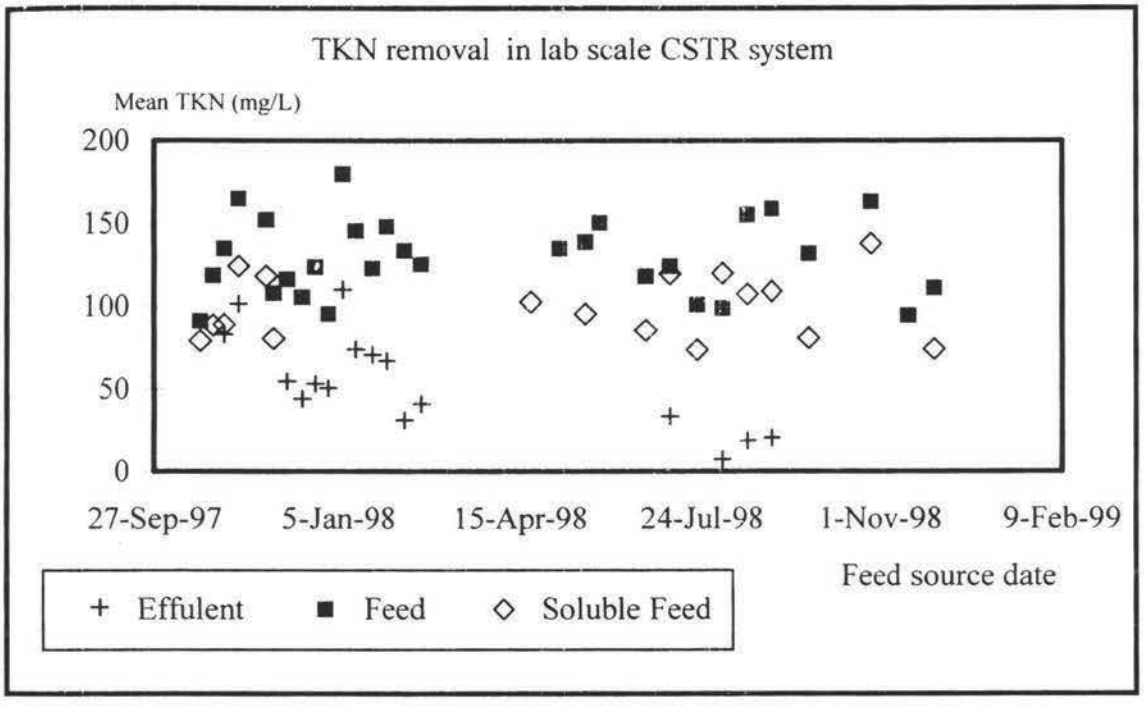


Figure 3-5 TKN removal in CSTR system – 93~38% (average 63%) removal

The overall average TKN ("Feed" in Figure 3-5) fed into the CSTR system was 127 mg/L (ranging from 62.1 to 209.7 mg/L). The system was able to remove 63% (in average) of TKN and reduced the TKN in effluents to 57.5 mg/L (in average).

3.3.3.4 Biomass and substrate preparation for batch experiments

To provide the bio-kinetics experiments with consistent and reliable biomass and substrate, standard preparation steps were complied as follows:

3.3.3.4.1 Biomass preparation – a serial dilution procedure

1. Four 50 ml of mixed liquors (at concentration of 200~3500mg/L) were taken from the lab scale CSTR system and each was transferred into a 250ml flask,
2. Two hundred ml of GF/C filtered meat-processing wastewater was added into each flask in step 1, the mixture was then shaken for 24 hours at $20^{o}C$,
3. On the next day, 50 ml of the shaken mixture was extracted and transferred into a new flask. Another 200ml of fresh GF/C was added into the flask. The mixture was shaken for another 24 hours. The same procedure was repeated for 3 days,
4. At the end of day 3, all four shaken mixtures were mixed together in a 1.5L flask and settle for 2 hours. Seven hundred and fifty ml of supernatant were decanted,
5. The settled portion was used for inoculating each kinetic experimental flask and the respirometer.

This serial dilution process is shown in Figure 3-6. The purpose of this process was to minimize particulate matter that is not biomass and to generate without adsorbed particulate substrate. The biomass by this stage was no longer identical to the CSTR biomass.

3.3.3.4.2 Substrate preparation -

1. Fresh primarily treated meat-processing wastewater was taken from the processing operation plant on the day the plant was conducting both killing and boning operations so that the obtained concentrated substrate can supply a wide range of initial substrate concentration S_o for varying the S_o/X_o ratio,
2. Total COD, soluble COD, TKN, and $NH_4 - N$ of the fresh wastewater were measured,
3. The fresh wastewater was centrifuged for 15 minutes using a RC5C Rotor at $20^{o}C$ and 9000 RPM,
4. The centrifuged feed was filtered with GF/C filter paper,
5. The COD, and TKN of GF/C filtered substrate was measured,
6. Th filtered substrate was diluted to the required initial substrate concentrations S_o for each experimental flask.

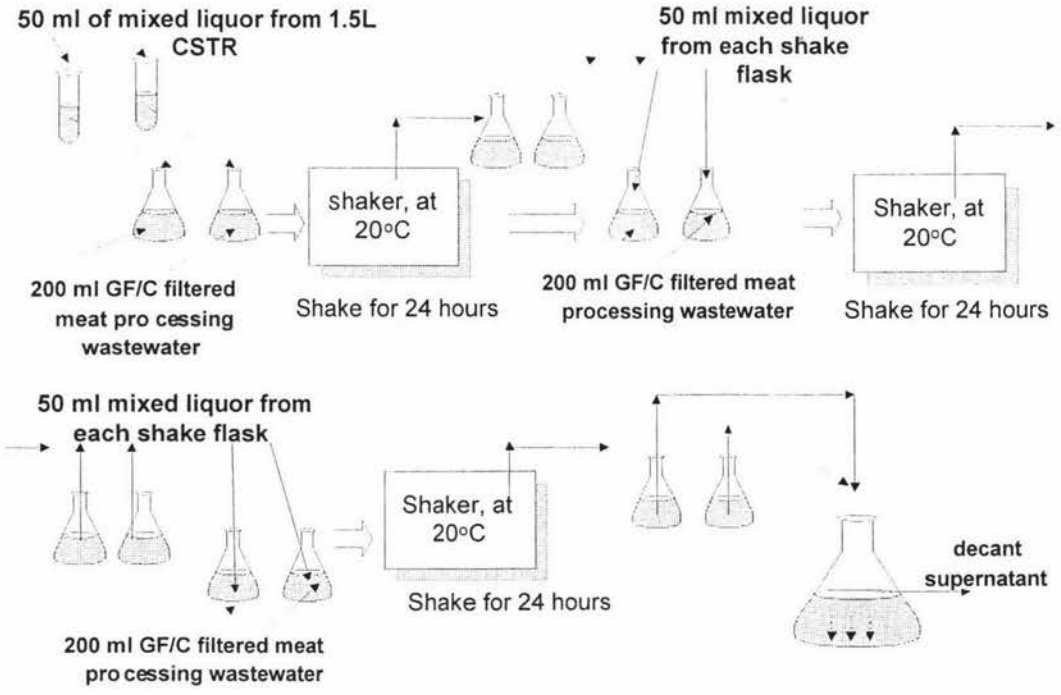


Figure 3-6 Biomass preparation –serial dilution

3.3.3.5 Processes to evaluate carbon removal bio-kinetic constants (Y_o, O_x, μ_H, $\mu_{H,MAX}$, K_s) in an activated sludge system

The experimental design for obtaining carbon removal kinetics includes the following steps:

(1) Cumulative oxygen uptake data was taken from a BI-100 Electrolytic Respirometer (Bioscience, Inc., Bethlehem PA 18017, USA) in which a volume of 2 to 30 ml biomass, prepared by the serial dilution, was inoculated into a volume of 500 to 1000ml unfiltered or GF/C filtered wastewater;

(2) Biomass growth and COD substrate utilization data were obtained by batch tests. Ten to forty ml of biomass, prepared by the serial dilution, was inoculated into a volume of 240 to 600ml GF/C filtered wastewater in three to five 1L-flasks. The observed yield coefficient (Y_o, mgVSS/mgCOD), COD of unit biomass (O_x, mgCOD/mgVSS), the COD based yield coefficient (Y_H, mgCOD/mgCOD), the specific substrate (COD) utilization

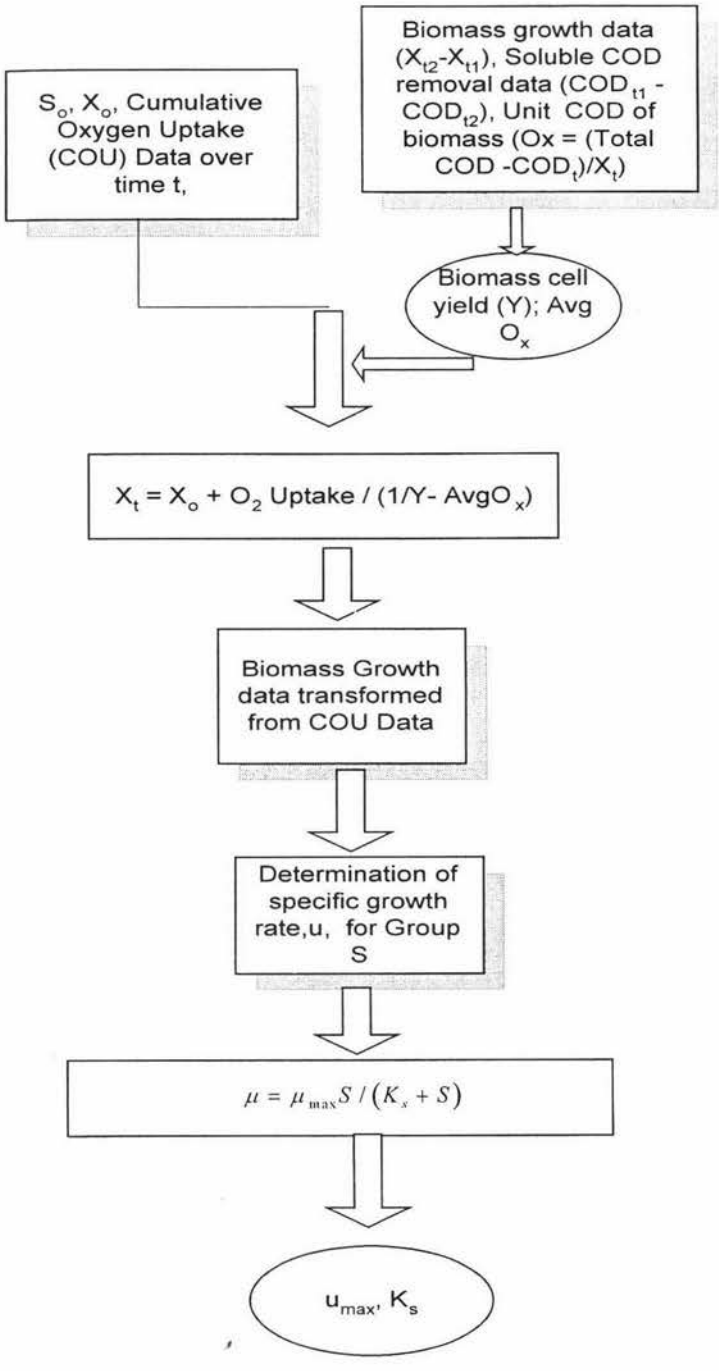
rate (q, d^{-1}), and the specific heterotrophic biomass growth rate (μ_H, d^{-1}) corresponding to its initial S_o/X_o ratio from each flask were calculated ;

(3) The initial biomass concentration (X_o), the values of Y_o and O_x obtained in (2), and the cumulative oxygen uptake data, were then applied to the equation of $X_t = X_o + O_2$ Uptake / ($1/Y_o$ – average O_x) (equation 3-3) estimate the biomass concentrations at time t for each flask;

(4) The specific heterotrophic biomass growth rate (μ_H, d^{-1}) corresponding to the initial substrate (S_o, mgCOD/L) was calculated;

(5) The data sets of S_o and its corresponding μ_H were then curve fit the Monod equation (equation 2-13, $\mu_H = \mu_{H,MAX} S/(K_s + S)$) to determine the constants of $\mu_{H,MAx}$ and K_s.

The above procedures are sketched as below, the details for the derivation of these procedures can be found in literature of Rozich & Gaudy, (1992): [85]



(Ref.: A.F. Rozich & AE Gaudy,Jr)

Figure 3-7 Processes of evaluating kinetics of μ_{MAX} and K_s.

3.3.3.5.1 Obtaining the yield coefficient (Y_o) and COD of unit biomass (O_x)

Three to five 1L-flasks reactors containing initial GF/C filtered treated meat-processing wastewater in amount ranging from 240 to 600 ml with inoculation from 10 to 40ml of initial acclaimed biomass prepared by the serial dilution procedure were run in a flask shaker for 1 to 2.5 days.

Some GF/C COD substrate was diluted to produce the desired levels of initial COD. The volumes of biomass added into the flasks controlled the initial biomass concentrations. By this means, a range of S_o/X_o ratio (from 2 to 24.5) was designed for monitoring the variations of the corresponding yield coefficients (Y_o and Y_H) and COD of unit biomass (O_x).

The temperature of the shaker was controlled at 20°C. Samples were taken during the exponential growth period every 1 to 2 hours for analysis (total COD, soluble COD, and MLSS/MLVSS) until the exponential growth phase had completed (i.e. when the slopes of COD decreasing and biomass increasing reduced). The exponential growth period was found to range from 4 hours to 24 hours depending on the initial conditions (S_o/X_o ratios). In general, the condition of higher S_o/X_o ratio had a longer exponential period. The design of using more than one flask in each experiment was to examine the variation of yield coefficient under different initial conditions (i.e. S_o/X_o ratios, where S_0 : initial substrate concentration, mgCOD /L; X_0: initial biomass concentration, mgcellCOD/L).

By monitoring the increase in biomass, X_t-X_{t0} (VSS mg/L) and the decrease in soluble carbonaceous substrate, $COD_{t0} - COD_t$ (mg/L), one can calculate the observed yield coefficient (Y_o) and COD of unit biomass (O_x) using the following equations:

$$Y_o(VSSbased) = \frac{(X_t - X_{to})}{(S_{to} - S_t)} \text{ (mgVSS/mgCOD)} \qquad (3\text{-}4)$$

$$O_x = (TotalCOD_t - Soluble COD_t) / X_t \text{ (mgCOD/mgVSS)} \qquad (3\text{-}5)$$

$$Y_H(CODbased) = Y_o(VSSbased) \bullet O_x \quad \text{(mgCOD/mgCOD)} \qquad (3\text{-}6)$$

3.3.3.5.2 Application of oxygen uptake data

The same source of biomass (prepared by the serial procedure) and wastewater were also inoculated into a respirometer to measure the cumulative oxygen uptake (COU) over the period of exponential growth period. Temperature was also controlled at 20°C in the respirometry experiments. The biomass growth in the respirometer can be estimated by applying the following equation:

$$X_t = X_0 + O_2 uptake / \left(1 / Y_{o(vssbased)} - O_x\right) \qquad \text{(mg/L)} \qquad (3\text{-}3)$$

Obtaining the maximum specific growth rate of heterotrophic biomass ($\mu_{H,\max}$) and half-saturation constant for utilization of soluble COD substrate (K_s):

Once biomass growth data, X_t, was estimated by equation 3-3, the specific growth rate (μ_H, day^{-1}) corresponding to the initial substrate in the reactor (S_o) was determined using the following equation:

$$\ln\left(\frac{X_{t2}}{X_{t1}}\right) / \left(t_2 - t_1\right) = \mu_H \ \text{(day}^{-1}\text{)} \qquad (3\text{-}7)$$

The data sets of μ_H and its initial S_o were curve fit (NLREG program [121]) the Monod function. The maximum specific growth rate ($\mu_{H,MAX}$) and half-saturation constant utilizing soluble COD substrate (K_s) were determined.

3.3.3.6 Determining the death-regeneration decay coefficient, b_H

The approach used to determine decay coefficient was the method suggested by the Task Group Model No.1. [19, 32] Biomass was removed from the CSTR system into a respirometer and the oxygen uptake rate was measured over a period of several days. The slope of the natural logarithm of the oxygen uptake rate versus time is the traditional decay coefficient, b'_H. The BI-100 Electrolytic Respirometer (Bioscience,

Inc., Bethlehem PA 18017, USA) was used to measure the endogenous respiration. Eight hundred ml of tap water and 200 ml of serial diluted biomass were put into cells of the respirometer. Nitrification was inhibited during the test by the addition of 20mg/L of thiourea. The death-regeneration decay coefficient b_H was calculated by equation 2-30: $b_H = \frac{b_H^{'}}{1 - Y_H(1 - f_p)}$, where f_p = the fraction of the biomass that ends up as inert particulate products following decay. The value of f_p is typically about 0.2, according to the task group. [19]

3.3.4 Evaluation of the maximum specific growth rate of nitrifying biomass for meat-processing wastewater in an activated sludge system

3.3.4.1 Batch approaches

From literature review, there was a preference of conducting batch studies for estimating kinetics of nitrification. Batch tests were carried out for evaluating the maximum specific growth rate of nitrifying biomass. To avoid free-ammonia loss, 1L-PVC bags (Intravenous Infusion BP, BAXTER HEALTHCARE PTY LTD NSW Australia) were used as close system reactors.

Traditionally, three methods (1) production rate of oxidized nitrogen, (2) removal gradients of ammonia nitrogen, and (3) oxygen uptake data have been used to estimate nitrification kinetic constants.

Amongst the above three approaches, measuring the rate of nitrification by means of monitoring the change in oxidized nitrogen concentration has been commonly used. This is because ammonia is taken up by heterotrophs for growth and also produced by hydrolysis in an activated sludge system. This study chooses to observe the production rate of oxidized nitrogen for evaluating rate of nitrification.

3.3.4.2 Source of biomass and wastewater

For data to be compatible with carbon removal kinetics which were based on utilization of GF/C filtered meat-processing wastewater, GF/C filtered meat-processing wastewater and the same source of biomass was used for nitrification kinetics studies.

3.3.4.3 Experimental procedure, sampling, and analysis

Experimental procedures include the following steps:

(1) Preparation processes for GF/C filtered substrate were stated earlier in this chapter, it is the same preparation procedures for carbon removal kinetic experiments;

(2) Each bag was cleaned thoroughly,

(3) 300 ml GF/C filtered wastewater was injected into each bag,

(4) 700 ml air was also injected into each bag,

(5) Each bag was closed and installed it onto a lab. scale rotating wheel, each bag was labeled and firmly attached to the wheel cell,

(6) 20 ml biomass, directly taken from the lab. scale CSTR, was then injected into each bag,

(7) The power of the wheel was switched on. The bags span so that the substrate, biomass, and air in each bag were completely mixed,

(8) Within the first 30 seconds of spinning, 8 ml mixed liquor sample was drawn from each bag for analyzing the oxidized nitrogen concentration and the biomass concentration at time zero,

(9) The wheel kept spinning throughout the course of experimental period, only being stopped for sampling,

(10) When a bag showed no further increase in observed oxidized nitrogen ($NO_x - N$), the sampling of this bag was terminated.

Samples (8ml each time) were taken from each bag every 3~7 hours over a period of 4~5 days for oxidized-nitrogen (i.e. NO_3-N and NO_2-N) analysis. When the observed concentration of oxidized nitrogen showed no increase, then the sampling was ended. The samples taken from each bag were filtered by 0.45 μm filters then injected into a DIONEX DX-100 Ion Chromatograph to measure the concentrations of NO_2^- and NO_3^-. Diluted samples were used when the expected NO_2^- and NO_3^- concentrations exceeded the range of calibration line. The concentrations of NO_2^- and NO_3^- were then used to calculated the oxidized nitrogen concentration (i.e. $NO_x - N$) that equals

to $NO_2 - N$ plus $NO_3 - N$.

It was suggested that monitoring of nitrification also includes tracking ammonia and nitrite concentrations to assist in mass balance of the nitrogen. [97] This study did not track ammonia concentrations when evaluated the nitrification kinetics by 1L PVC bags. The reasons for doing so include: (1) the small volume (1L) of the experimental bags and the small volume of substrate in each bag (approximately 300ml) which made tracking ammonia not feasible because ammonia analysis requires a relatively large volume of sample, i.e. 30 ~ 50 ml, and (2) the loss of ammonia was considered not significant due to the close-system design.

3.3.4.4 Temperature Control in nitrification

Temperature was controlled to within 2°C of the set point (20°C) in a temperature-controlled room.

A temperature coefficient of nitrification $\theta_{\mu nit}$, can be used for calibrating the designed temperature in the future. The following Arrhenius equation is commonly accepted: [15]

$$r_T = r_{20}\theta^{(T-20)} \qquad (3\text{-}8)$$

where

r_T = the reaction rate at temperatures T (°C)

r_{20} = the reaction rate at temperature 20°C

θ = temperature coefficient

T = temperature, °C

The temperature coefficient for nitrification rate of meat wastewater was suggested to be 1.083. [114]

3.3.4.5 Mass transfer

To obtain the intrinsic kinetic data, oxygen-limiting conditions need to be avoided. An experiment (Run ID 27) of mass transfer evaluation was carried out before conducting the nitrification experiments. Smith *et al.* (1998) [113] demonstrated that adequate mass transfer conditions for oxygen in the system could be verified by comparison of

samples having different biomass concentrations at a high substrate concentration. In other word, an increase in biomass concentration resulted in a proportional increase in the substrate reaction rate if the system was not mass transfer limited.

Chapter 4 Results and Discussions

The results of this study include:

(1) the characterization of primarily treated meat-processing wastewater that produced by a meat-processing plant in the period of October 1997 to January 1999;

(2) biodegradability study on meat-processing wastewater;

(3) evaluation of bio-kinetics for carbon removal in an activated sludge system; and

(4) the maximum growth rate of nitrifying biomass in an activated sludge system treating meat-processing wastewater.

4.1 Characteristics of Meat-processing Wastewater

Table 4-1 and Table 4-2 show the pollution load for both unfiltered (F as in Feed) and filtered (F-S as in Feed-Soluble) meat wastewater. These data were obtained from the characterization study during the period of October 1997 to February 1999.

4.1.1 Unfiltered meat-processing wastewater (F as in Feed):

COD concentration were found to range widely from 534mg/L to 3118mg/L. Ammonia nitrogen concentration was found in the range of 36 to 69.5 mg/L. TKN concentration ranged from 62 to 127 mg/L. The average concentration of total suspend solids was 322 mg/L, with a maximum of 1016 mg/L and a minimum of 66 mg/L. Alkalinity was observed to be around 400 mg/l (calculated as CaCO3 mg/L). Total suspended solids concentration ranged from 66 to 1016 mg/L. The total averaged phosphorus was 31mg/L. These concentrations figures characterized in this study were either in the range of or close to the characteristics found by previous researchers. [3]

4.1.2 GF/C filtered meat-processing wastewater (F-S as in Feed - Soluble):

Soluble COD accounted for 40% to 60 % of total COD. Soluble ammonia nitrogen concentration almost equaled the concentration of total ammonia nitrogen. Soluble TKN concentration was about 65% to 83% of total TKN. Alkalinity of the soluble wastewater was in the range of 240 to 412 mg/L (calculated as CaCO3 mg/L). The "n" in tables is number of observations.

Table 4-1 Characteristics of primarily treated meat-processing wastewater (1)

Parameter	type	mean	max	min	SD	n
COD, mg/L	F	1,352.2	3,118.1	534.3	490.7	208
COD, mg/L	F-S	707.7	1,020.3	146.0	188.5	80
H3-N, mg/L	F	69.5	119.8	36.0	21.1	36
NH3-N, mg/L	F-S	60.4	109.3	24.9	24.5	17
TKN, mg/L	F	127.1	209.7	62.1	25.8	63
TKN, mg/L	F-S	98.7	138.0	72.6	19.4	15
BOD_5/COD	F	0.72	0.83	0.61		15

COD (mg/L.hr into the CSTR system) = 131.69; N (mg/L.hr into the CSTR system) = 13, therefore, COD/N ratio = 10.1 in average

pH = 6.3~7.2

Table 4-2 Characteristics of primarily treated meat-processing wastewater (2)

Parameter	type	mean	max	min	SD	n
Particulate, mg/L	TSS	321.9	1,016.0	66.0	220.7	45
Particulate, mg/L	VSS	291.4	896.0	62.0	193.6	44
TP, mg/L	F	31.2	58.5	14.8	12.9	31
Alkalinity @$CaCO_3$, mg/L	F	432.5	435	430	3.53	2
Alkalinity @$CaCO_3$, mg/L	F-S	325.15	412	241.9		4
Hardness @$CaCO_3$, mg/L	F	88	88	88		1
Hardness @$CaCO_3$, mg/L	F-S	104	104	104		1
Oil and grease, mg/L	F	125	229.5	54.7	52.3	9

4.1.3 Quarterly variation

Table 4-3 presents the quarterly variation of COD of primarily treated meat-processing wastewater. The calving season in New Zealand started in summer and ended in late autumn. During the off peak seasons of summer and early autumn, the COD strengths varied much more than winter and spring, as shown by the standard deviation (SD) and ratios of maximum concentration to the average concentration (max./mean) in Table 4-3.

Table 4-3 Quarterly variation of COD (mg/L) of primarily treated meat-processing wastewater

Year	quarter	mean	max.	min.	S.D.	max./mean
1997 3:	spring	1043	1195	966	66	1.15
1997 4:	summer	1153	2501	560	356	2.17
1998 1:	autumn	1275	2647	534	489	2.08
1998 2:	winter	1035	1168	880	89	1.13
1998 3:	spring	1918	3118	1286	584	1.63
1998 4:	summer	1571	2724	794	619	1.73

The ammonia nitrogen of primarily treated meat-processing wastewater was within the range of 36 mg/L to 120 mg/L, with the seasonal averages ranging from 53 to 90mg/L. Whereas the total Kjeldahl nitrogen in the wastewater was in the range of 62 to 200 mg/L, with the seasonal averages ranging from 111 to 140 mg/L. Despite the off peak operation, the wastewater in summer (December and January) did not appear to be a low nitrogen season. (Figure 4-1 & Figure 4-2)

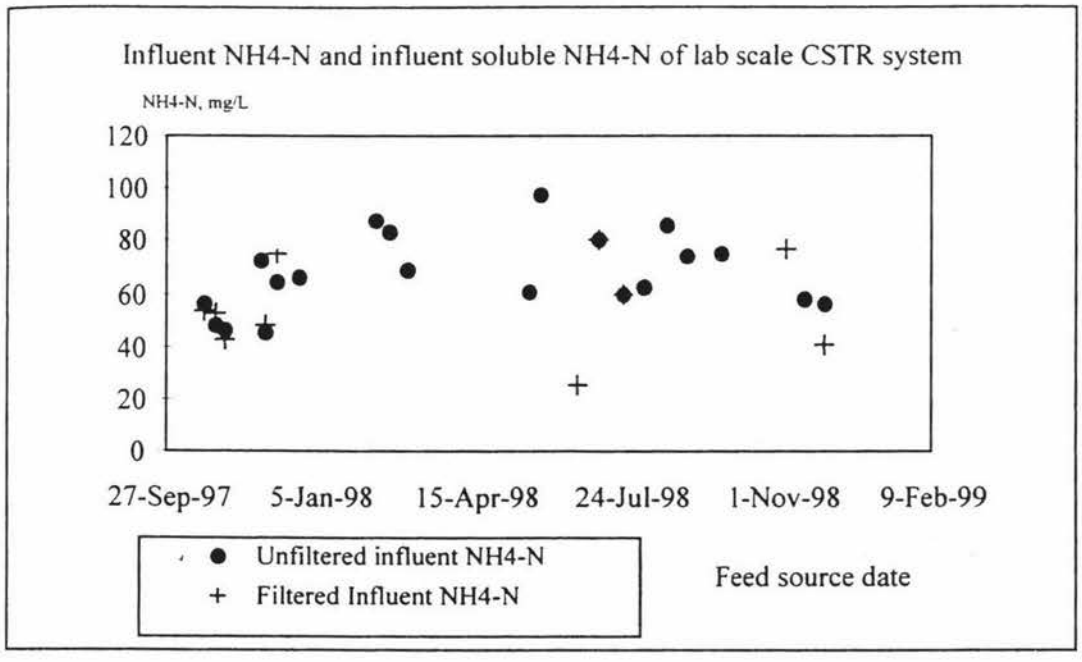


Figure 4-1 Ammonia nitrogen of primarily treated meat-processing wastewater

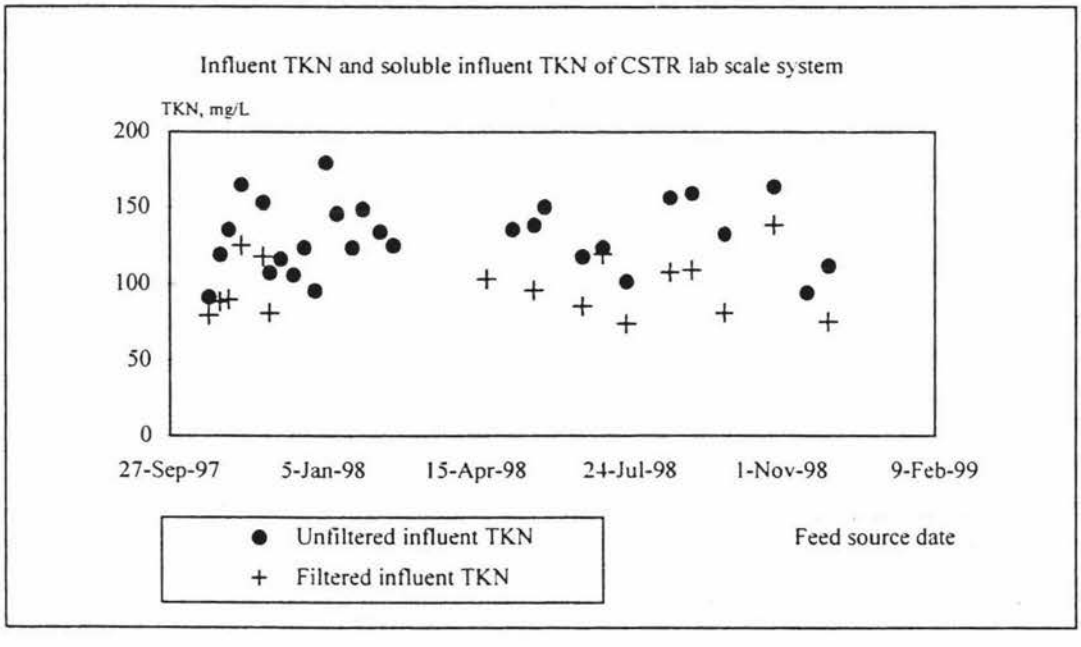


Figure 4-2 Total Kjeldahl Nitrogen of primarily treated meat-processing wastewater

4.2 The Biodegradability of Meat-processing Wastewater

4.2.1 Fraction of readily biodegradable COD

Fraction of readily biodegradable COD (RBCOD) in meat-processing wastewater is found to be 15%~17% of total COD (of unfiltered wastewater), while this fraction in GF/C filtered meat-processing wastewater is 25%~29%. Only about 31.5% of COD of primarily treated meat-processing wastewater was soluble biodegradable organic matter. These data show that not all of the soluble (GF/C filtered) organic matter in meat-processing wastewater was readily biodegradable. It implies that some slowly biodegradable or inert organic matter exists in meat-processing wastewater in soluble form, which may account for about 15% of total COD. The fraction of RBCOD defines the immediate oxygen uptake level in a batch reactor. The RBCOD is considered to be the most important component of wastewater that related to the exponential growth of heterotrophic biomass.

4.2.2 Fractions of inert matter and coefficients for microbial residues

Table 4-4 also shows that inert suspended organic matter accounts for 6.2% of the total COD of primarily treated meat-processing wastewater, while inert soluble organic matter only accounts for 3.2%. The coefficients for soluble microbial production and particulate microbial production are 0.14 and 0.089 respectively. This means that the formation of soluble and particulate inert products can be expressed as a constant fraction of the influent biodegradable COD for meat-processing wastewater, [19,14] and it is 0.14 and 0.089 respectively found by this study. Orhon and Artan [14] used data (associated with domestic sewage) of experiments conducted by Lesouf *et al*, and found the coefficient for inert soluble organic matter to be 0.062 and the coefficient for inert particulate organic matter to be 0.128. These values are not far away from the results of this study, i.e. 0.14 and 0.089 respectively. The fractions of inert suspended organic matter and inert soluble organic matter (associate with domestic sewage) found by Orhon and Artan [14] were 6.7% and 3.5% respectively. These values are similar to the results of this study (6.2% and 3.2%), and imply no significant difference between meat-processing wastewater and domestic sewage in terms of the inert fractions and the coefficients for formation of inert products.

Experimental data and analyses that produce the above results can be seen in Appendix A & Appendix B.

4.2.3 Summary

Table 4-4 Results of biodegradability study on primarily treated meat-processing wastewater

Characteristics and parameters	Results	Method	Reference of methodology
Fraction of RBCOD in GFC-F, fsi (for GFC-Feed)	0.25~0.29	Batch-OUR	32
Fraction of RBCOD in raw-F, fsi (for raw-Feed)	0.15~0.17	Batch-OUR	32
Fraction of inert suspended organic matter, fxi (for total COD)	0.062	Batch-flask-COD	14
Fraction of inert soluble organic matter, fsi (for total COD)	0.032	Batch-flask-COD	14
Fraction of soluble biodegradable COD, Cs (for total COD)	0.315	Batch-flask-COD	14
Coefficient for soluble microbial production, Ysp (or f_{es}, f_{sp} for soluble biodegradable COD)	0.14	Batch-flask-COD	14
Coefficient for particulate microbial production, Yxp (or f_p, f_{ex} for soluble biodegradable COD)	0.089	Batch-flask-COD	14

The readily biodegradable COD fraction accounts for the initial oxygen uptake of microorganisms. It showed the immediate impact associated with oxygen depletion on the receiving water, and the maximum rate level of oxygen requirement in the activated sludge system.

The inert COD fractions tell the COD level which is un-biodegradable entering the activated sludge system. The coefficients for inert COD products show the extent that inert COD produced when biodegradable COD was utilized by activated sludge. With this, one can predict the amount of inert residues produced from the metabolism of activated sludge.

The above result is found to be agreeable with the result of a meat-processing wastewater study conducted by Görgün *et al.* (1995). [33] as seen in Table 4-5.

Table 4-5 COD fractionation of meat-processing wastewater

Total COD	Soluble COD	Ssi, RBCOD	Xsi	Xp [fex; fp]	Sp [fes;fsp	Sii	Xii
1941	674	310	1448	157	86	63	120
100%	35%	16%	75%	0.089	0.014	3.2%	6.2%
This study							
2600	1140	380	1885			30	305
100%	44%	15%	72.5%	0.2	0.055	1.2%	12%
Görgün et al. (1995) results [33]							

[S_{si} is influent readily biodegradable COD; X_{si} is influent slowly biodegradable COD; S_{ii} is influent soluble inert COD; X_{ii} is influent particulate inert COD; X_p is the particulate microbial production (f_{ex} or f_p is its fraction); and S_p is the soluble microbial production (f_{es} or f_{sp} is its fraction)]

The major findings of this biodegradability study on meat-processing wastewater are:

- Although the soluble COD composes about 35% ~ 40 % of the total COD of the primarily treated wastewater, the readily biodegradable COD (RBCOD) only accounts for 16 % of total COD in the primarily treated mea-processing wastewater;
- Approximately, 1/3 of the wastewater COD composition is in soluble/fine colloidal form and the remaining 2/3 is in particulate form;
- The inert fraction, including soluble and particulate form, is about 10 % of total COD; this 10% of fraction is slightly lower than the level found in another meat wastewater biodegradation study conducted by Görgün *et al*; [33]
- When biodegradable COD of meat-processing wastewater was utilized by activated sludge, the extent that inert COD produced was found slightly lower than Görgün's results (also use meat wastewater as substrate), [133] but showed no

significant difference with the level found by Orhon and Artan [14] (use domestic sewage as substrate);

- Excluding the inert fraction (about 10%) and the readily biodegradable fraction (15 ~17%), over 70% of COD in primarily treated meat-processing wastewater is slowly biodegradable.

4.3 Bio-kinetics for Carbon Removal in an Activated Sludge System

4.3.1 Y_H and O_x for heterotrophos growing on soluble meat-processing wastewater

Results of observed yield coefficient, $Y_{,,}$, COD of unit biomass, O_x, and specific growth rate from five experiments with a total of 17 flasks are listed in Table 4-6.
An example of one experimental data is presented in Table 4-7 and Figure 4-3 ~ 4-6.

Table 4-6 The results of shake flasks experiments at 20 °C

Run ID	Date	S_o	S-type	X_o	S_o/X_o (COD base)	U(d^{-1})	Time period of straight line (hr) for ln(VSS)	Y_H (CODbased)	O_x	Y_o (vss based)	Time period of yield (hr)
16	27/5/98~28/5/9	429.92	GFC	64.86	4.67	2.89	0~6	0.71	1.46	0.41	0~6
16	27/5/98~28/5/9	401.73	GFC	60.07	4.71	3.14	0~6	0.7	1.36	0.43	0~6
16	27/5/98~28/5/9	496.87	GFC	66.76	5.24	3.52	0~6	0.87	1.27	0.52	0~6
16	27/5/98~28/5/9	604.35	GFC	70.36	6.05	3.88	0~6	0.79	1.34	0.56	0~6
21	4/6/98~16/6/9	263.92	GFC	28.33	6.56	1.91	0~16	0.53	1.51	0.36	0~16
21	4/06/98~16/6/9	397.02	GFC	35.83	7.80	2.06	0~16	0.66	1.46	0.35	0~16
21	4/6/98~16/6/9	526.68	GFC	40.83	9.08	2.20	0~16	0.71	1.5	0.36	0~16
21	4/6/98~16/6/9	632.25	GFC	40.83	10.90	1.89	0~21	0.56	1.52	0.36	0~21
22	27/6/98~28/6/9	392.93	GFC	14.85	18.64	2.25	0~24		1.49	0.41	0~24
22	27/6/98~28/6/9	441.61	GFC	13.89	22.40	2.41	0~24		1.43	0.43	0~24
22	27/6/98~28/6/9	561.53	GFC	17.33	22.80	2.42	0~22.5	0.63	1.4	0.41	0~22.5
22	27/6/98~28/6/9	493.77	GFC	14.18	24.53	2.43	0~24		1.41	0.41	0~24
23	8/7/98~19/7/9	24.78	GFC	16.28	1.07	2.48	0~6		1.22	0.47	0~4.5
23	8/7/98~19/7/9	43.48	GFC	18.60	1.65	2.24	0~6	0.64	1.23	0.33	0~4.5
23	8/7/98~19/7/9	74.34	GFC	20.93	2.50	3.39	0~6	0.51	1.23	0.31	0~6
23	8/7/98~19/7/9	99.12	GFC	18.60	3.75	3.17	0~8	0.44	1.53	0.39	0~8
23	8/7/98~19/7/9	221.25	GFC	27.90	5.58	2.94	0~8	0.45	1.47	0.32	0~8
Average								0.63	1.40	0.40	

The S_o/X_o ratios in those experimental flasks were in the range of 1.07 to 24.5. In such range, it was assumed no substrate-limiting phenomenon in each flask. The average yield coefficients of heterotrophic biomass growing on soluble meat-processing wastewater Y_H, COD based, and $Y_{,,}$, VSS based were determined as 0.63 and 0.40 in average respectively. The COD per unit biomass is in the range of 1.22 to 1.53 mgCOD/mgVSS, with an average of 1.40 mgCOD/mgVSS.

The found Y_H value of 0.63 in this study is the same as the typical value of Y_H (= 0.63) found for domestic waste by Sollfrank *et al.* (1991) [86] and Wanner *et al.*. (1992) [87] This value is also close to the finding, i.e. Y_H = 0.68 gcellCOD/gCOD, for meat-processing effluent conducted by Görgün *et al.* (1995), [33] and the Y_H = 0.67 gcellCOD/gCOD suggested by the Task Group [19] for domestic sewage. The averaged mass (VSS) based yield coefficient Y_o was determined as 0.40 mgVSS/mgCOD in this study. The Y_o value found here is similar to the values of 0.34 and 0.42 for abattoir wastewater determined by Lovett *et al.* (1984). [35] This yield coefficient Y_o (mgVSS/mgCOD) specifies the fraction of the biodegradable COD utilized for biomass production. The remaining part of biodegradable COD, i.e. 1-Y_o, is degraded for energy use.

Theoretically, the oxidization of 1 mole of biomass needs 5 moles of oxygen as electron acceptors, thus, a value close to 1.42 for O_x (COD of unit mass cell) should be obtained (as seen in equation 2-2, $5O_2/C_5H_7NO_2$ = 160/113 =1.416). The averaged O_x value observed in this study is 1.40 mgCOD/mgVSS, which is agreeable with the stoichiometry in equation 2-2 and also very close to the above theoretical value. This found O_x value is also similar to that of biomass growing on sewage, as specified in literature and Task Group Model No. 1, [15, 1419] i.e. 1.42 mgCOD/mgVSS, and comparable with the values previously reported associated with domestic sewage in the literature, e.g. 1.42 by Earais and Ekama (1976), 1.48 by Dold *et al.* (1980) and Haandel *et al.* (1981). [25, 28, 29]

While the ratio of Y_H/Y_o ratio should ideally equal to 1.4, the observed Y_H/Y_o ratio here is a little bit higher, i.e. 0.63/0.4 =1.575. This might be due to the adsorption of substrate onto biomass. By using the calculation of "Total $COD_{at\ time\ t}$ – soluble $COD_{at\ time\ t}$" to determine the increase of biomass COD at time t, some substrate COD might have been included in the increase of biomass COD. This can also explain the variation of observed O_x values in those flasks. On the other hand, these attached COD substrate might have made no significant bias in the increase of the weight of biomass. In general, the mass (VSS) based yield coefficient (Y_o) should be considered as more reliable than the COD based yield coefficient (Y_H) and the COD of unit biomass (O_x).

Table 4-7 Experimental data for evaluating yield coefficient, COD per unit biomass, and specific growth rate (resulting from a shake flask containing diluted soluble substrate in run21)

SK21-040 So/Xo (COD based)= 6.56

Time(hour)	Soluble COD (mg/L)	Total COD (mg/L)	SS Mean(mg/L)	VSS Mean(mg/L)	ln(VSS)	XCOD (mg/L)	Ox	d XCOD	dX	d SCOD	$Y_{H\ (COD\ based)}$	$Yo_{\ (VSS\ based)}$	U (d^{-1})	q, day^{-1}
0.0	263.9	302.9	35.0	28.3	3.3	39.0	1.38							
7.0	199.7	271.9	72.0	61.3	4.1	72.3	1.18	33.3	33.0	64.3	0.52	0.51		
10.0	122.1	293.7	108.0	97.3	4.6	171.7	1.76	132.6	69.0	141.8	0.94	0.49		
13.0	84.5	255.9	112.0	93.3	4.5	171.4	1.84	132.4	65.0	179.4	0.74	0.36		
16.0	63.4	209.0	108.0	101.3	4.6	145.6	1.44	106.6	73.0	200.5	0.53	0.36		2.7
21.0	50.5	180.8	96.0	89.3	4.5	130.3	1.46	91.3	61.0	213.4	0.43	0.29		
24.0	56.3	174.9	80.0	74.0	4.3	118.6	1.60	79.6	45.7	207.6	0.38	0.22		
36.0	55.2	157.3	76.7	71.7	4.3	102.1	1.43	63.1	43.3	208.7	0.30	0.21		
							1.51				0.53 (0~16 hour)	0.36 (0~16 hour)	1.91 0~16 hour)	

dX- Increase of MLVSS from time zero
XCOD- COD of Biomass = Total COD - Soluble COD
dXCOD Increase of XCOD from time zero
dSCOD- Decrease of soluble COD from time zero
$Yo_{\ (VSS\ based)}$ Yield coefficient, based on MLVSS
O_X- COD of biomass = (Total COD - Soluble COD)/(MLVSS) at time t
$Y_{H\ (COD\ based)}$ dXCOD/dSCOD, yield coefficient, based on COD
u (d^{-1}) specific growth rate calculated rom data of 0 ~ 16 hours
q (d^{-1}) specific substrate tuilization rate calculated rom data of 0 ~ 16 hours, = 24hours* ($SCOD_{t-0}$ -$SCOD_{t-16h}$)/(average of VSS from time 0 to 16 hours)/(16hours-0hour)

Table 4-7-1 Summary of results from 4 shake flasks of run21

Run ID	S_o	S-type	X_o	*S_o/X_o (COD based)	U(d^{-1})	Time period of straight line (hr)	$Y_{H\ (COD\ based)}$	O_x	$Y_{o\ (VSS\ based)}$	Time period of exponential growth (hr)	q, day^{-1}
21	263.9	GFC	28.3	6.56	1.91	0~16	0.53	1.51	0.36	0~16	2.77
21	397	GFC	35.8	7.80	2.06	0~16	0.66	1.46	0.35	0~16	3.26
21	526.7	GFC	40.8	9.08	2.2	0~16	0.71	1.5	0.36	0~16	3.26
21	632.3	GFC	40.8	10.90	1.89	0~21	0.56	1.52	0.36	0~21	2.55
average							0.62	1.50	0.36		

* COD per X_o was assumed = 1.42 mgCOD/mgVSS

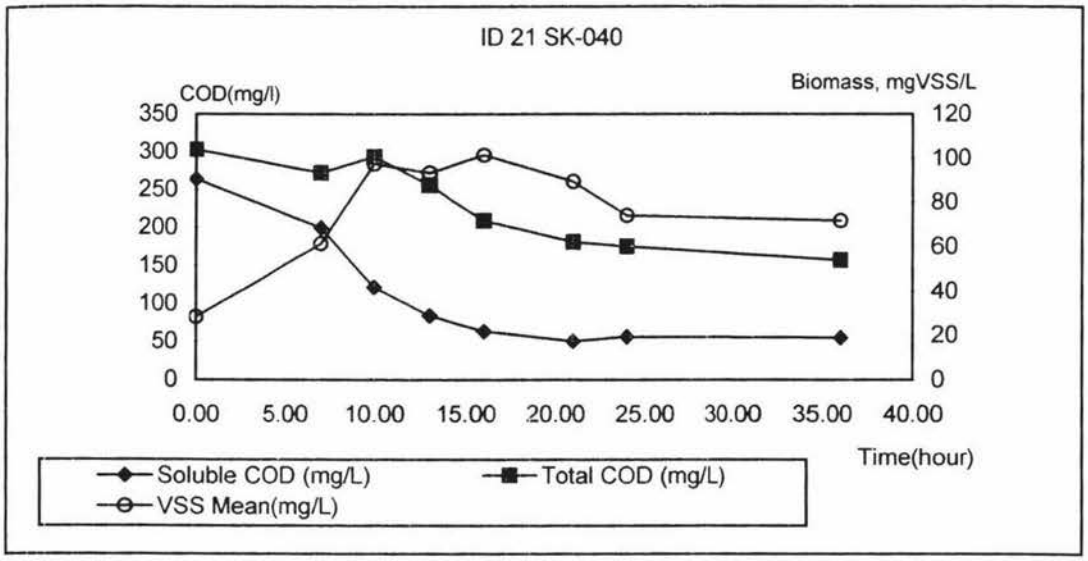


Figure 4-3 The growth of biomass in a shake flask -SK-040 of run 21

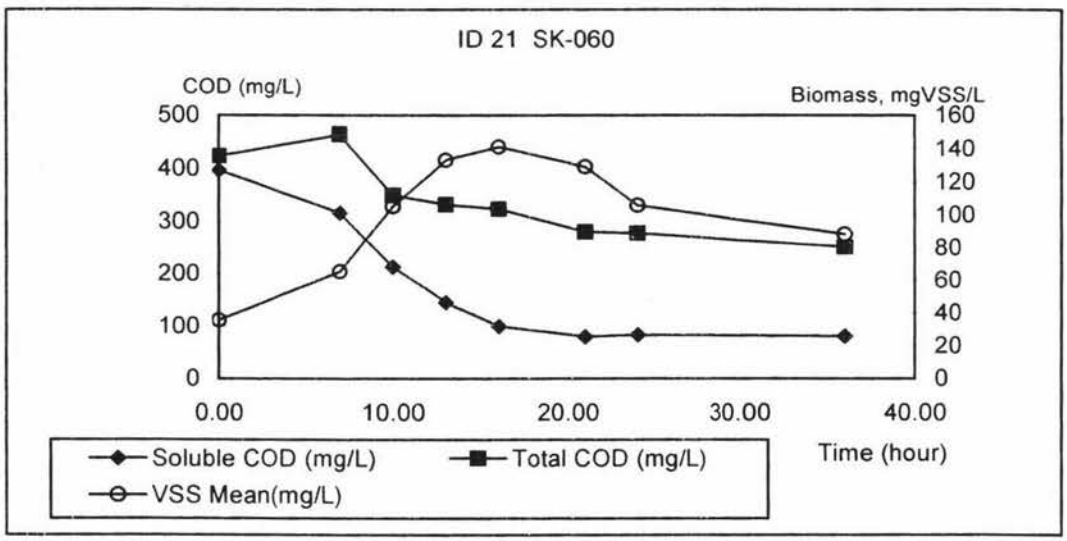


Figure 4-4 The growth of biomass in a shake flask -SK-060 of run 21

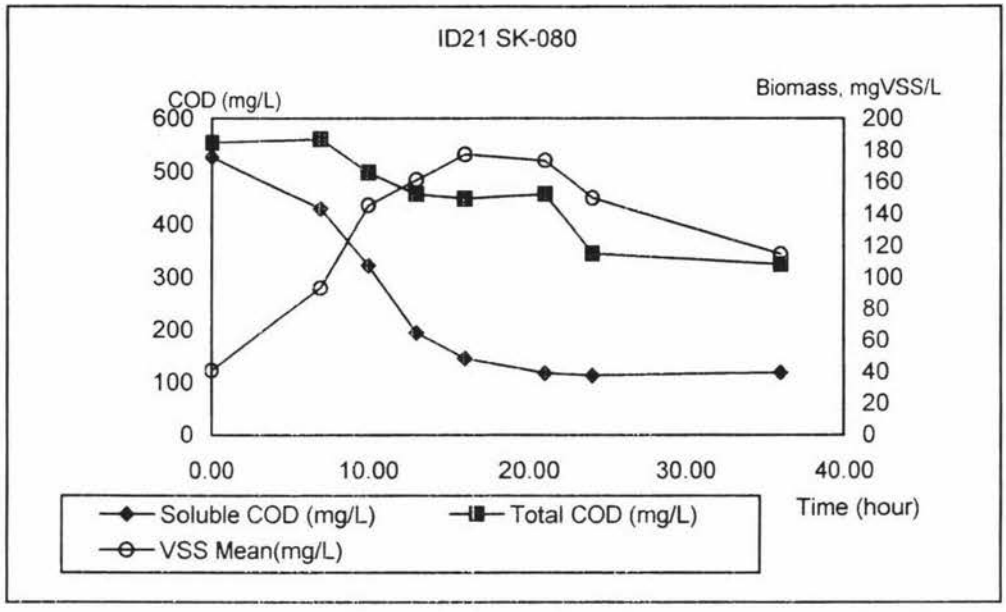


Figure 4-5 The growth of biomass in a shake flask -SK-080 of run 21

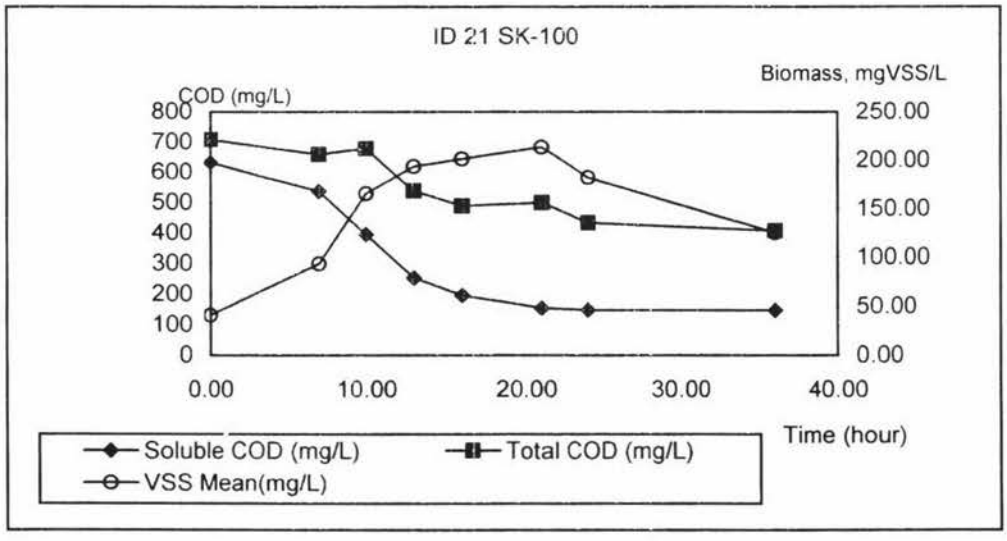


Figure 4-6 The growth of biomass in a shake flask -SK-100 of run 21

4.3.2 The influence of S_o/X_o on the value of yield coefficient

Chudoba *et al.* (1992) [69] suggested that the observed "yield" decreases with increasing S_o/X_o ratio, and explained the phenomenon by a theory that the quantity of substrate oxidized per unit of biomass synthesized would be greater for a growth response than for storage response. The yield coefficient of activated sludge growing on soluble meat-processing wastewater obtained from the above experiment showed no significant correlation with the S_o/X_o ratio in its batch cultivation. (See Figure 4-7)

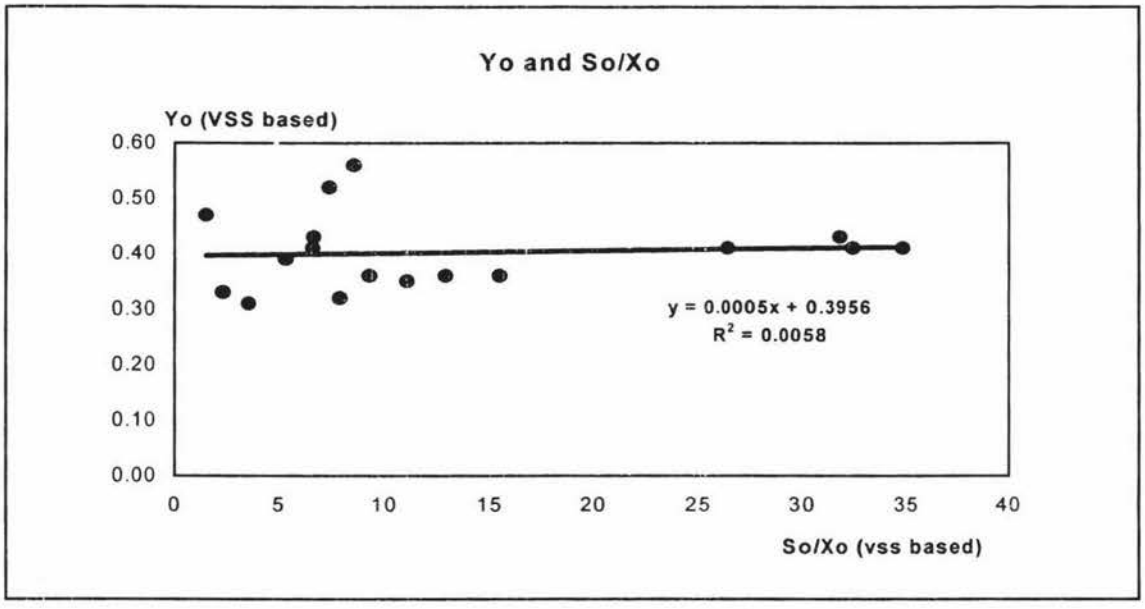


Figure 4-7 Parameter Y_0 and S_o/X_o

Unlike the finding of Chudoba *et al.* (1992), [69] varying the ratio of S_o/X_o caused no effect on Y_o. Similar to the finding of Payne (1970), [68] yield coefficient was found constant in regard of COD utilization in this study. The reason of finding no decreasing value of yield coefficient with increasing value of S_o/X_o in this study could be the sufficient amount of carbon and energy source provided in those batch-experiments. With the S_o/X_o value larger than 1, the observed growth of biomass might have been dominated by the increase in cell number (i.e. multiplication) rather than the increase in storage or accumulation. Consequently, the yields at the expenses of energy-efficient demands were not observed, instead, the yields at the expenses of high-energy demands were constantly observed in this study.

Since no significant correlation is found, this study assumes Y_0 to be a constant and

used the averaged value of Y_o (=0.40) for equation 3-3, $X_t = X_0 + Oxygen_{consumed} / \left(\frac{1}{Y_0} - O_x \right)$.

4.3.3 The specific growth rate of heterotrophos growing on soluble meat-processing wastewater

Both the data obtained from shake flask experiments and OUR data measured in respirometer were employed to evaluate the specific growth rate of heterotrophos growing on meat-processing wastewater.

Data collected from shake flasks (experiments for obtaining yield coefficient of heterotrophos growing on soluble meat-processing wastewater) were also used to calculate the specific growth rate of heterotrophos growing on soluble meat-processing wastewater, as seen in Table 4-6. The specific growth rate μ is calculated by equation 3-7 and was found to range from 1.91 to 4.07 day^{-1} for flasks with a S_o/X_o value greater than 1.

OUR data collected in each cell of the BI-100 Electrolytic Respirometer (Bioscience, Inc., Bethlehem PA 18017, USA) were also used to evaluate the specific growth rate μ (day^{-1}) of heterotrophos growing on meat-processing wastewater. The biomass preparation, substrate preparation, and experimental procedure have been described earlier in the methods and materials.

The treatment of OUR data included (1) collecting accumulated oxygen uptake data (2) biomass growth estimation using the equation 3-3, and (3) calculating the slope of $\ln(X_t)$, i.e. the specific growth rate μ, and is illustrated by the following experimental examples. (Figure 4-8 ~ Figure 4-10)

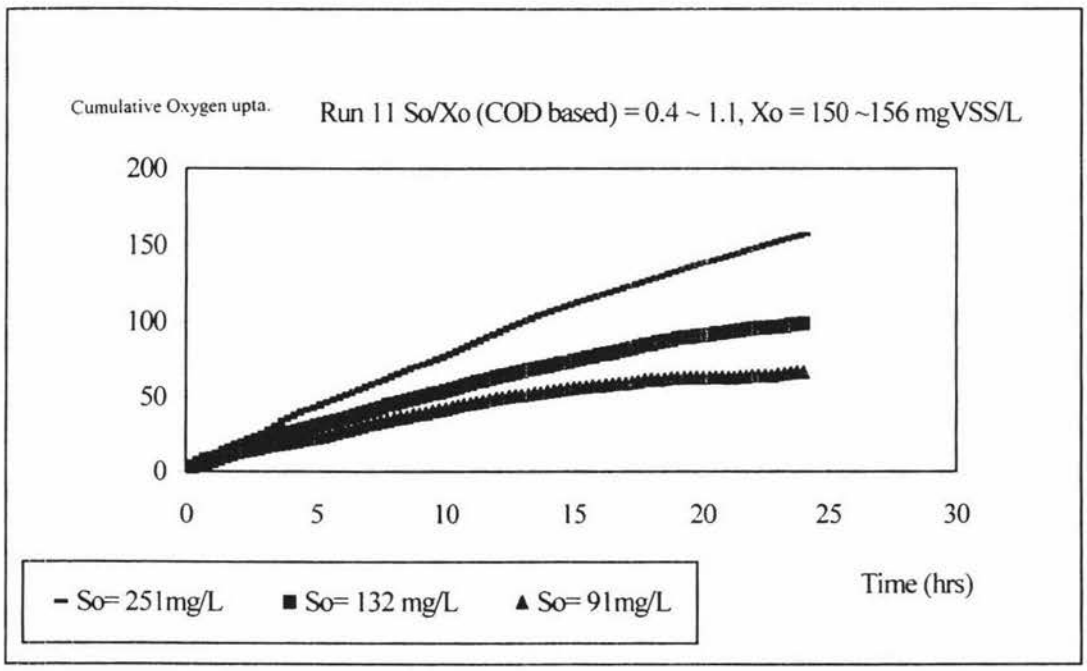


Figure 4-8 An example for determining μ using respirometric data. Step 1 – collecting accumulated oxygen uptake data (under experimental conditions of COD based $S_o/X_o = 0.4 \sim 1.1$, GF/C filtered substrate, and at $20^0 C$)

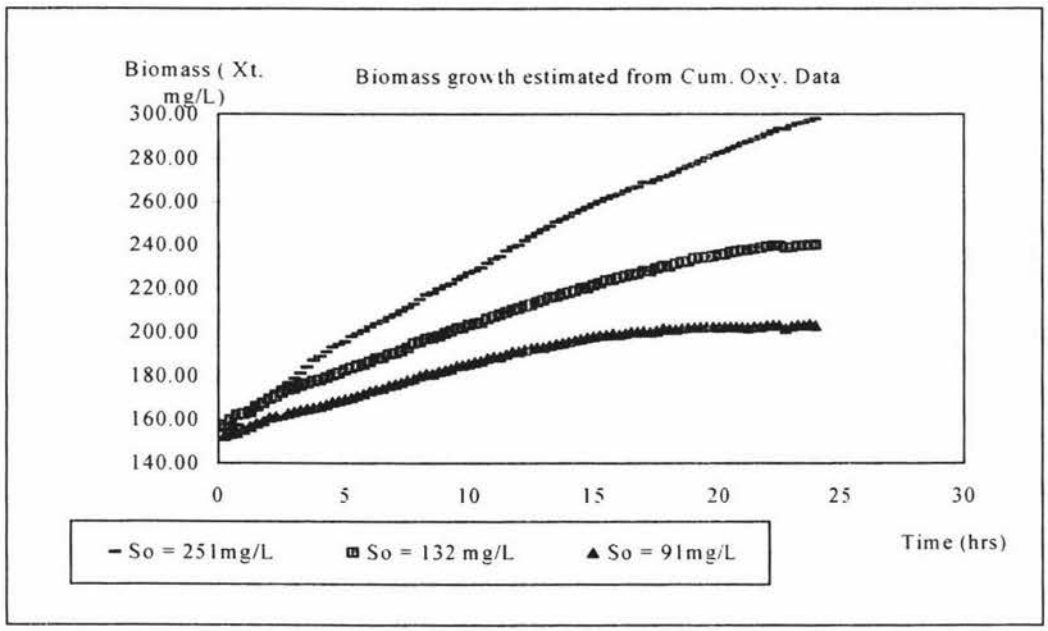


Figure 4-9 An example for determining μ using respirometric data. Step 2 – biomass growth estimation using the equation 3-3

In the above example, three batch reactors were installed in the respirometer, and the temperature was maintained at 20°C. Cell 1 had a $S_o/X_o = 1.135$, Cell 2 was 0.6 and Cell 3 was 0.43, the estimated biomass concentration at 24th hour was $X_{24hours} = 158.68 + 144.93 \times 0.324 = 205.5$ mg/L, which was very close to its observed biomass

concentration value, 225mg/L. The estimated biomass concentrations in the Cell 2 and 3 were 183.03 mg/L and 167.23 mg/L respectively, compared to observed biomass concentrations of 182.86 mg/L and 164.29 mg/L for each reactor. Such good agreement confirms the reliability of use of respirometric method for measuring the growth of biomass.

In step 3, the estimated biomass data was used to calculate the corresponding specific growth rate for each reactor by employing the equation 3-7, i.e.

$\ln\left(\frac{X_{t2}}{X_{t1}}\right)/(t_2 - t_1) = \mu_H$. The $t_1 - t_2$ was the time period of the straight line portion of the plot of $\ln(X_t)$ versus time, and X_t was the estimated biomass concentration for time t.

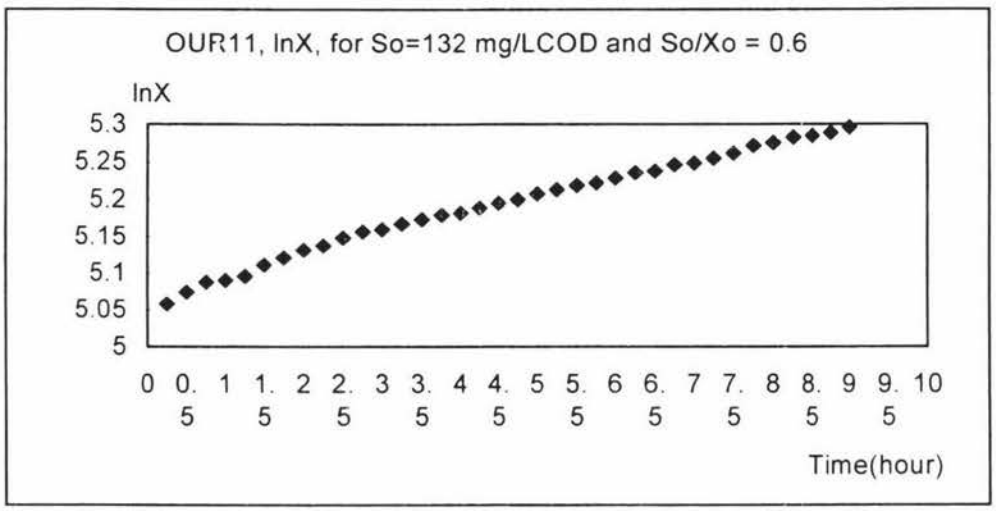


Figure 4-10 An example for determining μ. Step 3 – The slope of $\ln X_t$, the specific growth rate, μ (0~6hours), was calculated as 0.711 day^{-1}

A total of 15 respirometric experiments were carried out during the period 16/1/98~30/7/98, seven experiments were successful, as seen in Table 4-8.

Table 4-8 Results of respirometric experiments

Run ID	Date	S_o,mg/L	S-type	X_o, MLVSSm g/L	S_o/X_o (COD base)	U(d^{-1})	Time period of straight line (hr) for biomass growth
11	29/4/98-30/4/98	91.0	GFC	150	0.43	0.53	0~6
11	29/4/98-30/4/98	132.0	GFC	155	0.60	0.71	0~6
11	29/4/98-30/4/98	251.0	GFC	156	1.13	1.12	0~6
4	15/2/98~20/2/98	490.7	raw F	47.14	7.33	1.91	0~10
4	15/2/98~20/2/98	956.5	raw F	47.14	14.29	3.52	3~9
4	15/2/98~20/2/98	1215.3	raw F	47.14	18.15	4.13	3~9
4	15/2/98~20/2/98	1665.0	raw F	47.14	24.87	5.27	3~9
4	15/2/98~20/2/98	2377.0	raw F	47.14	35.51	5.17	3~10
18	1/6/98~4/6/98	821.5	raw F	11.5	51.60	6.31	2.5~8.5
18	1/6/98~4/6/98	1340.0	raw F	11.5	82.30	6.94	2.5~6
19	4/6/98~6/6/98	472.8	raw F	2.9	114.80	12.22	2~7
17	29/5/98~31/5/98	828.1	raw F	3.9	149.53	8.11	1.5~5.5
17	29/5/98~31/5/98	953.8	raw F	3.9	172.23	7.34	1.5~5.5
14	17/05/98~18/5/98	951.0	raw F	3.64	183.99	17.55	3~7
17	29/5/98~31/5/98	1118.3	raw F	3.9	201.93	6.22	2~8.5
17	29/5/98~31/5/98	1631.6	raw F	3.9	294.62	6.95	1.5~4.5
19	4/6/98~6/6/98	1469.7	raw F	3	345.00	12.01	2~6
19	4/6/98~6/6/98	1670.4	raw F	3	392.12	11.46	1.5~5

The results of Run 11 were obtained by respirometric experiments using GF/C filtered wastewater as substrate and in a condition of smaller S_o/X_o. The rest of the results were based on unfiltered meat-processing wastewater. Other OUR experiments failed mainly due to the malfunction of the electronic oxygen-generating electrodes in the respirometer.

In order to make a proper plot, 5 to 6 data points resulting from the same nature of substrate (GF/C substrate) are needed.

4.3.4 Determination of maximum specific growth rate $\mu_{H,MAX}$ and half-saturation constant K_s for heterotrophos growing on soluble peat-processing wastewater

Considering the shortage of available results upon GF/C filtered substrate in respirometric experiments, the data collected from flask experiments were used to determine the growth kinetics. Since the data of run 23 resulting from flask experiments have five μ and S data points, it was used for determining the maximum specific growth rate μ_{max} and half-saturation constant K_s for heterotrophos growing on soluble meat-processing wastewater by means of curve fitting. [121] (as shown in Figure 4-11)

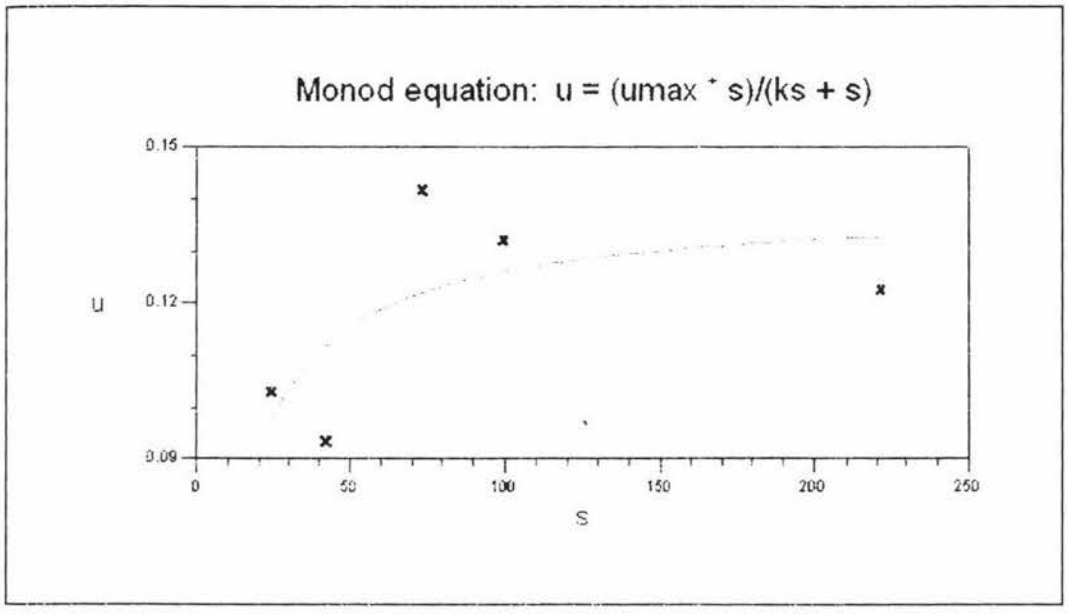


Figure 4-11 Data of μ_H and S (GF/C filtered meat-processing wastewater) of Run 23 curve fitting to Monod Equation. $R_a^2 = 0.2661$

R_a^2: The adjusted coefficient of multiple determination is a R_a^2 statistic adjusted for the number of parameters in the equation and the number of data observations. It is a more conservative estimate of the percent of variance explained, especially when the sample size is small compared to the number of parameters. It is computed as: $R_a^2 = 1-((n-1)/(n-p)\times(1-R^2)$, where n is the number of observations, p is the number of parameters, and R^2 is the unadjusted coefficient of multiple determination. [121]

Table 4-9 shows the result of μ_{max} and K_s for GF/C filtered meat-processing wastewater. The constants were derived from data of aerobic batch growth experiment (Run ID 23) and determined by a curve fitting technique (NLREG program [121]).

Table 4-9 $\mu_{H,MAX}$ and K_s for GF/C filtered meat-processing wastewater

Run ID	Range of S_o/X_{ao}	$u_{H,max}$, day^{-1} (p for μ_{max})	K_s, mgCOD/L (p for K_s)
23	1.1~5.6	3.31 (0.0039)	10.12 (0.302)

The results had a very good p-value for $\mu_{H,MAX}$. The $\mu_{H,MAX}$ values were found to be 3.3 d^{-1}.

The p-value for K_s is 0.302, indicating a 30 % probability that this result is not true, which is still too high. Further investigation on values of K_s is suggested.

In summary, the best estimated $\mu_{H,MAX}$ was found to be $3.3d^{-1}$ with a 95 % of possibility being true. The K_s value was estimated to be 10 mgCOD/L with a 30 % of possibility not being true. Further investigation on K_s is recommended.

4.3.5 Determination of decay coefficient of heterotrophic biomass

As indicated in the section of decay model in the literature review, the traditional decay coefficient b'_H (day^{-1}) in the endogenous respiration model can be related to decay coefficient b_H (day^{-1}) in the death regeneration model through the conversion equation 2-30. [28, 14, 19]

This study employed respirometric method used by Ekama *et al.* (1986) [32] to evaluate the decay coefficient of traditional decay coefficient, then calculated the death regeneration decay coefficient. The acclaimed biomass was removed from the lab CSTR reactor and put into a respirometer where the OUR was measured over a period of several days. The slope of a plot of the natural logarithm of the oxygen uptake rate versus time is the traditional decay coefficient b'_H. [32, 19] The traditional decay coefficient may then be converted to the decay coefficient b_H in the decay regeneration model by using equation 2-30.

Table 4-10 summarized the results of these values. Details of decay experiments (Run-24 and Run-25) are attached as Appendix C and Appendix A. An inhibitor of nitrification (thiourea) was added to cells of the respirometer to eliminate the effect of nitrification decay. The estimated values of decay coefficient for death regeneration b_H are 0.38 and 0.49 d^{-1}.

Table 4-10 Values of decay coefficient for meat-processing wastewater- by using the values of Y_H =0.63 (obtained from yield experiments) and f_p = 0.089 (obtained from the experiment of biodegradability) for calculation.

Experimental methods - Ekama *et al.*, 1986. [32] at 20° C

$b_H = b_H'/(1-Y_H(1-f_p))$

b_H': traditional decay coefficient, d^{-1}, endogenous respiration model

b_H: decay coefficient, d^{-1} in death regeneration model [183, 14, 19]

This study, meat processing wastewater

		b_H (d^{-1})	b_H' (d^{-1})
OUR24-	(without nitrification inhibitor)	0.57	0.024
	(with nitrification inhibitor)	0.38	0.016
OUR25-	(without nitrification inhibitor)	0.44	0.0182
	(with nitrification inhibitor)	0.49	0.020

Task Group Model (1987) [19] - Domestic wastewater b_H, d^{-1} = 0.62

The value of f_p (the fraction of the biomass that ends up as inert particulate products following decay) obtained in Run 24 of this study was 0.089, a typical value of 0.2 is indicated in Task Group Model No.1. [19]

The concept or meaning of the decay coefficient in the literature has not always been clearly addressed; care must be taken to avoid mistaking the values found in the literature.

4.3.6 Comparison of bio-kinetic constants of meat-processing wastewater

The obtained bio-kinetic constants were compared to the data of Görgün *et al.* (1995) [33] who used respirometric techniques [32, 92] to evaluate $\mu_{H,MAX}$, b_H, and bio-treatability of meat-processing wastewater, as summarized in Table 4-11. This study conducted shake flask experiments to determine $\mu_{H,MAX}$, while the techniques to evaluate b_H and biodegradation were the same with Görgün's.

Table 4-11 Bio-kinetic constants of meat-processing wastewater for aerobic bio-treatment system

Meat-processing wastewater	U_{max}, d^{-1}*	K_s, mgCOD/L*	b_H, d^{-1}	Y_H, g cell COD/COD*	f_{ex}	f_{es}
This study (at 20°C)	3.3	10	0.38, 0.49	0.63	0.14	0.089
techniques (reference)	shake flasks and curve fitting	shake flasks and curve fitting	respirometric measurements [32]	shake flasks	Procedure proposed by Orhon *et al.* [14]	Procedure proposed by Orhon *et al.* [14]
Görgün *et al.* (1995) [33]	4.2	30	b'_H, d^{-1} = 0.1	0.68	0.2 (assumed)	0.055
techniques (reference)	respirometric measurements [32] and curve fitting	respirometric measurements [32] and curve fitting	respirometric measurements [32]	Sözen (1995) [120]		Orhon *et al.* (1994a) in [33]
temperature	not specified	not specified	10-12°C	not specified	not specified	not specified

*The U_{max}, K_s, and Y_H of this study were based on GF/C filtered wastewater

The values of $\mu_{H,MAX}$ and K_s of this study were evaluated on GF/C filtered meat-processing wastewater. Görgün *et al.* (1995) [33] suggested that the $\mu_{H,MAX}$ is likely to be associated with readily biodegradable COD (RBCOD) fraction. Slowly biodegradable COD represented over 70% of total COD of the wastewater studied here and the fraction of readily biodegradable COD was 16% which is comparable with domestic sewage (8~25%). [90] Görgün *et al.* (1995) [33] suggested that the overall rate of COD removal is likely to be dictated by the hydrolysis rate, when the fraction of RBCOD is as small as the waste used for this study.

4.3.7 Activated sludge growing on unfiltered meat-processing wastewater

As mentioned above, the fraction of RBCOD of the waste in this study was found to be only 16%, in such case, Görgün *et al.* (1995) [33] suggested that the overall rate of COD removal for meat-processing wastewater is likely to be dictated by the hydrolysis rate.

In Activated Sludge Model No. 1, [19] the hydrolysis process of slowly biodegradable substrate is modelled by the expression:

$$\frac{dX_s}{dt} = -k_h \frac{X_s / X_a}{K_x + X_s / X_a} X_a \qquad (2\text{-}19)$$

where X_s = slowly biodegradable substrate concentration

X_a = active biomass concentration

k_h = maximum specific hydrolysis rate

K_x = half-saturation coefficient for hydrolysis

In this study, since the RBCOD is only 16 % of the total COD of meat-processing wastewater, and inert fraction is 10%, it was assumed that microbial growth on unfiltered meat-processing wastewater (mostly slowly biodegradable) follows a function similar to the above hydrolysis expression:

$$\mu = \frac{\mu_{H,MAX,overall} \bullet (S_o / X_o)}{K_{S_o / X_o} + (S_o / X_o)} \qquad (4\text{-}1)$$

where

μ = specific growth rate of activated sludge growing on unfiltered wastewater

$\mu_{H,MAX,overall}$ = maximum specific growth rate of activated sludge growing on unfiltered wastewater

S_o = initial COD concentration of unfiltered wastewater

X_o = initial concentration of biomass, COD based

K_{S_o / X_o} = half-saturation constant for utilizing unfiltered wastewater

The S_o/X_o and K_{S_o / X_o} here substitute the X_s/X_a and K_h in the hydrolysis equation (equation 2-19). This is because $S_o/X_o \approx X_s/X_a$ (S_o =RBCOD +Xs + inert COD matter, mgCOD/L, $\because$ 70 % of the substrate is X_s (slowly biodegradable COD), $\therefore S_o/X_o \approx X_s/X_a$)

The data collected from respirometric batch experiments, which used unfiltered wastewater as substrate, were analyzed by curve fitting for evaluating the overall growth kinetics. One OUR experiment (RUN ID 4) has five data sets of $\mu_{H,overall}$ and S_o/X_o, the data were curve fitting to equation 4-1, the S_o/X_o and the corresponding growth rate, μ, was found to fit equation 4-1with a adjusted coefficient of multiple determination (R_a^2) of 91%. (as shown in Figure 4-12)

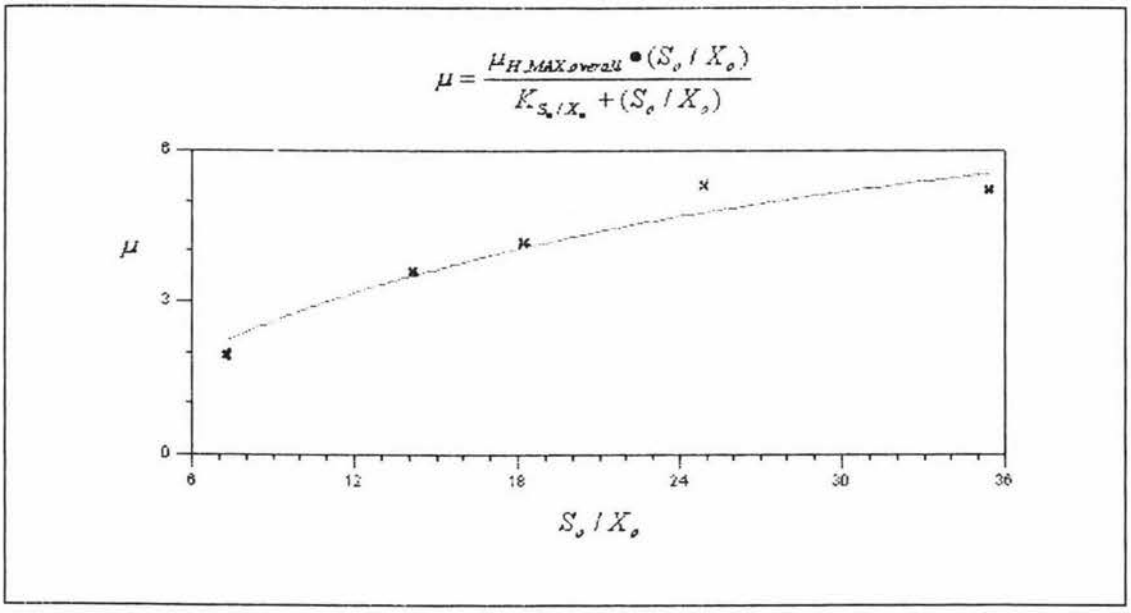


Figure 4-12 Data of S_o/X_o and $\mu_{H,overall}$ of OUR Run 4 for unfiltered meat-processing wastewater curve fitting to Equation 4-1 (R_a^2=0.9117)

The maximum specific growth rate of activated sludge growing on unfiltered meat-processing wastewater $\mu_{H,MAX,overall}$ and the half-saturation constant for utilizing unfiltered meat-processing wastewater K_{S_o/X_o} were determined as 9 day^{-1} and 22.22 respectively, and both values had a 90 % of possibility being true. As shown in Table 4-12:

Table 4-12 $\mu_{H,MAX,overall}$ and K_{S_o,X_o} of activated sludge growing on unfiltered meat-processing wastewater

Run ID	Type of meat-processing wastewater	Type of batch experiments	Range of S_o/X_0	$\mu_{H,MAX,overall}$	p-value for $\mu_{H,MAX,overall}$	K_{S_o/X_o}	p-value for K_{S_o/X_o}
4	Unfiltered	OURs	7.3~35.51	9.0	0.01858	22.22	0.101

For activated sludge growing on unfiltered meat-processing wastewater, when S_o/X_o (COD based) reaches 22, the specific growth rate will be higher than half of the maximum rate. The maximum specific growth rate of activated sludge is 3 times as fast for the unfiltered feed, and according to Görgün *et al.* (1995), [33] it is mainly depending on the process of hydrolysis.

4.4 Bio-kinetics for Nitrification in an Activated Sludge System

4.4.1 Oxygen demand calculation

An estimation of oxygen demand, which the biodegradation process in the experimental bag would require, was conducted to exam the sufficiency of oxygen supplied to the bag.

For nitrification study, some 700 ml air will be supplied into the experimental bag by adding compressed air in the preparation processes. The air contains 21% oxygen, which is 700*0.21=147 ml oxygen. This means each experimental bag contains some 196 mg oxygen, i.e.32mg * 147ml/[(293/273)*22.4ml/m.mole] = 196mg.

For bags contained 320ml of 500 mg/L COD GF/C substrate, the oxygen requirements would be 320ml×500mg/L÷1000ml =160 mg, if the carbonaceous GF/C filtered substrate were all biodegraded during the course of nitrification. If the bag also contains some 50 mgNH_4-N/L substrate, another 70mg oxygen (320ml×50mg/L×4.33mgO_2÷1000ml =69.28 mg) will be required for complete nitrification. That makes a total of 160 + 70 =230mg oxygen requirement for bags

containing 320ml of 500mgCOD/L and 50 mgNH4-N/L substrate, which is only about 34mg higher than the supplied oxygen amount (196mg).

From the above calculations of oxygen requirements, it was assumed that this type of experimental design (i.e. close system) is unlikely to be oxygen limited during the exponential growth period (first 2 to 3 days) of nitrifying biomass.

4.4.2 Mass transfer test

To confirm the above assumption, a preliminary test was carried. Three bags were fed with the same GF/C filtered meat-processing wastewater (COD $\approx$ 700 mg/L and $NH_4^+ - N \approx 69$ mg/L) and differing biomass concentrations. Bag No.1 contained a MLVSS concentration of 247 mg/L (named 1X), bag No.2 contained a MLVSS concentration of 124 mg/L (named 1/2/X), and bag No.3 contained a MLVSS concentration of 62mg/L (named 1/4X). This initial experiment to determine if this method would limit the mass transfer of oxygen is detailed in the methods and materials section.

The mass transfer test results are shown in Table 4-13 and Figure 4-13. On day 2, the oxidized nitrogen concentration in the three bags was 74.4mg/L, 46.6mg/L, and 19.4 mg/L for No.1, No.2 and No.3 bag respectively. With increasing biomass concentration (4:2:1), there was an increase in the nitrification rate (3.8:2.4:1). This indicated that, in the first two days of the batch experiment, the system was not likely to be mass transfer limited with the ranges of substrate and biomass tested.

Table 4-13 The result of mass transfer test for nitrification

	No.1 Bag	No.2 Bag	No.3 Bag
Time, day	$NO_x - N, mg/L$	$NO_x - N, mg/L$	$NO_x - N, mg/L$
0	0.26	0.26	0.26
0.5	8.87	2.46	1.19
1	28.24	9.39	3.33
1.5	48.55	21.54	8.46
2	74.45	46.60	19.39

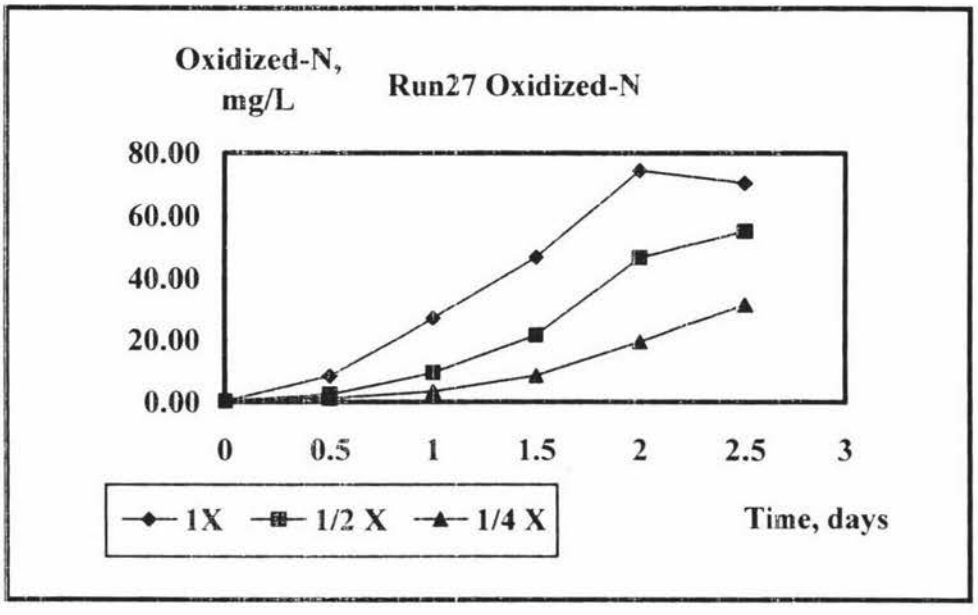


Figure 4-13 Increase of $NO_x - N$ over time in the mass transfer test

The specific nitrification rates of these bags were calculated as 0.15, 0.187 and 0.153 mgNO_x-N/mgVSS/d, indicating no mass transfer limiting in those three bags.

It was concluded that in a close system containing 700ml air and 300 ml substrate containing COD $\approx$ 700 mg/L and $NH_4^+ - N \approx 69$ mg/L, oxygen limiting is unlikely to occur during the first 2 days of biodegradation process.

4.4.3 Alkalinity control

As previously indicated, 7.07 g$CaCO_3$ of alkalinity will be consumed per g of $NH_4^+ - N$ oxidized. This means that 1.981 mole HCO_3^- will be consumed per mole of $NH_4^+ - N$ oxidized. For 69 mg /L $NH_4^+ - N$ in the bags of mass transfer evaluation to be oxidized, 9.7 millimoles of HCO_3^- alkalinity will be consumed. Alkalinity of 4.85 moles $CaCO_3$ is equivalent to 9.7 millimoles of HCO_3^- alkalinity. Therefore, 485mg $CaCO_3$ alkalinity was required in the system for 69 mg/L $NH_4^+ - N$ to be oxidized without alkalinity limitation.

The $NH_4^+ - N$ concentrations of GF/C filtered substrate of meat-processing wastewater was measured to be 60.4 mg/L in average. ranged from 35.5 to 84.9 mg/L for $\pm 1SD$. The alkalinity was also measured so that the required addition of alkalinity could be calculated. The alkalinity of GF/C filtered substrate was found to be 412, 400, 246.7, and 241.9 mg$CaCO_3$ /L, depending on the strength of the wastewater.

The alkalinity of the GF/C filtered substrate was corrected with $NaHCO_3$ when insufficient.

4.4.4 Nonlinear least-squares analysis

Linear approaches have some shortcomings: (1) the inversion of data violates several important assumptions of linear regression (Smith *et al.* (1998) [113]) and (2) neglecting some data and that may lead to unreliable estimated results. (Sözen *et al.* (1996) [99]) Therefore, the author adopted the nonlinear least-squared approach, which has been suggested by Sözen *et al.* (1996) and Smith *et al.* (1998). [99, 113]

The batch-approach experimental procedures were described in the methods and materials section. The equation 2-50 derived by Sözen *et al.* (1996) [99] was used for determining the constants "k" and "a" by nonlinear least-square analysis techniques.

The determined "k" and "a" were $\frac{Y_N(\mu_{N,MAX}-b_N)}{\mu_{N,MAX}X_{N0}}$ and $\mu_{N,MAX}-b_N$ in equation 2-48 respectively.

Given an initial guess of the value of k and a, together with appropriate constraints about k and a, such as $k>0, a>0$, the Excel "Solver" was able to obtain values of k and a, by means of minimizing the sum of squared differences between observed and modeled S_{NO} values during the course of nitrification.

The reliability of the results of "Solver" was verified by another regression analysis program called NLREG. [121] NLREG is a program for multivariate, linear, polynomial, exponential, logistic, and general nonlinear regression. It uses an iterative function optimization algorithm to minimize the squared residuals for the actual function so that the constants in the function can be determined.

4.4.5 The maximum specific growth rate of nitrifying biomass in an activated sludge system treating meat-processing wastewater

A nitrification kinetic study (Run ID 29) was conducted for obtaining the maximum specific growth rate of nitrifying biomass.

Amongst 6 bags of Run 29, 3 bags (named 2X-1, 2X-2, and 2X-3) have biomass concentrations of 130 to 140 mgMLVSS/L; while the other 3 bags (named 1X-4, 1X-5, and 1X-6) have biomass concentrations of 85.7 to 91.4mgMLVSS/L. Under conditions with no mass transfer limitation, the specific rate of growth and substrate utilization of the two groups should be the same.

There is one bag in each group with a synthetic substrate containing 40 ~50 mg NH_4^+-N/L as a substrate with no carbon for examining the effect of carbonaceous oxygen demand effect. Ten ml of a pH buffer and 10ml of mineral solutions were also added to the synthetic wastewater. The buffer and mineral solutions were prepared as described by Sözen *et al.* (1996). [99]

The observed S_{NO} were curve fit to equation 2-50 (i.e. $S_{NO} = S_{NO0} + \frac{1}{k}\left[e^{at} - 1\right]$), the constants, k and a, in equation 2-50 were then determined by means of nonlinear least-squares analysis. The initial guess of k and a was made by assuming that 5% of MLVSS was nitrifying biomass, $\mu_{N,MAX} - b_N = 0.5$, and $Y_N = 0.24$ mgCOD/mgN.

The observed $NO_x - N$ values and nonlinear regression derived $NO_x - N$ values (predicted by equation 2-50, with the least square determined "k" and "a") for each experimental bag are shown in Figure 4-14, Figure 4-15, and Appendix D.

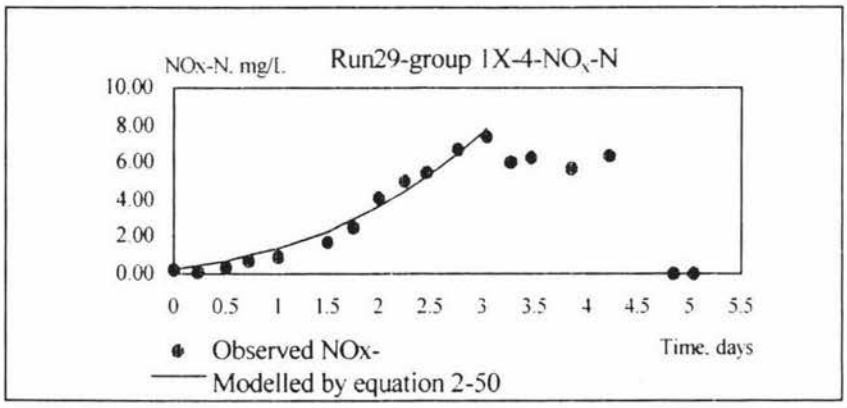


Figure 4-14 Observed and modeled $NO_x - N$ in Bag-1X-4 (Initial biomass concentration: 85.7 mg/L, Initial substrate concentrations: S_{NH4-N} = 52.2mg/L, S_{COD} = 534.7 mg/L)

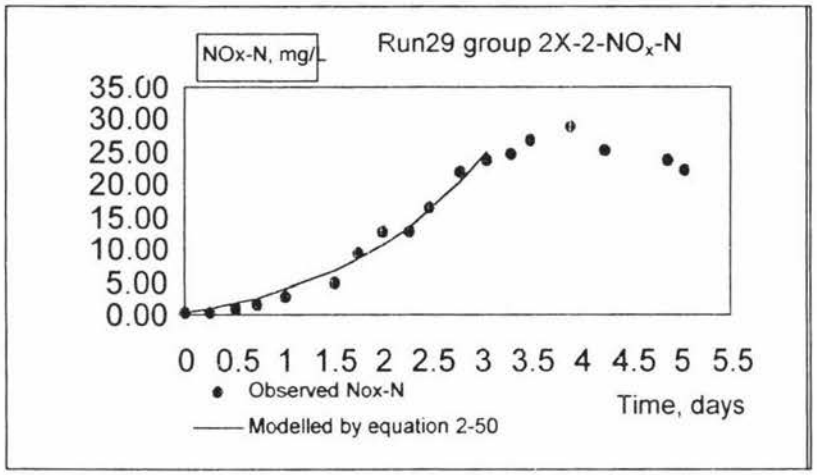


Figure 4-15 Observed and modeled $NO_x - N$ in Bag 2X-2 (Initial biomass concentration: 140 mg/L, Initial substrate concentrations: S_{NH4-N} = 50 mg/L, S_{COD} = 496 mg/L)

The best estimated maximum specific growth rates of nitrifying biomass are shown in Table 4-14:

Table 4-14 Maximum specific growth rate of nitrifying biomass (Result of Run 29)

	Bag ID. Type of substrate	1X-4. GF/C	1X-5. GF/C	1X-6. synthetic	2X-1. GF/C	2X-2. GF/C	2X-3. synthetic
	Parameters						
Initial substrate and biomass conditions	$S_{NH_4^+-N,I}, mg/L$	52.2	50.4	43.9	48.2	50.0	47.7
	$S_{NO_x-N,I} mg/L$	0.205	0.190	0.129	0.151	0.203	0.171
	$X_{a,I}, VSSmg/L$	85.7	91.4	88.5	134.3	140.0	134
	$S_{COD,I}, mg/L$	534.7	521.1	27.3	499.4	496.6	18.2
Solver	$\frac{Y_N(\mu_{N,MAX}-b_N)}{\mu_{N,MAX}X_{N0}}$	0.709	0.703	1.085	0.337	0.266	0.551
	$\mu_{N,MAX}-b_N$	0.613	0.688	0.608	0.714	0.665	0.646
	SSD (sum of squared residual)	1.894	2.375	0.104	14.468	14.598	0.172
NLREG	$\frac{Y_N(\mu_{N,MAX}-b_N)}{\mu_{N,MAX}X_{N0}}$	0.738	0.701	1.284	0.882	0.266	0.550
	$\mu_{N,MAX}-b_N$	0.626	0.686	0.674	0.560	0.665	0.645
	SSD (sum of squared residual)	1.890	2.369	0.103	9.533	1.460	0.172
	R_a^2	0.974	0.980	0.953	0.985	0.980	0.982

The maximum specific growth rate of nitrifying biomass was found to be in the range of 0.61 to 0.71 day^{-1} by means of "Solver" and 0.56 to 0.69 day^{-1} by means of MLREG.

The obtained "$\mu_{N\_MAx} - b_N$" values from the two nonlinear least-squared analysis for each bag are very much alike, as shown in Table 4 -14. The adjusted coefficient of multiple determination, R_a^2 values, in NLREG are very high (0.953 ~0.989). Therefore, these "$\mu_{N\_MAx} - b_N$" values are reliable.

4.5 Measurement Error of Experiments

To examine the variation among the duplicate samples, calculation of coefficient of variation was carried out as follows: [89]

$(C.V)_i = (SD)_i / (Mean)_i$

Where

$(C.V.)i$ = the coefficient of variance for duplicate sample set number i,

$(SD)i$ = standard deviation of values for duplicate sample set number i,

$(Mean)i$ = average of values for duplicate sample set number i

An overall C.V. was calculated for analysis of COD, TKN, NH3-N, TSS, and VSS, by averaging the C.V. of each duplicate sample set, as below:

$$(C.V) = \sum_{i=1}^{n} C.V./n$$

where

n = number of duplicate sample set

The overall C.V. was 5.5% for COD analysis, 8.2% for NH_3-N analysis, 7,1% for TKN analysis, 5.5% for analysis, and 6.75% for VSS analysis.

Chapter 5 Conclusions and Suggestions

5.1 Measurement Error

There were 5.5% to 8.2% measurement errors involved in the results of this study. This includes the operational errors (caused by the variation of analysis operation) and the systematic errors (caused by the variation of equipment itself and/or chemicals reagents). This means that the accuracy of the experimental analysis of this study is over 90%.

5.2 The Components and Biodegradation of Meat-processing Wastewater

In this study, the unfiltered meat-processing wastewater was characterized to contain COD ranging from 534 to 3118 mg/L, ammonia nitrogen from 36 to 69.5 mg/L, TKN from 62 to 127 mg/L, total suspended solid from 66 to 1016 mg/L, and the alkalinity averaged at 432.5 mg/L (as $CaCO_3$). The pH value of the meat-processing wastewater was mostly in the range of 6.3 to 7.2. These characteristics agree with Cooper and Russell (1991) and Cooper *et al.* (1979). [3.1]

The GF/C filtered meat-processing wastewater, defined as soluble substrate in this study, contains 40 to 60 % of the total COD and 60 to 80 % of the total TKN in unfiltered substrate. The concentration of ammonia nitrogen of the GF/C filtered wastewater is almost equal to that of the unfiltered substrate.

During the off peak operation seasons of summer and early autumn, the strength of carbonaceous matters of meat-processing wastewater varied much more than winter and spring. Despite the off peak operation, the wastewater in summer did not appear to be low nitrogenous strength

Approximately, 35% ~ 40% of total COD composition of the primarily treated meat-processing wastewater is in the soluble/fine colloidal form and the remaining 60% ~ 65% is in particulate form. Only 25~29% of soluble carbonaceous organic matters of meat-processing wastewater is readily biodegradable. In a sample of meat-processing

wastewater containing a total COD of 1941mg/L, the soluble COD was 674 mg/L, the readily biodegradable COD (RBCOD) was 310 mg/L. This shows that some slowly biodegradable or inert organic matter may exist in the primarily treated meat-processing wastewater as soluble form. The inert fraction, including particulate and soluble organic matter in the primarily treated meat-processing wastewater is about 10 % of total COD, which is similar to the range of 13% to 14% reported by Görgün *et al.* (1995) and Ekama and Marais (1977). [33, 26] The coefficients for soluble microbial soluble production (f_{sp}) and particulate microbial production (f_{xp}) of primarily treated meat-processing wastewater was found to be 0.14 and 0.089 respectively. These results resemble the values of 0.2 and 0.055 presented by Görgün *et al.* (1995). [33] The difference between the found f_{xp} value of 0.089 in this study and the listed values of 0.09 and 0.08 in Ekama and Marais (1977) [26] and Task Group Model No. 1[19] is very small, considering the error involved in the determinations.

5.3 Activated Sludge Treatment Processes and Bio-kinetic Constants

The activated sludge treatment process provides a promising approach to remove the carbonaceous and nitrogenous oxygen demand from the discharged wastewater thus mitigate the oxygen depletion in the receiving waters, when untreated waste is directly discharged.

Addition to the characteristics (components and biodegradability) of wastewater, kinetic constants such as the microbial growth rate, yield coefficient, decay rate and half-saturation constant are crucial elements to many bio-process expressions and models used to optimize the bio-treatment system and for predicting the outcome of the treatment.

5.4 Bio-kinetic Constants for Carbon Removal in an Activated Sludge System for Primarily Treated Meat-processing Wastewater

The yield coefficients of heterotrophic biomass for aerobic bio-treatment of meat-processing wastewater was found to be 0.63 mgcellCOD/mgCOD (Y_H) and 0.4 mgcellVSS/mgCOD (Y_o) without significant variation (as shown in Figure 4-7, R^2 = 0.0058). This Y_H value of 0.63 mgcellCOD/mgCOD is close to the yield coefficient

value of 0.68 mgcellCOD/mgCOD found by Görgün *et al.* [33] for meat-processing effluent, and the yield coefficient value of 0.67 mgcellCOD/mgCOD suggested by the Task Group Model No.1 [19] for domestic sewage. The Y_o value is similar to the values of 0.34 and 0.42 determined by Lovett *et al.* (1984) [35] for abattoir wastewater. The avearged COD per unit of biomass, O_X, determined in this study is 1.40 mgCOD/mgVSS. This O_X value is agreeable with the stoichiometry in equation 2-2 and comparable with the values previously reported associated with domestic sewage in the literature, e.g. 1.42 by Earais and Ekama (1976), 1.48 by Dold *et al.* (1980) and Haandel *et al.* (1981). [25, 28, 29] Due to the possible bias caused by the adsorbed substrate COD, the mass based yield coefficient Y_o should be considered more reliable than the COD based yield coefficient Y_H.

It has been suggested that the growth of heterotrophos may be limited, substrate storage phenomenon may dominate the yield, and the filamentous bacteria tend to dominate the system when S_o/X_o ratio is too low, [69,118] the constraint of "S_o/X_o ratio greater than 1" in this study was to remove these confounding effects of substrate limiting conditions. Resulting from flask experiments where S_o/X_o ratios were greater than 1, the specific growth rate of heterotrophic biomass (μ_H) was determined to range from 1.91 to 4.07 day^{-1}, and the specific substrate utilization rate was from 2.5 to 5.6 day^{-1}.

Unlike the finding of Chudoba *et al.* (1992), [69] varying the ratio of S_o/X_o caused no effect on Y_o in this study.

With the presence of nitrification inhibitor, the values of decay coefficient of the death regeneration concept, b_H, were estimated to be 0.38 day^{-1} and 0.44 day^{-1} (Run 24 and Run25). These values are not very different from that suggested (=0.62, day^{-1}) for domestic sewage by Task Group Model No.1 [19] where the same respirometric method used in this study was recommended and its "death regeneration" concept has been adopted in this study.

This study suggests the biomass oxygen uptake data taken in the respirometer to be

quite reliable for evaluation of the corresponding specific growth rate, as confirmed in the result section. However, the function of the electronic oxygen-generating electrodes of the respirometer needs to be well checked prior to the experiments.

The maximum specific growth rate $\mu_{H,MAx}$ (day^{-1}) and the half saturation constant K_s (mgCOD/L) were determined by curve fitting. Data used for this determination were derived from experiments applying the same GF/C filtered substrate and the same source of biomass. For heterotrophic biomass growing on the GF/C filtered meat-processing wastewater, the maximum specific growth rate ($\mu_{H,MAx}$) was determined to be 3.3 day^{-1} with a 95% of possibility being true, and the half-saturation constant (K_s) was found likely to be 10 mgCOD/L with a 30 % of possibility not being true. The determined $\mu_{H,MAx}$ value compares well with the value of 4.2 day^{-1} previously associated with meat-processing effluent. [33] The found K_s value is smaller than the value of 30 reported by Görgün *et al.* (1995) and the value of 20 by Task Group Model No.1. [33, 19] These obtained growth kinetic values are likely to be associated with the readily biodegradable COD fraction, and show good consistency with the literature associated with meat-processing effluent. [33]

Since the slowly biodegradable COD was found to account for over 65% of COD in the unfiltered meat-processing wastewater, the bio-kinetics of biomass utilizing unfiltered meat-processing wastewater were also investigated. The proposed expressions of Strenstrom [51] and Task Group Model No.1 [19] were applied to investigate the relationship between the S_o/X_o ratio (i.e. initial concentration of unfiltered substrate/ initial concentration of biomass) and the corresponding growth rate (μ). It was found to fit a Monod type function (i.e. $\mu = \frac{\mu_{H,MAX,overall} \bullet (S_o / X_o)}{K_{S_o/X_o} + (S_o / X_o)}$), and the maximum rate constant for COD removal of biomass utilizing unfiltered meat-processing wastewater was determined as 9 day^{-1}, while the half-saturation constant for removal of slowly biodegradable substrate was 22 (i.e. K_{S_o/X_o} =22.22). Both determined values of $\mu_{H,MAX,overall}$ and K_{S_o/X_o} had a 90 % of possibility being true.

5.5 Bio-kinetic Constants for Nitrification in an Activated Sludge System for Primarily Treated Meat-processing Wastewater

The obtained value of maximum specific growth rate of nitrifying biomass in an activated sludge system for primarily treated meat-processing wastewater ($\mu_{N,max,}$) was 0.65 day $^{-1}$. This value compares well with the values of 0.62 day^{-1} and 0.60 day^{-1} associated with domestic sewage, and the values of 0.56 to 0.63 day^{-1} for meat-processing effluent. [28,30,99]

5.6 Limitations and Suggestions

Since the obtained half-saturation constant (K_s = 10 mgCOD/L) for activated sludge utilizing GF/C filtered meat effluent was found with a 30 % of possibility not being true, further investigation on K_s is recommended.

About 70 % of the COD composition of meat-processing effluent is in solid/fine form, the kinetics of activated sludge utilizing the particulate portion of meat-processing effluent is recommended to be further investigated.

This study provide little information of temperature dependency coefficient for kinetics of aerobic bio-treatment. The kinetic values reported in this study were obtained under the conditions of $20^{o}C$. Most kinetic parameters are influenced by temperature. Although a number of temperature correction factors have been reported, it is suggested that further investigation on the temperature dependencies of kinetics of activated sludge utilizing meat-processing wastewater to be performed. This is essential to apply the bio-kinetics for conditions other than $20^{o}C$.

In regard of nitrification, this thesis provided collections of values for the decay constant, half-saturation constant from the literature. Few such values are associated with meat-processing wastewater. Further studies need to be carried out to acquire the comprehensive nitrification kinetic values for a complete design of an activated sludge system for the meat industry.

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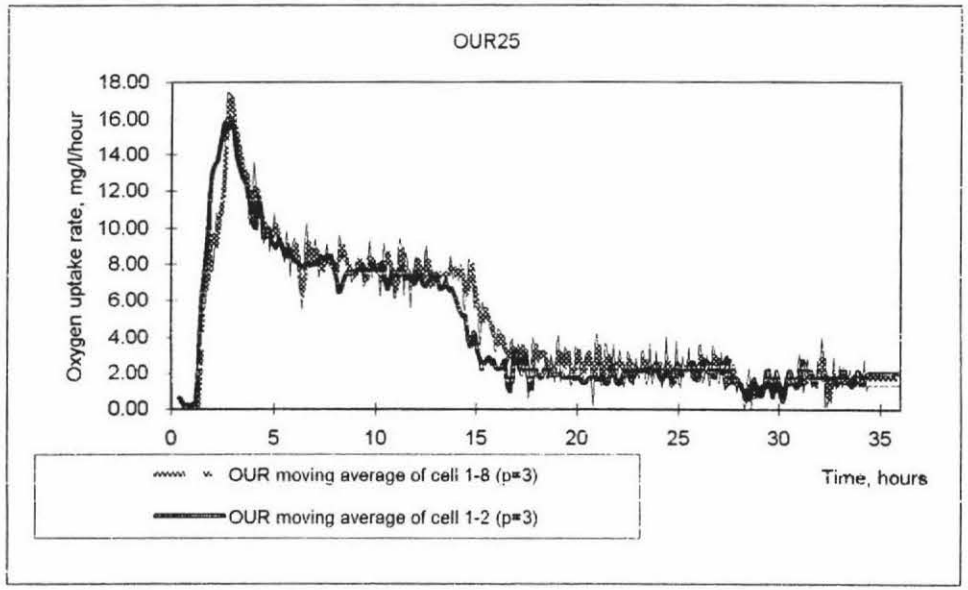
Appendix A

Calculation of the fraction of readily bio-degradable COD

Respirometric method for evaluation of readily bio-degradable COD (run25)

So (GFC substrate)= 200.028 mg COD in one litter reactor X_o= 548.4 mgVSS in one littre reactor

S_o/X_o= 0.26 COD based O_x= 1.57 mgCOD/mgVSS



Readily biodegradable substrate in two reactors which corresponds to the initial high oxygen consumptions (cell 1-2 and cell 1-8 are identical)

Ss, mg = $(1/(1-Y^*O_x))^*dO_2^*(V_{ww}+V_{mi})/V_{ww}$= 51.01 49.14 57.69

(Ekama et al., 1986) (1.5~5.5hours) (1.7~4.3hours) (1.5~4.5hours)

by applying average of 2 cells' O_2 comsumptions itno the equation

Substrate added, S_o = (543.6mg/L*0.33L+206.4mg/L*0.1L)/1L= 200.028 mg in each one littre reactor

where 543.6mg/L is GFC feed concentration in COD term;

206.4 mg/L is suloble solution concentration in the 100ml biomass portion

Ss=	51.01	49.14	57.69
Ss/So (GFC)=	0.26	0.25	0.29

Raw wastewater concentration of 6/7/98 feed souce was 1020mgCOD/L

Ssi/raw feed=	0.15	0.15	0.17
	(1.5~5.5hours)	(1.7~4.3hours)	(1.5~4.5hours)

Appendix B The Result of A Batch COD Method for Evaluating the Biodegradability - The Estimation of the Fraction of Inert Matter

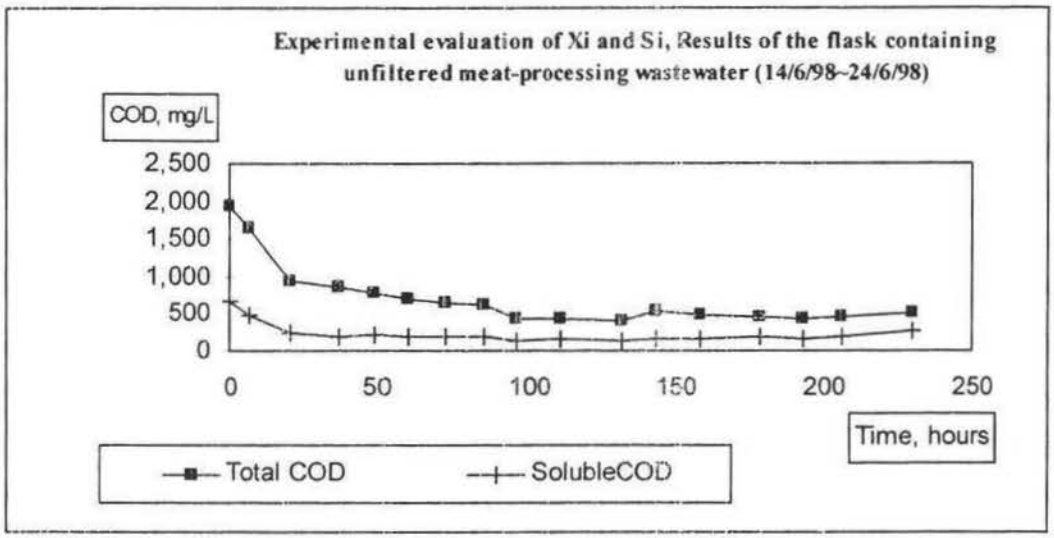


Figure B-1 Decrease of COD in flask containing unfiltered meat-processing wastewater

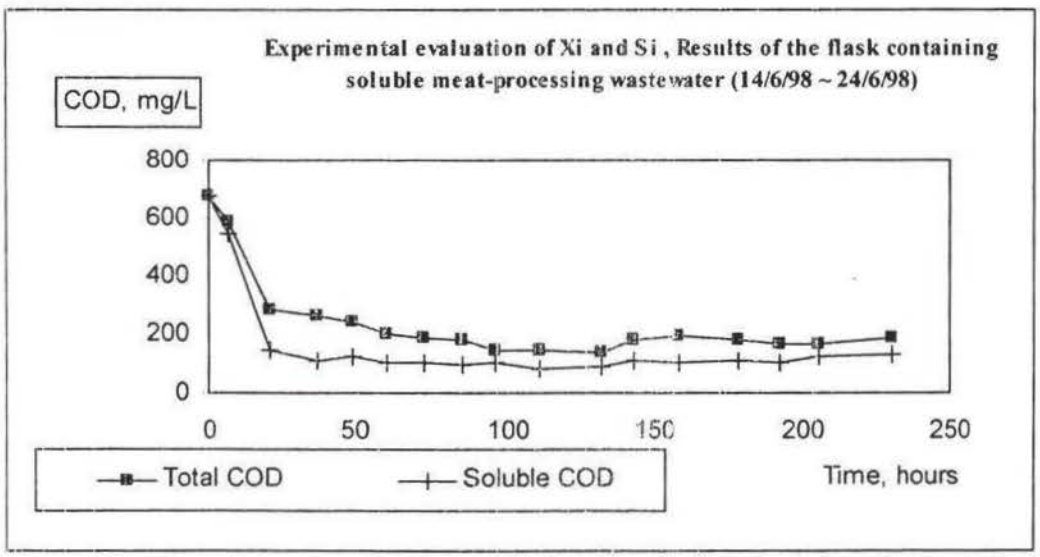


Figure B-2 Decrease of COD in flask containing fultered meat-processing wastewater

The calculation in this table follows the methods presented by Orhon and Artan (1994) [14]

Time(hour)	S-S-COD	S-T-COD	T-S-COD	T-T-COD
0	673.85	673.85	673.85	1,941.50
7	547.34	587.50	492.26	1,629.39
21	145.57	281.75	244.18	929.77
37	112.70	259.44	192.53	855.81
49	122.04	239.34	220.39	785.57
60	104.27	202.61	200.24	691.96
72	103.08	186.02	176.55	636.27
85	94.79	180.10	183.65	610.21
96	101.37	146.70	139.28	435.15
111	84.89	141.75	150.82	425.26
132	88.18	135.98	145.87	406.30
143	110.46	177.70	164.49	539.11
159	105.66	193.31	169.30	470.67
179	108.06	180.10	181.30	455.06
193	103.26	168.10	158.49	437.05
206	123.67	163.29	187.31	451.46
230	129.67	184.91	264.15	523.50
Avg of the smallest four value	92.31	146.93	148.62	425.94

CT1= 1941.5 (CT)1= 425.94 (CT)1-(ST)1= 277.32 (dCT)1= 1515.56

(ST)1= 148.62 (ST)1-(ST)2 = 56.31

ST1= 673.85 (CT)2= 146.93 (CT)2-(ST)2= 54.62 (d(CT)2= 526.92

(ST)2= 92.31

inert suspended organic matte

Xii (r) = [(CT)1-(ST)1]-[(CT)2-(ST)2](dCT)1/(dCT)2 = 120.21

inert soluble organic matter

Sii = (ST)1 - [(ST)1 - (ST)2]/[1-(dCT)2/(dCT)1] = 62.30

fxi= 0.062

fsi= 0.032

fxi+fsi = 0.094

Particulate product from decay in reactor 2

(Xp)2=YxpSsi=(CT)2-(ST)2 = 54.62440127

Yxp = 0.089

Soluble COD Ssi =ST1-Sii= 611.55

Yh =Yxp/fex, if fex=0.2 0.45 if Yh=0.6, fex=0.0534

Soluble microbial product in reactor 1, (Sp)1 = (ST)1 - Sii = 86.32

Biodegradable organic matter, Csi=CT1-Sii-Xii = 1758.99

Ysp =(Sp)1/Csi= 0.049

Y'sp =(Sp)1/Ssi= 0.141147

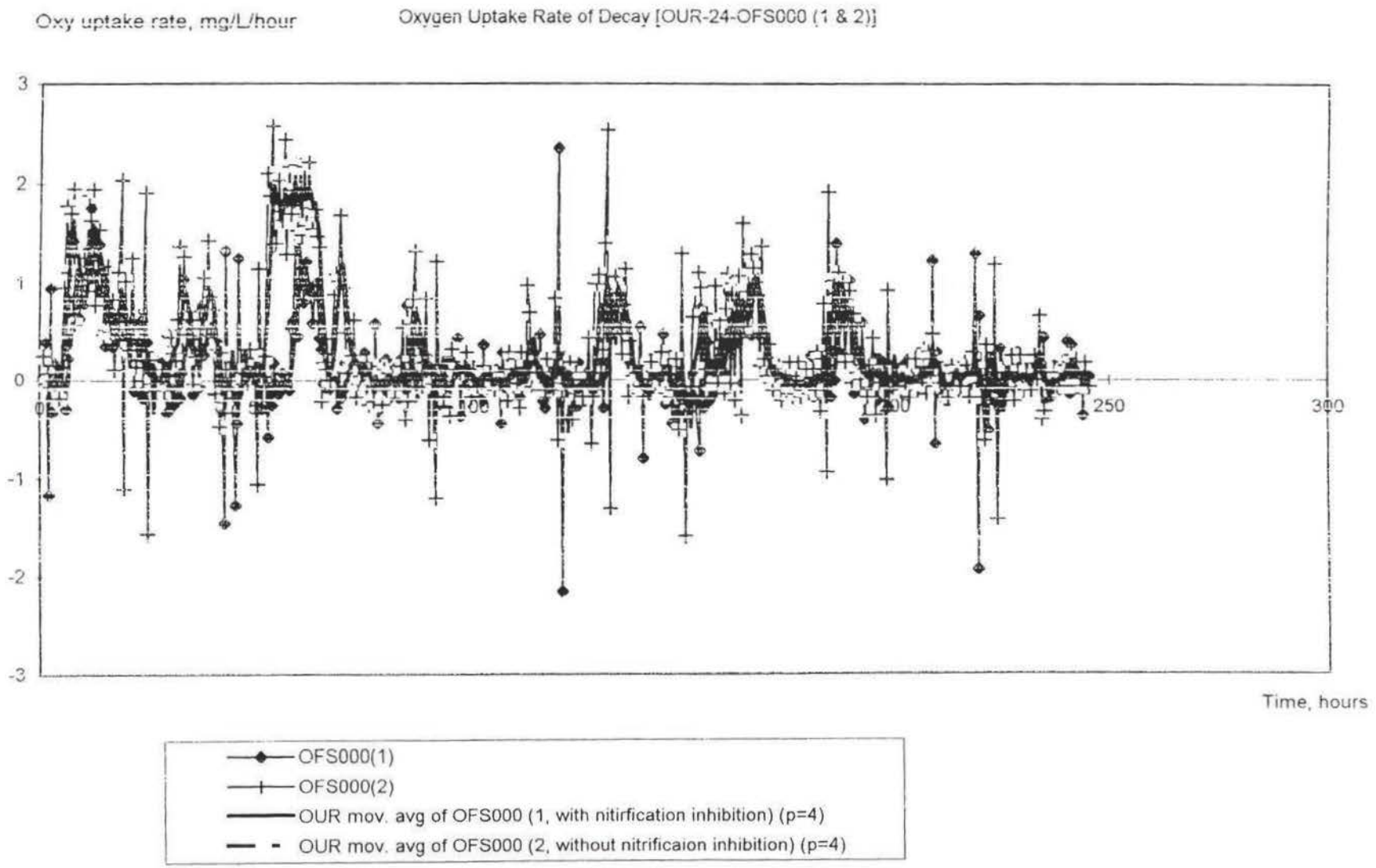
Coefficient in the death regeneration model= Ysp/Yh= 0.11

Particulate product from decay in reactor 1, (Xp)1 = (Xp)2 * Csi/Ssi= 157.11

Soluble microbial product in reactor 2, (Sp)2 = Ysp*Ssi= 30.01

Appendix c

Oxygen Uptake Rate of Decay



Appendix D The Observed and Modelled Oxidized Nitrogen Concentrations for Estimating the Maximum Specific Growth Rate Nitrifying Biomass

The observed oxidized-nitrogen concentrations–

Measured by DIONEX DX-100 Ion Chromatograph during the course of nitrification

The modelled oxidized-nitrogen concentrations –

Predicted by model $S_{NO} = S_{NO0} + \frac{1}{k}\left[e^{at} - 1\right]$ (equation 2-50), with the least square determined "k" and "a"

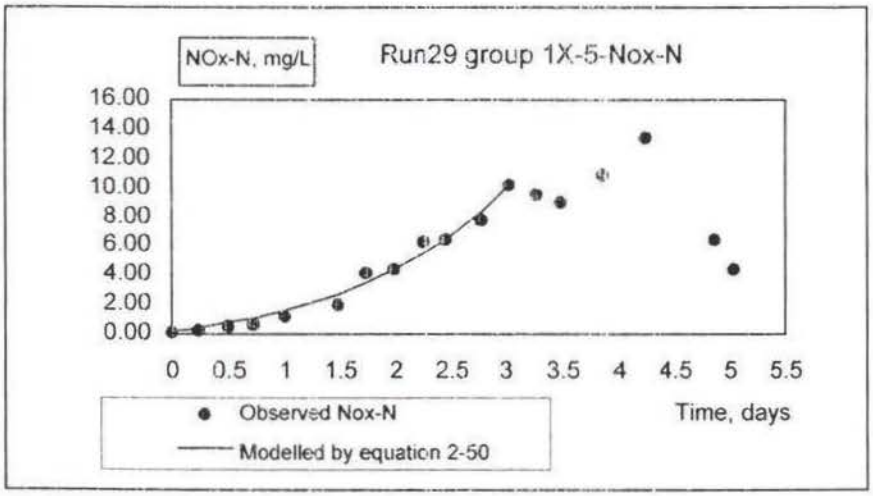


Figure 4-16 Observed and modeled $NO_x - N$ in Bag 1X-5

Appendix D

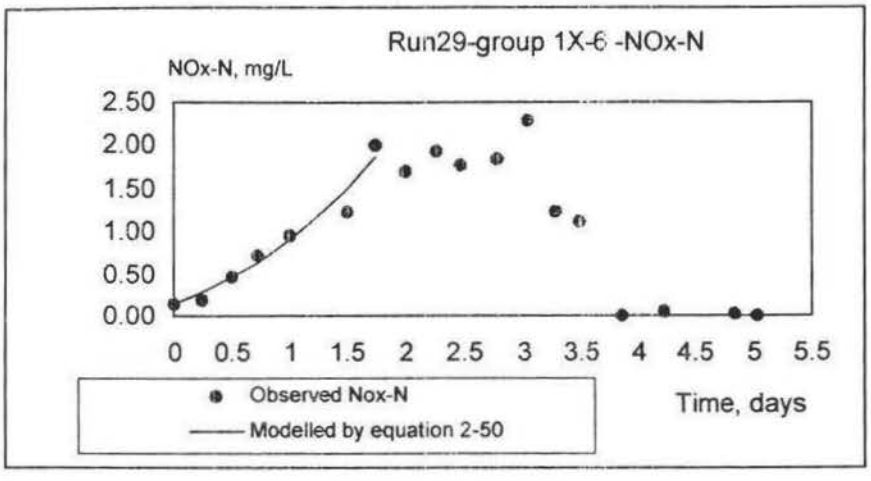


Figure 4-17 Observed and modeled $NO_x - N$ in Bag 1X-6

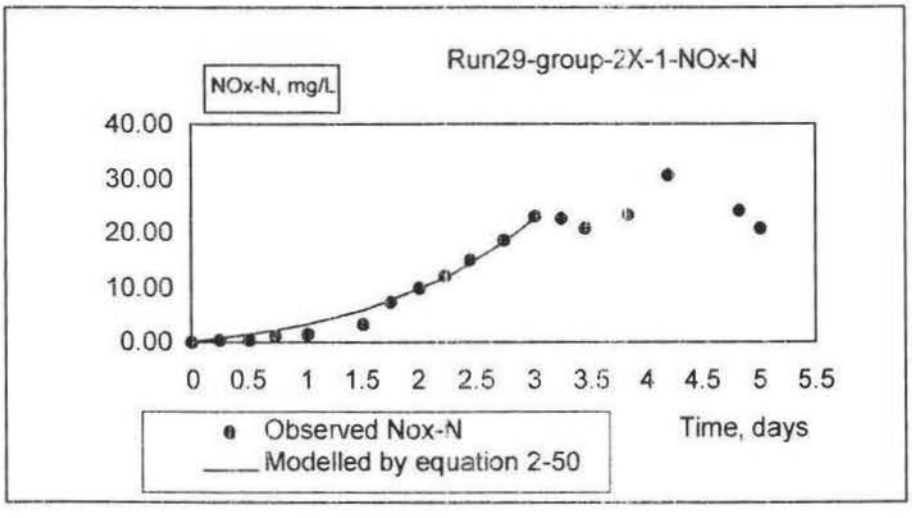


Figure 4-18 Observed and modeled $NO_x - N$ in Bag 2X-1

Appendix D

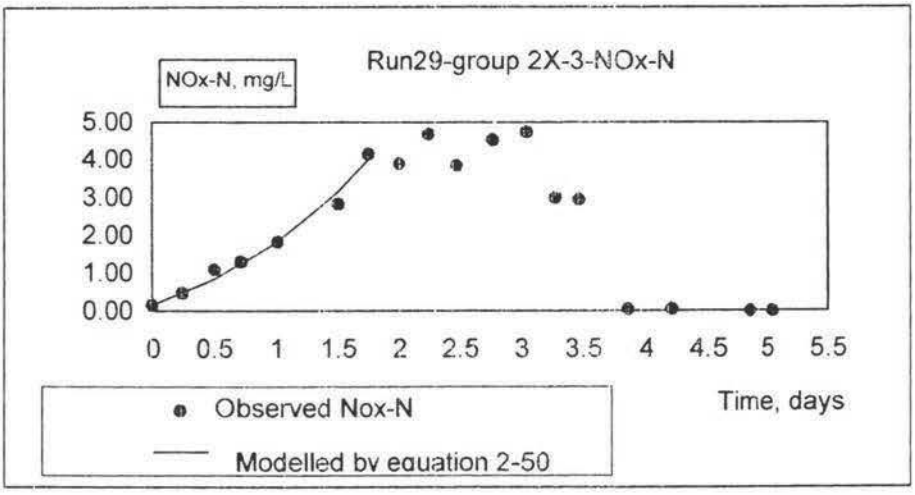


Figure 4-19 Observed and modeled $NO_x - N$ in Bag 2X-3