EFFECT OF SALINITY ON THE BIOLOGICAL TREATMENT OF WASTEWATER FROM FISH PROCESSING INDUSTRY

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FACULTY OF SCIENCE
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EFFECT OF SALINITY ON THE BIOLOGICAL TREATMENT OF WASTEWATER FROM FISH PROCESSING INDUSTRY

ABSTRACT

The fish processing industries produce large volumes of saline wastewater with high organic and nutrient concentrations. This research was aimed to study the effect of salt (NaCl) concentration on the biomass yield and biodegradation of fish processing wastewater in batch and continuous flow system by natural biodegradation. Three categories of synthetic wastewater were prepared according to random concentration which was diluted from the average concentration of fish processing wastewater; with the dilution at 30, 50 and 100 fold dilution. Each concentration of synthetic wastewater was later added with 12 series of salt concentration ranging from 0.0 g/L to 70.0 g/L of NaCl. All synthetic wastewater including the control were tested in the lab using 2.0 L bioreactor (oxygenated continuous stirred) with agitation speed at 250 rotation per minute (rpm) and operating temperature at 30^{o}C. Studies were carried out using batch and continuous operation with a 5-day cycle treatment. In continuous flow system, the first phase of the experiments were focused towards the evaluation of system performance via evaluating biomass yield and substrate removal at 9 different operating hydraulic retention time (HRT) range of 2-10 day in the 30-, 50-, and 100-fold diluted wastewater without salt content in the reactor. The second phase of the experiments was to investigate the effects of eleven different salt content (NaCl: 0.5-7%) in the 30-, 50-, and 100-fold diluted wastewater at the obtained optimal HRT of 10, 9 and 8 day. In each run of continuous system, daily samples are taken at set time to analyze the concentration of biomass and substrates for study the biomass growth and substrate removal efficiency. Organic and nutrient removal together with biomass yield were used indicators for its performance efficiency. In the batch operation, the efficiency of substrate removal and biomass yield dropped at 2.5% NaCl for all types of synthetic

fish processing wastewater. The efficiency of substrate removal and biomass yield dropped for continuous operation after 3.0% NaCl. The most efficient substrate removal and biomass yield took place at the salt concentration of 1.0% salt for batch and continuous operation in all types of synthetic fish processing wastewater. Continuous operation has shown that the longer hydraulic retention time (HRT) give the better efficiency of substrate removal up to 10-day HRT. Meanwhile for biomass yield, the HRT eight days has given the best results with 49.0% growth percentage found in the 30-fold diluted fish processing wastewater. The biomass yield and the substrate removal were decreased with bigger dilution factor of the diluted influent in both of the batch and continuous flow system. Results from this study could potentially increase fish processing saline wastewater handling and the treated wastewater reuse as an environmental friendly liquid fertilizer in agriculture, which is expected to yield high economic value.

Keywords: fish processing wastewater, salt concentration, batch and continuous operation, biodegradation, biomass yield

KESAN PARAS KANDUNGAN GARAM KE ATAS RAWATAN BIOLOGIKAL AIR SISA DARI INDUSTRI PEMPROSESAN IKAN

ABSTRAK

Industri pemprosesan ikan menghasilkan air sisa bergaram yang besar kuantiti dengan kepekatan organik dan nutrien yang tinggi. Penyelidikan ini bertujuan untuk mengkaji kesan kepekatan garam (NaCl) ke atas penghasilan biojisim dan biouraian air sisa pemprosesan ikan dalam sistem sesekumpul dan sistem selanjar secara proses biouraian semulajadi. Tiga kategori air sisa sintetik telah disediakan mengikut kepekatan rawak yang telah dicairkan dari kepekatan purata air sisa pemprosesan ikan, dengan 30, 50 dan 100 kali ganda pencairan. Setiap kepekatan air sisa sintetik kemudian ditambah dengan 12 siri kepekatan garam antara 0.0 g/L hingga 70.0 g/L NaCl. Semua air sisa sintetik termasuk kawalan diuji di makmal menggunakan 2.0 L bioreaktor (pengudaraan oksigen dan dikacau berterusan) dengan kelajuan penggoncangan pada 250 putaran per minit (rpm) dan operasi suhu pada 30^{o}C. Kajian telah dijalankan dengan menggunakan operasi sesekumpul dan selanjar dengan satu kitaran tempoh rawatan 5 hari. Dalam sistem selanjar, fasa pertama eksperimen difokuskan terhadap penilaian prestasi sistem melalui penilaian kadar penghasilan biojisim dan penurunan substrat pada 9 operasi masa penahanan hidraulik (HRT) yang berlainan dengan julat masa 2-10 hari dalam air sisa tanpa kandungan garam yang dicairkan 30-, 50-, dan 100-kali dalam reaktor. Fasa kedua eksperimen adalah untuk mengkaji kesan sebelas kandungan garam yang berlainan (NaCl: 0.5-7%) dalam air sisa yang dicairkan 30-, 50-, dan 100-kali pada HRT paling baik yang diperolehi iaitu HRT 10, 9 dan 8 hari. Dalam setiap operasi sistem selanjar, sampel harian diambil pada masa yang ditentukan untuk analisa kepekatan biojisim dan substrat bagi menganalisis keupayaan pertumbuhan biojisim dan penurunan substrat. Penurunan organik dan nutrien bersama dengan penghasilan biojisim merupakan penunjuk yang digunakan bagi penilaian prestasi sistem. Dalam

operasi sesekumpul, prestasi penurunan substrat dan penghasilan biojisim menurun pada 2.5% NaCl untuk semua jenis air sisa pemprosesan ikan sintetik. Prestasi penurunan substrat dan penghasilan biojisim menurun selepas 3.0% NaCl untuk operasi selanjar. Penurunan substrat dan penghasilan biojisim yang paling berkesan berlaku pada kepekatan garam sebanyak 1.0% bagi operasi sesekumpul dan operasi selanjar dalam semua jenis air sisa pemprosesan ikan sintetik. Operasi selanjar telah menunjukkan bahawa masa penahanan hidraulik (HRT) yang lebih lama akan memberi kecekapan penurunan substrat yang lebih baik sehingga HRT 10 hari. Manakala dalam penghasilan biojisim, HRT lapan hari telah memberikan hasil yang terbaik dengan 49.0% pertumbuhan biojisim didapati dalam air sisa pemprosesan ikan yang dicairkan 30-kali. Penghasilan biojisim dan penurunan substrat berkurang dengan faktor pencairan influen yang lebih besar dalam kedua-dua sistem sesekumpul dan selanjar. Keputusan daripada kajian ini berpotensi boleh meningkatkan pengendalian air sisa bergaram pemprosesan ikan dan penggunaan semula air sisa terolah sebagai baja cecair mesra alam dalam pertanian, yang dijangka menghasilkan nilai ekonomi yang tinggi.

Kata kunci: Air sisa pemprosesan ikan, kepekatan garam, operasi sesekumpul dan selanjar, penghasilan biojisim, proses biouraian

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LIST OF SYMBOLS AND ABBREVIATIONS

APHA : American Public Health Association

AWWA : American Water Works Association

BOD_5 : Biochemical Oxygen Demand (five days)

$CaCO_3$: Calcium carbonate

CH_4 : Methane

Cl^- : Chloride

CO_2 : Carbon dioxide

COD : Chemical Oxygen Demand

Cr^{+3} : Chromium ion

DVS : Dissolved Volatile Solids

EPA : Environmental Protection Agency

EQA : Environmental Quality Act

FAO : Food and Agricultural Organization of the United Nations

FAS : Ferrous Ammonium Sulphate

g/kg : gram per kilogram

g/m^3 : gram per cubic meter

GFC : glass microfiber filters

GPD : gallons per day

H_2O : water

HRT : Hydraulic Retention Time

H_2SO_4 : sulphuric acid / sulfuric acid

KHP : potassium acid phthalate

L : litre

m^3/ton : cubic meter per tonne

mg/L : milligram per litre

mL : milliliter

M : Molarity (moles/Litre)

MW : molecular weight

NaCl : Sodium Chloride

NaOH : Sodium hydroxide

NH_3-N : Ammoniacal nitrogen

nm : nanometer

NO_3^--N : Nitrate nitrogen

PO_4^{3-} : Orthophosphate

O_2 : Oxygen

P : Phosphorus

RM : Ringgit Malaysia

rpm : rotation per minute

SMEs : small and medium enterprises

TDS : Total Dissolved Solids

TS : Total Solids

TSS : Total Suspended Solids

TVS : Total Volatile Solids

VSS : Volatile Suspended Solids

WPCF : Water Pollution Control Federation

w/w : weight per weight

w/v : weight per volume

^oC : degree(s) Celsius (Centigrade)

µm : micrometer

% : percentage

Λ : wavelength

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CHAPTER 1

INTRODUCTION

1.1 World Fisheries Production and Consumption

The world fish processing industry plays a significant role in the economic and social well-being of nations, as well as in the feeding of a significant part of the world's population. As a highly perishable commodity, fish has a significant requirement for processing for human and animal consumption or non-edible purposes. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (preliminary estimate) (FAO, 2014). Fish is versatile as it can be processed into a wide array of products to increase its economic value. In 2000, more than 60% of total world fisheries production underwent some form of processing (FAO, 2002). It is generally distributed as live, fresh, chilled, frozen, heat-treated, fermented, dried, smoked, salted, pickled, boiled, fried, freeze-dried, minced, powdered or canned, or as a combination of two or more of these forms (FAO, 2002; Garibaldi, 2012).

1.1.1 Fisheries Production and Consumption in Malaysia

Malaysia is one of the few countries in world that consume more than 50 kg of fish per person per year (Department of Fisheries Malaysia, 2017). Fish processing industry has grown in Malaysia which is enriched with its fish resources since year 2006 during the implementation of the ninth Malaysian Plan (RMK9) (2006-2010) (Ali *et al.,* 2008). Malaysia is an important producer, market and trading nation for fish and fishery products in the region. In the year 2013, the fisheries' sector contributed RM 11.5 million to the nation's economy. The fisheries sector in Malaysia produced more than 1.5 million tonnes of fish, of which marine fisheries contributed more than 75% and the

balance was contributed by aquaculture (Department of Fisheries Malaysia, 2017; Department of Statistics Malaysia, 2017). The food fish sector which comprises of marine capture fisheries, inland fisheries and aquaculture (excluding seaweed) produced 1,749,314 tonnes worth RM 10,818.60 million (Department of Fisheries Malaysia, 2017). Malaysia's total export of fish and other seafood exceeded RM 2.5 billion per annum (MIDA, 2017).

The fish processing industry in Malaysia is largely export-oriented and encompasses the processing of prawns, the canning of fish, and the production of surimi products. Fish processing, such as the making of pickled fish, frozen fish, salted fish, dried fish, smoked fish, fermented fish, fish crackers, fish sauce, fish noodle, fish satay, fish powder, fish balls, fish cake, and shrimp paste, was traditionally a family-operated industry in fishing villages. However, the trend toward commercial operations has increased lately with industrial-scale setups. The trend is likely to continue with the slow phase out of small family businesses in the coming years. For example, the making of fish balls and surimi is mostly industrial in nature now (Department of Fisheries Malaysia, 2017).

1.2 Wastewater from Fish Processing

The opportunities for wastewater discharge from a fish processing plant are recognized as either discharge to a municipal system or discharge directly to a stream, estuary or the ocean (Boopathy *et al*., 2006). The fish processing wastewater are primarily generated from the operations of ice preservation, cleaning and grinding process in the fish processing plants. The wastewater has high loads of organic nutrients that originate primarily from carbonaceous compounds and nitrogen-containing compounds (protein, peptide, and volatile amines). In addition, the effluent may contain

suspended and dissolved solids, microbes, and variable pH. Another important aspect of this industrial wastewater is its high salinity ($Na+$, Cl^-, SO_4^{2-}).

The characteristics and generation rates of fish processing wastewater are highly variable, depending on the specific types of fish-based product processing operations, and the types of fish-based product's production processes vary widely (Suzuki, 1981). Characterization of wastewater is an important part of the initial work in the design of a treatment process. The characteristic and nature of the wastewater need to be understand and evaluated properly to determine the good treatment method with high efficiency. The wastewater can be categorized according to their characteristics such as physical, chemical and biological (Hammer, 2005).

1.2.1 Biological Treatment of Fish Processing Wastewater

Biological treatment technologies are in favor of wastewater treatment from fish processing industries since the wastewater rich in nutrients (Grady *et al*., 1999). The biological processes used for the treatment of wastewater are derived from processes occurring in nature. Agro-food wastewater treatment has been extensively studied in conventional biological treatment systems, mainly under aerobic conditions, in order to understand the process mechanisms and to improve the biodegradation efficiency of the system (Eric & Ronald, 2014). In most cases the substrates were not readily biodegradable and therefore high biomass concentrations and hydraulic retention times were required for degradation (Oren *et al*., 1992; Abbasi *et al*., 2015). Biodegradation is the most important process contributing to the natural attenuation of contaminants in wastewaters (FAO, 2016).

Aerobic natural biodegradation in batch and continuous system would be applied as a treatment option at small scale fish processing industries for the achievement of treatment technology and economics (Knapp, 2014). There are several benefits in utilizing natural biodegradation processes for the treatment of wastewaters. It can be implied potential benefits to the environmental, operational, and economic in reference to the optimal operational cost of the fish processing industry in our country. The research in this field is very beneficial in the future especially when energy cost and demand for water are steadily increasing; meanwhile environmental quality is degrading at exponential rate. Some action shall be taken to strike the balance between need to live and quality of life.

1.3 Problem Statement

The intensive production of fisheries products have carried an environmental burden, as some of the fish processing factories in this region discharge untreated or partially treated effluents into the receiving streams and rivers have resulted in water pollution problems. In addition, fish processing industries have been known to pollute nearby beaches and shores by releasing wastewater. In terms of numbers, small and medium-sized firms dominate the food industry in Malaysia. Noncompliance is a direct result of the lack of appropriate treatment technology, over utilized capacity, and poor maintenance of the treatment systems (Tay *et al*., 2006; Eric & Ronald, 2014). This industry is now facing both high treatment costs and problems in the operation of conventional wastewater treatment plants. These operational problems are linked to high organic loading, high salt content and bad smell (Len *et al*., 2008).

The fish processing wastewater is subject to bacterial decay, causes heavy pollution and bad smell to receiving water. The pollutants contained in wastewater can cause

eutrophication and oxygen depletion. Continual depletion of the oxygen in these waters would starve aquatic life of the oxygen it needs, and anaerobic decomposition of organic matter leads to the breakdown of proteins and other nitrogenous compounds, releasing hydrogen sulphide, methane, amines, diamines, and sometimes ammonia, all of which are potentially hazardous to the ecosystem, toxic to aquatic life in low concentrations, and also give rise to the development of obnoxious odors and unsightly scenes (Mostafa *et al*., 2006; Sherly *et al*., 2015). Odors are very important to the public perception and acceptance of any fish processing wastewater treatment plant. Although relatively harmless, it may affect general public life by inducing stress, nausea or sickness. The spatial and temporal scale of the impacts of fish processing wastewater may vary depending on the amount and nature of the waste output (Tay *et al*., 2004, 2006; Liu, 2007; Alrumman *et al*., 2016). However, local impacts are particularly obvious because wastewaters from fish processing industries are generally produced throughout the year, giving no chance for the environment to recover. Impacts are more likely to be detrimental when the same ecosystem receives wastewater from the cluster of processing industries.

1.4 Aim and Objectives

This research designed to study the effect of salt (NaCl) concentration on the treatment efficiencies of fish processing wastewater in batch and continuous flow system by natural aerobic biodegradation. The aim was to develop a low cost, simple operation and using biological process for wastewater treatment system that is needed by the local fish processing industries for the treatment of fish processing wastewater and the treated fish processing wastewater can be reuse as liquid fertilizer in agricultural.

Therefore, this research effort was undertaken to address the following objectives:

(i) To relate the substrate strength with the biomass yield in the biological treatment process of fish processing wastewater at different concentration of fish processing wastewater.

(ii) To study the effect of sodium chloride (NaCl) concentrations to substrate (organic and nutrient) removal and biomass yield using batch operation at different concentration of fish processing wastewater treatment.

(iii) To determine optimum operating HRT for continuous operation system by evaluate the pollutants removal and biomass growth at different concentration of fish processing wastewater at nine different operating HRT conditions.

(iv) To study the effect of sodium chloride (NaCl) concentrations to substrate (organic and nutrient) removal and biomass yield using continuous operation in three different concentration of fish processing wastewater treatment at the optimum HRT obtained.

1.5 Scope of Study

This study focused on the use of lab scale bioreactor, which was operated in aerobic batch and continuous flow systems for the treatment of three different concentrations of fish processing wastewater with salt concentrations varying from 0.0% – 7.0% w/v NaCl using naturally occurring microorganisms present in the fish processing wastewater.

Wastewater characterization study for the prepared raw concentrated synthetic wastewater, diluted influent and the final treated effluent of each operational cycle for

the batch and continuous flow system were performed. Parameters studied include measurement of organic matter together with solids matter and nutrients. Experiments were to be carried out using batch and continuous operation. Meanwhile the operational variable which were to be studied are substrate and salt concentration, and hydraulic retention time (HRT). The effect of operational variables would be observed on the removal efficiency, biomass yield and odor.

1.6 Importance of the Research

The fish industry is seeking cost effective ways including recycling options to treat wastewaters generated during fish processing (Tay *et al*., 2006; Knapp, 2014). The social and economic requirement for a low-cost, simple operation and a practical environmental technology wastewater treatment system has stimulated this study for designing an efficient, cost-effective, and low-maintenance requirement wastewater treatment system for fish processing industries; and the treated fish processing wastewater has high potential to be reused as a useful product in agriculture. Most small and medium enterprises (SMEs) of fish processing industries do not have treatment equipment installed or lack of proper wastewater treatment system. Those who have treatment systems face operation and maintenance problems and the systems often do not work efficiently (Lim *et al*., 1996; Tay *et al*., 2006; Len *at al*., 2008; Eric & Ronald, 2014). The stirred tank batch reactor is still the most widely used reactor type both in the laboratory and industry. Batch operation often are used because of their suitability and convenient use (Ferraro *et al*., 2011). Industrial practice generally favors processing continuously rather than in single batches, because overall investment and operating costs usually are less (Mojiri *et al*., 2014). Results from this study would be useful in determining the optimum operational conditions for fish processing wastewater treatment in biological treatment processes of batch and continuous operation, and

designing an efficient and cost-effective biodegradation system for the fish processing wastewater treatment in a biological treatment processes. The natural biodegradation in the continuous operation can be applied as a treatment option in small-scale fish processing industries. Utilizing natural biodegradation processes for the treatment of wastewater has several reasons or benefits that can be classified as environmental, economical, and operational benefits. The biological system should minimize the amounts of pollutants in the effluent water for reduction of environmental contaminant levels and improve the fish processing effluent water quality so that it can be reused satisfactorily as a liquid fertilizer for sustainable agriculture and the protection of environment quality (Oliver & Rene, 2006).

1.7 Outline of Thesis Structure

This thesis consists of seven chapters.

Chapter 1 Introduction

The introduction provides information on the world fisheries production and consumption, fisheries production and consumption in Malaysia, wastewater from fish processing, biological treatment of fish processing wastewater, identified problems associated with the wastewater produced from fish processing, introduce the aim and objectives of the research project, the scope of the study, the importance of the research, and the thesis outline.

Chapter 2 Literature Review

Literature review on fish processing, fish industry in Malaysia, wastewater from fish processing, environmental problems relating to fish processing wastewater, fish processing wastewater characteristic, the role of biological treatment technology in fish

processing wastewater, various published works about the effects of high salinity on biological wastewater treatment processes, and the reuse potential of the treated fish processing wastewater.

Chapter 3 Materials and Methods

This chapter describes and explains the materials and research methodology used to collect the data and generate the findings reported in this study. The preparation of synthetic fish processing wastewater, reactor set up and operating condition, experimental design and treatment operation methods, analytical methods for the measurement of wastewater parameters, materials (chemicals and reagents, apparatus and equipments) used in the experiments, and experimental procedures for this study are reported.

Chapter 4 Results

This chapter contain results for the characteristics of the fish processing wastewater used in the experimental works for this research; the system performance's results for three different concentrations of diluted fish processing wastewater (30-, 50-, and 100-fold) operated in batch operation with 0.0% - 7.0% w/v salt content, continuous operation of fish processing wastewater without salt addition at nine different operating HRT that range from 2-10 days, and continuous operation at the optimal HRTs obtained (HRT of 10, 9, and 8 days) with 11 different salt concentrations (0.5% -7.0% w/v NaCl) were reported in this chapter.

Chapter 5 Discussion

This chapter discuss all the results that have presented in chapter 4: Results. Observation of the pH value for the wastewater daily sample taken out from the reactor along the operation of batch and continuous system; the effects of different substrate concentration and salt concentration on the efficiency of biomass yield and pollutants

removal in three different concentration of diluted fish processing wastewater in batch and continuous operation, and the effects of different HRT on the efficiency performance of the continuous operation in three different concentration of diluted fish processing wastewater were presented. The findings from the experimental results are discussed and compared with those of previous studies presented in the literature review.

Chapter 6 Conclusion

In this chapter, the findings from the experimental results are summarized. Concluding remarks is presented.

Chapter 7 Recommendations for Future Works

Suggestion for future research works.

CHAPTER 2

LITERATURE REVIEW

2.1 Fish Processing Industry

Fishery industry represent one of the most-traded segments of the world food sector, with about 78 percent of seafood products estimated to be exposed to international trade competition (FAO, 2016). In 2012, about 200 countries reported exports of fish and fishery products (FAO, 2014). The fishery trade is especially important for developing nations, in some cases accounting for more than half of the total value of traded commodities. In 2012, it represented about 10% of total agricultural exports and 1% of the world merchandise trade in terms of value (FAO, 2014, 2016). The share of total fishery production exported in different product forms for human consumption or non-edible purposes grew from 25% in 1976 to 37% (58 million tonnes, live-weight equivalent) in 2012. In 2012, of the fish marketed for edible purpose, 46% (63 million tonnes) was in live, fresh or chilled forms, which in some markets are often the most preferred and highly priced products forms. In addition, 12% (16 million tonnes) was utilized in dried, salted, smoked or other cured forms, 13% (17 million tonnes) in prepared and preserved forms, and 29% (40 million tonnes) in frozen form. Freezing is the main processing method for fish for human consumption, accounting for 54% of total processed fish for human consumption and 25% of total fish production in 2012 (FAO, 2014, 2016).

2.1.1 Fish Industry in Malaysia

Fisheries product in Malaysia includes processed seafood products such as frozen and canned fish, dried fish, salted fish, pickled fish, fermented fish, smoked fish, fish

ball, fish cakes, fish powder, fish crackers, fish sauce, fish maruku, fish noodle, fish satar, fish satay, amplang, otak-otak, surimi, crustaceans and mollusks, shrimp and shrimp products (Department of Fisheries Malaysia, 2017).

2.2 Wastewater from Food Processing Sector

Wastewater is sewage, storm water and water that has been used for various purposes around the community and contains organic and inorganic materials in the form of dissolved and suspended particles (Pankaj *et al*., 2010). Unless properly treated, wastewater can harm public health and the environment. Most communities generate wastewater from both residential and nonresidential sources. A large percentage of the country's total wastewater effluent is released by food processing companies (Nadiah K., 2012). In terms of numbers, small and medium-sized firms dominate the food industry in Malaysia. Food processing sector accounts for about 10% of Malaysia's manufacturing output, the major sub-sector is the fish and fishery products, livestock and livestock products, processed fruits and vegetables and cocoa. Fish processing industry is one of the sub-sectors in the processed food and beverages industry which is an important component of the agro-based industry (MIDA, 2017).

The treated/processed effluent is referring to the cleaned wastewater whereby the water returns to nature. It can be discharged into local surface waters where it may be reused (Water Pollution Control Federation, 1994; Andreas & Shane, 2015).

2.3 Wastewater from Fish Processing

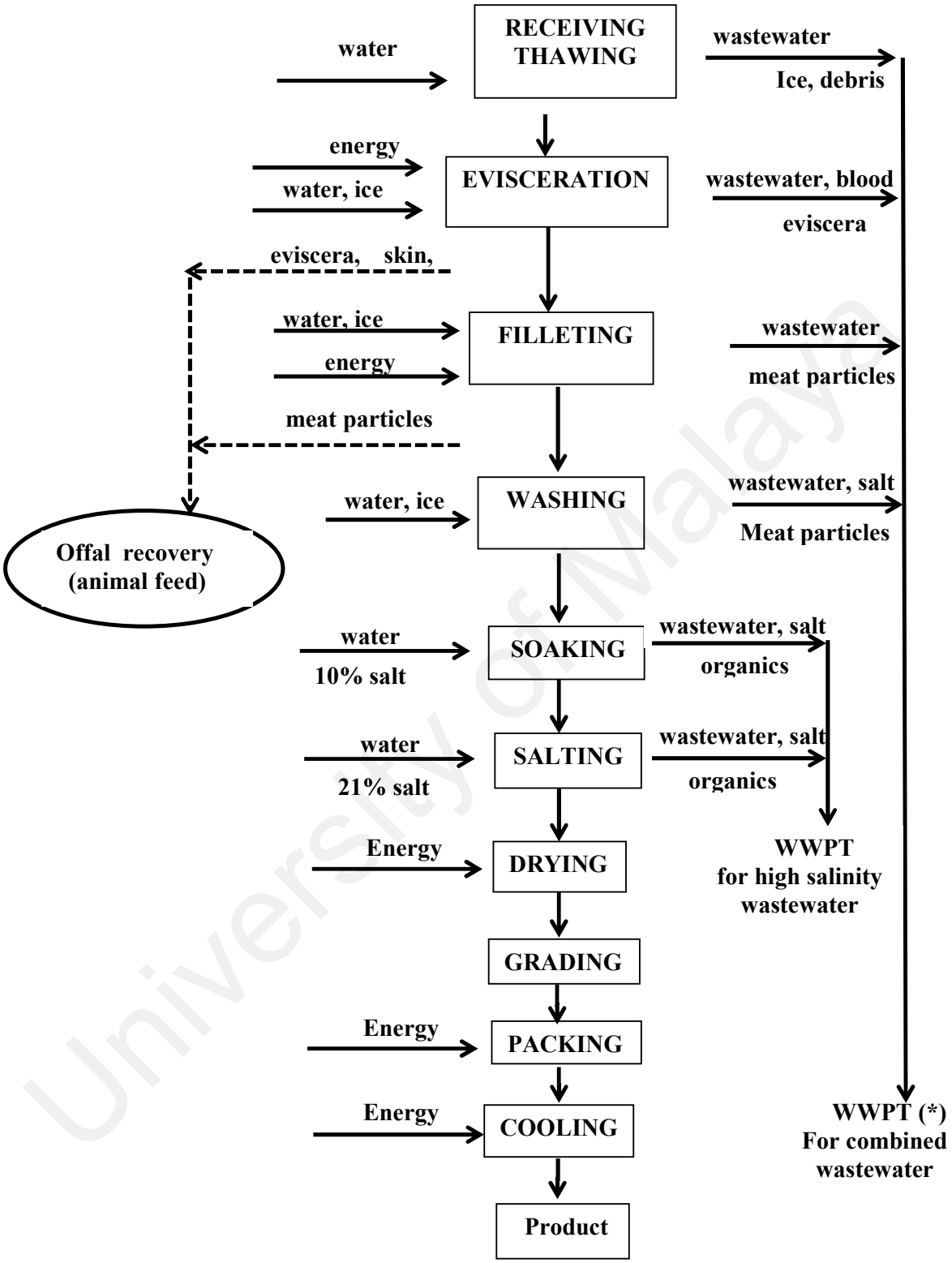
Fish processing requires large amounts of water for the operations of ice preservation, cleaning and grinding process in the fish processing plants. Washing is one of the most important steps in fish-based product processing. The cyclic washing and rinsing of the raw fish and fish mince with water are the central process in fish

processing. The washing process improves gel-forming ability due to leaching of a considerable amount of fat and sarcoplasmic proteins (Huidobro *et al*., 1998; Luo *et al*., 2010; Ismail *et al*., 2011). Washing also separates myofibrillar proteins (Huidobro *et al*., 1998; Luo *et al*., 2010), enzymes (proteases), blood and haem compounds causing lipid oxidation leading to protein denaturation (Hall & Ahmad, 1992; Kolhe & Pawar, 2011).

2.3.1 Salted Fish Processing

The typical processing of dried salted fish is schematized in Figure 2.1. Slime, blood and other contaminating substances of raw fish are washed off using a 3% solution of salt in water (Dan *et al*., 2003; Lliyasu *et al*., 2011). This reduces bacterial loads on the fish during subsequent salting. Large fish like mackerel are split open at the ventral side from the head down. All visceral matter and blood are removed. The fish is then cut into large pieces. Fishes have an odor of ammonia, the dressed fish, or fish fillets, are soaked in mild brine (10%) and crushed iced for 6 to 10h. This may be followed by salting. After washing in clean brine solution, the eviscerated fish is salted in 21% brine for about 15h. Salted fish is placed on bamboo trays and sun dried for two to three days in full sunshine, depending on the size of the fish. Salted fish can also be dried in ovens. Fishes are then packed and stored (Dan *et al*., 2003).

The characteristics of wastewater from the dried salted fish plant are shown in Table 2.1. This wastewater contains very high salt content, ranging from 17 g to 46 g NaCl/L. A large volume of wastewater is produced from soaking and washing operations. The volume ranges from 10 to 12 m^3/ton of preprocessed fish, and 20 - 30 m^3/ton of iced or fresh fish. The preprocessed fish, namely, is eviscerated or beheaded and cleaning at fishing boats or villages in coastal zone before it is transported to sea-food processing plant (Dan *et al*., 2003; Lliyasu *et al*., 2011; Ferraro *et al*., 2011)



(*) WWPT- Wastewater treatment plant

Figure 2.1: Flow diagram of dried and salted fish processing.

Table 2.1: Characteristics of wastewater from the dried salted fish plant (Dan, 2000; Ferraro *et al*., 2011).

Parameter	Unit	Concentration	
		Washing + soaking tank	Combine Wastewater
Chemical Oxygen Demand (COD)	mg/L	5,250	873
Suspended Solids (SS)	mg/L	371	119
Total Disolved Solids (TDS)	mg/L	46	17
Chloride (Cl^-)	g/L	27	10
Sulfate (SO_4^{2-})	mg/L	1,240	164
Total Kjeldal Nitrogen (TKN)	mg/L	747	128
Phosporus (P)	mg/L	5	5

2.3.2 Steamed Canned Shrimp Processing

Process for canning shrimp is shown in Figure 2.2. In this process, receiving, peeling and washing discharge large quantities of wastewater containing 90% of total COD. High salinity wastewater is generated from precooking or brine treatment. In the precooking operation, shrimp is boiled in brine solution for 3 - 5 minutes, or it is steamed. These operations curl the meat, extract moisture and develop the pink or red color of the finished product. The salt content of precooking wastewater is in range of 2 - 3% (UNEP, 1999; Ferraro *et al*., 2011).

The canning process for mollusks such as mussel, oysters, clams or scallops also generates large quantities of wastewater with salt content above 2%. The mollusks are shelled and washed with 3 to 6% salt solution. Then they are drained and steamed or cooked for 10 to 15 minutes at 100^{o}C. After inspection and grading, the cooked mollusks are packed in cans with 1 to 2% brine. Mendez *et al.* (1992) reported that wastewater from processing mollusks contained very high organic, nitrogen and salt content (18.5 g COD/L, 4.0 g N/L and above 2% salt).

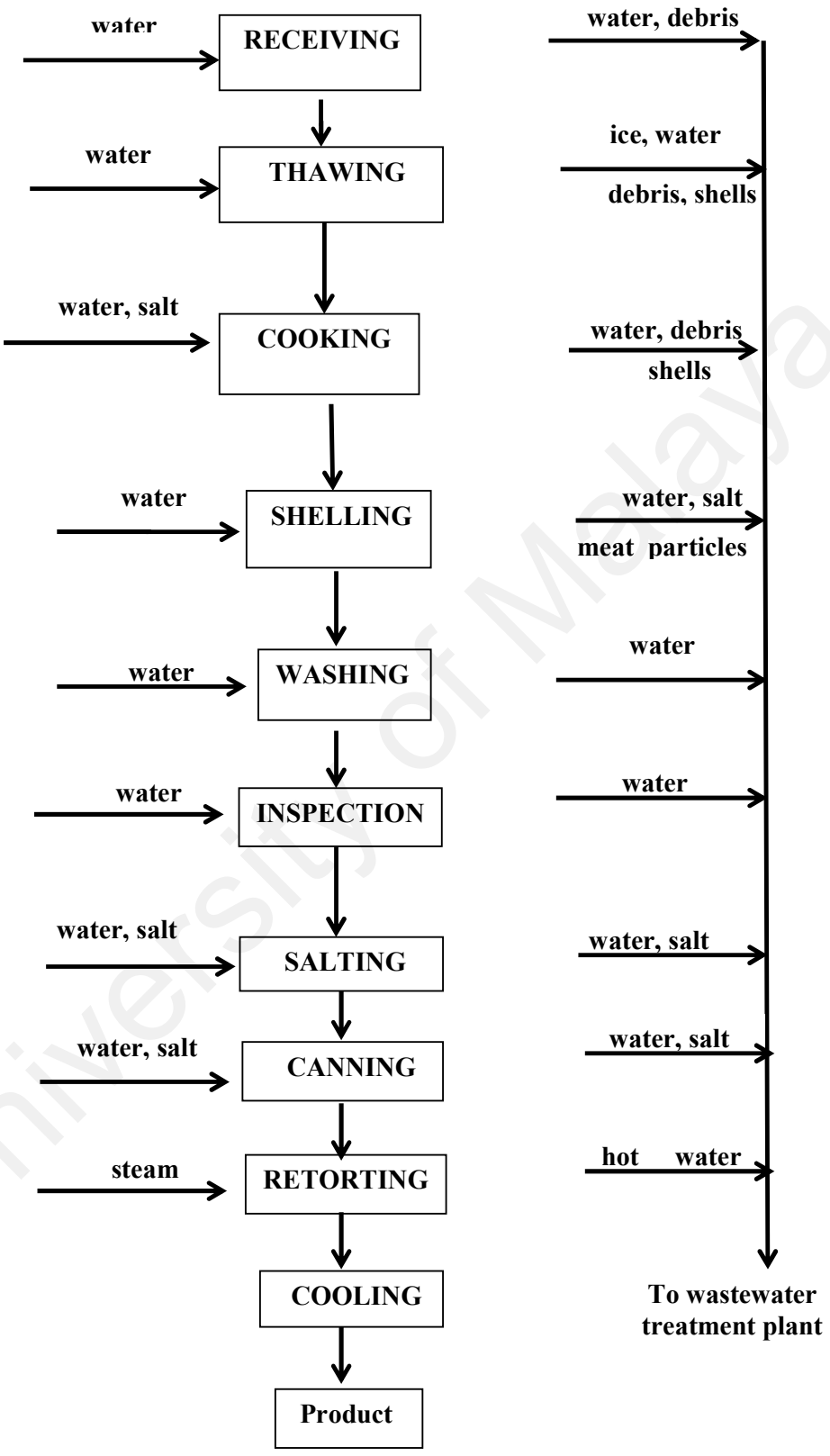


Figure 2.2: Flow diagram of steamed canned shrimp processing.

2.3.3 Commercial Fish Processing Industry

The commercial fish-processing industry generates large quantities of solid waste and wastewater. Solid waste includes whole waste fish, offal containing viscera and fish scrap, which are residues from filleting (Hwang & Hansen, 1998). Wastewater comes mainly from factory cleaning operations and washing of raw materials and contains organic contaminants in soluble, colloidal and particulate form. This wastewater has to be treated properly before discharge (Chowdhury *et al*., 2010). Because factories process a broad range of products with large seasonal variation, pollution characteristics vary significantly from plant to plant, and even within the same plant. Within a given fish processing plant, the wastewater discharged from different unit operations may vary with respect to flow rates and compositions. One important attribute is the general scale of the operations, since fish processing extends from small, local operations to large-scale national or international producers. This difference in scale is relevant not only in identifying sources of wastewater, but also in determining appropriate reduction or recycling options. Although the volume and characteristics of fish processing effluents often exhibit extreme variability, waste and wastewater production in fish processing industries are usually high in volume.

The wastewater generated by fish processing factories has high loads of organic and nutrients that originate primarily from carbonaceous compounds and nitrogen containing compounds (protein, peptide and volatile amines). In addition, effluent may contain suspended and dissolved solids, microbes, and variable pH. The presence of high chemical oxygen demand (COD) and organic nitrogen concentrations characterizes the wastewater produced in fish processing (Sirianuntapiboon & Nimnu, 1999; Alrumman *et al*., 2016). In general, the wastewater of fish processing wastewater can be characterized by its chemical and physical characteristics. Important pollutant parameters of the wastewater are chemical parameters of pH, five-day biochemical

oxygen demand (BOD_5), chemical oxygen demand (COD), ammoniacal nitrogen (NH_3-N), nitrate nitrogen (NO_3^--N), phosphorus (P); and physical parameter of temperature, odor, colour, and solids content (Mitchell, 1974; Mojiri *et al*., 2014; Abbasi *et al*., 2015; FAO, 2016). A comparison of wastewater pollutants concentrations in effluent from each fish processing stage has been presented in Table 2.2. Despite the substantial variation in results, these data provide a useful guideline for wastewater treatment system design and act as a starting point for wastewater minimization.

Table 2.2: Characteristics of raw wastewater at each step of seafood processing in North Carolina seafood processing plants (Carawan, 1991; Chowdhury *et al*., 2010).

Parameter	Rinsing	Scaling	Filleting	Cleaning	Total
Water Flow (WF)	5.5	3.2	4.6	0.16	13.4
Total Solids (TS)	2.08	4.74	3.44	0.27	10.53
Organic Solids (OS)	1.71	2.6	2.08	0.15	6.54
Total Suspended Solids (TSS)	0.38	2.59	0.86	0.09	1.96
Total Dissolved Solids (TDS)	0.71	1.85	2.96	0.13	5.65
Biochemical Oxygen Demand (BOD)	0.59	0.56	0.86	0.1	2.11

(Water flow is in liter and the other parameters are in g/kg of raw fish processed)

2.4 Fish Processing Wastewater Characteristics

In general, fish processing wastewater can be characterized by its chemical and physical characteristics. The presence of high chemical oxygen demand (COD) and organic nitrogen concentrations characterizes the wastewater in fish processing (Sirianuntapiboo & Nimnu, 1999). The important pollutant parameters of the wastewater are the chemical parameters of pH; the five-day biochemical oxygen demand (BOD_5); the COD, ammoniacal nitrogen (NH_3-N), nitrate nitrogen (NO_3^--N), and phosphorus (P); and the physical parameters, such as temperature, odor, color, and

solid contents (Mitchell, 1974; Mojiri *et al*., 2014). Therefore, a review of fish processing wastewater characterization studies is very important in this research project.

2.4.1 Physicochemical Parameter

(a) Temperature

With the exception of wastewaters from cooking and sterilization processes in a fish canning factory, fish processing industries do not discharge wastewater above ambient temperatures (Elena *et al*., 2007). The temperature of the receiving waterbody must not increase by more than 2°C or 3°C, since greater increases in temperature may affect the population balance and also reduce the solubility of oxygen, thereby threatening the survival of some forms of aquatic wildlife. Wastewaters from canning operations should be cooled if the receiving waterbody is not large enough to restrict the change in temperature to 3°C (Ferraro *et al*., 2011).

(b) pH value

The pH analysis is used to determine if the process is operating within the acceptable range of pH (Metcalf & Eddy, 1991; APHA, 1995, 2005; Radojevic & Bashkin, 1999). pH serves as one of the important parameters because it may reveal contamination of a wastewater or indicate the need for pH adjustment for biological treatment of the wastewater. It is possible to treat organic wastewater over a wide pH range; however the optimum pH for microbial growth is between 6.5 and 7.5. Bacteria grow best at slightly alkaline water. Similarly, algae and fungi grow best in slightly acidic water (Miroslav & Vladimir, 1999; Sagar & Pratap, 2012). The pH of wastewater needs to be remained in the range of 6-9 for organism's protection (Metcalf & Eddy, 1991; Efremenko *et al*., 2006; Zhao *et al*., 2013).

Effluent pH from seafood processing plants is usually close to neutral. For example, a study found that the average pH of effluents from blue crab processing industries was 7.63, with a standard deviation of 0.54; for non-Alaska bottom fish, it was about 6.89 with a standard deviation of 0.69 (Carawan *et al*., 1979; USEPA, 2010). The pH levels generally reflect the decomposition of proteinaceous matter and emission of ammonia compounds (Sagar & Pratap, 2012).

(c) Solids Content

Fish processing wastewater contains variety of solid materials. The solids consisted of blood, flesh, oil, and fat. Solids content in a wastewater can be divided into dissolved solids and suspended solids. However, suspended solids are the primary concern since they are objectionable on several grounds. Settleable solids may cause reduction of the wastewater duct capacity; when the solids settle in the receiving water body, they may affect the bottom-dwelling flora and the food chain. When they float, they may affect the aquatic life by reducing the amount of light that enters the water. Soluble solids are generally not inspected even though they are significant in effluents with a low degree of contamination. They depend not only on the degree of contamination but also on the quality of the supply water used for the treatment. In one analysis of fish filleting wastewater, it was found that 65% of the total solids present in the effluent were already in the supply water (Tay *et al*., 2006; Sagar & Pratap, 2012). A summary of the raw wastewater characteristics for the canned and preserved seafood processing industry is presented in Table 2.3.

Table 2.3: Solids content of the raw fish processing wastewater from various seafood processing industries.

Subcategory	TS	TSS	VSS	TDS	Reference
	(mg/L)				
Fish Processing	4721	918-1000	700-900		Del Valle & Aguilera, 1990; Jemli *et al*., 2015
Fish Processing	2905 -15600		875	488	Sagar & Pratap, 2012
Fish Processing			220-790		Avsan & Rao, 2001
Seafood processing		3640 -17000	900-1000		Cristóvão *et al*., 2015
Fishes and shrimps processing		235	3100	1276	Zeinaddine *et al*., 2013.
Herring	6966	1150-5310			Riddle & Shikaze, 1973
Herring	30000	600-5000			Cristóvão *et al*., 2015
Breaded/Non-Breaded Shrimp		800			Muthukumaran & Baskaran, 2013
Tuna processing	17900	500-1091			Tay *et al*., 2006
Tuna processing		1000-2100	700-1000	73-83	Artigaa *et al*., 2008
Salmon	1030-2500	320-1100	225-855		Battistoni & Fava, 1995
Salmon	88-3422	40-1824			Stone *et al*., 1981
Salmon	220-3640	11-2180			NovaTec & EVS, 1994
Salmon		120 -1400			Jemli *et al*., 2015
Nile perch fish	5580-6350	4500-5150	900		Gumisirizal *et al*., 2009
Fish salting		200 - 6,000	371	46	Ferraro *et al*., 2011
Fish Meal		70-20000			Carawan *et al*., 1979; Sunny & Mathai, 2013
Surimi	5120-7790				Green *et al*., 1984
Surimi	-	1500-2000			Olumura & Uetana, 1992
Surimi	5500	330-5300			Aquametrix, 1993
Surimi	3920-10800				Tay *et al*., 2006

i) Total Solids (TS)

There was significant variation for TS value between the samples studied where it ranges from 1,800 to 30,000 mg/L. The average value of TS was 17,900 mg/L in tuna fish processing of which 40% was organic, and the herring processing wastewater contained total solids concentration of 30,000 mg/L (Tay *et al*., 2006). While Sagar and

Pratap (2012) have reported the TS value ranges from 2,905 mg/L to 15,600 mg/L in the fish processing effluent released in Mirkarwada and Mandavi beach.

ii) Total Suspended Solids (TSS)

In fish processing wastewater, total suspended solids are primarily bacteria and bacterial flocs, algae and protozoa, and organic debris. Suspended solids can lead to the development of sludge deposits and anaerobic conditions when untreated wastewater is discharged in the aquatic environment (Porntip *et al*., 2014).

Fish meal plants wastewater were reported to have a TSS of 70 to 20,000 mg/L, and bottom fish was found to have a TSS of 100–800 mg/L (Carawan *et al*., 1979). Seafood processing plant wastewaters were reported to have a TSS of 3,640 to 17,000 mg/L of TSS (FREMP, 1993). Effluents from fish and crustacean processing plants are generally characterized by high total suspended solids concentration as high as 125,000 mg/L (AMEC Earth and Environmental Limited, 2003).

iii) Total Dissolved Solids (TDS)

Total dissolved solids are physical characteristics of wastewater, and also inorganic indicators of water quality chemical characteristics of wastewater. TDS is a measure of salt dissolved in a water sample after removal of suspended solids (Kuhn *et al*., 2007).

Wastewater from fish processing industries contain high fraction of dissolved solids. The size of colloidal particles in wastewater is typically in the range from 0.01 to 1.0 micrometer. Sagar and Pratap (1994) reported that total dissolved solids in range 488 mg/L in the wastewater.

vi) Volatile Suspended Solids (VSS)

Biomass in the bioreactor was mostly organic materials, thus an increase in biomass could be measured by volatile suspended solids (VSS) or particulate COD (total COD minus soluble COD). Other parameter that could be used to indicate biomass growth was protein content.

VSS was the parameter used most commonly to follow biomass growth in full-scale biological wastewater treatment system because its measurement was simple and minimal time was required for analysis. The biomass yield was determined as gram of VSS produced per gram of COD removed. Nevertheless, VSS measured include other particulate organic matter in addition to biomass. However, the VSS measurement was used as an apparent indicator of biomass production and also provided a useful measurement of reactor solids in general (Sharrer *et al*., 2007; Knapp, 2014).

VSS play an important role in wastewater treatment. VSS test results are routinely to assess the performance of conventional treatment processes and need for effluent filtration in reuse application. VSS is an indication of bacteria or biomass content in the wastewater, it is important for characterizing wastewater treatment basins (APHA, 2005). VSS are the samples under suspension and remains in water sample. The VSS analysis is applied mainly to wastewater sludge to measure their biological stability on the basis of their volatility at 550+/- 50^{o}C.

Sagar *et al*. (2006) has reported that the average VSS from fish processing wastewater was 875 mg/L. While Avsan and Rao (2001) observed the fish processing wastewater contained 220 to 790 mg/L VSS.

2.4.2 Organic Matters

The organic components in fish processing wastewater are highly biodegradable indicates by the COD/BOD_5 ratio in the range from 1.5 to 2 (Abbasi *et al*., 2015). In waterways, bacteria will consume the organic components of the waste. The process of biodegradation in waterways consumes oxygen according to Equation (2.1):

$$\text{Organic Material} + O_2 \rightarrow CO_2 + H_2O + \text{Bacteria} \quad (2.1)$$

Organic compounds normally are some combination of carbon, hydrogen, oxygen, nitrogen, and other elements. Many organics are proteins, carbohydrates, or fats and are biodegradable, which means they can be consumed and broken down by organisms. However, even biodegradable materials can cause pollution. In fact, too much organic matter in wastewater can be devastating to receiving waters (APHA–AWWA, 2005).

It is clear that the main impact will be due to high organic loads. The organic material in wastewater stimulates the growth of bacteria and fungi naturally present in water, which then consume dissolved oxygen. The concentration of oxygen in a river depends on both the rate at which oxygen is consumed by microorganisms and the rate of reaeration from the atmosphere. As a result of falling oxygen levels, fish and other creatures can suffocate (Mueller & Spahr, 2005; Olivier & René, 2006). A series of organic matters of the raw fish processing wastewater from the canned and preserved seafood processing industry is presented in Table 2.4.

Table 2.4: Organic matters of the raw fish processing wastewater from the canned and preserved seafood processing industry.

Subcategory	BOD (mg/L)	COD (mg/L)	References
Fish Canning	10000-50000	700	Mendez *et al.,* 1992
Fish Canning	5100	6000-9000	Chowdhury *et al*., 2010
Fish Processing	3300	3261-1432	Del Valle & Aguilera, 1990
Fishes and shrimps Processing	1050-1250	1730-1790	Zeinaddine *et al*., 2013.
Mechanized Blue Crab	600	1000	Andreas & Shane, 2015
Breaded/Non-Breaded Shrimp	720-1000	1200-2300	Noukeu *et al*., 2016
Fish Salting	2300	5400	Ziminska, 1985
Tuna processing	3300	5553	Chowdhury *et al*., 2010
Tuna Processing	700	1300-3250	Tay *et al*., 2006; Noukeu *et al*., 2016
Tuna Brine Steam injection		8000-12000 17000-26000	Artigaa *et al*., 2008
Fish Meal	100-24,000	150-42,000	Carawan *et al*., 1979
All Salmon	253-2600	300-5500	Cristóvão *et al*., 2015; Riddle & Shiikaze, 1973
All Salmon	665-2315	902-3177	Battistoni & Fava,1995
Bottom & Finfish (all)	200-1000	400-2000	Carawan *et al*., 1979; Sunny & Mathai, 2013
Herring	20-1745	25-4864	NovaTec, 1994
Mechanical Clams	500-1200	700-1500	Tay *et al*., 2006; Noukeu *et al*., 2016
All Oyster	250-800	500-2000	Tay *et al*., 2006
Surimi	5000-5500	1600-2200	Okumura & Uetana, 1992

a) Biochemical Oxygen Demand (BOD)

BOD estimates the degree of contamination by measuring the oxygen required for oxidation of organic matter by aerobic metabolism of the microbial flora. In seafood-processing wastewaters, this oxygen demand originates mainly from two sources. One is the carbonaceous compounds that are used as substrate by the aerobic microorganisms; the other source is the nitrogen-containing compounds that are normally present in seafood-processing wastewaters, such as proteins, peptides, and volatile amines. Standard BOD tests are conducted at 5-day incubation for

determination of BOD_5 concentrations (APHA, 1995, 2005; Sherly 2015; Zhao *et al*., 2013).

The BOD is generated primarily from the butchering process and from general cleaning (Sherly *et al*., 2015). Bottom fish was found to have a BOD_5 of 200–1,000 mg/L; fish meal plants were reported to have a BOD_5 of 100–24,000 mg/L, while the average BOD_5 value for fish processing stick water has been listed as ranging from 56,000 to 112,000 mg/L (Carawan *et al*., 1979). White fish filleting processes typically produce 12.5–37.5 kg BOD_5 for every ton of product. Fish canning industries have a high concentration of BOD_5 pollutants in the range of 10,000-50,000 mg/L (Mendez *et al*., 1992). Wastewaters from seafood-processing operations can be very high in BOD_5. Literature data for seafood processing operations show a BOD_5 production of one to 72.5 kg of BOD_5 per ton of product (Tay *et al*., 2006).

b) Chemical Oxygen Demand (COD)

Organic matter is the major pollutant in wastewater. The chemical oxygen demand (COD) is used as a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant. The COD test is used to determine the degree of pollution in both wastewater and natural water (HACH, 1999; Miroslav & Vladimir, 1999).

The COD is an important pollutant parameter for the seafood industry. This method is more convenient than BOD_5 since it needs only about 3 hours for determination compared with 5 days for BOD_5 determination. The COD analysis, by the dichromate method, is more commonly used to control and continuously monitor wastewater treatment systems. Because the number of compounds that can be chemically oxidized is greater than those that can be degraded biologically, the COD of an effluent is usually

higher than the BOD_5. Hence, it is common practice to correlate BOD_5 vs. COD and then use the analysis of COD as a rapid means of estimating the BOD_5 of a wastewater. Depending on the types of seafood processing, the COD of the wastewater can range from 150 to about 42,000 mg/L. One study examined a tuna-canning and byproduct rendering plant for five days and observed that the average daily COD ranged from 1,300–3,250 mg/L (Tay *et al*., 2006; Noukeu *et al*., 2014). Sagar *et al*., (2012) have reported 1,825 mg/l of COD in fish processed wastewater.

Basically, the higher the COD value, the higher the amount of pollution in the test sample (APHA, 2005; Andreas & Shane, 2015). This test allows measurement of a wastewater in terms of the total quantity of oxygen required for oxidation to carbon dioxide and water. The principal reaction using dichromate as the oxidizing agent may be represented in a general way by the following unbalanced Equation (2.2):

$$(C_aH_bO_c)\ \text{Organic matter} + Cr_2O_7^{-2} + H^+ \text{ ----- catalysts \& Heat } \rightarrow Cr^{+3} + CO_2 + H_2O \quad (2.2)$$

The measured wastewater sample is heated with a known amount of potassium dichromate-sulfuric acid solution, after which the concentration of unreacted oxidizing agent (chromate remaining) is determined by a redox back-titration or the amount of reduced chromium produced is determined colorimetrically (HACH, 1999; Miroslav & Vladimir, 1999; Andreas & Shane, 2015). Some of the dichromate is consumed in the oxidation of organic wastes. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence.

Chloride is the primary and the most common interference when determining COD concentration. It has the greatest effect on COD test results. The theoretical oxygen demand of chloride is expressed by the following Equation (2.3):

$$Cl^- + O_2 + 4\ H^+ \text{——>} 2\ H_2O + 2\ Cl_2 \quad (2.3)$$

One molecule of oxygen consumes 4 molecules of chloride ions. On a weight basis, the theoretical oxygen demand for chloride is 0.226 mg O_2 per mg Cl^-. A sample containing 1000 mg/L chloride will have a theoretical oxygen demand of 226 mg/L COD (Wayne, 1997; Andreas & Shane, 2015).

Chloride reacts with silver ion to precipitate silver chloride, and thus inhibits the catalytic activity of silver. Bromide, iodide, and any other reagent that inactivates the silver ion can interfere similarly. Such interferences are negative in that they tend to restrict the oxidizing action of the dichromate ion itself. However, under the rigorous digestion procedures for COD analyses, chloride, bromide, or iodide can react with dichromate to produce the elemental form of the halogen and the chromic ion. Results then are in error on the high side. The difficulties caused by the presence of the chloride can be overcome largely, though not completely, by complexing with mercuric sulfate ($HgSO_4$) before the refluxing procedure. Although 1 g $HgSO_4$ is specified for 50 mL sample, a lesser amount may be used where sample chloride concentration is known to be less than 2000 mg/L, as long as a 10:1 weight ratio of $HgSO_4$: Cl^- is maintained. This ties up the chloride ion as a soluble mercuric chloride complex and greatly reduces its ability to react further. Techniques designed to measure COD in saline waters are available (Ruttanagosrigit & Boyd, 1989; Wayne, 1997; HACH, 1999).

Mercuric sulfate is the most widely used reagent for masking chloride interference and is most effective when the ratio of mercuric sulfate to chloride is 10:1. For example, a 2 mL sample containing 2000 mg/L chloride (4 mg Cl^-) will require 40 mg mercuric sulfate to effectively mask the chloride interference. Hach Dichromate COD Reagents contain enough mercuric sulfate to eliminate the interference up to 2000 mg/L chloride.

The ultra high range COD procedure will eliminate up to 20,000 mg/L chloride, because the sample size used is 0.20 mL rather than the standard 2.00 mL (Wayne, 1997; HACH, 1996, 1999).

2.4.3 Nutrients

Nitrogen and phosphorus are nutrients that are of environmental concern. They may cause proliferation of algae and affect the aquatic life in a water body if they are present in excess (Chowdhury *et al*., 2010; Sherly *et al*., 2015). However, the concentration of nitrogen and phosphorus in the seafood-processing wastewater is minimal in most cases. It is recommended that a ratio of N to P of 5:1 be achieved for proper growth of the biomass in the biological treatment (Sherly *et al*., 2015). Sometimes the concentration of nitrogen may also be high in seafood-processing wastewaters. One study shows that high nitrogen levels are likely due to the high protein content (15–20% of wet weight) of fish and marine invertebrates (Sherly *et al*., 2015). Similarly, Sagar *et al*. (2012) has reported 150 mg/l of nitrate in fish processed waste water. Beside that, seafood-processing wastewater was noted to sometimes contain high concentrations of chlorides from processing water and brine solutions, and organic nitrogen of up to 300 mg/L from processing water. Phosphorus also partly originates from the seafood, but can also be introduced with processing and cleaning agents. Table 2.5 shows the nutrient contents from various types of seafood processing wastewaters.

Table 2.5: Nutrient contents of the raw seafood processing wastewater from different seafood processing industry.

Subcategory	Process	Nitrogen (mg/L)	Phosphorous (mg/L)	Reference
Blue crab	Conventional	1000		Del Valle & Aguilera, 1990; Sagar & Pratap, 2012
	Mechanical	3700		
Shrimp	Breaded	5900		Cristóvão *et al*., 2015
	Canned	9500		
	Frozen	10000		
Tuna	Conventional	2100		Cristóvão *et al*., 2015
Tuna	Brine Steam injection	1200-1800 2500-4000		Artigaa *et al*., 2008
Catfish	Conventional	650		Muthukumaran & Baskaran, 2013
Salmon	Conventional		0.26-1.12	Battistoni & Fava, 1995
Nile perch fish	Conventional	61-340	9-20	Gumisirizal *et al*., 2009
Fishes processing	Conventional	150-300		Jemli *et al*., 2015
Fishes and shrimps processing	Conventional	nitrate = 14-22 nitrite= 5.5-6.0	108-146	Zeinaddine *et al*., 2013.
Fishes processing	Conventional	4.79	0.72	Danh *et al*., 2013

Nitrogen is a very important component of the fish factory wastewaters. Some protein will lose to the waste streams. Bacteria convert the nitrogen in proteins to the inorganic forms including ammonia, and the ammonium, nitrite and nitrate ions. Each of these inorganic forms of nitrogen has different environmental effects. The nitrification process is used to convert the ammonia in the wastewater to nitrate. Nitrification is a two steps biological process utilizing two species of nitrogen converting bacteria. These species of bacteria are more active in the pH range of 7 to 8. During the nitrification process, hydrogen ions are released and alkalinity is consumed as the acid is neutralized (Ruiz *et al*., 2006; Zhao *et al.,* 2013). For every 1.0 mg of ammonia converted to nitrate, 8.14 mg of calcium carbonate ($CaCO_3$) equivalent are consumed. The generation of acid during ammonia conversion, and the need to maintain

the proper pH, is the reason why an alkali is added to the system (Zhao *et al.,* 2013). Nitrification of the wastewater is the single largest factor which leads to the consumption of alkalinity and the need to add alkali to the treatment system (Uygur & Kargi, 2004; Daniels *et al*., 2012).

Nitrogen pollution in water resources is a long existing environmental and public health problem. Nitrate ions are toxic in high concentrations to both humans and livestock. In young infants, nitrate can be converted to the nitrite form, absorbed into the bloodstream and convert haemoglobin to methaemoglobin (Alrumman *et al*., 2016). Methaemoglobin cannot transport oxygen. The condition of methaemoglobinaemia affects infants less than six months in age because they lack the necessary enzyme to reconvert the methaemoglobin back to haemoglobin. High levels of nitrates pollution in drinking water supply have been associated with (blue-baby) syndrome in infants (Susquehanna River Basin Commission, 1991). To protect humans the usual limit placed on drinking water supplies is 10 g/m^3 of nitrate-nitrogen. Livestock can also suffer from methaemoglobinaemia (Kavitha *et al*., 2012). Since ruminants have a more neutral stomach pH and rumen bacteria that reduce nitrates to nitrite, deaths from methaemoglobinaemia can occur. This usually results from the consumption of nitrate rich feed; although a limit of 30 g/ m^3 nitrate-nitrogen on drinking water for stock has been suggested (Lefebvre & Moletta, 2006; Pramanic *et al*., 2012).

Inorganic forms of nitrogen (nitrate, nitrite and ammonium ions) and inorganic phosphates act as plant nutrients and increase the chance of eutrophication occurring in waterways (Cho *et al*., 2006; Ferraro *et al*., 2011). This can result in an abundance of opportunistic algae, weeds and plants. The increase in total biomass also increases the amount of microorganisms, which are involved in breaking down dead matter. The overall result is a decrease in the amount of dissolved oxygen present in the water due to

the decomposition of plants, algae, bacteria and other microorganisms. This therefore has an adverse effect on any other organisms that reply on the dissolved oxygen to survive (Mueller & Spahr, 2005; Cho *et al*., 2006; Garibaldi, 2012).

High levels of phosphorus cause a similar impact on waterways to nitrogen. Nitrogen is more often the problem in salt waterways whereas phosphorus tends to affect fresh waterways. Phosphorus is found mainly in detergents (Amir *et al*., 2013). Phosphorus concentration in wastewater is also one of the parameter that needed to be monitored in the wastewater. An excess of phosphorus, however, can cause eutrophication of natural waters. This issue has become one of the most worrisome environmental problems worldwide. U.S. Environmental Protection Agency discussed on eutrophication as basically a problem caused by nutrient enrichment in surface water. Phosphorus, which has been identified as a nutrient limiting primary production is usually responsible for algal blooms and invasions of exotic species in most surface water ecosystems.

2.5 Environmental Impacts of Fish Processing Wastewater

The increased production of fish processing products may generate various types of environmental problems such as excessive nutrients and short oxygen levels in both the receiving soil and water body (Igbinosa & Okoh, 2009). The environmental consequences in not adequately removing the pollutants from the waste stream can have serious ecological ramifications (Amir *et al*., 2013). For example, if inadequately treated wastewater were to be discharged to a stream or river, an eutrophic condition would develop within the aquatic environment due to the discharge of biodegradable, oxygen consuming compounds. If this condition were sustained for a sufficient amount of time, the ecological balance of the receiving stream, river or lake (i.e., aquatic microflora, plants and animals) would be upset. Continual depletion of the oxygen in

these water systems would also result in the development of obnoxious odors and unsightly scenes (Kincannon & Gaudy, 1968; Carawan *et al*., 1979; Chowdhury *et al*., 2010; Cui *et al*., 2016). High organic pollution affects human and livestock health, the fisheries and aquaculture industries in Malaysia. General or common indicators, such as fish killing, odor generation, contamination of ground water and the decrease of crop production, occur because of improper management of agricultural and fish processing wastewater. These problems in environmental quality are closely associated with the wastewater from the agricultural and food production system (FAO, 2014, 2016).

2.6 Biological Treatment Processes

Biological treatment uses microorganism to remove organic solids and nutrients from wastewater. It is generally considered to be more cost effective and interesting method since it is friendly to the environment in treating high organic strength wastewaters, such as fish processing wastewater, as compared with physical and chemical treatment methods (Tay *et al*., 2006; Zhao *et al*., 2013; Sherly *et al*., 2015). Biological treatment involves the use of a biological reactor that contains wastewater rich in biodegradable organics and nutrients, in which organic matter is utilized by microorganisms for life-sustaining processes and as produce cell growth and reproduction. The nutrients available in the wastewater enhanced the growth of microorganisms and allow the biological treatment to be effective (Tay *et al*., 2006; Mojiri *et al*., 2014). The assimilatory pathway occurs when microorganisms utilize nitrate to produce ammonia, which is then utilized as a nitrogen source to generate biomass (Tay *et al*., 2004; Tay *et al*, 2006; Liu, 2007; Alrumman *et al*., 2016). Biological treatment technologies are in favor of wastewater treatment from fish processing industries since the wastewater is rich in nutrients. Biodegradation is often the most important process contributing to the natural attenuation of contaminants in wastewaters. Many different types of organisms

live in wastewater and some are essential contributors to treatment. A variety of bacteria, protozoa, and worms work to break down certain carbon-based (organic) pollutants in wastewater by consuming them (Igbinosa & Okoh, 2009; Knapp, 2014). Through this process, organisms turn wastes into carbon dioxide, water, or new cell growth. Bacteria and other microorganisms such as viruses and protozoa are particularly plentiful in wastewater and accomplish most of the treatment. Most wastewater treatment systems are designed to rely in large part on biological processes (Bassin *et al*., 2012; Eric & Ronald, 2014; Cui *et al*., 2016). In a single aerobic system, members of the genera Pseudomonas, Nocardia, Flavobacterium, Achromobacter, and Zooglea may be present, together with filamentous organisms (Andreas & Shane, 2015). In a wellfunctioning system, protozoas and rotifers are usually present and are useful in consuming dispersed bacteria or nonsettling particles (Tay *et al*., 2006; Andreas & Shane, 2015).

In wastewater treatment, there are three types of bacteria used to treat the waste that comes into the treatment plant: aerobic, anaerobic and facultative. The three conditions differ in the way they use oxygen. Aerobic microorganisms require oxygen for their metabolism, whereas anaerobic microorganisms grow in absence of oxygen; the facultative microorganism can proliferate either in absence or presence of oxygen although using different metabolic processes. Both aerobic and anaerobic technologies and a combination of both technologies have been applied in treating fish processing wastewaters (Ferraro *et al*., 2011; Knapp, 2014).

Biological treatment systems can convert approximately one-third of the colloidal and dissolved organic matter into stable endproducts and convert the remaining two-thirds into microbial cells that can be removed through gravity separation. The organic load present is incorporated in part as biomass by the microbial populations, and almost

all the rest is liberated gas. Carbon dioxide (CO_2) is produced in aerobic treatments, whereas anaerobic treatments produce both carbon dioxide and methane (CH_4). In seafood-processing wastewaters, the nonbiodegradable portion is very low.

2.6.1 Aerobic Treatment Process

Aerobic treatment is a biological process which uses the application of free or dissolved oxygen by microorganisms in degradation of organic wastes. The decomposition of contaminants in wastewater using aerobic method needs aeration; which will be the oxygen source for the growth of aerobic microbes that will treat the organic matter and form sludge as the by-product of the process (Yogalakshmi & Joseph, 2010; Nena *et al*., 2013).

Biological wastewater treatment with aerobic microorganisms has been performed for many years. The process was discovered in 1914 by Ardern and Lockett and came into heavy use throughout the 1950s and 1960s as models and design principles for the system were developed (Rittmann & McCarty, 2001; Alrmman *et al*., 2016). Wastewaters contain organic and other matter that can cause serious environmental problems if sent directly to natural bodies of water. In an aerobic biological treatment system, the wastewater is pumped into a tank or pond that contains a suspension of aerobic bacteria. The aerobic bacteria oxidize the organic matter forming CO_2, H_2O, and more bacteria (Campos *et al*., 2002; Qasim & Mane, 2013). Collectively, all the organic matter is referred to as BOD of the wastewater. BOD can be determined in the laboratory by measuring total oxygen consumption of aerobic bacteria digesting a waste over a defined period of time (Rittmann & McCarty, 2001; Vanerkar *et al*., 2013).

Aerobic process involves the reaction of the oxidation of organic matter in the wastes. Thus, the main reaction products for the aerobic process are CO_2, H_2O and

microbial biomass. The aerobic systems firstly applied to food processing wastewater included activated sludge, trickling filters, oxidation ditch, sequencing batch reactor, lagoons, irrigation and a combination of these processes (Zhu *et al*., 2008; Bouchra *et al*., 2014). The aerobic microorganisms in activated sludge and aerated basins exist in aggregates called flocs that are held together by biological polymers and electrostatic forces (Garibaldi, 2012; Eric & Ronald, 2014). The main microorganisms degrading waste are heterotrophic and are a diverse set of bacteria that breakdown a wide variety of organic matter. Most of the organisms are secondary consumers, consuming the products of primary consumers that are actually breaking down the organic matter of the wastewater (Ferraro *et al*., 2011). The species composition is continuously changing as bacteria turn-over and competes for organic matter. Accordingly, there seems to be a large redundancy in the species of microorganisms those breakdown organic substrates in wastewater (Zhu *et al*., 2008; Vymazal, 2014).

In fish processing wastewaters, the need for adding nutrients (the most common being nitrogen and phosphorus) seldom occurs, but an adequate provision of oxygen is essential for successful operation. The most common aerobic processes are activated sludge systems, lagoons, trickling filters and rotating disc contactors. The reactions occurring during the aerobic process can be summarized as follows:

$$\text{Organic} + O_2 \longrightarrow \text{cells} + CO_2 + H_2O \qquad (2.4)$$

Apart from economic considerations, several factors influence the choice of a particular aerobic treatment system. The major considerations are: the area availability; the ability to operate intermittently is critical for several seafood industries that do not operate in a continuous fashion or work only seasonally; the skill needed for operation of a particular treatment cannot be neglected; and finally the operating and capital costs are also sometimes decisive.

2.6.2 Effects of High Salinity on Biological Wastewater Treatment Processes

High salinity wastewaters are usually generated from industries such as fish processing, vegetable canning, pickling, tanning and chemical manufacturing. Fish processing wastewater was noted to contain high salinity (Na^+, Cl^-), caused both by the raw materials and seawater used in various processes, and salt is usually be added to a final wash to remove the last traces on the fish and improve water removal (Hall & Ahmad, 1992; Sherly *et al*., 2015; Cui *et al*., 2016), the salinity of the fish processing wastewater depending on the products or species being processed and type of processing undertaken. The wastewater generated from precooking or brine treatment for canning of shrimp process is in the range of 2–3% w/v NaCl (Vymazal, 2014). The canning process for mollusks such as mussels, oysters, clams or scallops also generates large quantities of wastewater with salt content above 2%. The mollusks are shelled and washed with 3 to 6% salt solution (Chowdhry *et al*., 2010; FAO, 2014). Characteristics of herring brine waste were found to contain 65 g/L chloride (Balslev *et al*., 1990). While the wastewater from the dried salted fish plant contains very high salt contents, ranging from 17 g to 46 g NaCl/L. A large volume of wastewater is produced from soaking and washing operations. The raw fish's slime, blood and other contaminating substances are washed off using a 3% solution of clean salt in water. The dressed fish, or fish fillets, are soaked in mild brine (10%). After washing in clean brine solution, the eviscerated fish is salted in 21% brine (Vallero *et al*., 2003; Len *et al*., 2008; Cui *et al*., 2016).

High salinity can cause high osmotic stress or the inhibition of the reaction pathways in the organic degradation process (Sherly *et al*., 2015; Cui *et al*., 2016). This has resulted in a significant decrease in biological treatment efficiency. In addition, high salt content induces cell lysis, which causes increased effluent solids. The populations of protozoa and filamentous organisms required for proper flocculation are also

significantly reduced by the elevated salt content (Sherly *et al*., 2015; Cui *et al*., 2016). Therefore, high salinity in fish processing wastewater will lead to difficulties in biological treatment processes (Sherly *et al*., 2015). In wastewater treatment, conflicting reports on the influence of salt or sodium chloride (NaCl) on the performance of biological treatment processes exist. Several reports indicated the adverse effects of high salinity or shocks of NaCl on organic removal efficiency and sludge settle ability (Burnett, 1974; Joong *et al*., 2007; Bassin *et al*., 2012). Others reported that the constant application of NaCl tobiological treatment systems does not upset the organic removal efficiency, thereby resulting in the good flocculation of the biomass. This shows that acclimation of the biomass and levels of salt are important factors that may explain these different observations (Harmoda & Al-Attar, 1995; Lefebvre & Moletta, 2006; Mostafa *et al*., 2006; Sherly *et al*., 2015).

Salt concentrations above 2% (20 g/L NaCl) in the wastewater will affect the growth of the bacteria. Study from Joong *et al*. (Joong *et al*., 2007) in the experiment for examination of the salt effect on cellular growth show that there is no effect on cellular growth at concentrations of 1% and 2% NaCl, but observed that there is an effect on cellular growth at the concentration of 3.5% NaCl. Burnett (1974) reported that operation of activated sludge process at salt contents higher than 20 g/L is characterized by poor flocculation, high effluent solids, and a severe decrease in substrate utilization rate. Hamoda and Al-Attar (1995) published on the effect of standard sodium chloride on aerobic activated sludge treatment processes. They demonstrate that no decrease in wastewater treatment performance is observed at concentrations up to 3% NaCl (w/w). Kargi and Uygur (1996) investigated the effects of high salinity on aerobic attach growth such as trickling filter and rotating biological contactors. The results indicated that the rate and efficiency of COD removal decrease significantly with the increase in salt content above 20 g/L. The COD removal efficiency with salt-free wastewater is

95%. The COD removal decreases to 60% at 5% salt content due to the adverse effect of salt on microorganisms. The increase in salt content causes a linear reduction in the COD removal rate. Kargi and Dincer (1999) reported that saline wastewaters with more than 2% salt resulted in low BOD removal and flocculation efficiencies of conventional biological processes such as activated sludge and attach-growth processes. It has been reported that a critical salinity concentration of approximately 1–2% (w/w) exists at which the mechanism governing bacteria aggregation and stability of sludge flocs changes. Below this critical concentration, double layer compression or the hydrophobic interactions improve inter bacterial interactions. However, as the concentration of salinity exceeds this limit, the tendency of bacteria aggregation or adsorption decreases Zhao *et al*., 2013; Andreas & Shane, 2015). Here, although salt acclimation can be expected from conventional processes, the extent of adaptation is limited, and thus conventional processes cannot be used to treat wastewaters containing more than 3% salt (Bassin *et al*., 2012; Andreas & Shane, 2015; Cui *et al*., 2016).

In nitrogen removal processes, the oxidation of ammonia to nitrite and then nitrite to nitrate (nitrification process) occurs under aerobic conditions (autotrophic bacteria), and the reduction of nitrate to nitrogen gas (denitrification process) occurs under anoxic conditions (hetetrophic bacteria) (Knapp, 2014). The system configuration, the instability in the experimental conditions with respect to temperature and pH, and the presence of inhibitory compounds are the factors that directly influence the extent of salt effect on the nitrification process (Moussa *et al*., 2006; Bassin *et al*., 2012; Cui *et al*., 2016). Nitrification is also susceptible to inhibition by salt, which has been considered as an instability factor in many wastewater treatment plants, especially in industrial settings (Moussa *et al*., 2006). Kargi and Dincer (1999) also reported that salt content reduces the rate and the efficiency of nitrification and denitrification at salt contents above 2% and 1%, respectively. Nitrobacter was more adversely affected by

high salinity than Nitrosomonas, resulting in the accumulation of nitrite in the effluent at salt contents above 2%. The denitrification rate seemed more sensitive to salt inhibition than nitrification (Ruiz *et al*., 2006; Andreas & Shane, 2015; Sherly *et al*, 2015).

Panswad and Anan (1999a) investigated the effects of various salinity levels on ammonia and nitrate uptake rates of the biological nutrient removal systems (anaerobic/anoxic/aerobic). In the steadystate, the specific ammonia and nitrate uptake rates decreased with increase in chloride concentrations. The total nitrogen removal dropped from 85% to 70% when the salt contents in wastewater have increased from 20 g/L to 30 g/L. Concurrently, when the salt contents in wastewater have increased from 5 g/L to 30 g/L, COD removal of the system also dropped from 90% to 71%. This indicated that the nitrifying and denitrifying bacteria are very sensitive to sudden high salt content even with a high degree of pre-acclimation. The phosphorous removal of this system decreased from 38% to 10% with gradually increase in salt content from 0 to 30 g/L. This indicates that poly-P bacteria have intense sensitivity to high salinity condition. As the summary, all the researchers agree that the salt concentration affects the effectiveness of the biodegradation process. Where there is a difference is the limiting salt concentration. This may be related to the differences in the properties of the aqueous system.

Currently, many saline wastewater treatment plants are able to overcome the technical problems associated with high salinity by diluting the saline waste stream with freshwater. Nevertheless, this practice is unsustainable due to the continuous pressure on industries to reduce freshwater consumption (Eric & Ronald, 2014). The fish processing industry seeks cost-effective reduction and recycling technologies for fish processing wastewaters (Chowdhury *et al*., 2010). Industrial practice generally favors

continuous processing rather than processing in single batches for relatively low overall investment and operating costs (Eric & Ronald, 2014). Aerobic natural biodegradation in continuous systems would be applied as a treatment option in small-scale fish processing industries to achieve treatment technology and economics (Chowdhury *et al*., 2010, Andreas & Shane, 2015). The social and economic requirements for a low cost, simple operation, and a practical environmental technology wastewater treatment system inspired this study to design an efficient, cost-effective, and low-maintenance wastewater treatment system that is required by local fish processing industries for the treatment of fish processing saline wastewater, and the treated fish processing wastewater has high potential to be reused as liquid fertilizer in agriculture.

2.7 Fish Processing Effluent Reuse

Water use has been growing at more than twice the rate of population increase in the last century (Andreas & Shane, 2015). Thus, the need for new water resources is inevitable. The reuse of treated wastewater is one of the permanent water resources that may have numerous applications (Amir *et al*., 2013). In addition to reusable water production, wastewater treatment is important for health and environment protection through the reduction of waste and production of natural fertilizer, energy and many other advantages (Igbinosa & Okoh, 2009; Abbasi *et al*., 2015). We can reuse the treated wastewater for beneficial purposes such as landscape irrigation and agricultural, toilet flushing, industrial processes, and replenishing a ground water basin. The reuse of treated wastewater offers water resource and financial savings. Wastewater treatment can be tailored to meet the water quality requirements of a planned reuse (Andreas & Shane, 2015).

The effluent of fish processing wastewater which contains organic matter, nitrogen, phosphorus and potassium may serve as plant nutrients when applied as irrigation water which discharged directly to the environment (Elena *et al*., 2007; Pankaj *et al*., 2010; Alrumman *et al*., 2016). Besides that, the organic matter that added through wastewater irrigation serves as a soil conditioner over period time which increases its water holing capacity. In addition through the soil humus build-up, preventing of land erosion and soil conservation could be achieved (Igbinosa & Okoh, 2009). Therefore this reduces requirements for artificial fertilizers or chemical fertilizers with a concomitant reduction in energy expenditure and industrial pollution elsewhere (Andreas & Shane, 2015).

2.8 Summary

Fish processing wastewater could be a valuable resource for agriculture. However, potential utilization of this fish wastewater has been limited because of its bad smell (Tay *et al*., 2004; Tay *et al*, 2006; Liu, 2007). Biological treatment process is a treatment that can make use of microbiology concept as a beneficial science for the destruction of contaminants in wastewater. Generally, biological treatment involves the use of a biological reactor that contains wastewater rich in biodegradable organics and nutrients, in which organic matter is utilized by microorganisms for life-sustaining processes and as a food source to produce cell growth and reproduction. The nutrients available in the wastewater enhance the growth of microorganisms and allow the biological treatment to be effective. The wastewater generated by fish processing factories after biological treatment has a high potential to be reused in agriculture since the main constituents are organic and nutrients substances capable of promoting plant growth and the wastewater do not contain known toxic or carcinogenic materials.

Aerobic natural biodegradation in continuous systems would be applied as a treatment option in small-scale fish processing industries to achieve treatment technology and economics. The utilization of natural biodegradation processes for the treatment of wastewaters has several reasons or benefits. It is expected that the use of aerobic natural biodegradation in continuous systems will bring benefits to the environment, operations, and economics in reference to the optimal operational cost of the fish processing industry in our country.

CHAPTER 3

MATERIALS AND METHODS

3.1 Preparation of Synthetic Fish Processing Wastewater

Fish processing wastewater were collected from the processing of common types of edible fish species commonly found within the Malaysian region. Fresh whole fish (white pomfret, fourfinger threadfin, goldstripe sardinella, wolf herring, milkfish, short mackerel, etc.) were purchased from a local fish market in Selangor and transported on ice to University of Malaya Environmental Lab. The synthetic raw wastewater with the same composition, with equivalent to the wastewater from fish industrial has been simulated in laboratory for the series of experiments in this project. The processing of fish involves hand-skinning, filleting, and washing with tap water without additional of salt for the wastewater to be added with various salt concentrations in the series of experiments to investigate the impact of increased salt concentration on a batch and continuous treatment operation. The fish processing wash water and fish blood were collected immediately in a beaker and homogenized by agitation on the stirrer plate for 30 min. The wastewater was then kept in a 1 L size polyethylene bottle and subsequently stored in the freezer below 0°C for further use. There were around 100 kg of fish had been collected and processed in this study and the total amount of water utilized to clean the fish was around 100kg/m^3 (Mitchell, 1974; Chapman, 1996; Dan, 2003; Chen *et al*., 2003).

Preparation of influent for feeding the bioreactor, the raw wastewater was diluted with distilled water to achieve the required concentration. Preparation included three different strength of wastewaters (30-, 50-, and 100-fold diluted fish processing

wastewater) at eleven different salt (NaCl) concentrations (0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 5.0%, 6.0% and 7.0% w/v) and without salt content (0.0% w/v NaCl). The wastewater used as feed was stored in a refrigerator at 4°C. It was maintained in a feed reservoir and mixing was applied manually at regular intervals of feeding.

3.1.1 Wastewater Characterization Study

Wastewater characterization was performed for the prepared raw concentrated synthetic wastewater, diluted influent, daily withdrawn samples, and also the treated effluent of each operational cycle. The physicochemical parameter of TS, TDS, TSS, VSS, and pH; organic contents of BOD_5 and COD; and nutrient contents of NH_3-N, NO_3^--N, and P were analyzed as shown in Table 3.1 according to the procedures outlined in Standard Methods for Water and Wastewater Examinations (APHA, 1995, 2005). All chemicals used were of analytical grade.

Table 3.1: Wastewater characterization study on 10 parameters.

Parameters		**Unit**
Physicochemical	TS	mg/L
	TDS	mg/L
	TSS	mg/L
	VSS	mg/L
	pH	pH value
Organic Contents	BOD_5	mg/L
	COD	mg/L
Nutrient Contents	NH_3-N NH_4^+	mg/L
	NO_3^--N NO_3^-	mg/L
	P PO_4^{3-}	mg/L

3.2 Biological Wastewater Operation

Duplicate study was performed in two identical reactors made of Perspex with a full volume of 2000 mL (120 mm diameter and 215 mm high), operated at 30°C. The working volume of the reactor was set to 1800 mL. Both reactors were operated continuously using an agitation speed of 250 rotations per minute (rpm) for uniform mixing and were supplied with air aeration using an aquarium air pump to achieve the aerobic environment as shown in Figure 3.1.

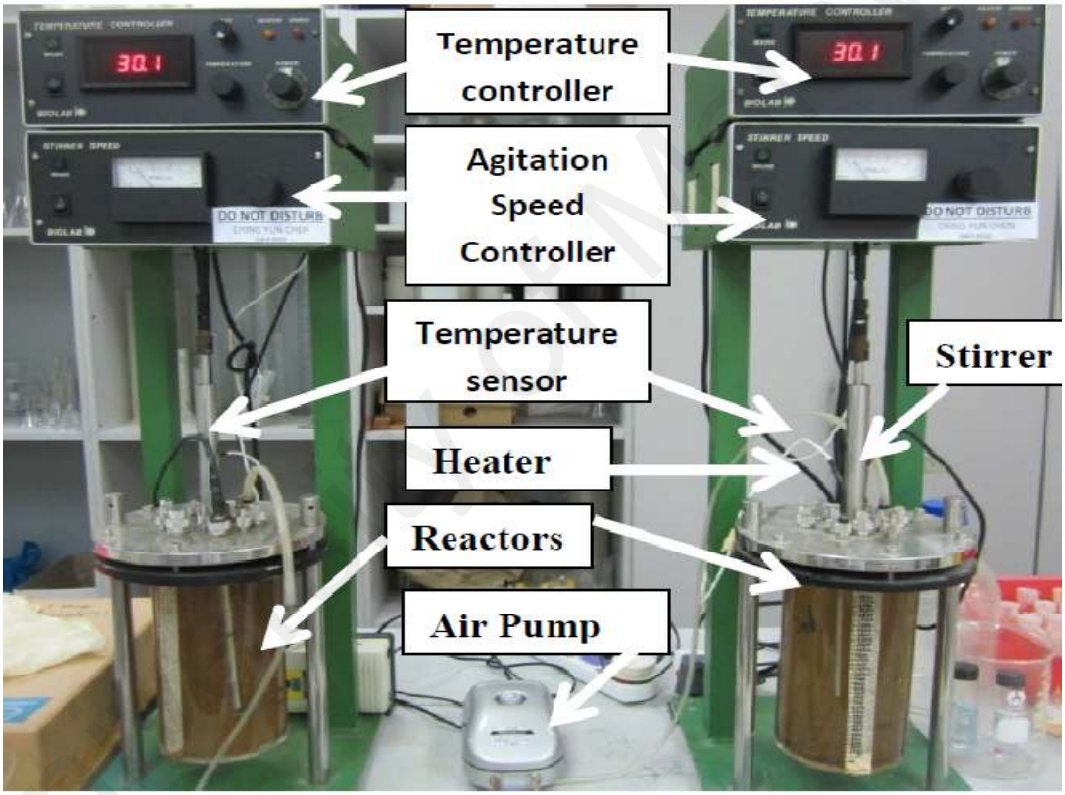


Figure 3.1: Operation of fish processing wastewater treatment in two identical laboratory scale bioreactor systems.

A heater in housing and a temperature sensor were embedded inside the reactor. This sensor is connected to a temperature controller and the reactor temperature was adjusted via a power control knob and set temperature switch/knob at the temperature controller. Temperature of the both reactor was set at 30°C for operation, as it is according to the daytime temperature in Malaysia's environment. For the convenient of data collection,

the cycle length of each treatment was set for 5 days (i.e. running the experiment from Monday to Friday), which inline with other experiment by Burnett (1974), Grady *et al*. 1999, Sagar and Pratap (2012). Feeding and withdrawal were done manually.

3.2.1 Batch Operation at Various Substrate and Salt Concentrations

In batch system operation, the influent, and the effluent after 5 days operation was studied by measuring the following parameter: pH value, TSS, TDS, TS, VSS, BOD_5, COD, NH_3-N, NO_3^--N, and P. All the experiments were performed using distilled water for dilution and final rinsed for the apparatus. All measurements of the parameters were performed in three replicates to obtain a consistent average value.

3.2.2 Continuous Operation at Various Substrate Concentrations

Continuous operation rans at several hydraulic retention time (HRT) in the continuous flow system. The first phase of the experiments focused on the evaluation of system performance by evaluating the biomass yield and the substrate removal at nine different operating HRTs that ranged from ten to two days of HRT. The experiments were carried out at different concentration of feed substrate/influent. The HRT was calculated as HRT = V/Q, where V = volume of the reactor (L), and Q = influent flow rate (L/day) as stated in Equation 3.1 (Metcalf & Eddy, 1991). Series of HRTs were set based on the working volume of the bioreactor. Range of the fresh feed flow rates were from 180 mL/day to 900 mL/day. The performance of the continuous operation was measured every day using the samples withdrawn daily from each reactor. The fresh medium was fed into the bioreactor at a specific flow rate based on the operating HRT. Each operating HRT was kept running for 5 days.

$$HRT = V/Q \tag{3.1}$$

Where:

V = volume of the reactor (L)

Q = influent flow rate (L/day)

Nine different operating HRTs were performed in order to determine the optimal retention time for the biological operation of fish processing wastewater. The degree of optimization was assed using the biomass yield and substrate removal.

3.2.3 Continuous Operation at Optimum HRT with Various Substrate and Salt Concentrations

At the second phase, the effects of 11 different salt contents (NaCl: 0.5%–7.0%) in the 30-, 50-, and 100-fold diluted wastewaters were then investigated in the continuous flow system at the obtained optimal HRT condition.

3.3 Wastewater Characterization

3.3.1 Physicochemical Parameters

(a) pH Value

Biological treatment processes have their own optimum range of pH (Len *et al*., 2008; Pankaj *et al*., 2010; Porntip *et al*., 2014). The pH analysis is used to determine if the process is operating within the acceptable range of pH. pH has a significant impact on wastewater treatment (Metcalf & Eddy, 1991; Radojevic & Bashkin, 1999; APHA, 1995, 2005). Although different processes have varying ranges, generally wastewater organisms in biological treatment systems function best at pH values from 6.5 to 7.5 (Metcalf & Eddy, 1991). The response to pH is largely due to changes in enzymatic activity (Pankaj *et al*., 2010; Porntip *et al*., 2014).

The pH value in this research was monitored by pH meter model Delta 320 (Mettler Toledo, Shanghai, P.R. China) using an integrated temperature electrode (ATC probe)

method with automatic temperature compensation (ATC) measurement. For measurement, the pH ATC electrode probe was taken out from the 3 mol/L KCL (Potassium Chloride) storage solution and rinse with distilled water. Two-point calibration of pH 7.00 and pH 4.01 was done daily using calibration buffer of pH 4.01 and pH 7.00 before measurement of the sample (APHA, 1995, 2005; Mettler Toledo, 2005). The procedure for the measurement of pH is stated in Appendix B-1.

(b) Total Solids (TS)

Total solids are the sum of dissolved and suspended solids in a water or wastewater. TS was determined by measured a volume of sample and transfer it into a pre-weighed porcelain. Evaporate to dryness in an oven at 105^{o}C, cool in desiccators with moisture indicate silica gel. The increase in weight over that of the empty porcelain dish represents the total solids. Analytically, TS was the matter that remained as residue upon evaporation at 103 to 105^{o}C (APHA, 1995, 2005). The procedure for the measurement of TS is stated in Appendix B-2. The TS was calculated using Equation 3.2:

$$\text{TS (mg/L)} = \frac{\{\text{Weight of the dish + dried residue (g)}\} - \text{Weight of the dish (g)}}{\text{Sample Volume (mL)}} \times 1000 \text{ mL/L} \quad (3.2)$$

Weight of the residue in mg (to convert W (g) to (mg), multiply w (g) with 1000)

Multiply the weight of the dry solids (in mg) by 1,000 mL/L to convert the sample size from mL to L.

(c) Total Suspended Solids (TSS)

Total suspended solids are typically composed of fine clay or silt particles, plankton, organic compounds, inorganic compounds or other microorganisms. TSS measure the particulate matter suspended in a sample of water or wastewater. These suspended

particles range in size from 10 nm to 0.1 mm, although in standardized laboratory tests, TSS is defined as the material that cannot pass through a 0.45 μm diameter filter (Radojevic & Bashkin, 1999; APHA, 1995, 2005). The procedure for the measurement of TSS is stated in Appendix B-3. Suspended solids were calculated after the soluble portion of the sample has passed through a filter by using Equation 3.3 (APHA, 1995, 2005).

$$\text{TSS (mg/L)} = (A - B)/C \times 1000 \text{ mL/L} \quad (3.3)$$

Where A = Mass of nonfilterable residue on Whatman GF/C filter after evaporation at 105^{o}C (mg)

B = Mass of the filter paper prior to sample filtration (mg)

C = volume of the sample used for filtration (mL)

(d) Total Dissolved Solids (TDS)

Total dissolved solids are physical characteristics of wastewater, and also inorganic indicators of water quality chemical characteristics of wastewater. TDS is a measure of salt dissolved in a water sample after removal of suspended solids (Kuhn *et al*., 2007). Total dissolved solids (TDS) are the weight per unit volume of all volatile and non-volatile solids dissolved in water or wastewater after a sample has been filtered to remove colloidal and suspended solids, the accumulated total of all solids that might be dissolved in water. TDS is defined (analytically) as all the matter that remains as residue upon evaporation at 180^{o}C ± 2^{o}C. The procedure for the measurement of TDS is stated in Appendix B-4. The TDS was calculated according to Equation 3.4 (APHA, 1995, 2005).

$$\text{TDS (mg/L)} = (A - B)/C \times 1000 \text{ mL/L} \quad (3.4)$$

Where A = Mass of dried porcelain plus filtrate after evaporation at 180^{o}C (mg)

B = Weight of the porcelain dish (mg)

C = volume of the sample used for filtration (mL)

(e) Volatile Suspended Solids (VSS) – Biomass Concentration

Solids remaining after the analysis for total solids, total dissolved solids or total suspended solids are ignited at 550 +/-50°C to a constant weight. The results are called total volatile solids (TVS), dissolved volatile solids (DVS) and total volatile suspended solids (TVSS). The weight loss as a result of the ignition represents the volatile portion of the solids. The difference in weight of the ash and support vessel remaining after ignition compared to the empty vessel represents the fixed solids.

VSS is a water quality measure obtained from the loss on ignition of TSS. The VSS analysis is applied most commonly to wastewater sludge to measure their biological stability on the basis of their volatility at 550 ± 50 ◦C. The organic fraction will be oxidized and will be driven off as gas at temperature of 550 ± 50 ◦C and are termed as "volatile suspended solids". The inorganic fraction remains as ash and is termed as "fixed suspended solids" (or mineral content of the suspended solids). Loss in weight is due to conversion of organic matter to CO_2 and H_2O (Metcalf & Eddy, 1991; Radojevic & Bashkin, 1999; APHA, 1995, 2005). The procedure for the measurement of VSS is stated in Appendix B-5. The VSS was calculated using Equation 3.5 as below (APHA 1995, 2005):

$$\text{VSS (mg/L)} = (A - B)/C \times 1000 \text{ mL/L} \quad (3.5)$$

Where A = Mass of nonfilterable residue on Whatman GF/C filter after evaporation at 550^{o}C (mg)

B = Mass of nonfilterable residue on Whatman GF/C filter after ignition at 150^{o}C (mg)

C = Sample size that have used (mL) for filtration.

3.3.2 Organic Contents

(a) 5-days Biochemical Oxygen Demand (BOD_5)

The most widely used parameter of organic pollution applied to both wastewater and surface water is the 5 day BOD (BOD_5). This determination involves the measurement of the dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter (Middlebrooks, 1965).

BOD test has its widest application in measuring waste loadings to treatment plants and in evaluating the BOD removal efficiency of such treatment systems. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron (APHA, 1998).

Two methods are widely used for BOD measurement. One method, the dilution method, is a standard method of the American Public Health Association (APHA) and is approved by the U.S. Environmental Protection Agency (USEPA). The other method, the manometric method, has been used for over 75 years in many sewage plants and other installations throughout the world. The USEPA denied approval of this method when it selected methods for wastewater analysis, although in certain cases the USEPA has approved the manometric method (Clifford *et al.*, 1997).

In this research, BOD was measured in a 5-day test period (BOD_5) using dilution method, and manometric method using BOD Trak by respirometric method. The procedure for the measurement of BOD_5 with dilution method is stated in Appendix C-1, and the procedure for the measurement of BOD_5 with BOD Trak by respirometric method is stated in Appendix B-6 and Appendix C-2.

(i) BOD Measurement with Dilution Method

Since most wastewater has BOD_5 that are much higher than the limited solubility of oxygen in water (8 mg/L at room temperature), it is necessary to make a series of dilutions containing varying amounts of sample in nutrient-containing, aerated dilution water. For samples whose BOD_5 does not exceed 7 mg/L, dilution is not necessary. The fish processing wastewater sample was diluted with prepared dilution water so that adequate nutrients and oxygen will be available during the incubation period. Several dilutions with the calculated dilution factor were prepared to cover the complete range of possible values. The dilution water was prepared by adding 1 mL of each of the phosphate buffer solution, magnesium sulfate ($MgSO_4$) solution, calcium chloride ($CaCl_2$) solution, and ferric chloride ($FeCl_3$) solution per liter of aerated water at 20^oC (Metcalf & Eddy, 1991; Radojevic & Bashkin, 1999; HACH, 1995, 2005).

The dilution method is conducted by placing various incremental portions of the sample into bottles and filling the bottles with dilution water, and measured with membrane electrode method. The dilution water contains a known amount of dissolved oxygen. The dilution water contains a portion of inorganic nutrients and a pH buffer. The bottles are completely filled, freed of air bubbles, sealed and allowed to stand for five days at a controlled temperature of 20°C (68°F) in the dark. During this period, bacteria oxidize the organic matter using the dissolved oxygen present in the water. At the end of the five-day period, the remaining dissolved oxygen is measured. The relationship of oxygen that was consumed during the five days and the volume of the sample increment are then used to calculate the BOD (Clifford *et al.*, 1997).

Before starting the BOD test, the amount of dissolved oxygen in the test sample is measured using Dissolved Oxygen Meter (YSI brand, Model No.: DO 200). At the end of the test, the oxygen concentration is measured again. To find the oxygen demand,

subtract the amount of dissolved oxygen on day five from the amount of dissolved oxygen on day one. Next, multiply the subtracted result with the dilution ratio and get the biochemical oxygen demand concentration in mg/L.

The wastewater sample that has diluted 50 times or more has insufficient bacteria to oxidize the organic matter that is present for carrying out the BOD test. Seeding of dilution water is required to test such sample. To make the seed solution, the contents of one dehydrated Polyseed capsule (Interlab®, The Woodlands, TX, USA, Environmental Protection Agency, EPA-approved BOD_5 seed inoculum) was poured into 500 mL dilution water to dissolve and rehydrate (aerate and stir for one hour, settle 15 min after aeration has stopped for the bran and undissolved material, decant the supernatant), and the decanted seeded dilution water (seed solution) was used to dilute the sample for making up 10% of the overall sample volume. A seed control was prepared by diluting the seed solution with dilution water for correcting the sample BOD measurement (Metcalf & Eddy, 1991; Radojevic & Bashkin, 1999; HACH, 1995, 2005).

To calculate the BOD (dilution method) using Equation (3.7) and Equation (3.8)

When dilution water is not seeded:

$$BOD_5, \text{ mg/L} = \frac{D1 - D2}{P} \quad (3.7)$$

When dilution water is seeded:

$$BOD_5, \text{ mg/L} = \frac{(D1 - D2) - (B1 - B2)\,f}{P} \quad (3.8)$$

Where:

D1 = DO of diluted sample immediately after preparation, mg/L

D2 = DO of diluted sample after 5 d incubation at $20^{o}C$, mg/L

P = decimal volumetric fraction of sample used

B1 = DO of seed control before incubation, mg/L

B2 = DO of seed control after incubation, mg/L

f = ratio of seed in diluted sample to seed in seed control =
(% seed in diluted sample) / (% seed in seed control)

(ii) BOD Measurement with Manometric Method

Biochemical Oxygen Demand, BOD_5 was determined by respirometric method using BOD Trak, and have accuracy check with a mixture of 150 mg/L each of glucose and glutamic acid. BOD seeding using BOD seed solution, BOD seed control have carried out for every batch of BOD experiment.

The Hach BOD Trak apparatus is based on the manometric principle of operation. This apparatus has been compared with the standard dilution method under controlled test conditions in the laboratory and in routine analysis. Results are equivalent in terms of both accuracy and precision.

BOD test was conducted at 20°C within a controlled environment, for a period of 5 days. Some of the advantages of using BOD Trak methods over the dilution method are as follows:

i. The sample is stirred constantly and maintained natural conditions. The BOD Trak has an initial head space containing 21% oxygen above the water sample. Continuous stirring replenishes dissolved oxygen to the sample. This makes the BOD Trak results similar to occurrences found in natural environment. The dilution method supplies no additional oxygen to the sample, this causes a higher percentage of oxygen depletion, sometimes as much as 89 %.

ii. The BOD rate can be observed daily without disturbing the sample. Pressure changes within the closed BOD Trak system are displayed graphically in

milligrams per liter (mg/L) on an LCD. The system supplies 480 evenly space data points over the selected period.

iii. The BOD Trak method continuously removes carbon dioxide from the system so that the pressure difference observed is proportional to the amount of oxygen used.

iv. Bubbles formed by degassing can cause errors when samples are warmed to the experimental temperature. The BOD Trak compensates for this occurrence by starting the test after the equilibrium temperature has been reached.

(b) Chemical Oxygen Demand (COD)

The chemical oxygen demand test is an index of water pollution measuring the mass concentration of oxygen consumed by the chemical breakdown of organic and inorganic matter. COD assays are generally used for the estimation of the chemically oxidizable organic carbon of samples varying and unknown composition, such as domestic and industrial wastes and natural waters (HACH, 1999). COD is significant in the control of the total content of pollution and the management of water surroundings (Chapman, 1996).

COD is defined as the amount of a specified oxidant that reacts with the sample under controlled conditions in an acidic medium. The quantity of oxidant consumed is expressed in terms of its oxygen equivalence. The COD test is used to determine the degree of pollution in water (APHA–AWWA, 2005). Either the open reflux method or closed reactor method is acceptable as the method of COD measurement (APHA, 1998).

In this research project, COD was measured using method 5220 C, closed reflux, and the titrimetric method according to procedures outlined in Standard Method for the Examination of Water and Wastewater (APHA, 1995). The procedure for the measurement of COD is stated in Appendix B-7, In addition, 2 mL diluted wastewater

sample (for blank - 2 mL distilled water, for check the accur - 2 mL 500 mg/L phthalic acid (potassium acid phthalate, KHP) solution as COD standard solution)was pipet into Hach Dichromate COD Reagent vial of High Range type (0 to 1500 mg/L) or 0.20 mL diluted wastewater sample (blank - 0.2 mL distilled water, accuracy check - 0.2 mL 10,000 mg/L KHP solution) was pipet into Hach Dichromate COD Reagents vial of High Range Plus type: 0 to 15,000 mg/L. Closed the prepared sample digestion reagent vial with the vial screw cap tightly. Mix the contents in the vial by shaking and invert the vial several times. The prepared vials were put into the block digester and heated for two hours at 150 ◦C. The digested sample was cooled to room temperature, added with 2 drops of ferroin indicator solution, and then titrated with standard ferrous ammonium sulphate (FAS) 0.01 M. The sample was titrated until a sharp colour changed from blue-green to reddish brown. Then the COD value was calculated using Equation 3.9 (APHA, 1995, 2005).

$$\text{mg/L COD} = \frac{(A - B) \times M \times 8000 \times \text{Dilution factor}}{\text{mL sample}} \qquad (3.9)$$

where: A = volume of FAS titrant used for blank, mL
B = volume of FAS titrant used for sample, mL
M = Molarity of FAS solution; **0.01 M** was used
8000 = milliequivalent weight of oxygen × 1000 ml/L.

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in column 1 in the Table 3.2 below. Samples with higher chloride concentrations should be diluted. Dilute the sample enough to reduce the chloride concentration to the level given in column 2. If sample dilution will cause the COD concentration to be too low for accurate determination, add 0.50 g of mercuric sulfate ($HgSO_4$) to each COD vial before the sample is added. The additional mercuric sulfate

will raise the maximum chloride concentration allowable to the level given in column 3 (HACH, 1996, 1999).

Table 3.2: Each type of Hach COD Digestion Reagent Vial and the maximum acceptable level of chloride concentration in the sample for pipette into each type of COD vial to perform COD test.

	Column 1	**Column 2**	**Column 3**
Vial type Used	Maximum Cl^- concentration in sample (mg/L)	Maximum Cl^- concentration of diluted samples (mg/L)	Maximum Cl^- concentration in sample when 0.50 $HgSO_4$ added (mg/L)
Low Range	2000	1000	8000
High Range	2000	1000	4000
High Range Plus	20,000	10,000	40,000

3.3.3 Nutrient Contents

(a) Ammoniacal Nitrogen (NH_3-N)

Concentration of NH_3-N was determined by DR/2000 spectrophotometer (HACH, Loveland, CO, USA) using the Nessler Method (Method 8038) at wavelength (λ) 425 nm, and this method is applicable over the range of 0 to 2.50 mg/L in 25 mL of water samples. The matched pair of square glass 25 mL (sample cell) was used, and one sample cell was filled with the wastewater sample, another sample cell (the blank) was filled with demineralized water, and both prepared sample cells were added with reagent HACH tests kits of Nessler reagent, mineral stabilizer, and polyvinyl alcohol dispersing agent. The blank was used to set the spectrophotometer to zero. A yellow colour was developed in proportion to the present of ammonia amount in the prepared sample cell (HACH, 2001). This procedure is equivalent to the USEPA Method 350.2 and Standard Methods for the Examination of Water and Wastewater, 4500-NH3 B and C for wastewater (HACH, 1995, 2005) (Refer to Appendix C-3 for the analysis method and detail procedure for the measurement of NH_3-N).

(b) Nitrate Nitrogen (NO_3^--N)

For this research, NO_3^--N determination employed Cadmium Reduction, High Range (HR) method (Method 8039) at wavelength (λ) 500 nm. One sample cell was filled with wastewater sample and added with the contents of one Nitra Ver 5 Nitrate reagent powder pillow, and another sample cell (the blank) was filled with wastewater sample without reagent (HACH, 2001). This method is applicable over the range of 0 to 30.0 mg/L in 25 mL of water samples. This procedure is adapted from Standard Methods for the Examination of Water and Wastewater (HACH, 1995, 2005) (Refer to Appendix C-4 for the analysis method and detail procedure for the measurement of NO_3^--N).

(c) Phosphorus (P) / Orthophosphate (PO_4^{3-})

P/PO_4^{3-} determination applied molybdovanadate method using molybdovanadate reagent (method 8114) where the wavelength (λ) used was 430 nm to detect mg/L PO_4 3− or mg/L P concentration. One sample cell was filled with wastewater sample, another sample cell (the blank) was filled with demineralized water, and both prepared sample cells were added with Molybdovanadate reagent. This method is applicable over the range of 0 to 45.0 mg/L in 25 mL of water samples and a yellow colour was developed in proportion to the presence of phosphate. This procedure is adapted from Standard Methods for the Examination of Water and Wastewater (HACH, 1995, 2005). The analysis method and experimental procedure for the measurement of Phosphorus is stated in Appendix C-5.

3.3.4 Analysis of Biomass Yield

Biomass concentrations in this research was measured as VSS. VSS is an indication of bacteria or biomass content in the wastewater, it is important for characterizing wastewater treatment basins (APHA, 1995, 2005; Grady et al., 1999; Radojevic &

Bashkin, 1999; Mettler Toledo, 2005). The biomass yield was determined as g VSS produced per g COD removed as shown in Equation 3.6 as below:

$$\text{Biomass Yield} = \frac{\text{g biomass (VSS) accumulated}}{\text{g COD removed}}$$

g biomass (VSS) accumulated/produced

= VSS at day 5 (mg/L) – VSS at day 0 (mg/L) X 1.8 L = VSS (mg)/1000

g COD removed/utilized

= COD at Day 0 (mg/L) – COD at day 5 (mg/L) X 1.8 L = COD (mg)/1000

Remarks : working volume of the fish processing wastewater in a bioreactor = 1.8 L

CHAPTER 4

RESULTS

4.1 Fish Processing Wastewater Characteristics

Wastewater characterization was performed for the prepared raw concentrated synthetic wastewater, diluted influent, daily withdrawn samples, and the final treated effluent of each operational cycle. Wastewater characteristics of the simulated synthetic original fish processing wastewater, and the influent of 30, 50 and 100-fold diluted liquid of the original fish processing wastewater are summarized in Table 4.1.

pH analysis is used to determine if a process is operating within the acceptable range of pH. In this study, the pH of the raw synthetic fish processing wastewater (simulated original, without dilution) with pH range of 6.65 ± 0.02, while the pH of the diluted raw wastewater with distilled water (30-, 50-, and 100-fold diluted) for feeding into the reactor (influent) have pH value ranged from 7.41-7.33. Therefore, the influent feed into the reactor for treatment processes was in the best pH value range of 6.5 to 7.5 for the function of biological treatment system (Tay *et al*., 2006; Zhu *et al*., 2008; Bassin *et al*., 2012).

Table 4.1: Experimental results for the characteristics of the simulated original (without dilution) and 30-, 50- and 100-fold diluted fish processing wastewater.

Parameter	Fish processing wastewater concentration			
	Simulated original	**30-fold dilution**	**50-fold dilution**	**100-fold dilution**
A. Physicochemical Parameter				
TSS (mg/L)	5,530 ± 638	184 ± 21	110 ± 13	55 ± 6
TDS (mg/L)	2,590 ± 450	86 ± 15	52 ± 9	26 ± 5
TS (mg/L)	22,350 ± 480	750 ± 16	450 ± 9	225 ± 5
VSS (mg/L)	905 ± 5.5	30 ± 0.20	18 ± 0.10	9 ± 0.06
pH (value)	6.65 ± 0.02	7.36 ± 0.03	7.38 ± 0.02	7.40 ± 0.01
B. Chemical Parameter : Organic Contents				
BOD (mg/L)	18,419 ± 660	614 ± 22	368 ± 12	184 ± 7
COD (mg/L)	30,000 ± 2,000	1,000 ± 66	600 ± 40	300 ± 20
C. Chemical Parameter: Nutrient Contents				
NH_3-N (mg/L)	504 ± 30	17 ± 1	10 ± 0.6	5 ± 0.3
NH_4^+ (mg/L)	650.16 ± 38.7	21.93 ± 1.29	12.9± 0.77	6.45 ± 0.39
NO_3^-N (mg/L)	51 ± 5	2.4 ± 0.2	1.0 ± 0.1	0.5 ± 0.05
NO_3^- (mg/L)	224.4 ± 22	7.48 ± 0.88	4.4±0.44	2.2 ± 0.22
P (mg/L)	95.5 ± 6.5	3.2 ± 0.20	1.91 ± 0.15	0.96 ± 0.06
PO_4^{3-} (mg/L)	293.19 ± 19.96	9.82 ± 0.61	5.86 ± 0.46	2.95 ± 0.18

4.2 Batch Operation at Various Substrate and Salt Concentrations

4.2.1 Solids Removal

(a) Total Solids (TS)

Figure 4.1 shows the TS removal in the batch system with three different concentration of fish processing wastewater. After 5 days of batch system operation, TS removal showed the highest percentage of TS removal of 30% in the 30-fold diluted fish processing wastewater at 7% salt content. The lowest percentage of TS removal of 4.5% was found in 30-fold diluted wastewater with 1% salt content.

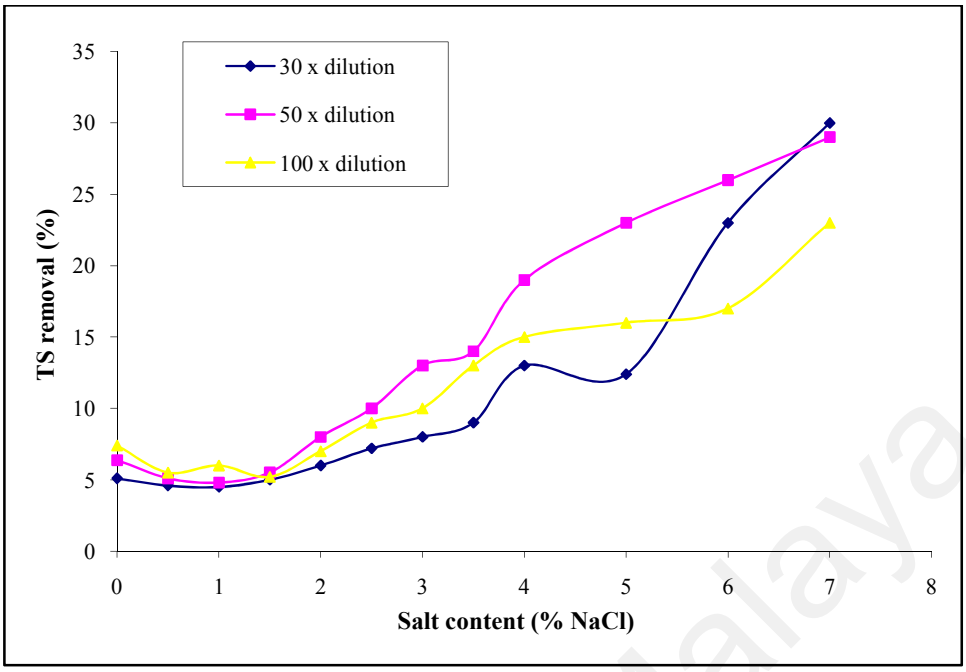


Figure 4.1: TS removal percentage in a batch treatment system for 30-, 50-, and 100-fold diluted fish processing wastewater with 0.0-7.0% of NaCl concentrations.

(b) Total Suspended solids (TSS)

Figure 4.2 shows the TSS removal percentage in batch system after 5 days of treatment operations. TSS has the lowest removal percentage of 5.0% in batch system at 1% salt content in 30-fold diluted fish processing wastewater. The highest percentage removal of 38.0% was found in 100-fold diluted fish processing wastewater at 7% salt contents.

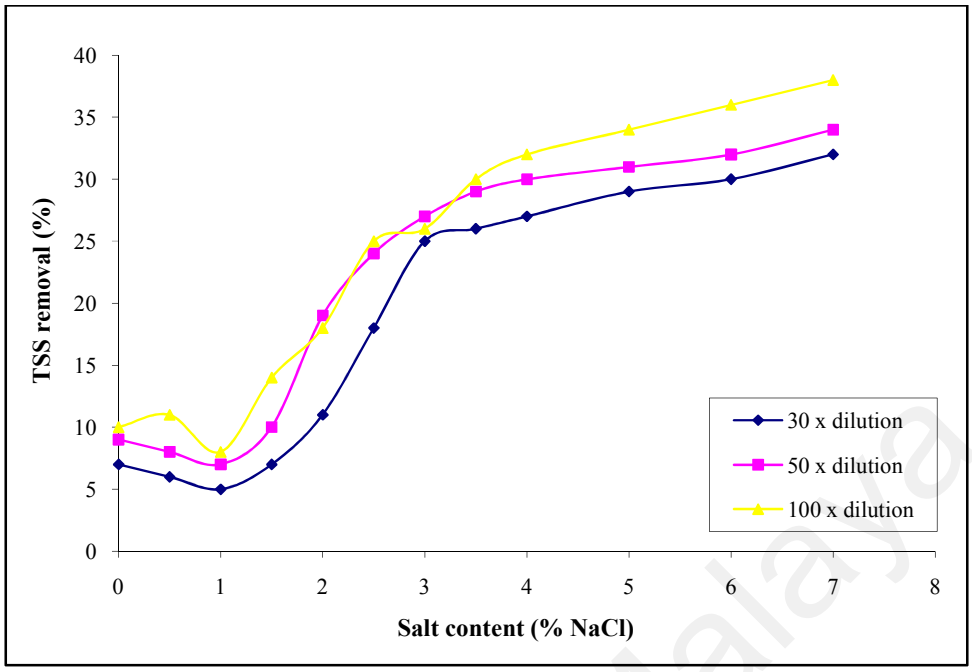


Figure 4.2: TSS removal percentage in a batch treatment system for 30-, 50-, and 100-fold diluted fish processing wastewaters with 0-7.0% of NaCl concentrations.

(c) Total Dissolved Solids (TDS)

Figure 4.3 shows the TDS percentage removal after 5 days of batch system operation for the three concentrations of fish processing wastewaters at twelve different concentration of NaCl.

The lowest percentage of TDS removal was 9.0% in 30-fold diluted fish processing wastewater containing 1% salt content. The highest percentage of TDS removal of 40.0% was found at 7% salt content in 100-fold diluted fish processing wastewater.

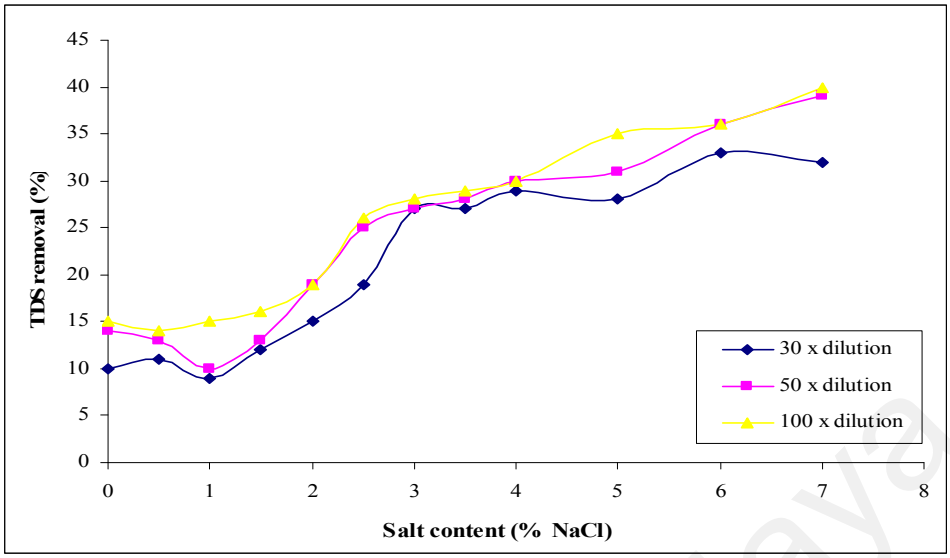


Figure 4.3: TDS removal percentage in a batch treatment system for three different concentrations of 0-, 50-, and 100-fold diluted fish processing wastewaters with 0-7.0% of NaCl concentrations.

(d) Volatile Suspended Solids (VSS)

Figure 4.4 shows the VSS increase percentage after 5 days of batch system operation for the three different type concentration of fish processing wastewaters at twelve different concentrations of NaCl.

The lowest increase percentage of VSS of 8.0%, was found in 100-fold and 50-fold diluted fish processing wastewater containing 7% of salt content. While the highest increase percentage of VSS of 46.0% was observed in 30-fold diluted fish processing wastewater at 1% salt content.

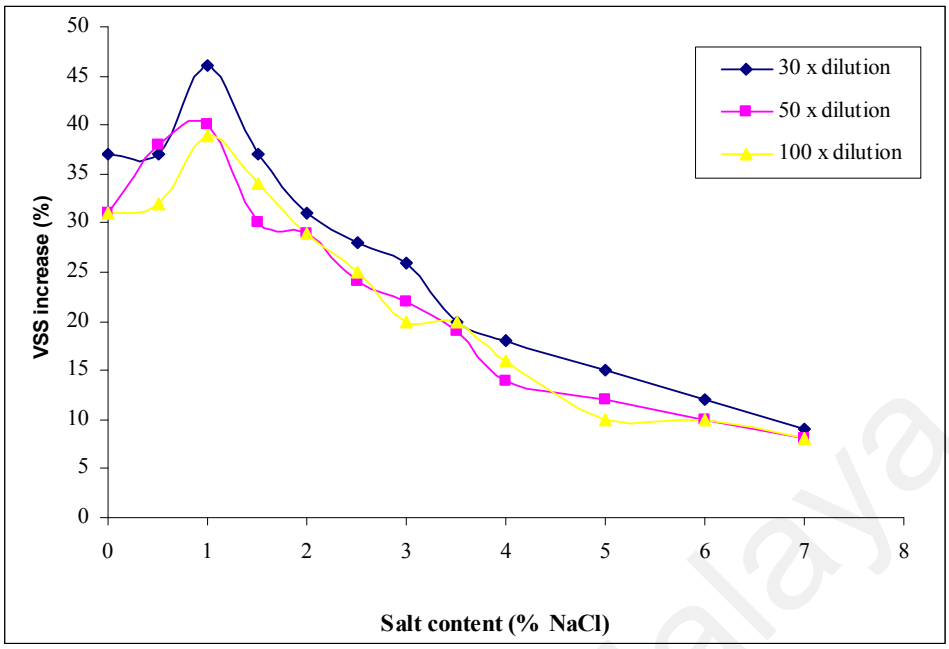


Figure 4.4: VSS increase percentage in a batch treatment system for three different concentrations of diluted fish processing wastewater with 0-7.0% of NaCl concentrations.

4.2.2 Organic Contents Removal

(a) 5-day Biochemical Oxygen Demand (BOD_5)

Figure 4.5 shows the BOD_5 removal percentage after 5-day batch system operation for the three different concentrations of fish processing wastewaters at twelve different NaCl concentrations.

In batch treatment system, the highest BOD_5 removal of 80.00% was achieved in 30-fold diluted wastewater at 1% NaCl content. The lowest BOD_5 removal of 11.54% was found in 100-fold diluted wastewater at 7% of salt content.

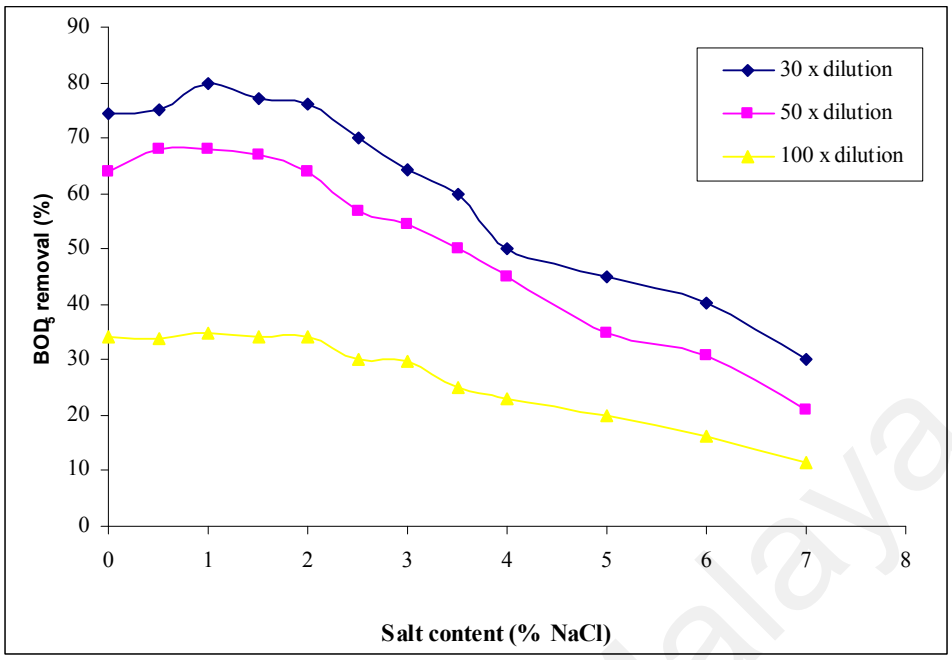


Figure 4.5: 5-day BOD removal percentage in a batch treatment system for three different concentrations of diluted fish processing wastewaters with 0-7.0% of NaCl concentrations.

(b) Chemical Oxygen Demand (COD)

Figure 4.6 shows the COD removal percentage after 5 days of batch system operation for the three different concentrations of fish processing wastewater at twelve different NaCl concentrations.

In batch treatment operation, COD removal achieved the highest value of 85.20% in 30-fold wastewater at 1% NaCl content. The lowest COD removal of 11.86% was found in 100-fold diluted wastewater at 7% salt content.

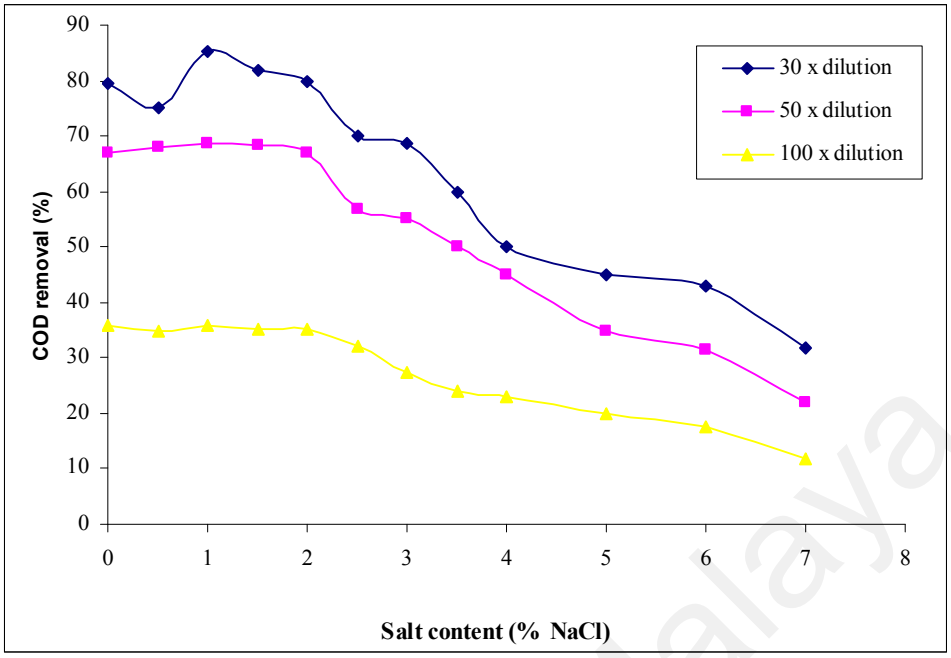


Figure 4.6: COD removal percentage in a batch system treatment for three different concentrations of diluted fish processing wastewater with 0-7.0 of NaCl concentrations.

4.2.3 Nutrient Contents Removal

(a) Ammoniacal Nitrogen (NH_3-N)

Figure 4.7 shows the percentage of ammoniacal nitrogen removal found in the effluent after 5 days of batch system operation of the three different concentrations of fish processing wastewaters with eleven different salt concentrations and without salt content as control.

After 5 days of batch system operation, the highest percentage of NH_3-N removal, 68.93% was found in 30-fold diluted fish processing wastewater at 1% NaCl. While NH_3-N removal showed the lowest value of 10% in 100-fold diluted fish processing wastewater at 7% of salt content.

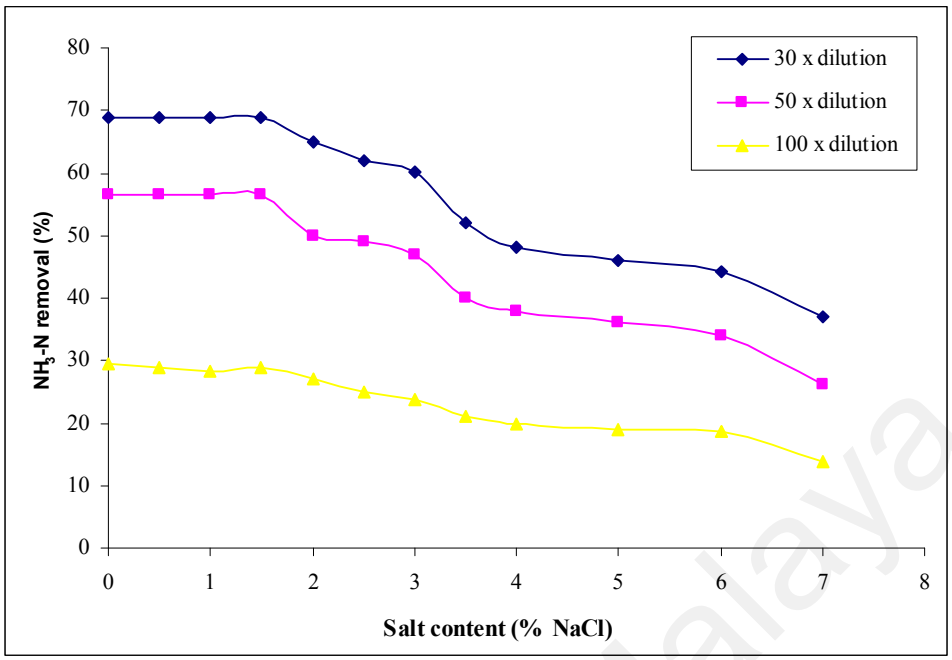


Figure 4.7: Ammoniacal nitrogen removal percentage in a batch treatment system for 30-, 50-, and 100-fold diluted fish processing wastewater with 0-7.0% of NaCl concentration.

(b) Nitrate Nitrogen (NO_3^--N)

Figure 4.8 shows the percentage of nitrate nitrogen removal found in the effluent after 5 days of batch system operation of the three different concentrations of fish processing wastewaters with eleven different salt concentrations and without salt content.

After 5 days of batch system operation, the highest percentage of NO_3^--N removal was 66% removed in 30-fold diluted fish processing wastewater containing 0.5% and 1.0% salt content. While the lowest percentage of NO_3^--N removal was 10% found in 100-fold diluted fish processing wastewater at 7% salt content.

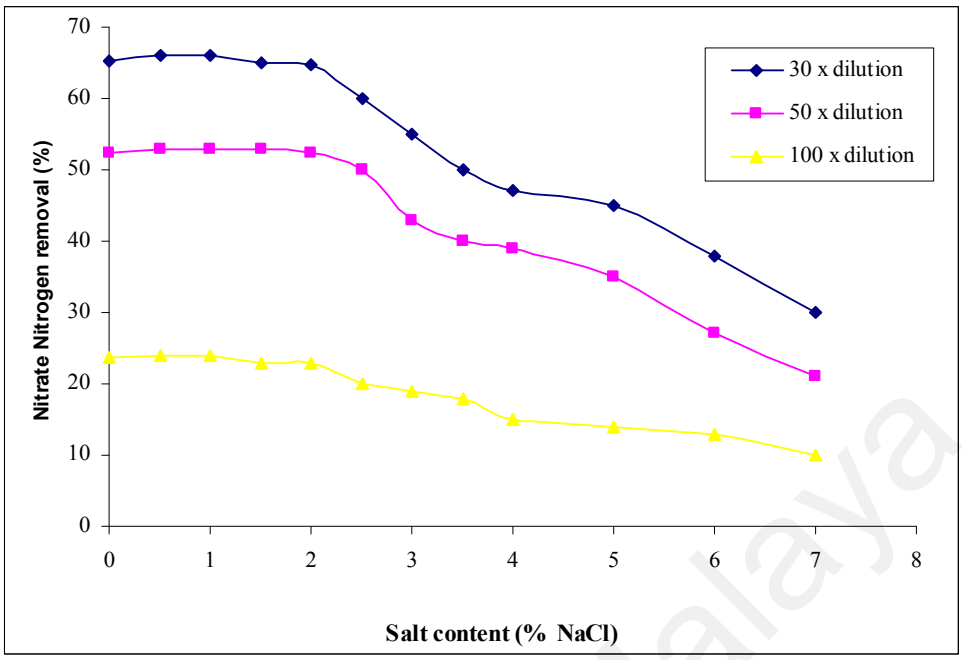


Figure 4.8: NO_3^--N removal percentage in a batch treatment system for three different concentrations of diluted fish processing wastewaters with 0-7.0% of NaCl concentrations.

(c) Phosphorus (P)

Figure 4.9 shows the percentage of phosphorus removal found in the effluent after 5 days of batch system operation of the three different concentrations of fish processing wastewaters with various salt contents.

After 5 days of batch system operation, the highest percentage of phosphorus removal of 61% was found in 30-fold fish processing wastewater with the addition of 1.0% salt content. The lowest percentage of P removal was 4% had been recorded in 100-fold diluted fish processing wastewater at 7% salt content.

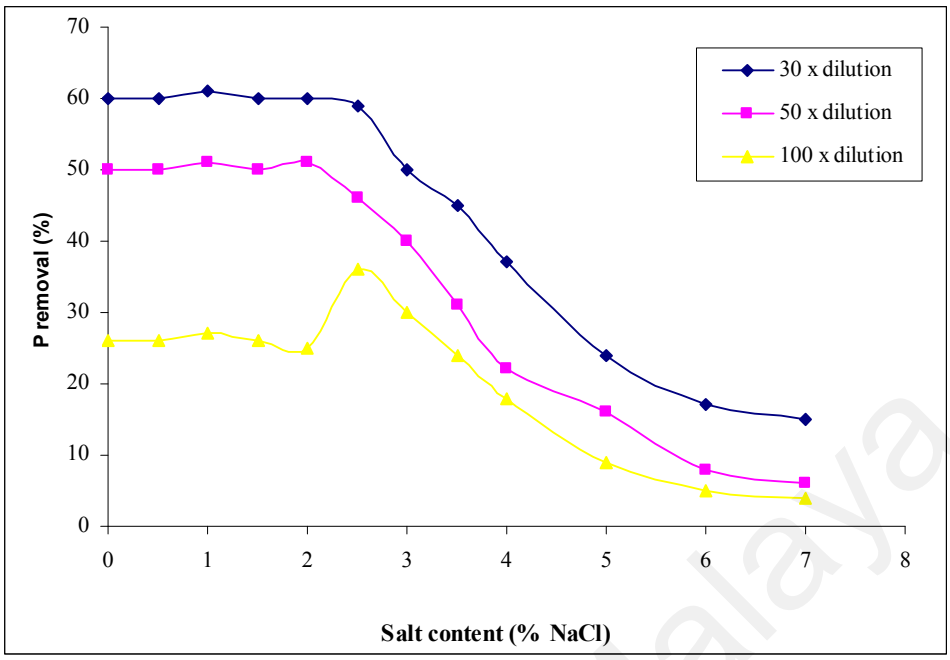


Figure 4.9: Phosphorus removal percentage in a batch treatment system for three different concentrations of diluted fish processing wastewater with 0-7.0% of NaCl concentrations.

4.2.4 Biomass Yield

Figure 4.10 shows the percentage of biomass yield obtained in the effluent after 5-day batch system operation of the three different concentrations of fish processing wastewater with eleven different salt concentrations and without salt content as control.

After 5 days batch system operation, the highest biomass yield percentage, 60.0% was found in 50-fold fish processing wastewater at 1.0% salt content. While the lowest percentage of biomass yields percentage of 8.0% was found in 100-fold diluted fish processing wastewater at 7% salt content.

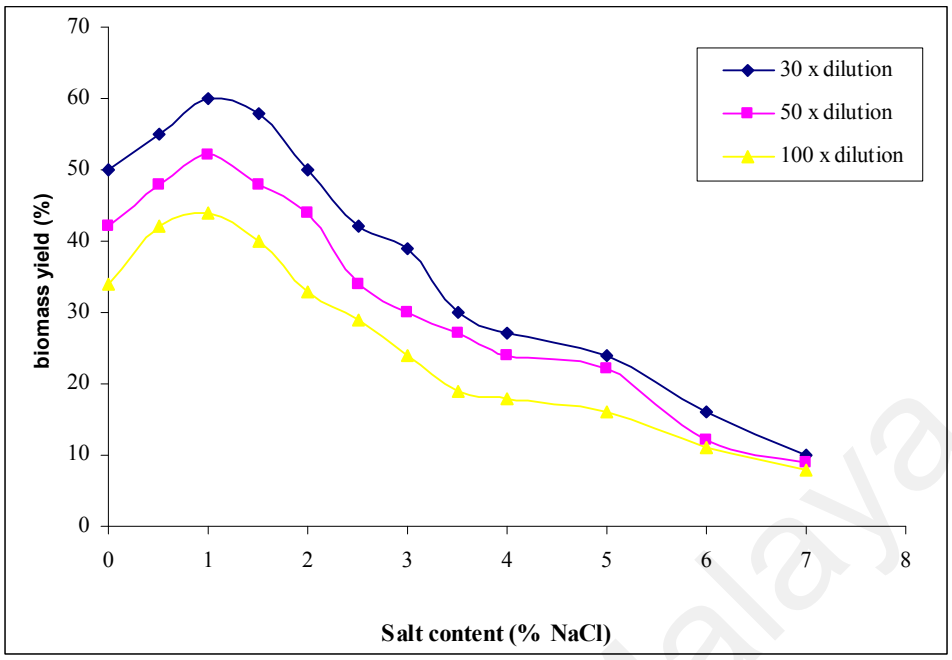


Figure 4.10: Biomass yield percentage in a batch treatment system for 30-, 50-, and 100-fold diluted fish processing wastewater with 0-7.0% of NaCl concentration.

4.3 Continuous Operation at Different Substrate Concentrations and HRTs

4.3.1 Solids Removal

(a) Total Solids (TS)

Figure 4.11 shows the TS removal in the continuous system at various HRTs. For the continuous system at nine different operating HRT, the highest percentage of TS removal, 32.0% was found in 50-fold diluted fish processing wastewater at 2-day HRT, and the lowest percentage of removal, 5.0% was found in 30-fold diluted fish processing wastewater at 8-day HRT.

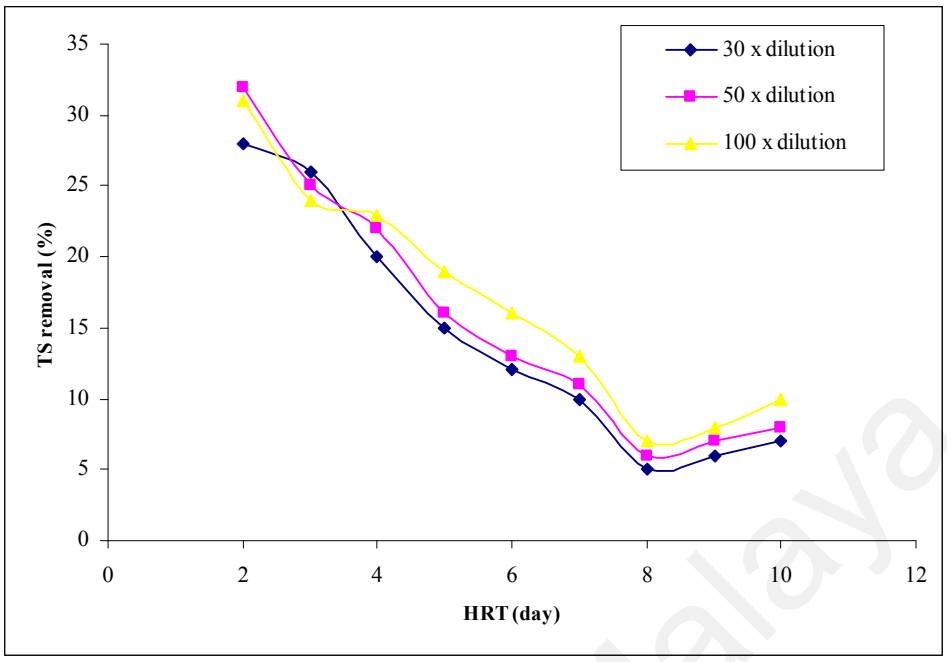


Figure 4.11: TS removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with nine different operating HRTs.

(b) Total Suspended solids (TSS)

Figure 4.12 shows the TSS removal percentage in the effluent of the continuous system without salt content at nine different operating HRTs after the 5-day treatment operations. The lowest percentage of TSS removal (7.0%) was found at the 8-day HRT in the 30-fold diluted fish processing wastewater. The highest percentage of TSS removal was 33.0% found in the 100-fold diluted wastewater at the 2-day HRT.

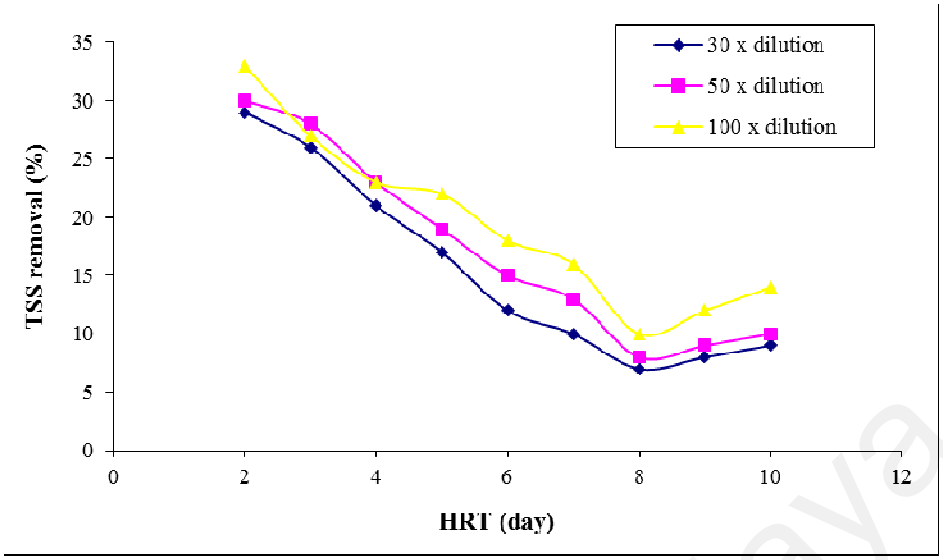


Figure 4.12: TSS removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with nine different operating HRTs.

(c) Total Dissolved Solids (TDS)

Figure 4.13 shows the percentage removal after 5 day continuous system operation with nine different operating HRT range from 10 to 2 days HRT (10, 9, 8, 7, 6, 5, 4, 3, 2 day) for the three different concentration of fish processing wastewater.

The lowest percentage removal of TDS, 9.0% was found at 8 days HRT in 30-fold diluted fish processing wastewater. While the highest percentage of TDS removal of 35.0% was found in the 100-fold diluted fish processing wastewater at 2-day HRT.

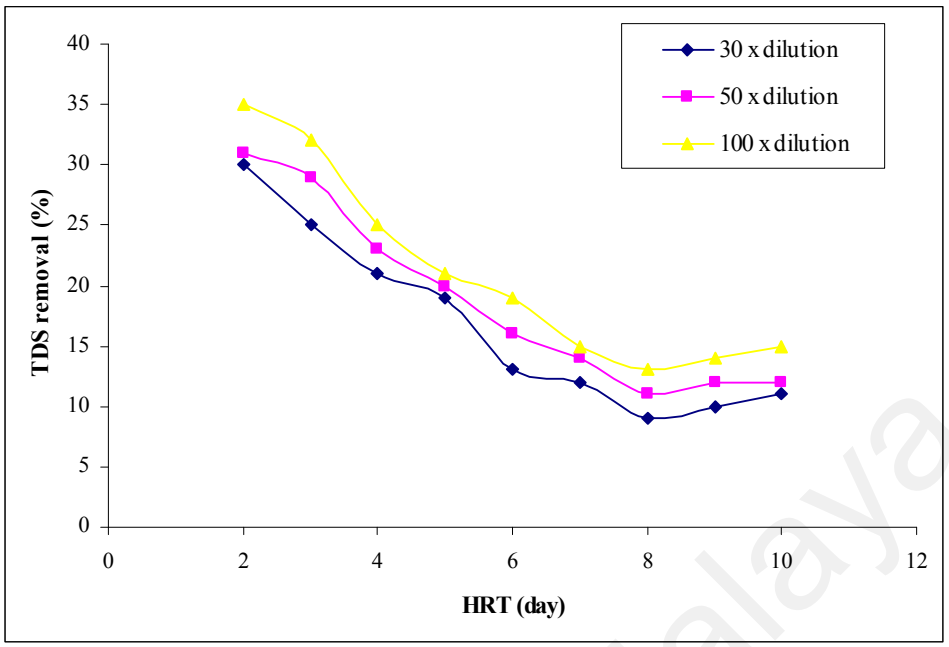


Figure 4.13: TDS removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with nine different operating HRTs.

(d) Volatile Suspended Solids (VSS)

Figure 4.14 shows the VSS increase percentage after five days of continuous system operation with nine different operating HRTs (10, 9, 8, 7, 6, 5, 4, 3, and 2 days) for the three different concentrations of fish processing wastewaters. In the reactor with continuous feeding and removal of substrates, the lowest VSS increase percentage of 9.0% was found at the 2-day HRT in the 100-fold diluted fish processing wastewater, while the highest VSS increase percentage of 49.0% was found at the 8-day HRT in the 30-fold diluted fish processing wastewater.

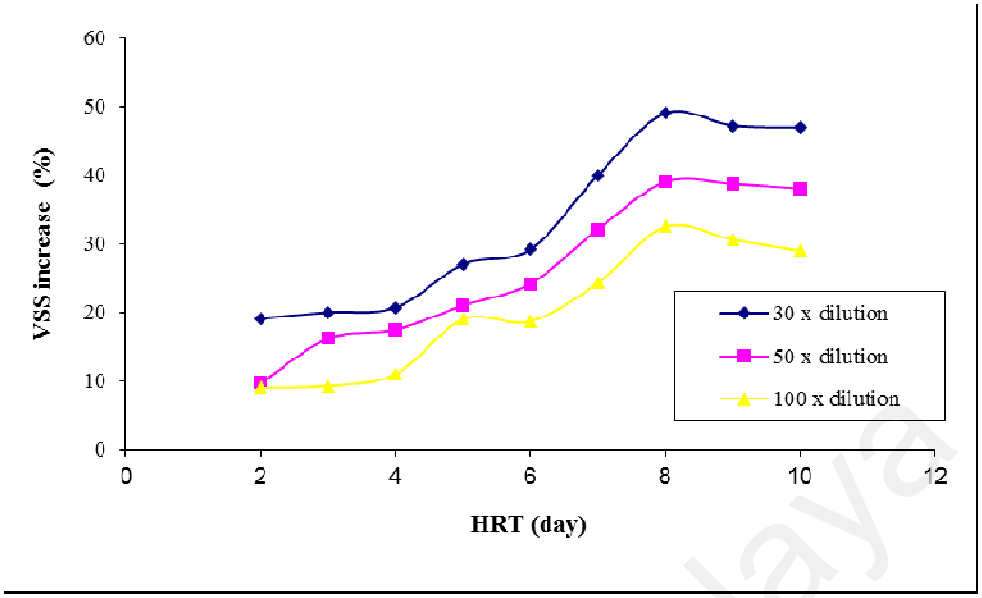


Figure 4.14: VSS increase percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters at nine different operating HRTs.

4.3.2 Organic Contents Removal

(a) 5-day Biochemical Oxygen Demand (BOD_5)

Figure 4.15 shows the BOD removal percentage after five days of continuous system operation at nine different operating HRTs for the three different concentrations of fish processing wastewaters without salt content. In the continuous system treatment, 10-day HRT attained the highest BOD removal rate of 81.0% in the 30-fold diluted wastewater and the lowest removal rate of 15.0% was found at the 2-day HRT in the 100-fold diluted wastewater.

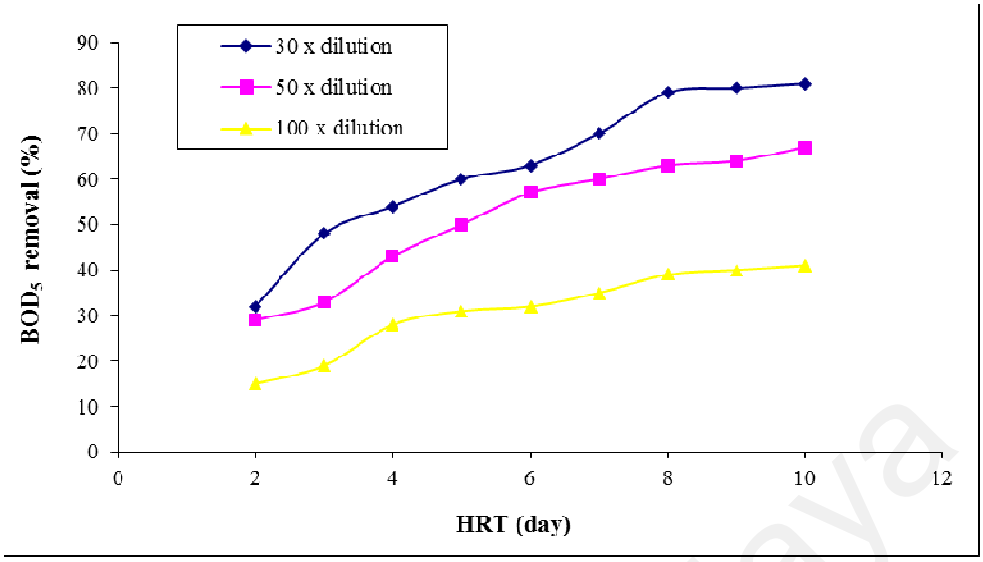


Figure 4.15: BOD_5 removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with nine different operating HRTs.

(b) Chemical Oxygen Demand (COD)

Figure 4.16 shows the COD removal percentage after five days of continuous system operation at nine different operating HRTs for the three different concentrations of fish processing wastewaters without salt content. In the continuous flow treatment system, the 10-day HRT attained the highest COD removal rate of 85.5% in the 30-fold diluted wastewater and the lowest removal rate of 16.0% was found at the 2-day HRT in the 100-fold diluted wastewater.

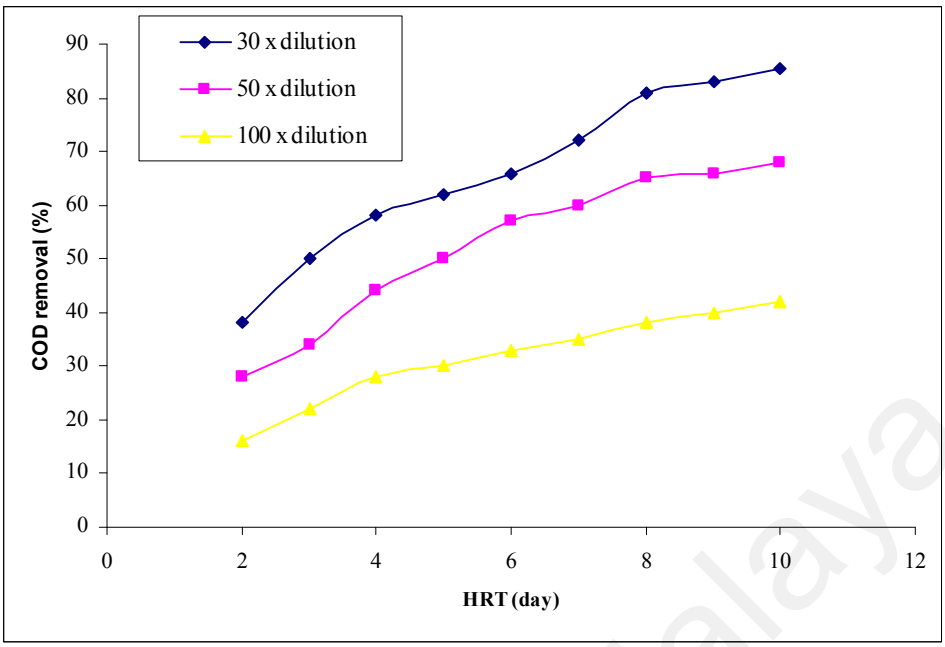


Figure 4.16: COD removal percentage in a continuous flow treatment system for three different concentrations of diluted fish processing wastewaters with nine different operating HRTs.

4.3.3 Nutrient Contents Removal

(a) Ammoniacal Nitrogen (NH_3-N)

Figure 4.17 shows the percentages of NH_3-N removal found in the effluent after five days of continuous flow system operation for the three concentrations of fish processing wastewaters without salt content at nine different operating HRTs. The efficiency of NH_3-N removal decreased with the increasing dilution fold of the original fish processing wastewater and decreasing of HRT from 7-day HRT to 2-day HRT. At HRTs of 8, 9, and 10 days, the removal of NH_3-N was more than 70.0% in 30-fold diluted fish processing wastewater. The highest percentage of NH_3-N removal, 72.5% was found in 30-fold diluted fish processing wastewater at 10 day HRT, while the lowest percentage of NH_3-N removal, and 14.0% was found in 100-fold diluted fish processing wastewater at 2-day HRT.

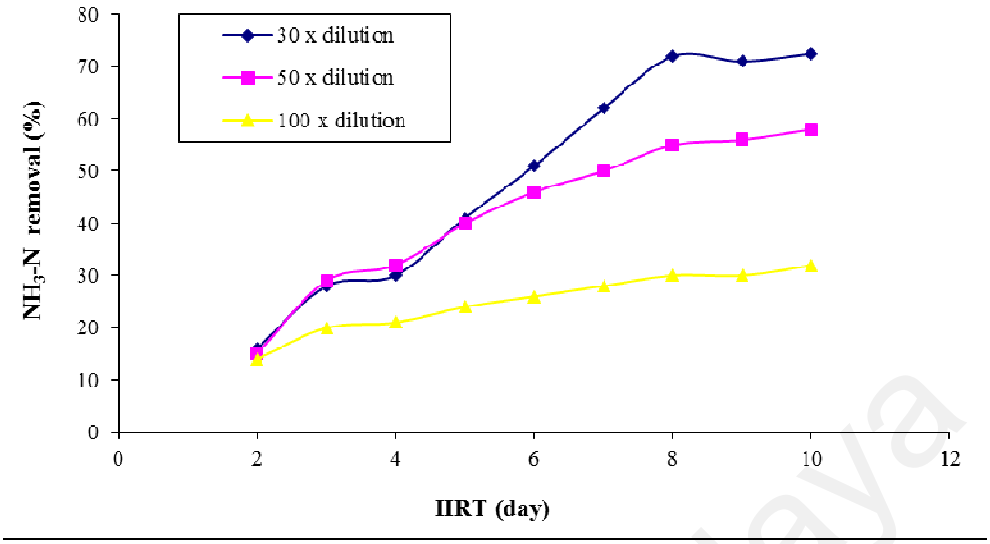


Figure 4.17: NH_3-N removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with nine different operating HRT conditions.

(b) Nitrate Nitrogen (NO_3^--N)

Figure 4.18 shows the percentage of NO_3^--N removal found in the effluent after five days of continuous flow system operation of the three different concentrations of fish processing wastewaters at nine different HRT conditions. After five days of continuous flow operation, the highest percentage of NO_3^--N removal of 70.0% was found in the 30-fold diluted fish processing wastewater at the 10-day HRT. The lowest percentage of NO_3^--N removal of 10.0% was found in the 30-fold diluted fish processing wastewater at the 2-day HRT.

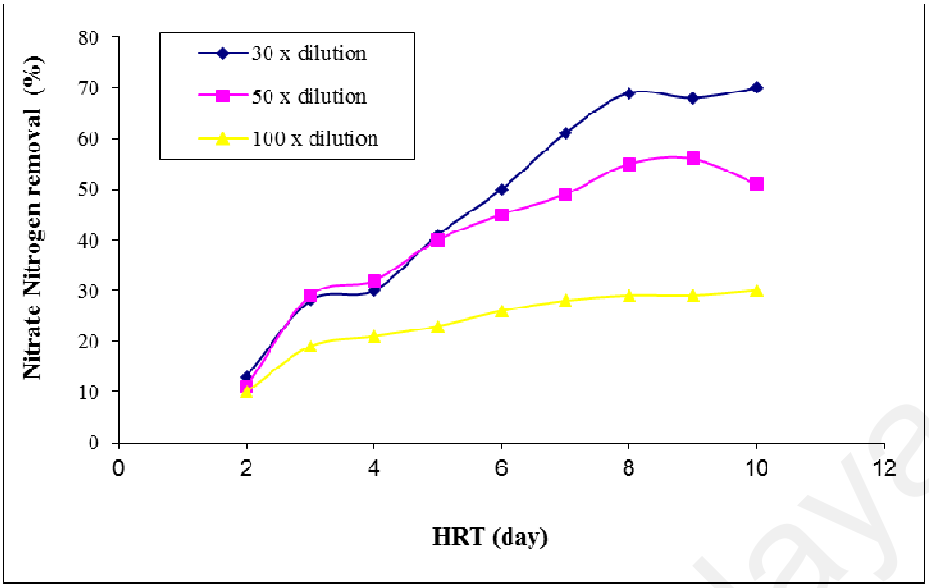


Figure 4.18: NO_3^--N removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with 9 different HRT operating conditions.

(c) Phosphorus (P)

Figure 4.19 shows the percentages of phosphorus removal found in the effluent after five days of continuous system operation of the three different concentrations of fish processing wastewaters without salt content. After five days of continuous flow operation, the highest percentage of P removal of 62.0% was found in the 30-fold diluted fish processing wastewater at the 10-day HRT, while the lowest percentage of P removal of 4.0% was found in the 100-fold diluted fish processing wastewater at the 2-day HRT.

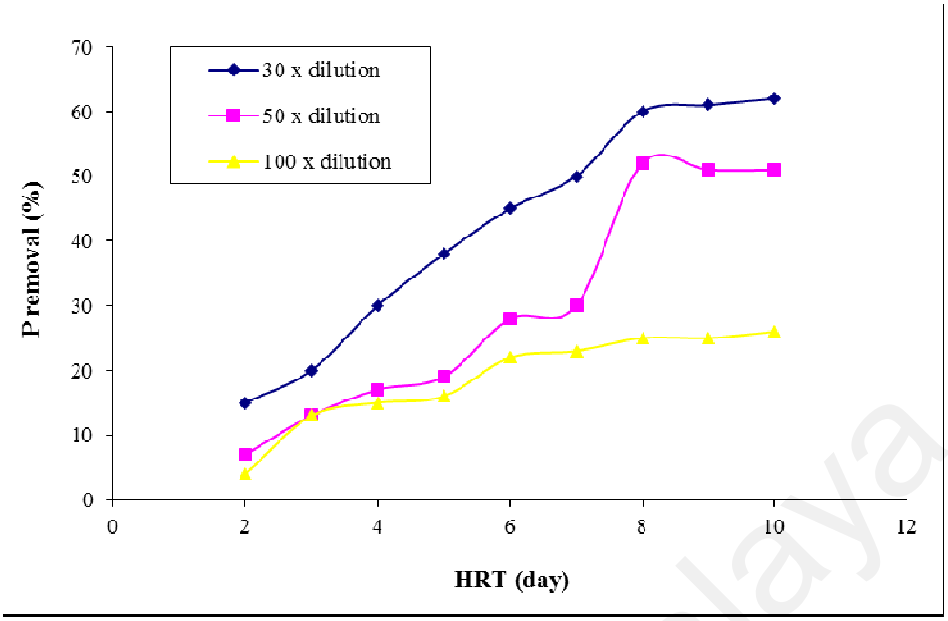


Figure 4.19: P removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters at nine different HRT operating conditions.

4.3.4 Biomass Yield

Figure 4.20 shows the percentage of biomass yield obtained in the effluent after five days of continuous system operation of the three different concentrations of fish processing wastewaters at nine different operating HRTs. In the figure, three different diluted concentrations of salt-free fish processing wastewaters increased with the HRTs. The biomass yield decreased with the increasing dilution fold of the original fish processing wastewater. In the continuous system, the lowest biomass yield percentage of 10.0% was found at the 2-day HRT in the 100-fold diluted wastewater, while the highest biomass yield percentage of 65.0% was found in the 30-fold diluted fish processing wastewater at the 8-day HRT.

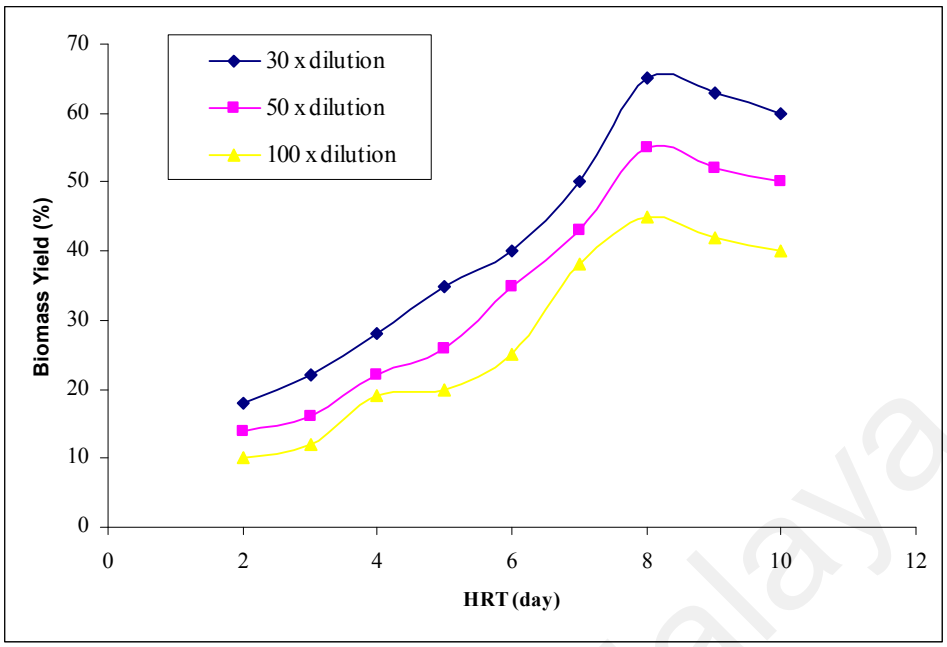


Figure 4.20: Biomass yield percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with nine different operating HRT conditions.

4.4 Continuous Operation at Different Substrate and Salt Concentrations

4.4.1 Solids Removal

(a) Total Solids (TS)

Figure 4.21 shows the TS removal in the continuous system at continuous system with various salt contents at the optimum HRT of 8-, 9-, and 10-day, respectively.

In continuous system operation with various salt contents at HRT of 8, 9 and 10 days, the highest percentage of TS removal, 32.0% was found in 50-fold diluted fish processing wastewater at HRT 9 day with 7% salt content. The lowest percentage removal of 4.0% was found in 30-fold diluted wastewater added with 1% salt content at 8 days of HRT.

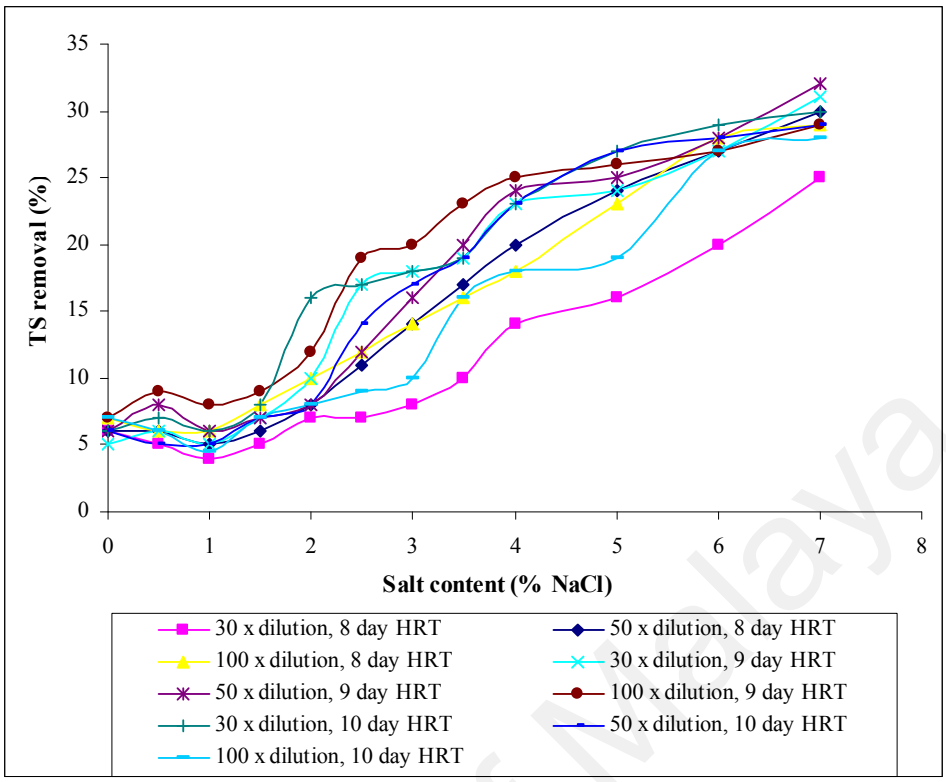


Figure 4.21: TS removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with twelve different salt concentrations at HRT of 8, 9 and 10 days.

(b) Total Suspended solids (TSS)

The TSS removal percentages in the continuous system at 12 different salt contents after five days of treatment operations are shown in Figure 4.22. The highest percentage of TSS removal was 37.0% and was achieved at the 8-day HRT in the 100-fold diluted fish processing wastewater that was added with 7% NaCl. The lowest percentage removal of 6.0% was found in the 30- and 50-fold diluted fish processing wastewaters with 1% salt concentration at the 8-day HRT and in the 30-fold diluted wastewater without salt concentration at the 8- and 9-day HRTs.

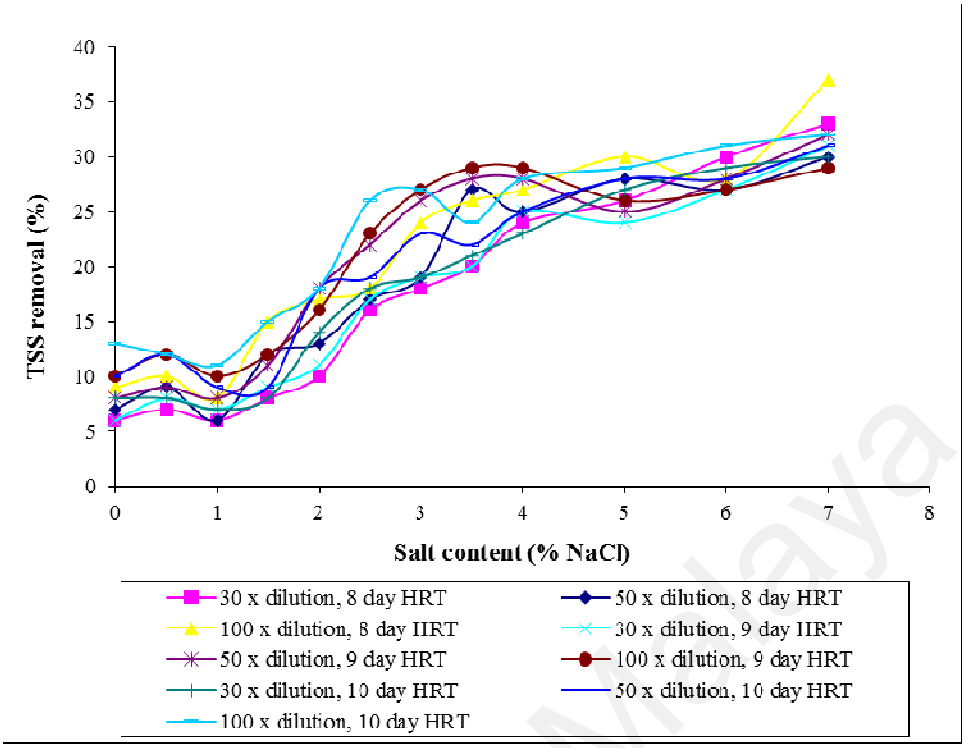


Figure 4.22: TSS removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with 12 different salt concentrations at the HRTs of 8, 9 and 10 days.

(c) Total Dissolved Solids (TDS)

Figure 4.23 shows the percentage removal after 5-day continuous system operation at 3 optimum operating HRT range from 10 to 8 days of HRT for the three different concentration of fish processing wastewater at twelve different NaCl concentrations.

The lowest percentage of TDS removal was 8.0% found at 9-day HRT in 30-fold diluted fish processing wastewater with 1% salt content. While the highest percentage of TDS removal, 35.0% was found at 8-day HRT in 100-fold diluted fish processing wastewater with 7% salt content.

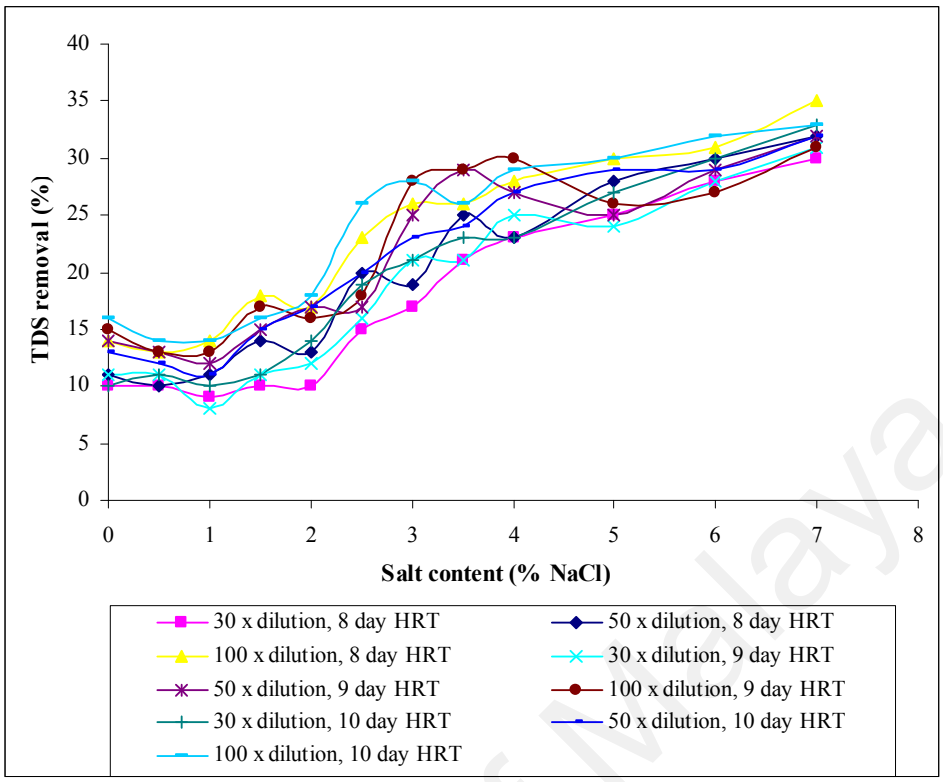


Figure 4.23: TDS removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with twelve different salt concentrations at HRTs of 8, 9 and 10 days.

(d) Volatile Suspended Solids (VSS)

Figure 4.24 shows the VSS increase percentage after five days of continuous system operation with three different operating HRTs (10-, 9-, and 8-day HRTs) for the three different concentrations of fish processing wastewaters at 12 different salt contents. In the continuous reactor with various salt concentrations, the lowest VSS increase percentage of 9.0% was found at the 9- and 10-day HRTs in the 100-fold diluted fish processing wastewater with 7% salt content, while the highest VSS increase percentage of 50.0% was found at the 8-day HRT in the 30-fold diluted fish processing wastewater with 1% salt contents.

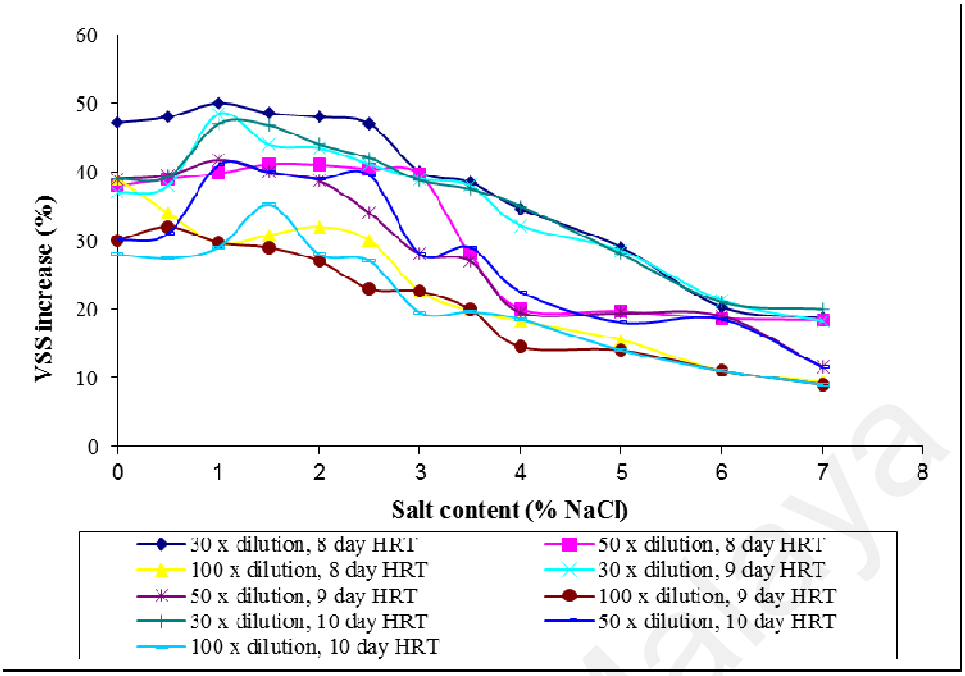


Figure 4.24: VSS increase percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with 12 different salt concentrations at the HRTs of 8, 9 and 10 days.

4.4.2 Organic Contents Removal

(a) 5-day Biochemical Oxygen Demand (BOD_5)

Figure 4.25 shows the BOD removal percentage after five days of continuous system operation with three different operating HRTs (10-, 9-, and 8-day HRTs) for the three different concentrations of fish processing wastewaters at 12 different salt contents. In the continuous treatment system, the average BOD removal rates of the HRTs 8, 9 and 10 days in the 30-, 50-, and 100-fold diluted fish processing wastewaters with 1% salt content were 80.3%, 65.0%, and 38.7%, respectively; at 7% salt content, the average BOD removal rates of the three operating HRT were down to 21.3%, 20.3%, and 15.3%, respectively. The lowest BOD removal of 14.0% was found at the 8-day HRT in the 100-fold diluted wastewater with 7% NaCl content. The highest BOD removal of 82.0% was achieved at the 10-day HRT in the 30-fold diluted wastewater

with 1% NaCl content. The high bacteria growth rate in the reactor was able to remove more biochemical oxygen demand in the reactor.

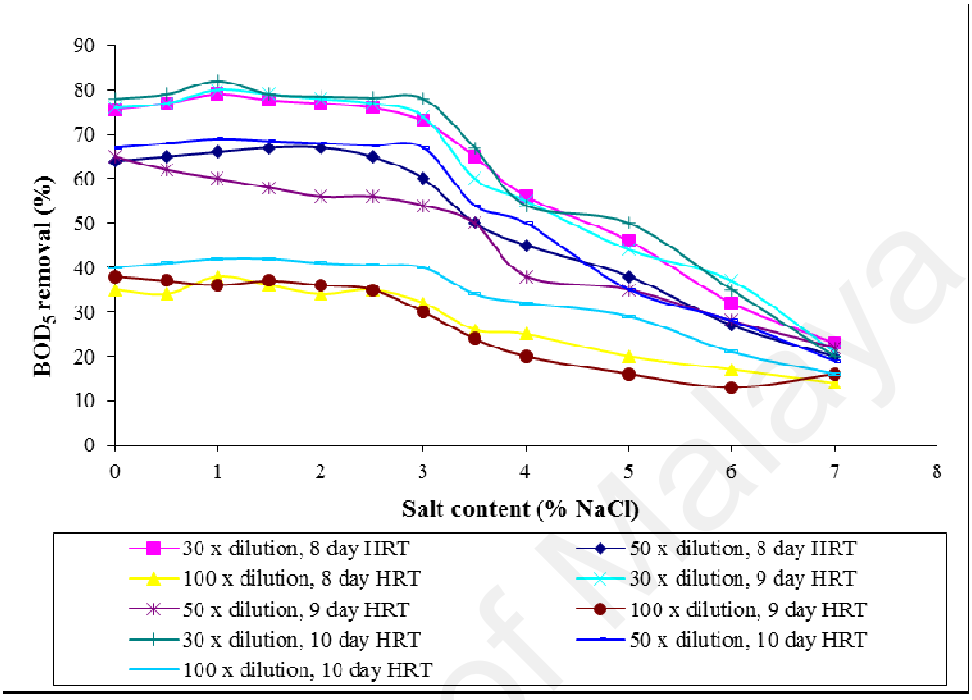


Figure 4.25: BOD_5 removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with 12 different salt concentrations at HRTs of 8, 9 and 10 days.

(c) Chemical Oxygen Demand (COD)

Figure 4.26 shows the COD removal percentage after five days of continuous system operation with three different operating HRTs (10-, 9-, and 8-day HRTs) for the three different concentrations of fish processing wastewaters at 12 different salt contents. In the continuous flow treatment system, the highest COD removal of 86.0% was observed at the 10-day HRT in the 30-fold diluted wastewater with 1% NaCl content. The lowest COD removal of 15.0% was found at the 9-day HRT in the 100-fold diluted wastewater with 7% salt content. The 10-day HRT achieved the highest COD removal due to the longer contact time between the biomass and the substrate

value. The COD removal rate increased with the increase in HRT. COD was the most affected parameter by the increase in salt concentrations in the fish processing wastewater. The average COD removal rates of the HRTs 8, 9, and 10 days for the 30-, 50-, and 100-fold diluted fish processing wastewaters dropped from 84.0% to 23.3%, 68.3% to 21.7%, and 38.7% to 16.0%, respectively, when the salinity increased from 1 to 7%. The low COD removal efficiency might be due to the adverse effect of salt on microbial activity. High salinity is generally known to cause plasmolysis and loss of cell activity (Campos *et al.,* 2002; Rene *et al*., 2008).

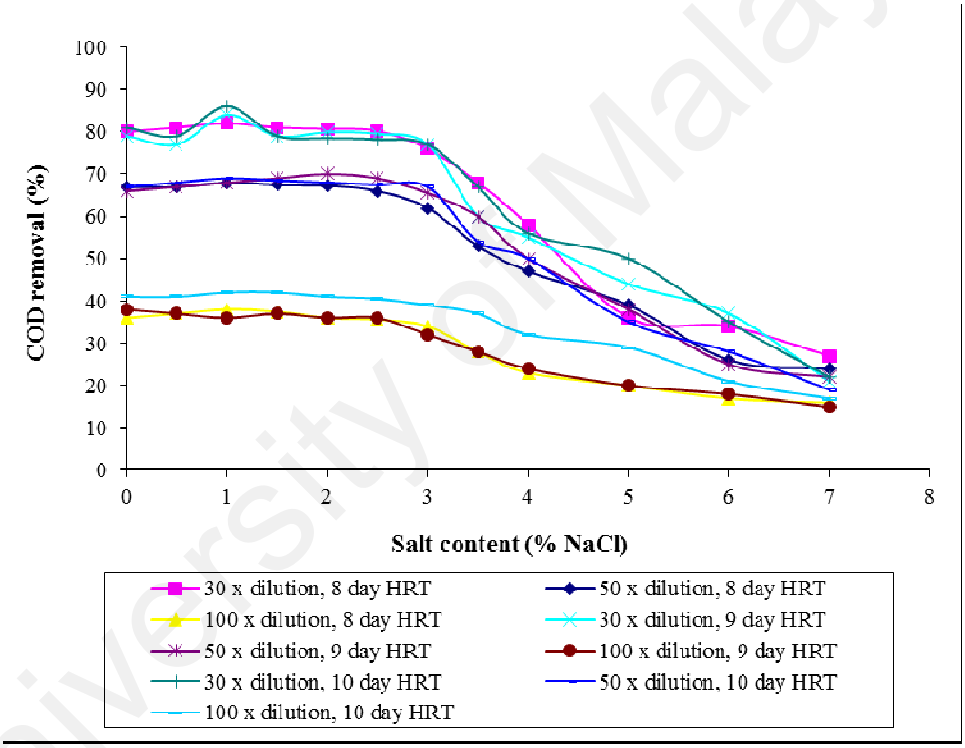


Figure 4.26: COD removal percentage in a continuous system for three different concentrations of diluted fish processing wastewater with 0-7.0% of salt concentrations at the HRTs of 8, 9 and 10 days.

4.4.3 Nutrients Removal

(a) Ammoniacal Nitrogen (NH_3-N)

Figure 4.27 shows the percentage of NH_3-N removal found in the effluent after five days of continuous system operation of the three different concentrations of fish processing wastewaters with 11 different salt concentrations and without salt content as the control at three different optimum HRT operating conditions. After five days of continuous flow operation, the highest percentage of NH_3-N removal of 73.0% was found at the 10-day HRT in the 30-fold diluted fish processing wastewater with 1% NaCl, while the lowest percentage of NH_3-N removal of 15.0% was found in the 100-fold diluted fish processing wastewater with 7% salt content at the 8-day HRT.

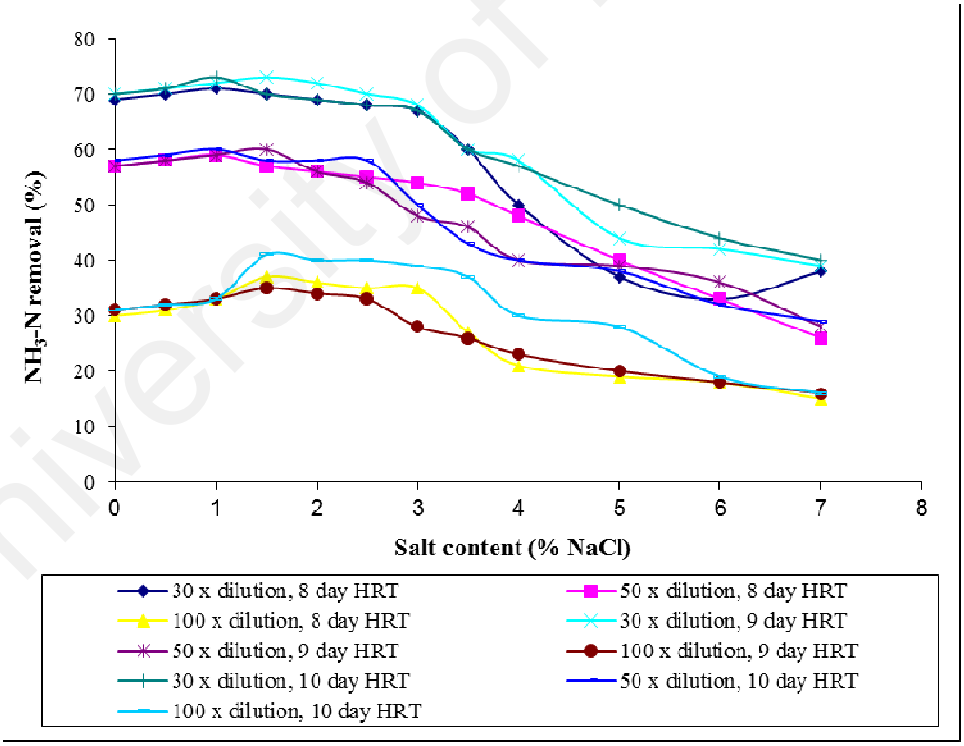


Figure 4.27: NH_3-N removal percentage in continuous treatment system for three different concentrations of diluted fish processing wastewaters with 12 different salt concentrations at the HRTs of 8, 9, and 10 days.

(b) Nitrate Nitrogen (NO_3^--N)

Figure 4.28 shows the percentage of NO_3^--N removal found in the effluent after five days of continuous flow operation of the three different concentrations of fish processing wastewaters with 11 different salt concentrations and without salt content as the control at three different optimum HRT operating conditions. After five days of continuous flow operation, the highest percentage of NO_3^--N removal of 72.0% was found at the 9-day HRT in the 30-fold diluted fish processing wastewater with 1% NaCl, while the lowest percentage of NO_3^--N removal of 12.0% was found in the 100-fold diluted fish processing wastewater with 7% salt contents at the 9-day HRT.

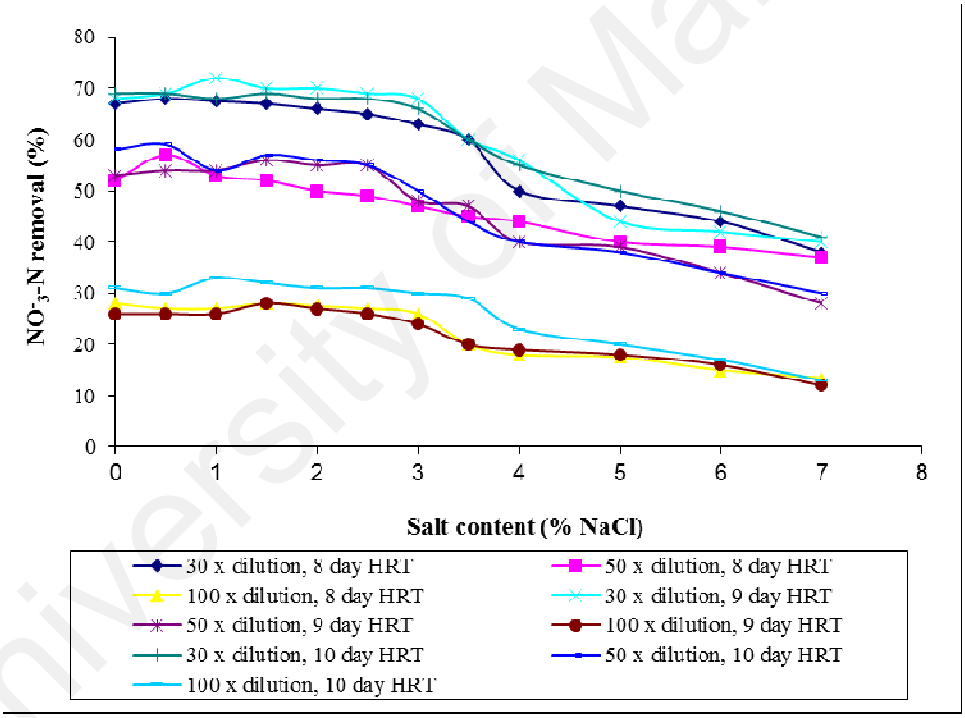


Figure 4.28: NO_3^--N removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with 12 different salt concentrations at HRTs of 8, 9 and 10 days.

(c) Phosphorus (P)

Figure 4.29 shows the percentage of P removal found in the effluent after five days of continuous operation of the three different concentrations of fish processing

wastewaters with 11 different salt concentrations and without salt content as the control at three different optimum HRT operating conditions. The results demonstrated that phosphate removal was severely affected by the increase in salt concentrations from 3.0%, particularly in the 30- and 50-fold diluted fish processing wastewater. When the salt content increased from 2.5% to 7.0%,the highest drops of P removal efficiency rates was found in the 30-fold diluted fish processing wastewater, with 45.0% at 8-day HRT, 43.0% at 9-day HRT and 42.0% at 10-day HRT. The second highest drops of P removal rates was found in the 50-fold diluted wastewater with removal rates of 43.0% at 8-day HRT, 40.0% at 9 day-HRT and 40.0% at 10-day HRT. While the lowest removal rates of P was observed in the 100-fold diluted fish processing wastewater with 21.0% at 8-day HRT, 19.0% at 9-day HRT and 21.0% at 10-day HRT.

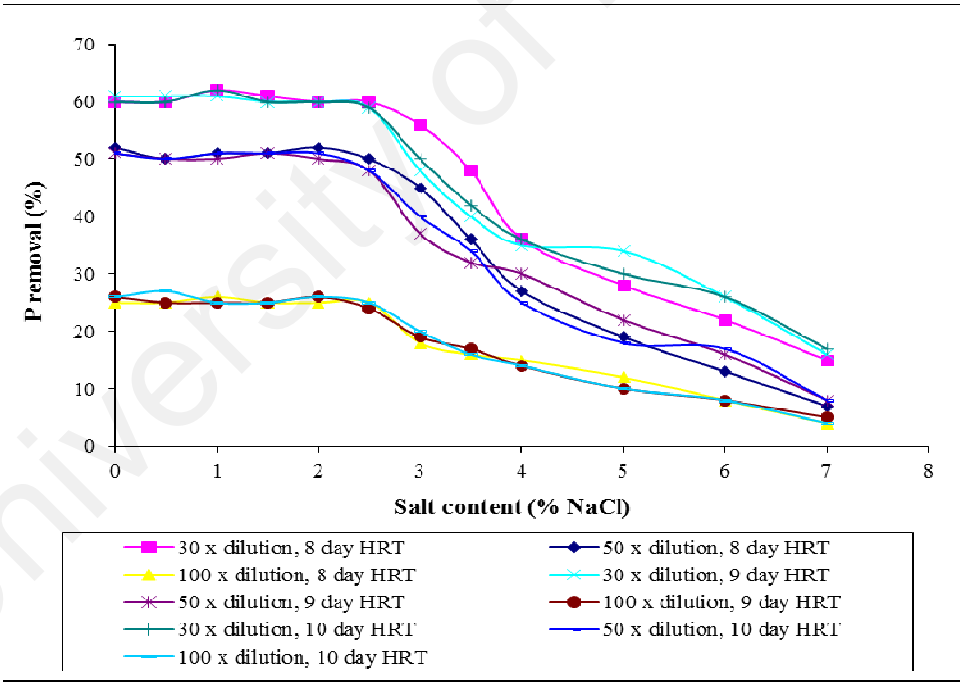


Figure 4.29: P removal percentage in a continuous system treatment for three different concentrations of diluted fish processing wastewaters with 12 different salt concentrations at the HRTs of 8, 9 and 10 days.

After five days of continuous operation, the highest percentage of P removal of 62.0% was found at the 8-day HRT in the 30-fold diluted fish processing wastewater

with 1% NaCl, while the lowest percentage of P removal of 4.0% was found in the 100-fold diluted fish processing wastewater with 7% salt contents at the 8- and 10-day HRTs.

4.4.4 Biomass Yield

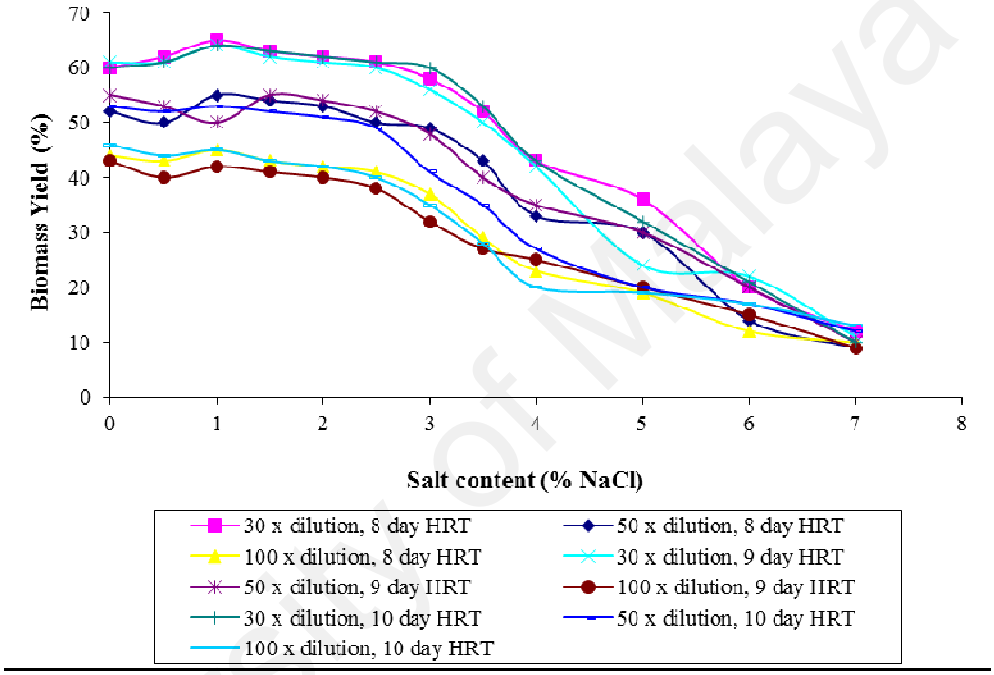


Figure 4.30: Biomass yield removal percentage in a continuous treatment system for three different concentrations of diluted fish processing wastewaters with 12 different salt concentrations at the HRTs of 8, 9 and 10 days.

Figure 4.30 shows the biomass yield percentage in the effluent after five days of continuous system operation of the three different concentrations of fish processing wastewaters at three different operating HRTs of 10, 9, and 8 days with 12 different salt concentrations. In the continuous reactor with various salt concentrations, the lowest biomass yield percentage of 9% was found at the 8-day HRT in the 50-fold diluted wastewater and at the 9-day HRT in the 100-fold diluted wastewater with 7% salt content. The highest biomass yield percentage of 65.0% was found at 1% salt content in

the 30-fold diluted fish processing wastewater at the 8-day HRT. As the salt content in the 30-, 50-, and 100-fold diluted fish processing wastewaters increased to 7%, the average biomass growth rates of HRTs 8, 9 and 10 days were decreased to 11.0%, 10.3%, and 10.7%, respectively.

CHAPTER 5

DISCUSSION

5.1 Introduction

The treatment system performances in this research project were studied by evaluating the data of the influent feed into the reactor and the daily effluent from day one to day five treatment on the temperature and pH value, biomass accumulated, solids removal, organic degradation of BOD and COD, and nutrients removal of NH_3-N, NO_3^--N and P for analyzing the biomass yield and the efficiency of microorganisms in treating fish processing wastewater in batch operation with the variables of different substrate concentrations (30-, 50-, and 100-fold diluted fish processing wastewater) and different salt concentrations (0.0% - 7.0%), and in continuous operation with the variables of different substrate concentrations, different HRT (ten to 2 days), and different salt concentrations. Batch operation was performed in three different concentration of fish processing wastewater at 12 different salt concentrations (0.0%, 0.05%, 0.10%, 0.15%, 0.20%, 0.25%, 0.30%, 0.35%, 0.40%, 0.50%, 0.60%, 0.70% NaCl *w/v* NaCl), while continuous operation was operated in three different concentration of fish processing wastewater without salt content at nine different HRT (ten to two days) in the first phase of the experiments, and the second phase of experiments was performed in three different concentration of fish processing wastewater added with 11 different salt concentrations varying from 0.05% to 0.70% at the optimal HRT obtained from 8-day, 9-day, and 10-day HRTs. On the basis of the results of the laboratory investigation, the occurrence and effectiveness of the natural aerobic biodegradation processes were confirmed.

5.2 pH Observation during Batch and Continuous Operation

Generally, wastewater microorganisms in biological treatment systems function best in pH values from 6.5 to 7.5 for optimal biological growth although growth will occur over the range of pH 4.0 to 9.5 (Metcalf & Eddy, 1991). In this study, the pH of the fish processing wastewater in reactor of the batch and continuous operation increasing along the treatment processes, at the range of pH 7.33 to pH 8.86.

In batch operation, the highest increase percentage of pH was 19.3% found in the effluent of 30-fold diluted fish processing wastewater with 1.0% NaCl after five days operation. While the lowest increase percentage of pH value of 9.6% was found in the 100-fold diluted fish processing effluent with 7.0% salt content.

In continuous flow operation at nine different HRT, the highest pH increase percentage of 19.5% was observed at 8-day HRT in the effluent of 30-fold diluted fish processing wastewater. The lowest increase percentage of 10.0% was found at the 2-day HRT in the 100-fold diluted fish processing wastewater.

In continuous flow operation at 11 different salt concentrations, the pH value increased along the 5-day treatment operation. The highest pH percentage increase of 19.6% was observed at the 8-day HRT in the effluent of 30-fold diluted fish processing wastewater with 1.0% salt content. The lowest percentage increase of 8.0% was found at the 8-day HRT in the 100-fold diluted fish processing wastewater with 7.0% salt content. The increase of pH value indicated that the wastewater has high biomass growth rate of the naturally occurring microorganisms (Tay *et al*., 2006; Lefebvre & Moletta, 2006; Zhu *et al.,* 2008; Bassin *et al*., 2012; Sherly *et al*., 2015).

5.3 Effects of Substrate and Salt Concentrations on Batch Operation Efficiency

After 5 day aerobic batch treatment system with natural biodegradation, the three different diluted concentration of salt free fish processing wastewater were found to have higher percentage of biomass growth and substrate removal compared to the wastewater added with salt content range from 2.5 to 7% in batch system. However, the wastewater added until 2.0% salt concentration was found to have a positive to no effect on the biomass growth and substrates removal rates. Inhibition process was found in the wastewater added with salt concentrations of 2.5 to 7% NaCl. The biomass growth and the substrate removal of BOD, COD, NH_3-N, NO_3^--N and P show significant reduction rate from 3 to 7% salt concentrations. It has been found that the addition of salt, in the form of NaCl, increases the respiration rate of microorganisms until a specific salt concentration is reached, thereafter a decrease is observed (Hamoda & Al-Attar, 1995).

5.4 Effects of HRT and Substrate Concentrations on Continuous Operation Efficiency

In this study, the effect of nine different hydraulic retention times (HRT) on biomass growth and pollutants removal in the fish processing wastewater were studied. The experiments' results showed that the pH value, biomass growth, biomass yield, TSS concentration, and pollutant removal rates of BOD, COD, NH3-N, NO_3^--N and P in the reactor were decreased with lower concentration of fish processing wastewater influent, and showed significant linear decreases in HRT from 7-day HRT to 2-day HRT. The optimal pH value, biomass growth, biomass yield, TSS concentration, and pollutant removal rates condition were found at HRTs of 8-day, 9-day, and 10-day HRT. The optimum pH value, TSS concentration, biomass growth, and biomass yield were found at 8-day HRT in 30-fold diluted wastewater, and the lowest were verified in the 100-fold diluted wastewater at 2-day HRT. While the optimum removal percentages of

pollutants were found at 10-day HRT in 30-fold diluted wastewater, and the lowest were observed at 2-day HRT in 100-fold diluted wastewater. According to Zhu *et al*. (2008), the increase in HRT increases the wastewater contact time in the reactor and improves the removal efficiency of organic matter by promoting the growth of aerobic microorganisms.

5.5 Effects of Salt and Substrate Concentrations on Continuous Operation Efficiency

After five days of aerobic continuous flow treatment system operation with natural biodegradation at the optimal HRT of 8-day, 9-day, and 10-day HRT, the results obtained for this study have shown that the three different concentrations (30-, 50-, and 100-fold diluted) of salt-free fish processing wastewater had higher percentages of pH value, biomass growth, biomass yield, TSS concentration, and substrate removal of BOD_5, COD, NH_3-N, NO_3^--N, and P than the wastewater with 3.0% to 7.0% salt content. However, the wastewater with ≤ 2.5% salt concentration had a positive to no effect on the pH value, biomass growth, biomass yield, TSS concentration and the substrate removal rates. Inhibition was observed in the wastewater added with salt concentrations of 3.0% to 7.0%.

The pH value, biomass growth, biomass yield, TSS concentration, and the substrate removal showed significant linear reduction rates from 3.0% to 7.0% salt concentrations and decreased with the increasing influent's dilution fold of the original fish processing wastewater. The lowest substrates removal efficiencies, pH value, biomass growth, biomass yield, and TSS concentration were found in the greatest dilution of 100-fold diluted wastewater containing 7.0% NaCl, while the highest percentage was verified in the 30-fold diluted wastewater containing 1.0% NaCl. The reduced efficiencies with salt additions could have been caused by the adverse effect of salt on microbial flora and the

plasmolysis of organisms resulting in the loss of metabolic activity. The addition of salt increased the respiration rate of microorganisms until it reached a specific salt concentration; thereafter, a decrease was observed (Vallero *et al*. 2003).

5.6 Effects of Salt Concentrations on the Solids, Organic and Nutrients Removal in Batch and Continuous Operation

The TSS concentration decreased when the salt concentration increased from 1.0% to 7.0% NaCl. The decrease in TSS concentration indicates the death and washout of biomass. Elevated salt concentrations are known to increase water density, which can potentially cause the washout of small and poor settling sludge flocs. According to Woolard and Irvine (1995b), biological systems subjected to salt shock loads respond with increased effluent suspended solids and loss of volatile suspended solids, which are similar to those observed in this work. Panswad and Anan (1993b) also observed a decrease in TSS with the salt concentration increase. These authors observed a TSS reduction of 60.0% with the increase in salinity from 0 g/L to 30g/L.

Among the ten analyzed parameters in this study, COD was the most affected parameter with the increase in salt concentrations in the fish processing wastewater, followed by BOD. The results show that the batch system can be effectively used to treat high salinity fish processing wastewater to 586 conform to effluent standards of COD lower than 80 mg/L and BOD lower than 20 mg/L. The results show that phosphorus-accumulating bacteria are more sensitive to the high salinity than nitrifiers and denitrifiers. The foul wastewater odor was completely removed, and the strong reddish-brown color of the wastewater was reduced after five days of treatment.

When salt concentration was increase from 3.0 to 7%, there was a significant decrease in NH_3-N removal efficiency. This result demonstrates the effect of high salt

concentration on nitrification. This finding is accordance with the findings of previous study (Münch *et al*., 1996; Chen *et al*., 2003; Lyssenko & Weaton, 2006; Moussa *et al*., 2006; Sharrer *et al*., 2007; Yogalakshmi & Josep, 2010; Bassin *et al*., 2012). Yogalakshmi and Joseph (2010) have reported lower removal of ammonia at high salt concentration. They found that the ammonia removal rate was 64.0% to 84.0% at the NaCl loading of 5–30 g/L. The decrease in nitrification efficiency can be related to the more sensitive short- and long-term salt stresses of nitrite and ammonia oxidizers, to the repression of the synthesis of enzymes that utilize nitrate and nitrite as electron acceptor, and to the significant changes in osmotic pressure. Decrease in removal of NH_4-N at high salt concentration could be due to the effect of plasmolysis or lower availability of saline-resistant nitrifiers (Chen *et al*., 2003). The decrease in nitrification efficiency can be related to the more sensitive short- and long-term salt stresses of nitrite and ammonia oxidizers (Moussa *et al*., 2006; Rene *et al*., 2008), to the repression of the synthesis of enzymes that utilize nitrate and nitrite as electron acceptor (Munch et al., 1996), and to the significant changes in osmotic pressure (Zhao *et al*., 2013).

The NO_3^--N removal efficiency generally decreased with the gradual increase in salt concentration from 3% to 7% NaCl. This means that the gradual increment of salt concentration above 3% NaCl showed inhibitory effect on NO_3^--N removal efficiency in continuous flow reactor. Chen *et al*. (2003) reported that nitrification was good up to Cl concentration of 2.5 g/L and beyond that the nitrification rate started to decrease. The decrease in NO_3^--N removal efficiency with the increase in salt concentration was due to the washed out dead biomass and lysed cell constituents (Lefebvre & Moletta, 2006), the nitrification inhibition (Lefebvre and Moletta, 2006; Rene *et al*., 2008; Zhu *et al*., 2008), and the denitrification process (Zhao *et al*., 2013).

The high salt concentration was clearly seen as having an adverse impact on P removal. The effect of salt on phosphorus removal was also reported by Uygur and Kargi (2004), who described a decrease in P removal from 84% to 22% when the NaCl concentration increased from 0% to 6% (w/v).

5.7 Summary

In summary, the results from this study reveal that the fish processing wastewater with 0.5–7.0% NaCl and without salt content in a well-stirred aerobic continuous flow reactor system of batch and continuous operation could be biodegraded by the natural microorganisms in the wastewater without seeded with sludge (a well-grown sediment sample) taken from the fish or food processing effluent for process startup. Pollutants in the fish processing wastewater could be biodegraded by natural microbial populations in the wastewater under an aerobic condition with the supplement of sufficient oxygen using an aquarium air pump.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The fish processing sector contributes serious organic pollution loads and high salinity to receiving waters. This project study the effect of various salt concentrations in three different concentration of fish processing wastewater (30-, 50-, and 100-fold diluted wastewater concentration) in batch, and in continuous flow system at the optimal HRT obtained. The performance of the systems were studied based on the efficiency of pollutants removal and the biomass yield in the wastewater containing 0.0% to 7.0% w/v NaCl with the aid of the natural microorganisms in the wastewater and the microorganisms developed inside the reactors. A better understanding of the potential inhibitory effect of salt content on biological treatment processes is necessary to consider the feasibility of fish processing saline wastewater disposal in the treatment systems.

In summary, the experimental results showed that the biomass accumulated and the removal of 5-day BOD (BOD_5), COD, NH_3-N, NO_3^--N, and P substrates in both of the batch and continuous flow system were increased with higher concentration of influent feed into the bioreactor. The evaluation of system performance at nine different operating hydraulic retention times (HRT) that range from 2–10 days indicated that the substrate removal rates increase with the increase in HRT along with the increasing wastewater concentration. The optimum biomass yield was found at the 8-day HRT, and the highest substrate removal was obtained at 10-day HRT.

The analyzed results have shown that the highest percentage for biomass growth and substrate removal are verified in the wastewater contained 1% NaCl in batch and continuous systems. Experimental results showed that the biomass growth and substrate removal rates in batch system were not affected up to 2.0% salt concentration, while the continuous system results show the existence of inhibition effects at salt concentration from 3.0%. This means that the biomass is able to reduce the organic load on the biological batch system with a salt concentration as high as 2.0% w/v (20 g/L), and beyond that started to decrease; while continuous flow system can successfully treat the wastewater with a salt concentration as high as 2.5% (25 g/L) without inhibition by the salt.

It was also found that the stinky odour of the wastewater was completely removed, and the strong reddish-brown colour of the wastewater was reduced after 5 days treatment. The treated wastewater without offensive odor and remained with the nitrogen, protein and phosphorus content is feasible for reutilization as an environmental friendly liquid fertilizer.

6.2 Recommendations

As future works, the following ideas are proposed:

Results from this study could potentially increase effluent handling and its reuse. Wastewater from fish processing industries has high potential to be reused as a liquid fertilizer in agriculture since the main constituents are organic substance. Hence, the potential of using fish processing treated effluent as an organic fertilizer to enhance growth of plants need to be further confirmed through further analysis. Further studies should involve the investigation for the impacts on the soil, the plants and the different yields of the crops when irrigated with the treated fish processing saline wastewater of different salt content.

It is also believed that the the main constituents in fish processing wastewater are organic substance and do not contain known toxic or carcinogenic materials. However, there is still a series of characterization tests are required in future works to determine the substances are free from any toxic or carcinogenic elements. The analysis works in this direction is a must to confirm the potential use of fish processing treated effluent as an organic fertilizer to enhance growth of plants.

Research work also needs to be carried out to determine the potential of the effluent to serve as nutrient source to the plant and develop the cells of the plants. It is believed that using effluent as a feed source to the plant can reduce the demand of water resource in agriculture. It is targeted that the effluents can substitute the fertilizer usage upon plant. The success of effluent to serves as nutrient source can help the farmer to reduce the cost of synthetic fertilizer periodically and less pollution will take place on land and in stream due to short term usage of synthetic fertilizer.

Closing the loop for nutrients in wastewater is a necessary sustainable development objective to reduce resource consumption and greenhouse gas emissions. A new paradigm is emerging, globally. Commercial marketing of recovered nutrients as "green" fertilisers, and recycling of nutrients through biomass production to new outlets such as biofuels, are taking off.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

ISI Paper publication:

Ching, Y. C. & Redzwan, G. (2017). Biological Treatment of Fish Processing Saline Wastewater for Reuse as Liquid Fertilizer. *Sustainability*, 9(7), 1062.

Paper presented at conference:

Title: Biomass Growth and Substrate Removal in Fish Processing Saline Wastewater by Aerobic Biodegradation

Ching Yun Chen and Ghufran Redzwan

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Ching Y. C. & Redzwan, G. (2017). Biomass Growth and Substrate Removal in Fish Processing Saline Wastewater by Aerobic Biodegradation. In M. A. Ashraf & W. S. Aqma (Eds.), *Environmental Conservation, Clean Water, Air & Soil (CleanWAS) International Conference Proceedings, China.* (pp.102-105). China: IWA publishing.

APPENDIX A

Fish Processing Wastewater Treatment Operation in Lab

Appendix A-1 Operation of fish processing wastewater treatment in two identical laboratory scale reactor.

Appendix A-2 The reactor was operated continuously with agitation speed set at 250 rpm and have supplied with air aeration using aquarium air pump. Temperature of the reactor was set at 30^{o}C for operation.

Appendix A-3 Aerobic biodegradation of fish processing wastewater were examined in a 2 liter reactor with 1800 mL working volume.
(wastewater treatment in day-1 operation).

Appendix A-4 Measure pH value of treated fish processing effluent using Mettler Toledo Delta 320 pH meter.

APPENDIX B

Analysis Methods for Wastewater Parameter of pH, TS, TSS, TDS, VSS, BOD_5, and COD

Appendix B-1 : pH Meter Calibration and Measurement Procedure:

1. Take out the pH ATC electrode probe from the 3 mol/L KCL (Potassium Chloride) storage solution.

2. Rinse pH electrode with distilled water.

3. Start a two-point calibration by put the electrode in pH 7 buffer solution, press **Cal**, the display shows **Cal 1, 7.00 pH, 25.0^{o}C, ATC.**

4. Rinse electrode with distilled water; put the electrode in pH 4 buffer solution, press **Cal**, the display shows **Cal 2, 4.01 pH, 98 % slope, ATC**, press **Read, 4.01pH, 25.0^{o}C, ATC**.

5. Rinse pH electrode with distilled water.

6. Measure distilled water sample, record the pH value and temperature.

7. Measure wastewater sample, record the pH value and temperature.

Appendix B-2 : TS Measurement Procedure

1. Clean evaporating (porcelain) dish with distilled water and heat in oven at 105^{o}C for 1 hour.

2. Take out the porcelain dish from oven, store in desiccators and weigh immediately before use using analytical balance.

3. Measure accurately a volume of well mixed sample using cylinder.

4. Pour the measured sample into the pre-weighed evaporating porcelain dish.

5. Put in a preheated drying oven set at 105^{o}C, evaporate to dryness overnight one or two days.

6. Take the dish out of the oven and cool in a desiccators.

7. Weigh the dish with dried residue on an analytical balance (4 decimal points).

8. Calculate the Total Solids using Equation (3.2):

$$TS\ (mg/L) = \frac{\{\text{Weight of the dish + dried residue (mg)}\} - \text{Weight of the dish (mg)}}{\text{Sample Volume (mL)}} \times 1000 \qquad (3.2)$$

Weight of the residue in mg (to convert W (g) to (mg), multiply w (g) with 1000)

Multiply the weight of the dry solids (in mg) by 1,000 mL/L to convert the sample size from mL to L.

Appendix B-3: TSS Measurement Procedure

1. Wash GFC filter paper in filter holder under suction with successive small volumes of laboratory water.

2. Remove filter paper from assembly, place in a petri dish that have cover with aluminium foil and dry in oven at 105^{o}C for 1 hour. Cool in desiccators and weigh.

3. Put the dried filter paper in the filtration assembly, and pour the prepared well-mixed sample (100 - 500 mL) into the filter holder on vacuum pump for filter the sample.

4. Remove the filter; put the filter paper with nonfilterable residue in a petri dish that has covered with aluminium foil. Dry in oven at 105^{o}C for 1 hour or overnight. Cool in desiccator and weigh until constant weight is obtained (+/- 0.5 mg).

5. Calculate the TSS using Equation (3.3) as follow:

$$TSS\ (mg/L) = (A - B)/C \times 1000 \qquad (3.3)$$

Where A = Mass of nonfilterable residue on Whatman GF/C filter after evaporation at 105^{o}C (mg)

B = Mass of the filter paper prior to sample filtration (mg)

C = volume of the sample used for filtration (mL)

Appendix B-4: TDS Measurement Procedure

1. Dry the GFC filter paper and a porcelain dish that will be using for filtration in an oven at temperature of 180^{o}C for 1 hour.

2. Cool down the filter paper and the porcelain dish in a desiccators after take out from oven.

3. Weigh the tare mass of the filter paper and the porcelain.

4. Filter the wastewater sample (100 – 500 mL) by using the weighed filter paper and vaccum filtration set on vacuum pump for filter the sample.

5. After the filtration process is completed, take the vacuum filter flask with filtrate (filterable) and pour it into the pre-weighed evaporating porcelain dish and dry at 180^{o}C in oven for at least 1 hour or overnight.

6. Take the porcelain dish with filtrate from oven and cool down in desiccators.

7. Weigh the mass of porcelain with filtrate by using electronic weighing balance after take out from desiccators.

8. Calculate the TDS using Equation (3.4):

$$\text{TDS (mg/L)} = (A - B)/C \times 1000 \qquad (3.4)$$

Where A = Mass of dried porcelain plus filtrate after evaporation at 180^{o}C (mg)

B = Weight of the porcelain dish (mg)

C = volume of the sample used for filtration (mL)

Appendix B-5: VSS Measurement Procedure

1. Dry the filter paper and a beaker that will be using for filtration in an oven at temperature 105^{o}C overnight.

2. Cool down the filter paper and the beaker in desiccators after take out from oven.

3. Weigh the tare mass of the filter paper.

4. Filter wastewater sample (according to the sample size required, 100 – 500 mL) by using the weighed filter paper, vaccum filtration set, a vacuum pump, and the beaker.

5. After the filtration process is completed, carefully take out the filter paper that have nonfilterable residue on it from vacuum pump.

6. Dry the filter paper with nonfilterable residue in an oven at temperature of 105^{o}C overnight.

7. After overnight, take the filter paper with nonfilterable residue from the oven and cool down in a desiccators.

8. Weigh the mass of filter paper plus nonfilterable residue by using electronic weighing balance after take out from desiccators.

9. Have the muffle furnace up to temperature before inserting the sample (usually, 15 to 20 min ignition are required).

10. Ignite the filter paper with nonfilterable residue produced to constants weight in a muffle furnace at temperature of 550 +/- 50^{o}C for at least one hour.

11. Allow the filter paper with nonfilterable residue to cool partially in air until most of the heat has been dissipated and transfer to desiccators for final cooling in a dry atmosphere.

12. Weigh the filter paper with nonfilterable residue as soon as it has cooled completely.

13. Report the loss of weight on ignition as total volatile suspended residue and the weighed residue as total fixed residue.

14. Calculate the VSS using Equation 3.5 as below:

$$VSS\ (mg/L) = (A - B)/C \times 1000 \qquad (3.5)$$

Where A = Mass of nonfilterable residue on Whatman GF/C filter after evaporation at 550^{o}C (mg)

B = Mass of nonfilterable residue on Whatman GF/C filter after ignition at 150^{o}C (mg)

C = Sample size that have used (mL) for filtration.

Appendix B-6: Operation of Hach BOD Trak™ Apparatus, 115/230 Vac, 50/60 Hz.

A measured sample of wastewater is placed in one of the amber bottles on the apparatus and the bottle is connected to the instrument. Above the water sample is a quantity of air, which contains 21 percent oxygen. Over a period of time, bacteria in the wastewater consume dissolved oxygen to oxidize organic matter present in the sample. The air in the closed sample bottle replenishes the used oxygen, resulting in a drop in air pressure in the sample bottle. The BOD Trak Apparatus measures the drop in pressure and displays results directly as mg/L BOD. During the test period (usually five days) the sample is continually agitated by a magnetic stirring bar. Carbon dioxide is produced by the oxidation of organic matter and must be removed from the system so that the pressure difference observed is proportional only to the amount of oxygen used. This is accomplished by the addition of a few crystals of lithium hydroxide in the seal cup of each sample bottle. The electromagnetic stirring mechanism provides adequate agitation to effectively maintain rapid transfer of oxygen from the liquid sample to the air above. The BOD Trak Apparatus is free of leaks and has an effective carbon dioxide absorption system. The instrument also has accurate pressure sensors for reading pressure changes. The BOD Trak Apparatus is a practical, convenient and economical answer to BOD testing.

Appendix B-7: COD Measurement Procedure

i. For blank – pipette 2 mL distilled water into a Hach COD Digestion Reagent Vial (High Range or High Range Plus). Prepare 3 sample tubes, each fill with 2 mL sample for triplicate study.

ii. For standard solution – pipette 2 mL 500 mg/L COD standard solution into a Hach COD Digestion Reagent Vial (High Range), or pipette 0.2 mL 10,000 mg/L COD standard solution into a Hach COD Digestion Reagent Vial (High Range Plus). Prepare 3 sample tubes, each fill with 2 mL sample for triplicate study.

Preparation of COD standard solution, for control and accuracy check

1. Prepare 500 mg/L standard solution for check the accuracy of the 0 to 1500 mg/L range. Prepare a 500 mg/L Phthalic acid solution by dissolving 0.425 g of dried (120^{o}C, overnight) potassium acid phthalate (KHP) in some distilled water, pour the dissolved KHP solution into a 1000 mL volumetric flask, add distilled water into the volumetric flask until the solution level. Close the cap of volumetric flask and shake it evenly. Ready for use.

2. Prepare 10,000 mg/L standard solution for check the accuracy of the 0 to 15000 mg/L range. Prepare a 10,000 mg/L Phthalic acid solution by dissolving 8.500 g of dried (120^{o}C, overnight) potassium acid phthalate (KHP) in some distilled water, pour the dissolved KHP solution into a 1000 mL volumetric flask, add distilled water into the volumetric flask until the solution level. Close the cap of volumetric flask and shake it evenly. Ready for use.

3. For sample measurement – pipette 2 mL wastewater sample (diluted wastewater sample) into a Hach COD Digestion Reagent Vial (High Range), or 0.2 mL wastewater sample (diluted wastewater sample) into a Hach COD Digestion Reagent Vial (High Range Plus). Prepare 3 sample tubes, each fill with 2 mL sample for triplicate study.

4. Closed the prepared sample digestion reagent vial with the vial screw cap tightly. Mix the contents in the vial by shaking and invert the vial several times.

5. Placed the prepared vial into the reactor block, set at 150^{o}C, refluxed for 2 hours.

6. Cooled to room temperature. (if titration will not be done immediately, keep the digested sample vial in dark condition).

7. Add 1 or 2 drops ferroin indicator solution (add the same drops for all the sample tube) into the digested solution, pour the solution into a shake flask, rinse the sample tube with distilled water, and add the rinsed distilled water into the shake flask.

8. Titrated with Standard Ferrous Ammonium Sulphate (FAS) titrant, 0.01 M.

Prepared by dissolve 3.92 g FAS (MW = 392.13) in distilled water, add 20 mL concentrated H_2SO_4 (Merck Sulfuric Acid 95 - 97%, M = 98.08 g/mol), made up to 1000 mL with distilled water.

9. The end point for titration is a sharp colour change from blue-green to reddish brown.

Calculation using Equation (3.9):

$$\text{mg/L COD} = \frac{(A - B) \times M \times 8000 \times \text{Dilution factor}}{\text{mL sample}} \quad (3.9)$$

where: A = volume of FAS titrant used for blank, mL
B = volume of FAS titrant used for sample, mL
M = Molarity of FAS solution; **0.01 M** was used
8000 = milliequivalent weight of oxygen × 1000 ml/L.

APPENDIX C

Analysis Methods for Wastewater Parameter of BOD_5, NH_3-N, NO_3^--N, PO_4^{3-}

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 1 of 16)

Source: HACH. (1999). *Wastewater and Biosolids Analysis Manual: Digestion and Selected Methods for Determining Metals, Minerals, and Other Related Parameters* (1st ed.). U.S.A.: Hach Company.

Method 8043

OXYGEN DEMAND, BIOCHEMICAL

Dilution Method* (USEPA Accepted)

1. Prepare sample dilution water using a BOD Nutrient Buffer Pillow; see *Dilution Water Preparation* following this procedure.

2. Determine the range of sample volumes required for your sample; see *Choosing Sample Size* following this procedure.

Note: *If the minimum sample volume is 3 mL or more, determine the dissolved oxygen in the undiluted sample. This can be omitted when analyzing sewage and settled effluents known to have a dissolved oxygen content near 0 mg/L.*

3. With a serological pipet, measure a graduated series of at least four, but preferably five or six, portions of well mixed sample and transfer to separate 300-mL glass-stoppered BOD bottles. Stir the sample with the pipet before pipetting each portion.

Note: *See Interferences following this procedure when analyzing chlorinated or industrial effluents.*

Note: *Do not add sample to one BOD bottle. This will be the dilution water blank. For additional proof of accuracy, see Accuracy Check following these steps.*

4. Add two shots of Nitrification Inhibitor (approx. 0.16 g) to each bottle, if desired.

Note: *Use of Nitrification Inhibitor will inhibit the oxidation of nitrogen compounds if only carbonaceous oxygen demand is desired. It is especially recommended for samples with low BODs.*

* Adapted from *Standard Methods for the Examination of Water and Wastewater* and from Klein, R.L., Gibbs, C., *Journal Water Pollution Control Federation*, 51 (9), 2257 (1979).

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 2 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

5. Fill each bottle to just below the lip with seeded or unseeded dilution water. When adding the water, allow it to flow slowly down the sides of the bottle to prevent formation of bubbles.

6. Stopper the bottle, being careful not to trap any air bubbles. Press on the stopper of the bottle with your finger; then invert the bottle several times to mix.

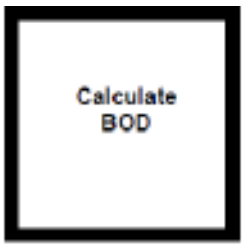
Note: *Determine initial DO's if following the Standard Methods' procedure. This is not necessary if using the graphical method.*

7. Add enough dilution water to the lip of the BOD bottle to make a water seal.

8. Place a plastic overcap over the lip of each bottle and place bottles in an incubator at 20 ± 1° C. Incubate in the dark for five days.

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 3 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued



9. When the five-day incubation period is complete, determine the dissolved oxygen content (mg/L DO remaining) in each bottle as described in the *Dissolved Oxygen, Procedure* in this manual, or potentiometrically by using a dissolved oxygen probe.

Note: *This procedure has been EPA approved. But, the graphical method outlined in step 10 has not. See Calculating Results: Standard Methods (following these steps), for the EPA Approved calculation.*

10. Determine the BOD using the graphical method as follows; see Calculating Results: Grapical Method for more information.

a. Plot the mg/L DO remaining in each diluted sample versus the mL sample taken; then draw the best straight line through the plotted points.

Note: *An erroneous point is visually evident and can be disregarded. However, at least three points should be on the line or very close to it. For unseeded dilution water, the line should cross the "mg/L oxygen remaining" scale near or below the oxygen saturation value for the altitude of the laboratory (see* Dilution Water Preparation*).*

b. To calculate the BOD, use the following equation which is mathematically equivalent to the BOD equation in Standard Methods.

$$\text{mg/L BOD} = (A \times 300) - B + C$$

Where:

A = the slope. The slope of the line is equal to the mg/L DO consumed per mL of sample taken. Take any point on the line and subtract the mg/L DO remaining at that point from the mg/L DO where the line crosses the DO scale (Y intercept, mg/L DO remaining). Divide the difference by the mL of sample at the point chosen.

300 = the volume of the BOD bottle

B = the Y intercept. This is the DO value where the line crosses the "DO remaining" scale. (This should be very close to the actual dilution water blank value.)

C = the sample DO. This is the DO of the undiluted sample.

Another way to write this equation is:

mg/L BOD =

(Slope x 300) -

Y intercept + Sample DO

Note: *If the best straight line is obtained by linear regression through use of a calculator, the sign (-) of the slope must be changed (+) before multiplying by 300.*

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 4 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

Dilution Water Preparation

Note: *The DO uptake in five days at 20 °C should not exceed 0.2 mg/L.*

The BOD test requires very high quality water be used for diluting samples. Water must be free of all toxic substances, such as small amounts of chlorine, copper and mercury, as well as free of organic matter. If organic matter is present in dilution water, it will create an oxygen demand.

The most practical way to produce water of low organic content on a consistent basis is by distillation from alkaline permanganate (Sodium Hydroxide Pellets and Potassium Permanganate). Commercial stills which automatically produce high quality distilled water are available.

Direct use of deionized water from ion exchange columns is not recommended because of the erratic release of organic materials from the cartridges, especially new ones. These organic materials will not be detected with conductivity measurements but may show up in the final results as an oxygen demand. Bacterial growth also may be present on the column.

Distilled water, as it is produced from a still, is usually and not saturated with oxygen. The temperature of the BOD dilution water must be 20 °C at the time of use and near or at saturation with oxygen. It is recommended that distilled water be stored in a BOD incubator until it reaches 20 °C and dilution water be prepared immediately before use. The distilled water can be placed in one-gallon jugs by filling each of them with three liters or by filling two-gallon jugs with six liters. The jugs should be capped and placed in the incubator for storage. After 24 hours or more, the temperature will be 20 °C and the water will be saturated or nearly saturated with oxygen furnished by the air above the water in the jugs.

Note: *The bubbling apparatus should be cleaned before and after use.*

If five-gallon containers are used, the distilled water should be saturated with oxygen by bubbling in filtered air from a hose connected to an aquarium pump or air compressor.

It is not necessary to use seeded dilution water when analyzing sewage, sewage plant effluent (unless it has been chlorinated) or river water. However, there are certain samples such as industrial or trade wastes or chlorinated sewage which do not contain sufficient bacteria to oxidize the organic matter that is present.

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 5 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

To test such samples, some bacterial seed must be added to the samples. This is done by adding a small, measured volume of water known to contain a good bacterial population to the dilution water.

Raw sewage is recommended as a source of seed. This material should be stored at 20°C for 24 to 36 hours before use. When using domestic sewage as seed, it should be allowed to stand undisturbed until most solids settle. Pipet from the upper portion of the bottle of seed material. It has been found that the addition of 3.0 mL of raw domestic sewage seed to each liter of dilution water is ample. Seed that has a BOD of 200 mg/L (a typical range for domestic sewage) when added at the rate of 3 mL per liter of dilution water will deplete 0.6 mg/L DO. An alternative to raw sewage as a source of seed is USEPA-approved Polyseed® BOD Seed Inoculum (Cat. No. 24712-00), a dehydrated seed inoculum.

Using BOD Nutrient Buffer Pillows

To prepare dilution water, select the BOD Nutrient Buffer Pillow for the amount of dilution water you wish to prepare; see *Table 1*. Shake the pillow, cut it open, and add the contents to a jug containing the proper amount of 20 °C distilled water. Choose a container which will be only partially filled by the solution. Cap the jug and shake vigorously for one minute to dissolve the slurry and to saturate the water with oxygen.

Table 1

Description		Cat. No.
BOD Nutrient Buffer Pillows:		
	for preparing 300 mL of dilution water	14160-66
	for preparing 3 liters of dilution water	14861-66
	for preparing 6 liters of dilution water	14862-66
	for preparing 19 liters of dilution water	14863-98

Following Conventional Method

To prepare dilution water by the conventional method, pipet 1 mL of each of the following solutions per liter of distilled water at 20 °C: Calcium Chloride Solution, Ferric Chloride Solution, Magnesium Sulfate Solution, and Phosphate Buffer Solution. Cap the bottle and shake vigorously for one minute.
The Phosphate Buffer Solution should be refrigerated to decrease

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 6 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

the rate of biological growth. Use care with all solutions to avoid contamination.

Choosing Sample Size

The range of sample volumes to be diluted depends on two factors: type of sample and the laboratory's elevation.

If the sample contains high levels of organic material, such as raw sewage, its BOD will be high and small portions must be diluted in the test. If a sample has a low BOD, such as polluted river water, larger portions will be necessary; see *Table 2*.

The laboratory's elevation influences the amount of oxygen that can be dissolved in the dilution water. At sea level and normal barometric pressure, water can be saturated with up to 9.2 mg/L DO at 20 °C.

At higher elevations, the amount of oxygen that can dissolve in water decreases, so less oxygen is available to microorganisms. Refer to *Table 4*. Smaller portions of sample must be taken so there will be dissolved oxygen remaining in the BOD bottle after five days of incubation. For most accurate results, sample sizes should be chosen so that at least 2.0 mg/L of dissolved oxygen are consumed during the incubation period, but 1.0 mg/L DO is left in the BOD bottle. (*Table 2* has done this for you).

Follow these steps to determine the range of sample volumes to use:

a. Estimate the BOD of your particular sample type (see *Table 2*). Sewage has approximately 300 mg/L BOD; oxidized effluents have about 50 mg/L or less.

b. Determine the minimum sample volume that can be used for the estimated BOD of your sample from *Table 2*. For example, if a sewage sample is estimated to contain 300 mg/L BOD, the smallest allowable sample volume is 2 mL. For sewage effluent with an estimated BOD of 40 mg/L, the volume is 15 mL.

c. Determine the laboratory's altitude.

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 7 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

Table 2 Determining Minimum Sample Volume

Sample Type	Estimated BOD mg/L	mL of Sample*
Strong Trade Waste	600	1
Raw and Settled Sewage	300 200 150 120 100 75 60	2 3 4 5 6 8 10
Oxidized Effluents	50 40 30 20 10	12 15 20 30 60
Polluted River Waters	6 4 2	100 200 300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

d. Determine the maximum sample volume for the altitude of your laboratory from *Table 3*. At 1,000 feet an estimated BOD of 300 mg/L, the largest sample portion should be 8 mL. For a BOD of 40 mg/L the maximum volume is 60 mL.

e. Choose three other sample volumes between the minimum and maximum volumes so the total number of portions is five or greater. In the two cases given in *step d* above, a series of 2, 4, 5, 6 and 8 mL portions is suggested for an estimated BOD of 300 mg/L and a series of 15, 25, 35, 45 and 60 mL portions for a BOD of 40 mg/L.

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 8 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

Table 3 Determining Maximum Sample Volume

Estimated BOD at			mL of Sample*
sea level	1000 ft. 300 m	5000 ft. 1500 m	
2460	2380	2032	1
1230	1189	1016	2
820	793	677	3
615	595	508	4
492	476	406	5
410	397	339	6
304	294	251	8
246	238	203	10
205	198	169	12
164	158	135	15
123	119	101	20
82	79	68	30
41	40	34	60
25	24	21	100
12	12	10	200
8	8	7	300

* mL of sample taken and diluted to 300 mL in standard BOD bottle

Table 4 Oxygen Saturation Values at Various Altitudes

Oxygen Saturation Value	Sea Level	1000 ft. 300 m	2000 ft. 600 m	3000 ft. 900 m	4000 ft. 1200m	5000 ft. 1500 m	6000 ft. 1800 m
(at 20 °C)	9.2 mg/L	8.9 mg/L	8.6 mg/L	8.2 mg/L	7.9 mg/L	7.6 mg/L	7.4 mg/L

Interferences

Many chlorinated and industrial effluents require special handling to ensure reliable BOD results. Usually, careful experimentation with the particular sample will indicate what modifications should be made to the test procedure.

Toxins in the sample will adversely affect any microorganisms present and result in lower BODs.

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Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 9 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

1. To eliminate small amounts of residual chlorine*, allow the sample to stand for one to two hours at room temperature. For larger quantities, determine the amount of sodium thiosulfate to add to the sample as follows:

   a. Measure 100 mL of sample into a 250-mL Erlenmeyer flask. Using a 10-mL serological pipet and a pipet filler, add 10 mL of 0.020 N Sulfuric Acid Standard Solution and 10 mL of Potassium Iodide Solution, 100 g/L, to the flask.

   b. Add three full droppers of Starch Indicator Solution and swirl to mix.

   c. Fill a 25-mL buret with 0.025 N Sodium Thiosulfate Standard Solution and titrate the sample from dark blue to colorless.

   d. Calculate the amount of 0.025 N Sodium Thiosulfate Standard Solution to add to the sample:

$$\text{mL 0.025N sodium thiosulfate required} = \frac{\text{mL titrant used} \times \text{volume of remaining sample}}{100}$$

   e. Add the required amount of 0.025 N Sodium Thiosulfate Standard Solution to the sample. Mix well. Wait 10 to 20 minutes before running the BOD test.

2. To eliminate the effect of phenols, heavy metals or cyanide, dilute the sample with high quality distilled water. Alternately, the seed used in the dilution water may be acclimatized to tolerate such materials. Acclimatize seed as follows:

   a. Fill a one-gallon stainless steel or plastic container with domestic sewage and aerate for 24 hours. Allow the heavier material to settle.

   b. After settling for one hour, siphon off three quarts of material and discard.

* Measure chlorine with Hach Water Quality Chlorine Test Strips (Cat. No. 27450-50).

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Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 10 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

c. Fill the container with a mixture of 90% sewage and 10% wastes containing the toxic material.

d. Aerate for 24 hours. Repeat *steps b* and *c* with increasing amounts of waste until the container holds 100% toxic waste material.

3. Optimum pH for BOD test is between 6.5 and 7.5. Adjust samples to pH 7.2 with Phosphate Buffer Solution or 1 N (or more dilute) Sulfuric Acid or Sodium Hydroxide Standard Solution if the pH is not in this range.

4. Cold samples may be supersaturated with oxygen and will have low BOD results. Fill a one-quart bottle about halfway with cold sample and shake vigorously for two minutes. Allow sample temperature to reach 20 °C before testing.

Calculating Results

Graphical Method

The mg/L DO remaining was determined for a series of four dilutions of domestic sewage after five days of incubation. Results were as follows:

mL of sample taken	mg/L DO remaining
2.0	7.50
3.0	6.75
6.0	4.50
9.0	2.25

The DO values were plotted versus the mL of sample taken and a straight line drawn as in *Figure 1*. If a set of BOD dilutions is run correctly with a homogeneous sample, a graph of the mg/L DO remaining versus the sample volume should result in a straight line. The value where the line intersects the y axis is equal to the DO content of the dilution water after incubation, although this is not actually measured. In this case, it was equal to 9.0 mg/L and the DO of the domestic sewage sample was assumed to be zero. If another type of sample is used, the DO of an undiluted sample should be measured either by the Winkler titration or potentiometrically.

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 11 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

The American Public Health Association formula for calculating BOD also can be written as follows (not approved for reporting purposes):

$$\frac{\text{mg/L DO remaining (smaller sample)} - \text{mg/L DO remaining (larger sample)}}{\text{mL (larger sample)} - \text{mL (smaller sample)}} \times 300 - DO_D + S = BOD$$

Using this information in the previous example:

mg/L DO remaining with smaller sample volume = 7.50

mg/L DO remaining with larger sample volume = 2.25

mL of larger sample volume = 9.0

mL of smaller sample volume = 2.0

300 = volume (mL) of BOD bottle

DO_D = mg/L DO of dilution water = 9.0

S = mg/L DO of sample = assumed in this case to be zero

Therefore:

$$\frac{(7.50 - 2.25)}{(9.0 - 2.0)} \times 300 - 9 + 0 = \text{mg/L BOD}$$

$$\frac{5.25}{7} \times 300 - 9 =$$

$$0.75 \times 300 - 9 =$$

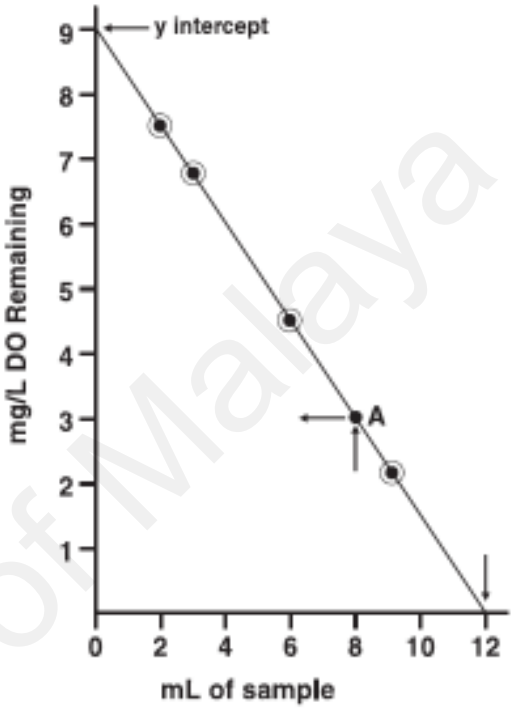
$$225 - 9 =$$

$$216 = \text{mg/L BOD}$$

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 12 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

Figure 1



Using the equation in step 10:

(slope × 300) – Y intercept + sample DO = mg/L BOD

Where:

slope = we arbitrarily select point A in Figure 1. At this point the mg/L DO remaining is equal to 3.0 mg/L. The mL of sample at this point is 8 mL. The difference between the Y intercept of 9.0 mg/L and 3.0 mg/L equals 6 mg/L. 6 mg/L divided by 8 mL = 0.75 mg/L per mL.

Y intercept = 9.0 mg/L

sample DO = 0; because the sample is domestic sewage, this was assumed to be 0.

Therefore:

(0.75 × 300) – 9.0 + 0 = mg/L BOD

225 – 9 = 216 mg/L BOD

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Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 13 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

Standard Methods*

When dilution water is not seeded:

$$BOD_5, mg/L = \frac{D_1 - D_2}{P}$$

When dilution water is seeded:

$$BOD_5, mg/L = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

Where:

D_1 = DO of diluted sample immediately after preparation, mg/L

D_2 = DO of diluted sample after 5 d incubation at 20 °C, mg/L

P = decimal volumetric fraction of sample used

B_1 = DO of seed control before incubation, mg/L

B_2 = DO of seed control after incubation, mg/L

f = ratio of seed in diluted sample to seed in seed control = (% seed in diluted sample)/(% seed in seed control)

If seed material is added directly to sample or to seed control bottles:

f = (volume of seed in diluted sample)/(volume of seed in seed control)

Report results as $CBOD_5$ if nitrification is inhibited. If more than one sample dilution meets the criteria of a residual DO of at least 1 mg/L and a DO depletion of at least 2 mg/L and there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, average results in the acceptable range.

* Adapted from *Standard Methods for the Examination of Water and Wastewater*

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 14 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

Accuracy Check

A standard mixture of glucose and glutamic acid is available to check the accuracy of BOD results. Follow the directions below:

a. Snap the neck off a BOD Voluette® Ampule Standard Solution for BOD, dilution method.

b. Using Class A volumetric pipets and a pipet filler, pipet 1.00, 2.00, 3.00, and 4.00 mL of standard into four BOD bottles.

c. Fill bottles with seeded dilution water and incubate at 20°C for five days.

d. Determine the DO remaining in each bottle; then plot the mg/L remaining DO versus the volume of standard used.

e. Draw the best straight line through the plotted points.

f. Determine the BOD of the standard according to *step 10* of the procedure.

g. Divide the value by two.

Note: *Since the BOD standard contains 300 mg/L each of glucose and glutamic acid, the BOD value determined from the graph must be divided by 2 to correspond with values reported in Standard Methods. Your result should be within the standard deviation listed.*

Note: *On the basis of a mixed primary standard containing 150 mg/L each of glucose and glutamic acid, Standard Methods determines that the average five-day BOD would be 198± 30.5 mg/L*.*

* Data taken from *Standard Methods for the Examination of Water and Wastewater, 19th ed., p.5-6* (**1995**).

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 15 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

Summary of Methods

Biochemical Oxygen Demand (BOD) is an empirical measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters. The BOD test is of limited value in measuring the actual oxygen demand because temperature change, biological population, water movement, sunlight, oxygen concentration and other environmental factors cannot be reproduced accurately in the laboratory. The BOD test is of greatest value after patterns of oxygen uptake for a specific effluent and receiving water have been established.

The BOD is performed by incubating a sealed wastewater sample (or a prepared dilution) for the standard five day period and then determining the change in dissolved oxygen content. The BOD value can then be calculated from the results of the dissolved oxygen tests.

REQUIRED REAGENTS AND APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
BOD Nutrient Buffer Pillows, preparing 3 liters of dilution water	1	50/pkg	14861-66
Bottle, glass-stoppered, 300 mL	6	each	621-00
Bottle, wash, 500 mL	1	each	620-11
Clippers, large	1	each	968-00
BOD Bottle Cap	6	6/pkg	2419-06
Pipet Filler	1	each	12189-00
Select one or more based on sample volume:			
Pipet, serological, 1 mL	varies	each	532-35
Pipet, serological, 5 mL	varies	each	532-37
Pipet, serological, 10 mL	varies	each	532-38

Appendix C-1 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Dilution Method Test Procedure, pp. 281-296. (1998). (page 16 of 16)

OXYGEN DEMAND, BIOCHEMICAL, continued

OPTIONAL REAGENTS

Description	Unit	Cat. No.
BOD Standard Solution, Voluette™ ampule, 300 mg/L, 10 mL, dilution method	16/pkg	14865-10
BOD Nutrient Buffer Pillows		
for preparing 300 mL of dilution water	50/pkg	14160-66
for preparing 6 liters of dilution water	50/pkg	14862-66
for preparing 19 liters of dilution water	25/pkg	14863-98
Buffer Solution, APHA, for BOD, pH 7.2, phosphate type	1 L	431-53
Calcium Chloride Solution, APHA, for BOD	1 L	428-53
Ferric Chloride Solution, APHA, for BOD	1 L	429-53
Magnesium Sulfate Solution, APHA, for BOD	1 L	430-53
Nitrification Inhibitor	35 g	2533-35
Polyseed® BOD Inoculum	50/pkg	24712-00
Potassium Iodide Solution, 100 g/L	500 mL	12289-49
Potassium Permanganate	454 g	168-01
Sodium Hydroxide, pellets	500 g	187-34
Sodium Hydroxide Standard Solution, 1.000 N	100 mL MDB	1045-32
Sodium Thiosulfate Standard Solution, 0.025 N	1 L	352-53
Starch Indicator Solution	100 mL MDB	349-32
Sulfuric Acid Standard Solution, 0.020 N	1 L	203-53
Sulfuric Acid Standard Solution, 1.000 N	1 L	1270-53

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Bottle, 4 L, with spigot	each	14868-17
Bottle, 10 L, with spigot	each	14868-58
Buret, Teflon stopcock, 25 mL	each	14681-40
Clamp, buret double	each	328-00
Cylinder, graduated, 100 mL	each	508-42
Dispenser Cap, for Nitrification Inhibitor (35 bottle only)	each	459-01
Flask, Erlenmeyer, 250 mL	each	505-46
Meter, Dissolved Oxygen, *sension*™*6*, portable, w/ probe	each	51850-10
Pipet, serological, 25 mL	each	2066-40
Sampler, sewage	each	427-00
Still, laboratory, 2–3 liter/hour, 110 V	each	26318-00
Support Base and Rod	each	329-00
Thermometer, -10 to 110 °C	each	1877-01

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 1 of 15)

Source: HACH. (1999). *Wastewater and Biosolids Analysis Manual: Digestion and Selected Methods for Determining Metals, Minerals, and Other Related Parameters* (1st ed.). U.S.A.: Hach Company.

Method 10099

OXYGEN DEMAND, BIOCHEMICAL

Respirometric Method (using the BODTrak apparatus)

1. Heat or cool the sample to within 2 °C of its incubation temperature (typically 20 °C (68 °F).

2. Using a clean graduated cylinder, pour the correct sample volume into a BODTrak sample bottle (see *Table 1*). See the *Sample Dilutions* section for more information on BOD range selection.

3. Place a 3.8-cm (1½-in.) magnetic stir bar in each sample bottle.

4. Add the contents of one BOD Nutrient Buffer Pillow to each bottle for optimum bacteria growth.

Note: *Step 4 is optional. If simulation of original sample characteristics is required, do not add the BOD Nutrient Buffer.*

Table 1 Selection of Sample Volume

BOD Range (mg/L)	Required Volume (mL)
0-35	420
0-70	355
0-350	160
0-700	95

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Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 2 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

5. Apply stopcock grease to the seal lip of each bottle and to the top of each seal cup.

6. Place a seal cup in the neck of each bottle.

7. Using the funnel, add the contents of one Lithium Hydroxide Powder Pillow to each seal cup. Do not allow lithium hydroxide particles to fall into the sample. If this occurs, discard the sample and prepare a fresh one.

8. Place the bottles on the base of the BODTrak. Connect the appropriate tube to the sample bottle and firmly tighten the cap. Each tube is tagged with the channel number, and the channel number setup will be reflected on the control panel.

9. Place the instrument in the incubator.

Note: *The American Public Health Association (APHA) recommends a solution incubation temperature of 20 ±1 °C (68 ±1 °F) for the BOD test. Adjust your incubator to the appropriate temperature setting for each sample volume used in this test. This temperature value varies with incubator circulation.*

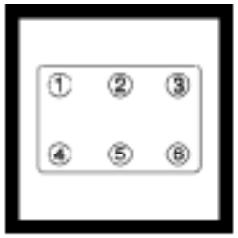
10. Start the instrument (connect the electrical plug and turn the instrument on).

11. Make sure all stir bars are rotating. If a stir bar slides to the side of the bottle, lift the bottle off the unit and gently replace. Do not start the channel until the stir bar is rotating properly.

12. To select a test duration, simultaneously press and hold the left and the right arrow keys until the time menu appears. Press the **CHANNEL 6** key to activate the test length parameter. Use the arrow keys to choose a 5-, 7-, or 10-day test (test length is shown on the last line of the screen). Press **OFF** to save selections and exit the menu.

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 3 of 15)

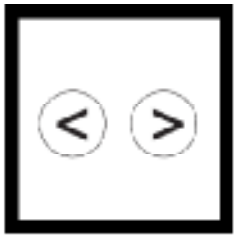
OXYGEN DEMAND, BIOCHEMICAL, continued



13. To start a test press the channel number corresponding to the sample bottle.

Note: *Each channel (1–6) must be started individually.*

14. Press the **ON** key. A menu for selecting the BOD range will be displayed.



15. For 0–350 mg/L range, press the right arrow key. For 0–700 mg/L press the right arrow key a second time.

For 0–35 mg/L range, press the left key.

For 0–70 mg/L press the left arrow key a second time.

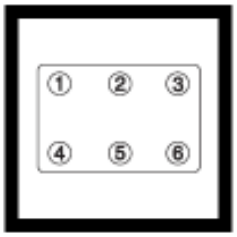
16. Press and hold the **ON** key to start a test. A graph will be displayed. To cancel a test, press **OFF**.

Note: *Repeat steps 13 to 16 for each channel used.*

Note: *The BODTrak automatically stops each channel after the selected time has passed. A channel can be manually stopped by depressing the* **OFF** *key for several seconds. The display status will change from RUN to END.*

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 4 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued



17. Read the BOD results directly from the BODTrak display by pressing the key corresponding to each sample channel.

Note: *See Downloading Test Results in the Instrument Manual for information on transferring data to a computer or printer.*

18. Use a brush and hot soapy water to clean all bottles, stir bars, and seal cups. Rinse thoroughly with distilled water.

Sampling and Storage

For best results, analyze samples immediately after collection. If this is not possible, preserve samples at low temperature (4 °C) for no longer than 24 hours.

Sample Dilutions

If the sample's BOD is unknown, you can generally assume that effluent is normally in the 0-70 mg/L range while influent is usually in the 0-700 mg/L range.

If a sample does not contain sufficient nutrients for optimum bacteria growth, add the contents of one BOD nutrient Buffer Pillow to each bottle. Do not add the BOD Nutrient Buffer Pillow if close simulation of original sample characteristics is required.

When Oxygen Demand Exceeds 700 mg/L

When the O_2 demand of a sample exceeds 700 mg/L, dilute the sample with high-quality dilution water. Make the dilution water with distilled water that does not contain organic matter or traces of toxic substances such as chlorine, copper, and mercury.

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 5 of 15)

Demineralizers can release undetected organic matter that will create an objectionable oxygen demand. The most practical way to consistently produce water of low organic content is by distillation from alkaline permanganate. (For example, add 2 g $KMnO_4$ and 4 g NaOH for every liter of water.)

After distillation, place 3 L of distilled water in a jug and bring the water temperature to 20 °C. Add the contents of one BOD Nutrient Buffer Pillow for 3 L to ensure sufficient nutrient concentration for diluted samples. Cap the jug and shake it vigorously for one minute to saturate the water with oxygen.

Do not store the solution.

Preparing Several Identical Samples
Perform a single dilution for all samples when several identical samples are needed. After the dilution, multiply the reading by the dilution factor.

Example:
Prepare a 1:5 dilution by multiplying the original sample volume by 5 and adding dilution water until the new volume is obtained. If the sample volume is 200 mL:

$5 \times 200 = 1000$ mL

Dilute the 200-mL sample to 1000 mL using the dilution water. Multiply the reading corresponding to the diluted sample by 5.

After sample dilution, refer to *Table 1* to select volume and range.

Sample Seeding

Determining BOD of Seed
Certain types of BOD samples, such as many industrial discharges, do not contain sufficient bacteria to oxidize organic matter present in the sample. Some sewage treatment plant effluents are chlorinated to the extent that they are essentially sterile, making it impossible to perform a direct BOD test. To test such samples, seed each bottle with water known to contain an abundant bacterial population (e.g., domestic sewage or Polyseed Inoculum, Cat. No. 24712-00).

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 6 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

The BOD of the seed must be known in order to calculate the BOD of the sample. To determine the BOD of the seed, follow the same procedure used to determine the BOD of the sample. Run a BOD test on the seed and sample at the same time. For seed acclimation information, see *Seed Acclimatization on page 307.*

Determining Sample BOD

After determining the BOD of the seed, apply the following formula to determine the sample BOD.

$$\text{BOD sample} = \frac{\text{BOD observed} - (\text{Decimal fraction of seed used} \times \text{BOD seed})}{\text{Decimal fraction of sample used}}$$

Example:

A seeded sample is 10% seed and 90% sample (by volume). The observed BOD is 60 mg/L, and the pure seed BOD is 150 mg/L.

$$\text{BOD sample} = \frac{(60\ \text{mg/L}) - (0.10 \times 150\ \text{mg/L})}{0.90} = 50\ \text{mg/L}$$

Variations in Initial Bacterial Populations

Low seed concentrations are more critical than those that are too high. They delay the start of oxidation and cause low BOD results. Use the trial and error method to determine the optimum concentration of seed for a specific waste material.

Choose the seed concentration yielding the highest corrected waste sample BOD. This seed percentage can range from 2–30%, depending on the waste material tested.

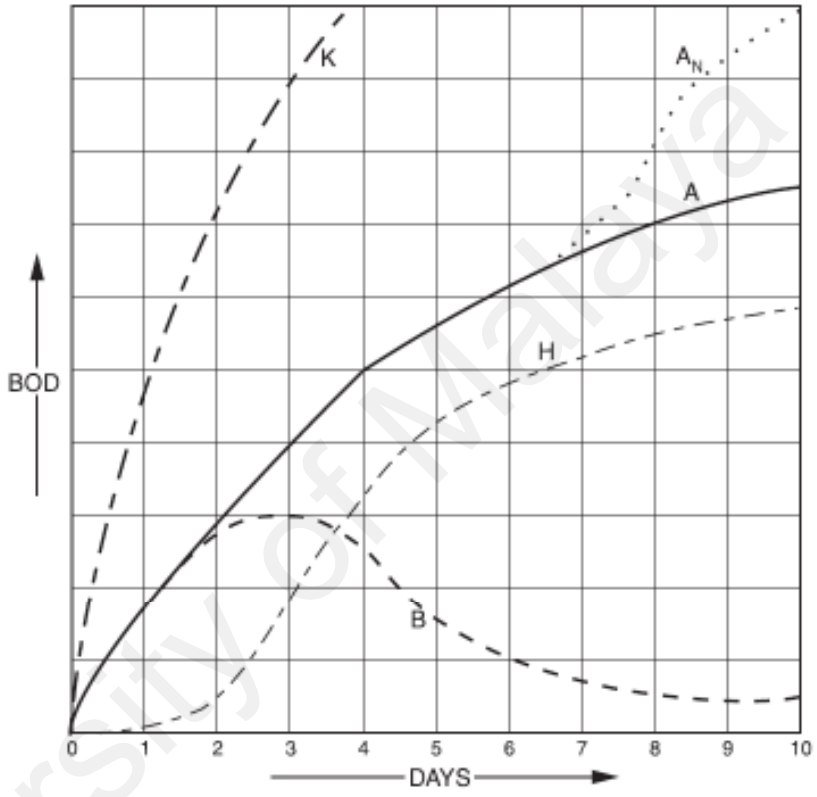
Interpreting Test Results

If the test proceeds correctly, the display should produce a curve similar to Curve A in *Figure 1.*

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 7 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

Figure 1 Example of BOD curves



If such a curve does not occur, one or more of the following problems may have occurred: bottle leak, time lag, high oxygen demand, or nitrification. These potential problems are discussed in detail in the next four sections.

Bottle Leak

A leak between the bottle cap and seal cup may cause readings similar to those plotted as Curve B in *Figure 1*, or may cause no response from the system. If such a response to BOD changes occurs, check for dirt inside the bottle cap and under the seal cup.

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 8 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

Time Lag

Tests that begin with insufficient bacteria during the incubation period produce data similar to that of Curve H in *Figure 1*. For sample processing when insufficient bacteria are present, seed the sample as described in *Sample Seeding* on page *301*.

Bacterial acclimation also produces conditions that could generate Curve H in *Figure 1*. This sometimes occurs when running standards, even though seed has been added.

High Oxygen Demand

Samples that are over range (for example, a BOD over 350 mg/L when a 160-mL sample is taken) will produce results as shown in Curve K in *Figure 1*. Dilute the sample according to the *Sample Dilutions* section, or use a higher BOD range and a different sample volume as directed in *Table 1*.

When the BOD range of a sample is unknown, use the results from the Chemical Oxygen Demand (COD) test, or the results from a series of BOD tests using the same sample but different volumes, or dilution ratios to select an appropriate BOD range. Generally, effluent normally is in the 0–70 mg/L range while influent is in the 0–700 mg/L range. When the BOD of the sample is greater than 700 mg/L, prepare a sample dilution (see *Sample Dilutions on page 300*).

Nitrification

The condition shown by Curve A_N in *Figure 1* is an example of nitrification. Biological oxidation of organic nitrogen usually occurs after five days with normal domestic waste because it takes that long for the nitrifying bacteria to develop; however, an abnormally high uptake of oxygen (especially when testing final effluent) is evidence of nitrifying bacteria adding appreciably to the oxygen demand.

Control nitrification problems with Hach Nitrification Inhibitor (Cat. No. 2533-35). Dispense the inhibitor powder into an empty sample bottle and then add the sample. When using the Hach Dispenser Cap (Cat No. 459-01), dispense two shots (approximately 0.16 grams) into the empty bottle.

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 9 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

Accuracy Check

To check test accuracy and proper performance of the BODTrak Apparatus, test a standard BOD sample and evaluate it for one or more of the mechanical, physical, or biological effects described in *Interpreting Test Results*.

Use a mixture of 150 mg/L each of glucose and glutamic acid as the BOD standard. A prepared BOD standard solution, 3000 mg/L each of glucose and glutamic acid, is available as the Voluette Ampule Standard for the Manometric Method (Cat. No. 14866-10). A 1:20 dilution of this standard (to 150 mg/L) is incorporated in the following procedure. Follow this procedure to analyze the 3000 mg/L prepared standard sample:

1. Shake three liters of distilled water in a partly filled container for one minute to saturate the water with oxygen.
2. Add the contents of one BOD Nutrient Buffer Pillow (Cat. No. 14861-98) for 3 L, and invert several times to mix.
3. Snap the neck off a Voluette Ampule Standard for BOD (Cat. No. 14866-10) and pipet 7 mL of standard into a sample bottle.
4. Add 133 mL of the nutrient buffer solution, prepared in step *2*, and 15 mL seed. A 10% by volume of seed in solution will result. See *Sample Seeding*.
5. Follow the general procedure for the BOD test using the 0–350 mg/L BOD range and a five-day test period.
6. Also perform a full strength BOD test on the pure seed to determine its BOD while determining the BOD of the standard.
7. To determine the BOD result, see *Determining Sample BOD*. The corrected BOD of the standard solution should be 198 ±30.5 mg/L.

Sample Temperature

The American Public Health Association (APHA) recommends a solution temperature of 20 ±1 °C (68 °F) for conducting the BOD test. Obtain this temperature by placing the BODTrak instrument

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 10 of 15)

in an appropriate incubator and adjusting the temperature until the solution reaches 20 ±1 °C. An undercounter BOD Incubator and a combination BOD Incubator/Refrigerator are available (see *OPTIONAL APPARATUS*).

Samples should be cooled to incubation temperature. Seeding of samples with initially high sample temperatures may also be necessary because samples may have insufficient bacteria (see *Sample Seeding*). Determine the BOD of the seed and the BOD of the sample at the same time.

Other BOD Test Temperatures

The BOD test can be conducted at temperatures other than 20 °C. Tool's[1] results indicate that the five-day, 20 °C value can be obtained in 2.5 days at 35 °C. Middlebrooks[2] presents nomographs for converting BOD tests to temperatures other than 20 °C.

Interferences

Industrial and chlorinated samples often contain toxic substances and require special considerations when running BOD tests. The presence of toxic substances in the sample will cause decreased BOD values. Either remove the toxic substances or eliminate their effects by diluting the sample.

Chlorine

Low chlorine[3] concentrations may be dissipated by maintaining the sample at room temperature for 1–2 hours before testing. Remove the chlorine from samples with high chlorine levels by adding sodium thiosulfate as described below:

1. Add 10 mL of 0.02 N Sulfuric Acid Standard Solution and 10 mL of 100 mg/L Potassium Iodide Solution to a 100-mL portion of sample in a 250-mL Erlenmeyer flask.

1 H. R. Tool, Manometric Measurement of the Biochemical Oxygen Demand, *Water and Sewage Works Journal*, 114: 211-218, 1967.
2 E.J. Middlebrooks, A Nomograph for Solution of the BOD Equation, *Water and Sewage Works Journal*, 112: R230, 1965.
3 Measure chlorine with Hach Water Quality Chlorine Test Strips (Cat No. 27450-50).

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 11 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

2. Add three full droppers of Starch Indicator Solution and swirl to mix.

3. Titrate from dark blue to colorless with 0.025 N Sodium Thiosulfate Standard Solution.

4. Calculate the amount of Sodium Thiosulfate Standard Solution necessary to dechlorinate the remaining sample:

$$\text{mL of Sodium Thiosulfate} = \frac{(\text{mL used})(\text{mL sample to be dechlorinated})}{100}$$

5. Add the required amount of 0.025 N Sodium Thiosulfate Standard Solution to the sample and mix thoroughly. Wait 10 to 20 minutes before running the BOD test.

Other Toxic Materials

Determine the concentrations of other toxic materials such as phenols, heavy metals, and cyanides.

Dilute the sample with distilled water to eliminate the effect of these materials. The correct BOD is obtained when two successive dilutions result in the same sample BOD value. Or, the seed used in the dilution water may be acclimatized to tolerate such materials.

Seed Acclimatization

Domestic sewage or Polyseed Inoculum can provide seed for most samples. Polyseed Inoculum is ideally suited for domestic and industrial wastewater because it provides a constant seed source and is free of nitrifying microorganisms.

Pour the contents of one polyseed capsule into dilution water to rehydrate (refer to the procedure packaged with the Polyseed). Aerate and stir for one hour. Use enough of this solution so that it makes up 10 to 30% of the overall sample volume. The exact percentage of seed must be determined for each sample type.

For more information, *Standard Methods for the Examination of Water and Wastewater, 19th edition* emphasizes the importance of selecting the proper seed for specific wastes.

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 12 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

If the waste sample to be tested contains toxic materials such as phenol, formaldehyde, or other microbic inhibitory agents, use acclimated seed. Acclimate the seed in any non-metal or stainless steel gallon container fitted with an aeration system. Proceed as follows:

1. Aerate domestic sewage for about 24 hours. Allow one hour settling time for heavier materials to settle.
2. After the one hour settling, siphon and discard the top two-thirds of the volume.
3. Refill the container to the original volume with domestic sewage containing 10% of the waste material in question.
4. Repeat **steps 1–3**, increasing the addition of waste material by 10%. Stop the procedure when 100% waste material has been reached.

pH Effect

Low BOD test results occur when the pH of a test waste material exceeds the 6–8 range. The operator may maintain this pH to simulate original sample conditions or may adjust the pH to approach neutrality (buffered at pH 7). Neutralize samples containing caustic alkalinity or acidity by using 1.0 N (or weaker) sulfuric acid or sodium hydroxide, respectively.

Supersaturation

Reduce supersaturated cold samples (containing more than 9 mg/L dissolved oxygen at 20 °C) to saturation. To do so, first bring the sample temperature to about 20 °C. Then partly fill a sample bottle with sample and shake vigorously for two minutes, or aerate with filtered compressed air for two hours.

Summary of Method

Biochemical Oxygen Demand (BOD) is an empirical measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters.

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 13 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

BOD measures the amount of oxygen used by bacteria as they oxidize organic matter in the sample. The waste sample is put in an amber BOD bottle with an ample amount of air left above the sample. The bottle is connected to a pressure sensor. The bacteria use dissolved oxygen, which is replaced by the air above the sample. This causes a drop in air pressure in the bottle, which is registered by the pressure sensor. The drop can be read directly as mg/L BOD off the graphical display. Carbon dioxide produced by oxidation is removed by the lithium hydroxide crystals in the seal cup.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
BOD Nutrient Buffer Pillows, for preparing 300 ml	1	50/pkg	14160-66
BOD Nutrient Buffer Pillows, for preparing 3 L	1	50/pkg	14861-66
Grease, stopcock, tube	varies	75 g	562-75
Lithium Hydroxide, Powder Pillows	1	100/pkg	14163-69

REQUIRED APPARATUS

BODTrak™ Apparatus, 115/230V	1	each	26197-00
Bottle, BOD, amber	1	6/pkg	7144-21
Funnel, powder	1	each	22644-67
Seal cup	1	6/pkg	10977 52
Stir bar, magnetic	1	6/pkg	10764-16
Power cord, 115V	1	each	18010-00
Power cord, 230V	1	each	46836-00
Power Supply, 110/230 VAC	1	each	26249-00

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 14 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

OPTIONAL REAGENTS

Description	Unit	Cat. No.
BOD Nutrient Buffer Pillows, for preparing 6 L	50/pkg	14862-66
BOD Nutrient Buffer Pillows, for preparing 19 L	25/pkg	14863-98
Buffer Solution, phosphate type, pH 7.2	1000 mL	431-53
Calcium Chloride Solution	1 L	428-53
Ferric Chloride Solution	1 L	429-53
Magnesium Sulfate Solution	1 L	430-53
Chromic Acid Cleaning Solution	500 mL	1233-49
Nitrification Inhibitor	35 g	2533-35
Polyseed Inoculum	pkg/50	24712-00
Potassium Iodide Solution, 100 g/L	500 mL	12289-49
Potassium Permanganate	454 g	168-01
Sodium Hydroxide ACS Pellets	500 g	187-34
Sodium Hydroxide Standard Solution, 1.0 N	900 mL	1045-53
Sodium Sulfite Anhydrous, ACS	454 g	195-01
Sodium Thiosulfate Standard Solution, 0.025 N	1000 mL	352-53
Starch Indicator Solution	100 mL, MDB	349-32
Sulfuric Acid, ACS:		
Concentrated	500 mL	979-49
0.02 N Standard Solution	1000 mL	203-53
1.0 N Standard Solution	1000 mL	1270-53
Voluette Ampule Standard for BOD, for manometric, 3000 mg/L 10 mL ampule	16/pkg	14866-10
Water Quality Test strips, chlorine	50/pkg	27450-50

Appendix C-2 Analysis method for BOD test - according to the *Wastewater and Biosolids Analysis Manual*: Oxygen Demand, Biochemical. Respirometric Method (using the BODTrak apparatus) Test Procedure, pp. 297-311. (1998). (page 15 of 15)

OXYGEN DEMAND, BIOCHEMICAL, continued

OPTIONAL APPARATUS

Description	Unit	Cat. No.
Bottle, polyethylene, with spigot	4 L	14868-17
Brush, cylinder, size 2	each	687-00
Buret, straight stopcock, Teflon plug, 25 mL	each	14681-40
Clamp, buret, double	each	328-00
Cylinders, graduated:		
10 mL	each	508-38
25 mL	each	508-40
50 mL	each	508-41
100 mL	each	508-42
250 mL	each	508-46
500 mL	each	508-49
1000 mL	each	508-53
Dispenser Cap for 35g bottle (used with Nitrification Inhibitor)	each	459-01
Flask, Erlenmeyer, 500 mL	each	505-49
Flask, volumetric, 1000 mL	each	547-53
Incubator, under counter, Model 205, 120V	each	26162-00
Incubator, under counter, Model 205, 240V	each	26162-02
Incubator/Refrigerator, Model 207, 120V	each	26198-00
Incubator/Refrigerator, Model 207, 240V	each	26198-02
IncuTrol®/2, Temperature Regulator, 115V	each	2597-00
IncuTrol®/2, Temperature Regulator, 220V	each	2597-02
Pipet Filler	each	12189-00
Pipet, serological:		
1.0 mL	each	532-35
10 mL	each	532-38
Printer, 115 V, Epson Model LQ-570+ (graphic)	each	48236-00
Printer cable	each	26582-00
Sampler, sewage	each	427-00

Appendix C-3 Analysis method for Nitrogen, Ammonia test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 343 – 346. (1996). (page 1 of 4)

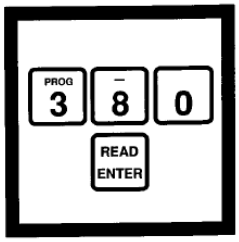
Source: HACH. (1996). DR/2000 Spectrophotometer Instrument Manual (7-21-95 3rd ed., Rev. 2, 01/96).U.S.A.: Hach Company.

Method 8038

NITROGEN, AMMONIA (0 to 2.50 mg/L NH_3–N)

For water, wastewater* and seawater*

Nessler Method, USEPA accepted for reporting wastewater analysis (distillation is required)*****



1. Enter the stored program number for ammonia nitrogen (NH_3–N).

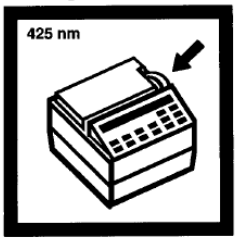
Press: **3 8 0 READ/ENTER**

The display will show:
DIAL nm TO 425

Note: *DR/2000s with software versions 3.0 and greater will display "P" and the program number.*

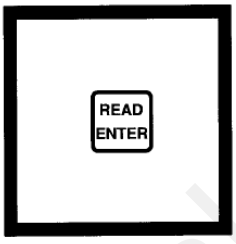
Note: *Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.*

Note: *If samples cannot be analyzed immediately, see* Sampling and Storage *following these steps. Adjust pH of stored samples before analysis.*



2. Rotate the wavelength dial until the small display shows:
425 nm

Note: *This test is sensitive to the wavelength setting. To assure accuracy, run the test using a 1.0 mg/L standard solution and demineralized water blank. Repeat Steps 9 to 12 at slightly different wavelengths, setting the dial from higher to lower values, until the correct result is obtained. The wavelength should be 425 ±2 nm. Always set this wavelength by approaching from high to low values.*



3. Press: **READ/ENTER**

The display will show:
mg/l N NH_3 Ness

4. Fill a 25–mL graduated mixing cylinder to the 25–mL mark with sample (the prepared sample).

Note: *For proof of accuracy, use a 1.0-mg/L Nitrogen Ammonia Standard Solution (listed under* Optional Reagents*) in place of the sample.*

*Requires distillation

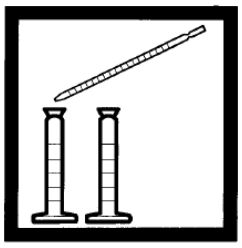
** Adapted from *Standard Methods for the Examination of Water and Wastewater.*

***Procedure is equivalent to USEPA method 350.2 and Standard Method 4500–NH_3 B and C for wastewater.

NITROGEN, AMMONIA, continued

5. Fill another 25–mL mixing graduated cylinder with demineralized water (the blank).

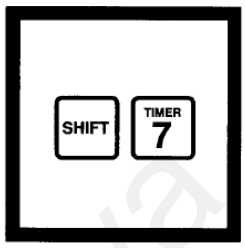
6. Add three drops of Mineral Stabilizer to each cylinder. Invert several times to mix. Add three drops of Polyvinyl Alcohol Dispersing Agent to each cylinder (hold the dropping bottle exactly vertical). Invert several times to mix.



7. Pipet 1.0 mL of Nessler Reagent into each cylinder. Stopper. Invert several times to mix.

Note: *Nessler Reagent is toxic and corrosive. Pipet carefully and use a pipet filler.*

Note: *A yellow color will develop if ammonia is present. (The reagent will cause a faint yellow color in the blank.)*



8. Press: **SHIFT TIMER**

A 1–minute reaction period will begin.

Note: *Continue with Step 9 while timer is running.*

9. Pour each solution into respective blank and prepared sample cells.

Note: *The Pour–Thru Cell can be used with this procedure. If the Pour–Thru Cell Assembly Kit is used, periodically clean the cell by pouring a few sodium thiosulfate pentahydrate crystals into the cell funnel. Flush it through the funnel and cell with enough demineralized water to dissolve. Rinse out the crystals.*

10. When the timer beeps, the display will show:

mg/l N NH_3 Ness

Place the blank into the cell holder. Close the light shield.

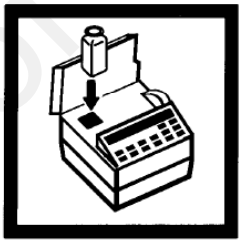
Press: **ZERO**

The display will show:

WAIT

then:

0.00 mg/l N NH_3 Ness



11. Place the prepared sample into the cell holder. Close the light shield.

12. Press: **READ/ENTER**

The display will show:

WAIT

then the result in mg/L ammonia expressed as nitrogen (NH_3–N) will be displayed.

Note: *Do not wait more than five minutes after reagent addition (Step 7) before performing Step 12.*

Note: *The results may be expressed as mg/L ammonia (NH_3) or mg/L ammonium (NH_4^+) by multiplying the result by 1.22 or 1.29 respectively.*

Note: *In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.*

Appendix C-3 Analysis method for Nitrogen, Ammonia test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 343 – 346. (1996). (page 3 of 4)

NITROGEN, AMMONIA, continued

SAMPLING AND STORAGE

Collect samples in clean glass or plastic bottles. If chlorine is present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl_2 in a 1–liter sample. Preserve the sample by reducing the pH to 2 or less with sulfuric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Before analysis, warm samples to room temperature and neutralize with 5 N sodium hydroxide. Correct the test result for volume additions *(see Correction for Volume Additions in Section 1)*.

ACCURACY CHECK

Standard Additions Method

a) Snap the neck off a Nitrogen Ammonia Voluette Ampule Standard Solution, 50 mg/L NH_3-N.

b) Use the TenSette Pipet to add 0.1, 0.2 and 0.3 mL of standard to three 25–mL samples. Mix each thoroughly.

c) Analyze each sample as described above. The nitrogen concentration should increase 0.20 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see *Standard Additions* in *Section 1* for more information.

Standard Solution Method

To check accuracy, use a 1.0-mg/L NH_3–N Nitrogen Ammonia Standard Solution listed under *Optional Reagents*. Or, this can be prepared by diluting 1.00 mL of solution from a Voluette Ampule Standard For Nitrogen Ammonia to 50.0 mL with demineralized water.

PRECISION

In a single laboratory, using standard solutions of 1.00 mg/L ammonia nitrogen (NH_3–N) and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.015 mg/L.

INTERFERENCES

A solution containing a mixture of 500 mg/L $CaCO_3$ and 500 mg/L Mg as $CaCO_3$ does not interfere. If the hardness concentration exceeds these concentrations, extra Mineral Stabilizer should be added.

Iron and sulfide interfere by causing a turbidity with Nessler Reagent.

Residual chlorine must be removed by addition of sodium arsenite solution. Use two drops to remove each mg/L Cl from a 250–mL sample. Sodium thiosulfate can be used in place of sodium arsenite. See *Sampling and Storage* section.

Less common interferences, such as glycine, various aliphatic and aromatic amines, organic chloramines, acetone, aldehydes and alcohols may cause greenish or other off colors or turbidity. It may be necessary to distill the sample if these compounds are present.

Seawater samples may be analyzed by addition of 1.0 mL (27 drops) of Mineral Stabilizer to the sample before analysis. This will complex the high magnesium concentrations found in sea water, but the sensitivity of the test will be reduced by 30 percent due to the high chloride concentration. For best results, perform a calibration, using standards spiked to the equivalent chloride concentration, or distill the sample as described below.

DISTILLATION

a) Measure 250 mL of sample into a 250–mL graduated cylinder and pour into a 400–mL beaker. Destroy chlorine, if necessary, by adding 2 drops of Sodium Arsenite Solution per mg/L Cl_2.

b) Add 25 mL of Borate Buffer Solution and mix. Adjust the pH to about 9.5 with 1.0 N Sodium Hydroxide Standard Solution. Use a pH meter.

c) Set up the general purpose distillation apparatus as shown in the *Hach Distillation Apparatus Manual*. Pour the solution into the distillation flask. Add a stir bar.

d) Use a graduated cylinder to measure 25 mL of demineralized water into a 250–mL erlenmeyer flask. Add the contents of one Boric Acid Powder Pillow. Mix thoroughly. Place the flask under the still drip tube. Elevate so the end of the tube is immersed in the solution.

e) Turn on the heater power switch. Set the stir control to 5 and the heat control to 10. Turn on the water and adjust to maintain a constant flow through the condenser.

f) Turn off the heater after collecting 150 mL of distillate. Immediately remove the collection flask to avoid sucking solution into the still. Measure the distillate to assure 150 mL was collected (total volume 175 mL).

g) Adjust the pH of the distillate to about 7 with 1.0 N Sodium Hydroxide Standard Solution. Use a pH meter.

h) Pour the distillate into a 250–mL volumetric flask. Rinse the erlenmeyer with several small volumes of demineralized water and add the rinsings to the volumetric flask.

Appendix C-3 Analysis method for Nitrogen, Ammonia test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 343 – 346. (1996). (page 4 of 4)

NITROGEN, AMMONIA, continued

i) Dilute to the mark with ammonia–free demineralized water. Stopper. Mix thoroughly. Analyze as described above.

SUMMARY OF METHOD

The Mineral Stabilizer complexes hardness in the sample. The Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonium ions. A yellow color is formed proportional to the ammonia concentration.

REQUIRED REAGENTS

Description	Quantity Required Per Test	Units	Cat. No.
Nessler Reagent	2 mL	500 mL	21194–49
Mineral Stabilizer	6 drops	59 mL* SCDB	23766–26
Polyvinyl Alcohol Dispersing Agent	6 drops	59 mL* SCDB	23765–26
Water, demineralized	25 mL	4 L	272–56

REQUIRED APPARATUS

Cylinder, graduated, mixing, tall form, 25 mL	2	each	21190–40
Pipet, serological, 1 mL	2	each	532–35
Pipet Filler, safety bulb	1	each	14651–00

OPTIONAL REAGENTS

Borate Buffer Solution	1000 mL	14709–53
Boric Acid Powder Pillows	50/pkg	14817–66
Nitrogen, Ammonia Standard Solution, 1 mg/L NH_3-N	500 mL	1891–49
Nitrogen, Ammonia Standard Solution, Voluette Ampule, 50 mg/L NH_3–N	16/pkg	14791–10
Sodium Arsenite Solution, 5 g/L	100 mL MDB	1047–32
Sodium Hydroxide Standard Solution, 5.0 N	100 mL* MDB	2450–32
Sodium Hydroxide Standard Solution, 1.0 N	100 mL* MDB	1045–32
Sodium Thiosulfate Solution, 0.1 N	100 mL* MDB	323–32
Sulfuric Acid, ACS	500 mL*	979–49

OPTIONAL APPARATUS

Ampule Breaker Kit	each	21968–00
Beaker, 400 mL	each	500–48
Cylinder, graduated, 25 mL	each	508–40
Cylinder, graduated, 250 mL	each	508–46
Distillation apparatus general purpose accessories	each	22653–00
Distillation heater and support apparatus set, 115 V	each	22744–00
Distillation heater and support apparatus set, 230 V	each	22744–02
Dropper, plastic, 0.5 and 1.0–mL marks	10/pkg	21247–10
Flask, erlenmeyer, 250–mL	each	505–46
Flask, volumetric, 50 mL	each	547–41
Flask, volumetric, 250 mL	each	547–46
pH Meter, EC10, portable	each	50050–00
Pipet, serological, 2 mL	each	532–36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700–01
Pipet Tips, for 19700–01 TenSette Pipet	50/pkg	21856–96
Pipet, volumetric, Class A, 1 mL	each	14515–35
Pour–Thru Cell Assembly Kit	each	45215–00
Thermometer, –20 to 105 °C	each	1877–01

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes.

Appendix C-4 Analysis method for Nitrate, High Range (HR) test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 293-301. (1996). (page 1 of 9)

Source: HACH. (1996). DR/2000 Spectrophotometer Instrument Manual (7-21-95 3rd ed., Rev. 2, 01/96).U.S.A.: Hach Company.

Method 8039

NITRATE, HR (0 to 30.0 mg/L NO_3^-–N)

For water, wastewater and seawater*

Cadmium Reduction Method (Powder Pillows or AccuVac Ampuls)

USING POWDER PILLOWS

1. Enter the stored program number for high range nitrate nitrogen (NO_3^-–N)–powder pillows.

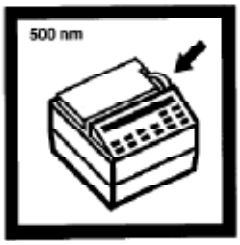
Press: **3 5 5 READ/ENTER**

The display will show:
DIAL nm TO 500

Note: DR/2000s with software versions 3.0 and greater will display "F" and the program number.

Note: Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.

Note: If sample cannot be analyzed immediately, see Sampling and Storage *following these steps. Adjust the pH of stored samples before analysis.*



2. Rotate the wavelength dial until the small display shows:

500 nm

3. Press: **READ/ENTER**

The display will show:
mg/l N NO_3^- H

4. Fill a sample cell with 25 mL of sample.

Note: For proof of accuracy, use a 10 mg/L Nitrate Nitrogen Standard Solution (listed under Optional Reagents*) in place of the sample.*

Note: A reagent blank must be determined on each new lot of NitraVer 5. Perform Steps 4 to 12 using demineralized water as the sample. Subtract this value from each result obtained with this lot of reagent.

*For seawater, a manual calibration is required; see Interferences.

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Appendix C-4 Analysis method for Nitrate, High Range (HR) test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 293-301. (1996). (page 2 of 9)

NITRATE, HR, continued

5. Add the contents of one NitraVer 5 Nitrate Reagent Powder Pillow to the cell (the prepared sample). Stopper.

6. Press: **SHIFT TIMER**

Shake the cell vigorously until the timer beeps in one minute.

Note: *A deposit of unoxidized metal will remain after the NitraVer 5 Nitrate Reagent Powder dissolves. This deposit will have no effect on test results.*

Note: *Shaking time and technique influence color development. For most accurate results, make successive tests on a 10 mg/L Nitrate Nitrogen Standard Solution listed under* Optional Reagents. *Adjust the shaking time to obtain the correct result.*

7. When the timer beeps, press: **SHIFT TIMER**

A 5–minute reaction period will begin.

Note: *An amber color will develop if nitrate nitrogen is present.*

8. Fill another sample cell with 25 mL of sample (the blank).

Appendix C-4 Analysis method for Nitrate, High Range (HR) test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 293-301. (1996). (page 3 of 9)

NITRATE, HR, continued

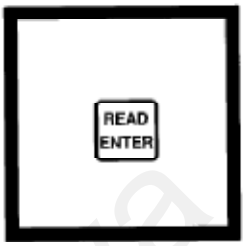
9. When the timer beeps, the display will show:
mg/l N NO_3^- H
Place the blank into the cell holder. Close the light shield.

Note: The Pour-Thru Cell can be used if rinsed well with demineralized water after use. Avoid pouring any cadmium particles into the cell.

10. Press: **ZERO**

The display will show:
WAIT
then:
0.0 mg/l N NO_3^- H

11. Remove the stopper. Place the prepared sample into the cell holder. Close the light shield.



12. Press: **READ/ENTER**

The display will show:
WAIT
then the result in mg/L nitrate nitrogen (NO_3^-–N) will be displayed.

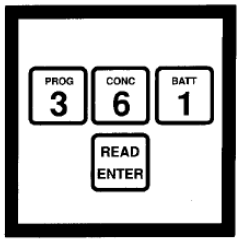
Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

Note: The results can be expressed as mg/L nitrate (NO_3^-) by multiplying the mg/L nitrate nitrogen (NO_3^--N) by 4.4.

Note: Rinse the sample cell immediately after use to remove all cadmium particles.

NITRATE, HR, continued

USING ACCUVAC AMPULS



1. Enter the stored program number for high range nitrate nitrogen (NO_3^-–N)–AccuVac ampuls.

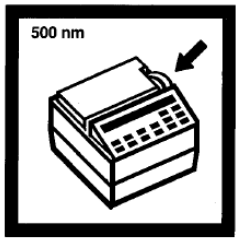
Press: **3 6 1 READ/ENTER**

The display will show:
DIAL nm TO 500

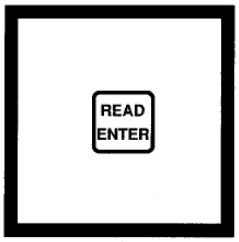
Note: *DR/2000s with software versions 3.0 and greater will display "P" and the program number.*

Note: *Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.*

Note: *If your instrument does not have program number 361, see* Instrument Setup *following these steps.*



2. Rotate the wavelength dial until the small display shows:
500 nm



3. Press: **READ/ENTER**

The display will show:
mg/l N NO_3^- H AV

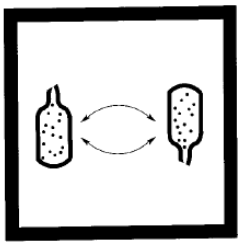
4. Collect at least 40 mL of sample in a 50–mL beaker. Fill a NitraVer 5 Nitrate AccuVac Ampul with sample.

Note: *Keep the tip immersed while the ampul fills completely.*

Note: *For proof of accuracy, use a 10 mg/L Nitrate Nitrogen Standard Solution (listed under* Optional Reagents*) in place of the sample.*

Note: *A reagent blank must be determined on each new lot of NitraVer 5. Repeat Steps 4 to 12 using demineralized water as the sample. Subtract this value from each result obtained with this lot of reagent.*

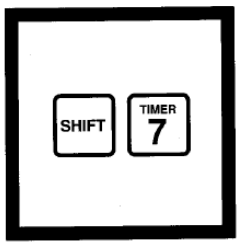
NITRATE, HR, continued



5. Press: **SHIFT TIMER**

A one–minute mixing period will begin. Invert the ampul repeatedly until the timer beeps. Wipe off any liquid or fingerprints.

Note: *Shaking time and technique influence color development. For most accurate results, make successive tests on a 10 mg/L Nitrate Nitrogen Standard Solution listed under* Optional Reagents. *Adjust the shaking time to obtain the correct result.*



6. When the timer beeps, press: **SHIFT TIMER**

A 5–minute reaction period will begin.

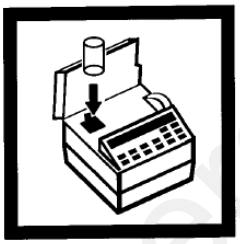
Note: *A deposit of unoxidized metal will remain after the NitraVer 5 Nitrate Reagent Powder dissolves. this deposit will have no effect on test results.*

Note: *An amber color will develop if nitrate nitrogen is present.*

7. Fill a zeroing vial with at least 10 mL of sample (the blank).

8. Place the AccuVac Vial Adapter into the cell holder.

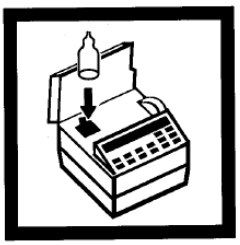
Note: *Place the grip tab at the rear of the cell holder.*



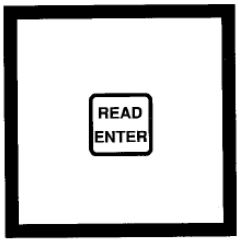
9. When the timer beeps, the display will show:
mg/l N NO_3^- H AV
Place the blank into the cell holder. close the light shield.

10. Press: **ZERO**

The display will show:
WAIT
then
0.0 mg/l N NO_3^- H AV



11. Place the AccuVac ampul into the cell holder. Close the light shield.



12. Press: **READ/ENTER**

The display will show:
WAIT
then the nitrate result in mg/L nitrate nitrogen (NO_3^--N) will be displayed.

Note: *The results can be expressed as mg/L nitrate (NO_3^-) by multiplying the mg/L nitrate nitrogen (NO_3^--N) by 4.4.*

Note: *In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.*

NITRATE, HR, continued

INSTRUMENT SETUP

For a DR/2000 with software versions 1.27 or 1.265, enter the following calibration as an operator–programmed calibration for high range nitrate nitrogen AccuVac ampuls (method 361). Follow steps in the *Operation* section of the *DR/2000 Instrument Manual*. Store the method as follows:

nm = 500
Decimal = 000.0
Units = mg/l
Symbol = NO_3-N
Timer 1 = 01:00
Timer 2 = 05:00

At first, enter the calibration with 0.000 absorbance values for zero and standards #1–4. To do this, do not place anything in the sample compartment. Begin by storing zero, #1 standard, #2 standard, #3 standard and #4 standard as concentrations of 0, 6.0, 12.5, 20.0 and 35.0, respectively, with nothing in the sample compartment. Accept 0.000 Abs. as the value for all standards. Next, the values for the standards must be changed to the values given below.

Standard	Concentration	Absorbance
0	0	0.000
1	6.0	0.125
2	12.5	0.250
3	20.0	0.375
4	35.0	0.563

The method is now stored as an operator–programmed method with a method number between 950 and 999. Record the method number for future reference.

For a DR/2000 with software version 2.0 and 2.2, enter the calibration as an update to Hach–stored programs. (Stored program number 360 has been replaced with number 361.)

1. Press: [I/O]

2. Press: [SHIFT] [CONFIG METH]

3. Press: [PROG 3] [CONC 6] [BATT 1] [READ ENTER]

4. Within 3 seconds, press: [SHIFT] [PROG 3] [CONFIG METH]

The display will show:
ENTER nm

If the display returns to the METHOD prompt, repeat the sequence.

5. Press: [ABS 5] [0] [0]

If you make an error, press **SHIFT CLEAR** and re–enter the number. When the number is correct, press **READ/ENTER**. The display will show:
DECIMAL? 00.00

6. Use the arrow keys to correctly position the decimal point. For this method, press the **RIGHT/DOWN ARROW** key once. The display will show:
DECIMAL? 000.0

7. When the decimal point is correctly positioned, press: **READ/ENTER**. The display will show:
UNITS?

8. Use the arrow keys to select the appropriate unit of measure. For this method, press the **RIGHT/DOWN ARROW** key twice. The display will show:
mg/l

9. With the proper unit of measure displayed, press **READ/ENTER**. The display will show:
SYMBOL?

10. Use the arrow keys to construct the correct symbol display. For this method, press the **RIGHT/DOWN ARROW** key repeatedly until you see:
mg/l n

11. Press **SHIFT** to make the "n" uppercase. The display will show:
mg/l N

12. Press the **READ/ENTER** key to accept the capital "N."

13. Using the arrow keys, continue to construct the display:
mg/l N NO_3^- H AV
The space is the "character" displayed after one press of the **RIGHT/DOWN ARROW** key. To enter subscript 3, press the number 3 key. It will enter as a subscript.

NITRATE, HR, continued

14. When the last character of the symbol is accepted with the **READ/ENTER** key, the display will show:
TIMER?

15. There are two timers for this method, so press **SHIFT TIMER**. The display will show:
MM:SS TIME 1?

16. To enter the first timer value of 1:00 minute, press:

The display will then read:
01:00 TIME 1 ?

17. Press **READ/ENTER** to accept the timer value. The display will show:
MM:SS TIME 2 ?

18. To enter the second timer value of 5:00 minutes, press:

The display will then read:
05:00 TIME 2 ?

19. Press **READ/ENTER** to accept the timer value.

20. The display will then read:
MM:SS TIME 3 ?

21. Press **READ/ENTER** to complete the timer entry. The display will show:
 # 1 Data 0

22. Enter the following 12 numbers as shown. Complete each number entry by pressing **READ/ENTER**.

# 1 Data	**0**
# 2 Data	**15425**
# 3 Data	**20324**
# 4 Data	**25855**
# 5 Data	**65535**
# 6 Data	**65535**
# 7 Data	**65535**
# 8 Data	**65535**
# 9 Data	**65535**
# 10 Data	**8192**
# 11 Data	**512**
Checksum	**60769**

The final number is a check value which is used to determine if the data sequence was correctly entered. If an error is made during number entry, the display will return to the prompt for data number 1 and the entire sequence must be re–entered. If all numbers are correctly entered, the display will return to the method prompt and is ready for use:
METHOD #?

23. Once the new method 361 has been successfully entered, block access to the now obsolete method 360. Press:

Press:

Within 3 seconds press:

The display will show:
800 CONFIGURE

Press **READ/ENTER** three times to return to:
METHOD #?

Access to method 360 is blocked. Method 361 is now a stored method.

SAMPLING AND STORAGE

Collect samples in clean plastic or glass bottles. Store at 4 °C (39 °F) or lower if the sample is to be analyzed within 24 to 48 hours. Warm to room temperature before running the test. For longer storage periods, up to 14 days, adjust sample pH to 2 or less with sulfuric acid, ACS, (about 2 mL per liter). Sample refrigeration is still required.

Before testing the stored sample, warm to room temperature. Neutralize the sample with 5.0 N Sodium Hydroxide Standard Solution.

Do not use mercury compounds as preservatives.

Correct test results for volume additions *(see Correction for Volume Additions in Section I).*

Appendix C-4 Analysis method for Nitrate, High Range (HR) test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 293-301. (1996). (page 8 of 9)

NITRATE, HR, continued

ACCURACY CHECK

Standard Additions Method

a) Snap the neck off a fresh High Range Nitrate Nitrogen Voluette Ampule Standard, 500 mg/L NO_3^--N.

b) Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard to three 25–mL samples. Mix each thoroughly. (For AccuVac ampuls, use 50–mL beakers.)

c) Analyze each sample as described above. The nitrogen concentration should increase 2.0 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see *Standard Additions* in *Section I* for more information.

Standard Solution Method

Use a 10.0 mg/L Nitrate Nitrogen Standard Solution listed under *Optional Reagents* to check test accuracy. Or, this can be prepared by diluting 1.00 mL of solution from a High Range Nitrate Nitrogen Voluette Ampule Standard Solution, 500 mg/L NO_3^-–N, to 50.0 mL with demineralized water.

PRECISION

In a single laboratory, using standard solutions of 20.0 mg/L nitrate nitrogen (NO_3^--N) and two representative lots of reagent with the DR/2000, a single operator obtained a standard deviation of ±0.8 mg/L nitrate nitrogen.

Using standard solutions of 30.0 mg/L (NO_3^-–N) and one representative lot of AccuVac ampuls with the DR/2000, a single operator obtained a standard deviation of ±2.3 mg/L nitrate nitrogen.

INTERFERENCES

Compensate for nitrite interference as follows:

a) Add Bromine Water, 30 g/L, drop–wise to the sample in Step 4 until a yellow color remains.

b) Add one drop of Phenol Solution, 30 g/L, to destroy the color.

c) Proceed with Step 4. Report results as total nitrate and nitrite.

Strong oxidizing and reducing substances will interfere. Ferric iron causes high results and must be absent. Chloride concentrations above 100 mg/L will cause low results. The test may be used at high chloride levels (i.e., seawater), but a calibration must be performed using standards spiked to the same chloride concentration. See *User Stored Programs* in the *DR/2000 Instrument Manual* for more information.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment *(see pH Interference in Section I)*.

SUMMARY OF METHOD

Cadmium metal reduces nitrates present in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. This salt couples to gentisic acid to form an amber–colored product. Nitrate can be determined directly using the Nitrate Ion Selective Electrode (Cat. No. 44560–71).

REQUIRED REAGENTS (Using Powder Pillows)

Description	Quantity Required Per Test	Units	Cat. No.
NitraVer 5 Nitrate Reagent Powder Pillows	1 pillow	50/pkg	14034–66

REQUIRED REAGENTS (Using AccuVac Ampuls)

NitraVer 5 Nitrate Reagent AccuVac Ampul	1 ampul	25/pkg	25110–25

REQUIRED APPARATUS (Using Powder Pillows)

Clippers, for opening powder pillows	1	each	968–00
Stopper, rubber, size 2	1	12/pkg	2118–02

REQUIRED APPARATUS (Using AccuVac Ampuls)

Adapter, AccuVac Vial	1	each	43784–00
Beaker, 50 mL	1	each	500–41
Zeroing Vial	1	each	21228–00

NITRATE, HR, continued

OPTIONAL REAGENTS

Bromine Water, 30 g/L	29 mL*	2211–20
Nitrate Nitrogen standard Solution, 10 mg/L NO_3^--N	500 mL	307–49
Nitrate Nitrogen Standard Solution, Voluette Ampule, 500 mg/L (NO_3^--N), 10 mL	16/pkg	14260–10
Phenol Solution, 30 g/L	29 mL	2112–20
Sodium Hydroxide Standard Solution, 5.0 N	59 mL*	2450–26
Sulfuric Acid, ACS	500 mL*	979–49
Water, demineralized	4 L	272–56

OPTIONAL APPARATUS

AccuVac Snapper Kit	each	24052–00
Ampule Breaker Kit	each	21968–00
Cylinder, graduated, 25 mL	each	1081–40
Dropper, for 1–oz bottle	each	2258–00
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391–33
Pipet, serological, 2 mL	each	532–36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700–01
Pipet Tips, for 19700–01 TenSette Pipet	50/pkg	21856–96
Pipet, volumetric, 1.0 mL, Class A	each	14515–35
Pipet Filler, safety bulb	each	14651–00
Pour–Thru Cell Assembly Kit	each	45215–00
Sample Cells, 1–inch, polystyrene, disposable	12/pkg	24102–12

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes

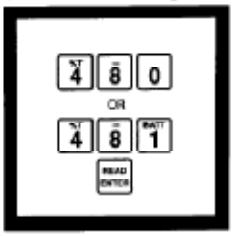
Appendix C-5: Analysis method for Phosphorus, Reactive test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 511-522. (1996). (page 1 of 12)

Source: HACH. (1996). DR/2000 Spectrophotometer Instrument Manual (7-21-95 3rd ed., Rev. 2, 01/96).U.S.A.: Hach Company.

Method 8114

PHOSPHORUS, REACTIVE (0 to 45.0 mg/L PO_4^{3-}) For water and wastewater

(also called orthophosphate) Molybdovanadate Method*



1. Enter the stored program number for reactive phosphorus, molybdovanadate method.

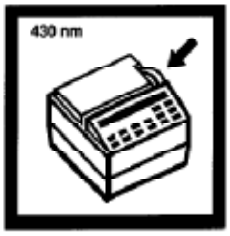
Press: **4 8 0 READ/ENTER** for units of mg/L PO_4^{3-}
OR
Press: **4 8 1 READ/ENTER** for units of mg/L P

The display will show:
DIAL nm to 430

Note: *DR/2000s with software versions 5.0 and greater will display "P" and the program number.*

Note: *Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.*

Note: *If sample cannot be analyzed immediately, see* Sampling and Storage *following these steps.*



2. Rotate the wavelength dial until the small display shows:
430 nm

3. Press: **READ/ENTER**

The display will show:
mg/l PO_4^{3-} MoV
OR
mg/l P MoV

4. Fill a sample cell (the blank) with 25 mL of demineralized water with a 25-mL graduated cylinder.

*Adapted from *Standard Methods for the Examination of Water and Wastewater*

Appendix C-5: Analysis method for Phosphorus, Reactive test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 511-522. (1996). (page 2 of 12)

PHOSPHORUS, REACTIVE, continued

5. Fill a second sample cell (the prepared sample) with 25 mL of sample with a 25–mL graduated cylinder.

Note: For proof of accuracy, use a 10.0 mg/L phosphate (3.3 mg/L phosphorus) standard solution (preparation given in the Accuracy Check) in place of the sample.

6. Add 1.0 mL of Molybdovanadate Reagent to each sample cell. Swirl to mix.

Note: A yellow color will form if phosphate is present. A small amount of yellow will be present in the blank because of the reagent.

7. Press: **SHIFT TIMER**

A 3–minute reaction period will begin.

Note: If the sample concentration is greater than 24 mg/L PO_4^{3-}, read at exactly 3 minutes or make a 1:1 dilution of the sample.

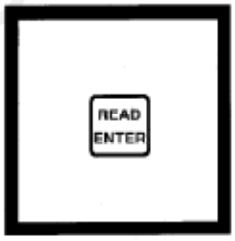
8. When the timer beeps, the display will show:
mg/l PO_4^{3-} MoV
OR
mg/l P MoV
Place the blank into the cell holder. Close the light shield.

Note: The Four–Thru Cell can be used with this procedure.

9. Press: **ZERO**

The display will show:
WAIT
then:
0.0 mg/l PO_4^{3-} MoV
OR
0.0 mg/l P MoV

10. Place the prepared sample into the cell holder. Close the light shield.



11. Press: **READ/ENTER**

The display will show:
WAIT
then the result in mg/L PO_4^{3-} or mg/L P will be displayed.

Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

Note: Phosphorus Conversions
mg/L X PO_4^{3-} = mg/L P X 3.07
mg/L P_2O_5 = mg/L P X 2.25
mg/L P_2O_5 = mg/L PO_4^{3-} X 0.75

Appendix C-5: Analysis method for Phosphorus, Reactive test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 511-522. (1996). (page 3 of 12)

PHOSPHORUS, REACTIVE, continued

SAMPLING AND STORAGE

Collect samples in clean plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution and rinsed with demineralized water. Do not use a commercial detergent because the phosphate content will contaminate the sample.

If samples cannot be analyzed the same day, adjust the pH to 2 or less by adding about 2 mL of sulfuric acid, ACS, per liter of sample. Store the sample at 4 °C (39 °F) or below. Samples can be stored up to 24 hours. For longer storage periods, add 4.0 mL of Mercuric Chloride Solution for each liter of sample taken and mix. Use of mercuric chloride is discouraged to minimize the amount of mercury released to the environment. Sample refrigeration is still required. Sample preserved with mercuric chloride must be spiked with 0.1 g sodium chloride level to 50 mg/L or more if the sample is low in chloride. The addition of chloride prevents mercury interference in the test.

Before analysis, adjust the acidified sample to about pH 7 by adding 5.0 N Sodium Hydroxide Standard Solution. Mix thoroughly. Warm to room temperature before analyzing.

ACCURACY CHECK

Standard Additions Method

a) Snap the neck off a Phosphate Voluette Ampule Standard Solution, 500 mg/L as PO_4^{3-}.

b) Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to three 25-mL water samples. Mix well.

c) Analyze each sample as described in the procedure and compare the results with that of the original test sample. Each 0.1–mL addition of standard should cause an increase of 2.0 mg/L PO_4^{3-} or 0.67 mg/L P.

d) If these increases do not occur, see Standard Additions in Section I for more information.

Standard Solution Method

A 10.0 mg/L phosphate standard can be prepared by pipetting 10.0 mL of a Phosphate Standard Solution, 50 mg/L PO_4^{3-}, into a 50 mL volumetric flask. Dilute to volume with demineralized water.

Wavelength Check

This test is sensitive to the wavelength setting. To ensure accuracy, the test should be run on a 10–mg/L standard solution and blank. Repeat Step 8 to 11 at slightly different wavelengths, setting the dial from higher to lower values until the correct result is obtained. The wavelength should be 430 ±2 nm. Always set this wavelength from high to low values.

PRECISION

In a single laboratory, using standards of 20.0 mg/L PO_4^{3-}, two lots of reagent and the DR/2000, a single operator obtained a standard deviation of ±0.08 mg/L PO_4^{3-}.

INTERFERENCES

Sulfide interference may be removed by oxidation with Bromine Water as follows:

a) Measure 25 mL of sample into a sample cell.

b) Add Bromine Water drop–wise with constant swirling until permanent yellow color develops.

c) Add Phenol Solution drop–wise until the yellow color just disappears. Proceed with Step 5 using this treated sample.

Positive interferences are caused by silica and arsenate only if the sample is heated. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate or excess molybdate. Blue color is caused by ferrous iron but this does not affect results if ferrous iron concentration is less than 100 mg/L. Ions that do not interfere in concentrations up to 1000 mg/L are pyrophosphate, molybdate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, salicylate, Al^{3+}, Fe^{3+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Sr^{3+}, Li^{+}, Na^{+}, K^{+}, NH_4^{+}, Cd^{2+}, Mn^{2+}, NO_3^{-}, NO_2^{-}, SO_4^{2-}, SO_3^{2-}, Pb^{2+}, Hg^{+}, Hg^{2+}, Sn^{2+}, Cu^{2+}, Ni^{2+}, Ag^{+}, Ti^{4+}, Zr^{4+}, AsO_3^{-}, Br^{-}, CO_3^{2-}, ClO_4^{-}, CN^{-}, IO_3^{-}, SiO_4^{4-}.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment *(see pH Interference in Section I)*.

SUMMARY OF METHOD

In the molybdovanadate method, orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to the phosphate concentration.

PHOSPHORUS, REACTIVE, continued

REQUIRED REAGENTS

Description	Quantity Required Per Test	Units	Cat. No.
Molybdovanadate Reagent	2.0 mL	100 mL* MDB	20760–32
Water, demineralized	25 mL	4 L	272–56

REQUIRED APPARATUS

Cylinder, graduated, 25 mL	1	each	508–40

OPTIONAL REAGENTS

Bromine Water	29 mL*	2211–20
Hydrochloric Acid Solution, 1:1	500 mL	884–49
Mercuric Chloride Solution, 10 g/L	100 mL	14994–42
Phenol Solution, 30 g/L	29 mL	2112–20
Phosphate Standard Solution, 50 mg/L as PO_4^{3-}	500 mL	171–49
Phosphate Standard Solution, Voluette ampule, 500 mg/L as PO_4^{3-}, 10 mL	16/pkg	14242–10
Sodium Chloride, ACS	454 g	182–01
Sodium Hydroxide Standard Solution, 5.0 N	100 mL** MDB	2450–32
Sulfuric Acid, ACS	500 mL*	979–49

OPTIONAL APPARATUS

Ampule Breaker Kit	each	21968–00
Dispenser, fixed volume, 1.0 mL Repipet Jr.	each	21113–02
Flask, erlenmeyer, 50 mL	each	505–41
Flask, volumetric, Class A, 50 mL	each	14574–41
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391–33
pH Meter, EC10, portable	each	50050–00
Pipet, serological, 2.0 mL	each	532–36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700–01
Pipet Tips, for 19700–01 TenSette Pipet	50/pkg	21856–96
Pipet, volumetric, Class A, 10.00 mL	each	14515–38
Pipet Filler	each	12189–00
Pour–Thru Cell Assembly Kit	each	45215–00
Spoon, measuring, 0.1 g	each	511–00

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Contact Hach for larger sizes

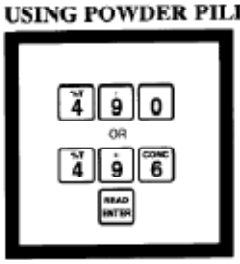
Appendix C-5: Analysis method for Phosphorus, Reactive test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 511-522. (1996). (page 5 of 12)

Method 8048

PHOSPHORUS, REACTIVE (0 to 2.50 mg/L PO_4^{3-}) For water, wastewater and seawater

(also called Orthophosphate) PhosVer 3 (Ascorbic Acid) Method* (Powder Pillows or AccuVac Ampuls), USEPA accepted for reporting**

USING POWDER PILLOWS



1. Enter a stored program number for reactive phosphorus powder pillows.

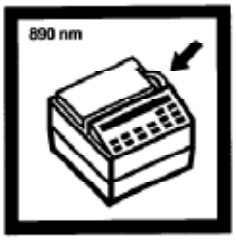
Press: **4 9 0 READ/ENTER** for units of mg/L PO_4^{3-}
OR
Press: **4 9 6 READ/ENTER** for units of mg/L P

The display will show:
DIAL nm TO 890

Note: *DR/2000s with software versions 3.0 and greater will display "P" and the program number.*

Note: *Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.*

Note: *Measurement range for P is 0 to 0.83 mg/L*



2. Rotate the wavelength dial until the small display shows:
890 nm

Note: *For instruments with software versions that do not have stored program method 496, refer to* Instrument Setup *following these steps.*

3. Press: **READ/ENTER**

The display will show:
mg/l PO_4^{3-} PV
OR
mg/l P PV

4. Fill a sample cell with 25 mL of sample.

Note: *For proof of accuracy, use a 1.0 mg/L Phosphate (0.33 mg/L P) Standard Solution listed under* Optional Reagents *in place of the sample.*

*Adapted from *Standard Methods for the Examination of Water and Wastewater*
** Procedure is equivalent to USEPA method 365.2 and Standard Method 4500–P–E for wastewater.

515

Appendix C-5: Analysis method for Phosphorus, Reactive test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 511-522. (1996). (page 6 of 12)

PHOSPHORUS, REACTIVE, continued

5. Add the contents of one PhosVer 3 phosphate Powder Pillow to the sample cell (the prepared sample). Swirl immediately to mix.

Note: A blue color will form if phosphate is present.

6. Press: **SHIFT TIMER**

A 2–minute reaction period will begin.

Note: An 8–10 minute reaction period should be used if determining total phosphate following the acid–persulfate digestion.

Note: If the sample temperature is less than 15 °C (59 °F), allow 4 minutes of reaction time.

7. Fill another sample cell (the blank) with 25 mL of sample. Place it into the cell holder.

Note: The Pour–Thru Cell can be used with this procedure.

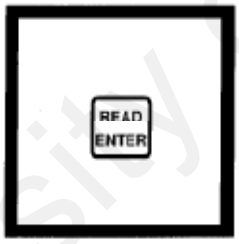
8. When the timer beeps, the display will show:
mg/l P PV

Press: **ZERO**

The display will show:
WAIT
then:
0.00 mg/l PO_4^{3-} PV
OR
0.00 mg/l P PV

9. Place the prepared sample into the cell holder. Close the light shield.

Note: Run a reagent blank for this test. Use demineralized water in place of the sample in Step 4. Subtract this result from all test results run with this lot of PhosVer 3.



10. Press: **READ/ENTER**

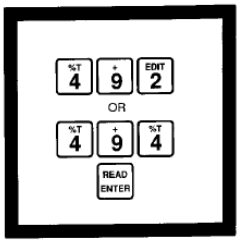
The display will show:
WAIT
then the results in mg/L PO_4^{3-} or mg/L P will be displayed.

Note: mg/L PO_4^{3-} results can be expressed as mg/L phosphorus by dividing by 3 or as mg/L phosphorus pentoxide (P_2O_5) by multiplying by 0.75.

Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

PHOSPHORUS, REACTIVE, continued

USING ACCUVAC AMPULS



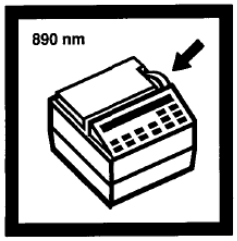
1. Enter the stored program number for reactive phosphorus AccuVac ampuls.

Press: **4 9 2 READ/ENTER** for units of mg/L PO_4^{3-}
OR
Press: **4 9 4 READ/ENTER** for units of mg/L P

The display will show:
DIAL nm TO 890

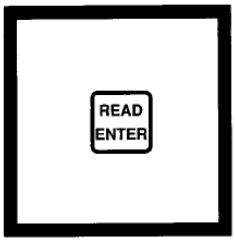
Note: *DR/2000s with software versions 3.0 and greater will display "P" and the program number.*

Note: *Instruments with software versions 3.0 and greater will not display "DIAL nm TO" message if the wavelength is already set correctly. The display will show the message in Step 3. Proceed with Step 4.*



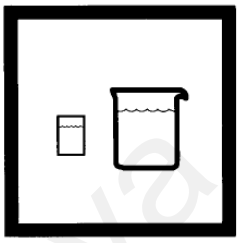
2. Rotate the wavelength dial until the small display shows:
890 nm

Note: *For instruments with software versions that do not have stored program method 494, refer to* Instrument Setup.



3. Press: **READ/ENTER**

The display will show:
mg/l PO_4^{3-} PV AV
OR
mg/l P PV AV



4. Fill a zeroing vial (the blank) with at least 10 mL of sample. Collect at least 40 mL of sample in a 50–mL beaker.

Appendix C-5: Analysis method for Phosphorus, Reactive test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 511-522. (1996). (page 8 of 12)

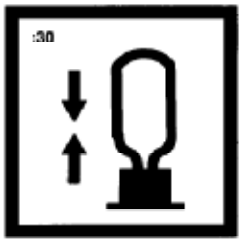
PHOSPHORUS, REACTIVE, continued

5. Place the AccuVac Vial Adapter into the cell holder.

Note: Place the grip tab at the rear of the cell holder.

6. Fill a PhosVer 3 Phosphate AccuVac ampul with sample.

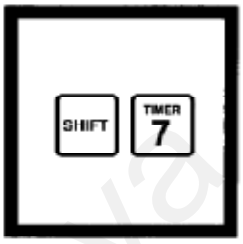
Note: Keep the tip immersed while the ampul fills completely.



7. Place an ampul cap securely over the tip of the ampul. Shake the ampul for approximately 30 seconds. Wipe off any liquid and fingerprints.

Note: A blue color will form if phosphate is present.

Note: Accuracy is unaffected by undissolved powder.



8. Press: **SHIFT TIMER**

A 2–minute reaction period will begin.

9. When the timer beeps, the display will show:

mg/l PO_4^{3-} PV AV

OR

mg/l P PV AV

Place the zeroing vial into the cell holder.

10. Press: **ZERO**

The display will show:

WAIT

then:

0.00 mg/l PO_4^{3-} PV AV

OR

0.00 mg/l P PV AV

11. Place the AccuVac ampul into the cell holder.

Note: Run a reagent blank for the test. Use demineralized water in place of the sample in Step 4. Subtract this result from all results with this lot of ampuls.

12. Press: **READ/ENTER**

The display will show:

WAIT

then the result in mg/L PO_4^{3-} or mg/L P will be displayed.

Note: mg/L PO_4^{3-} results can be expressed as mg/L phosphorus by dividing by 3 or as mg/L phosphorus pentoxide (P_2O_5) by multiplying by 0.75.

Note: In the constant–on mode, pressing READ/ENTER is not required. WAIT will not appear. When the display stabilizes, read the result.

PHOSPHORUS, REACTIVE, continued

INSTRUMENT SETUP

For a DR/2000 with a software version before 2.0 which does not have Phosphorus, Reactive, Ascorbic Acid Method, Stored Method 496 and Stored Method 494, enter the following calibrations as operator–programmed calibrations. Follow the steps in the *Operation* section of the *DR/2000 Instrument Manual*. Store the method as follows:

Stored Method 496	**Stored Method 494**
nm = 890	nm = 890
Decimal = 00.00	Decimal = 00.00
Units = mg/l	Units = mg/l
Symbol = P PV	Symbol = p PV AV
Timer 1 = 02:00	Timer 1 = 02:00

The calibrations are first entered with 0.000 absorbance values for zero and #1 standards. To do this, leave the sample cell compartment empty, and begin by storing zero and #1 standard as concentrations of 0 and 0.79 mg/L, respectively, for Stored Method 496 and 0 and 0.77 mg/L for Stored Method 494. Accept 0.000 Abs as the value for all standards. Next, the values for the zero standard and #1 standard must be changed to the values given below:

Stored Method 496

Standard	Absorbance	Concentration
0	0.019	0
1	1.375	0.79

Stored Method 494

Standard	Absorbance	Concentration
0	0.003	0
1	1.250	0.77

The methods are now stored as operator programmed methods with numbers between 950 and 999. Record the method numbers for future reference.

For a DR/2000 with a software version of 2.0 or 2.2 that does not have the Phosphorus, Reactive, Ascorbic Acid Method, Stored Method, Stored Methods #496 and #494, enter the calibrations as Hach–entered programs.

1. Press: I/O

2. Press: SHIFT, CONFIG METH

3. Press: %T 4, + 9, CONFIG 6, READ ENTER

OR

Press: %T 4, + 9, %T 4, READ ENTER

4. Within 3 seconds, press: SHIFT, PRGM 3, CONFIG METH

The display will show:

ENTER nm

If the display returns to the METHOD prompt, repeat the sequence.

5. Press: − 8, + 9, 0

Note: If an error is made, press SHIFT, CLEAR and re–enter the number. When the number is correct, press READ/ENTER.

The display will show:

DECIMAL? 00.00

6. The decimal point is already correctly positioned. Press: **READ/ENTER.** The display will read:

UNITS?

7. Use the arrow keys to select the appropriate unit of measure. For this method, press the **DOWN ARROW** key twice. The display will show:

mg/l

8. With the proper unit of measure displayed, press **READ/ENTER.** The display will read:

SYMBOL?

9. Use the arrow keys to construct the correct symbol display. For this method, press the **DOWN ARROW** key repeatedly until the display reads:

mg/l p

10. Press **SHIFT** to make the "p" upper case. The display will show:

mg/l P

11. Press **READ/ENTER** to accept the capital "P". Continue to construct the display to read:

mg/l P PV

OR

mg/l P PV AV

PHOSPHORUS, REACTIVE, continued

The space is the "character" displayed after one press of the **DOWN ARROW** key.

12. When the last character of the symbol is accepted with the **READ/ENTER** key, the display will show:

TIMER?

There is one time selection for this method. Press **SHIFT, TIMER.** The display will read:

MM:SS TIME 1?

13. To enter the first timer value of 02:00 minutes, press:

The display will read:

02:00 TIME 1?

14. Press **READ/ENTER** to accept the value. The display will then read:

MM:SS TIME 2?

15. Press **READ/ENTER** to complete timer entry. The display will show:

 # 1 DATA 0

16. Enter the following 12 numbers as shown. Complete each number with the **READ/ENTER** key.

Stored Method 496

# 1 Data	0
# 2 Data	1544
# 3 Data	1799
# 4 Data	1800
# 5 Data	1799
# 6 Data	2055
# 7 Data	1799
# 8 Data	65535
# 9 Data	65535
# 10 Data	3276
# 11 Data	512
CHECKSUM	50954

Stored Method 494

# 1 Data	0
# 2 Data	1800
# 3 Data	2056
# 4 Data	2055
# 5 Data	2056
# 6 Data	2055
# 7 Data	2303
# 8 Data	65535
# 9 Data	65535
# 10 Data	3276
# 11 Data	512
CHECKSUM	49425

The final number in each group is a check value which is used to determine that the data sequence was correctly entered. If an error is made during number entry, the display will return to the prompt for data number 1 and the entire sequence must be re-entered. If all numbers are correctly entered, the update procedure is complete and the display will return to the method prompt:

METHOD?

With the new method 496 and 494 successfully entered, block access to the now obsolete methods 491 and 493.

Press:

Within 3 seconds, press:

Press:

Within 3 seconds, press:

Access to methods 491 and 493 are now blocked.

SAMPLING AND STORAGE

Collect sample in plastic or glass bottles that have been cleaned with 1:1 Hydrochloric Acid Solution and rinsed with demineralized water. Do not use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis. Most reliable results are obtained when samples are analyzed as soon as possible after collection. If prompt analysis is impossible, preserve samples up to 24 hours by storing at or below 4 °C. For longer storage periods, add 4.0 mL of Mercuric Chloride Solution to each liter of sample taken and mix. Use of mercuric chloride is discouraged whenever possible for health and environmental considerations. Sample refrigeration is still required. Samples preserved with mercuric chloride must have a sodium chloride level of 50 mg/L or more to prevent mercury interference. Samples low in chloride should be spiked with 0.1 g sodium chloride per liter of sample.

PHOSPHORUS, REACTIVE, continued

ACCURACY CHECK

Standard Additions Method

a) Snap the neck off a Phosphate Voluette Ampule Standard Solution, 50 mg/L PO_4.

b) Use the TenSette Pipet to add 0.1 mL, 0.2 mL and 0.3 mL of standard, respectively, to three 25–mL water sample. Mix each thoroughly. (For AccuVac ampuls use 50–mL beakers.)

c) Analyze each sample as described above. The phosphate concentration should increase 0.2 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see *Standard Additions* in *Section I*.

INTERFERENCES

Large amounts of turbidity may cause inconsistent results in the phosphate tests because the acid present in the powder pillow may dissolve some of the suspended particles and because of variable desorption of orthophosphate from the particles. For highly turbid or colored samples, add the contents of one Phosphate Pretreatment Powder Pillow to 25 mL of sample. Mix well. Use this solution to zero the instrument.

The PhosVer 3 Reagent Powder Pillows shoud be stored in a cool, dry place.

The following may interfere when present in concentrations exceeding these listed below:

Aluminum	200 mg/L
Chromium	100 mg/L
Copper	10 mg/L
Iron	100 mg/L
Nickel	300 mg/L
Silica	50 mg/L
Silicate	10 mg/L
Zinc	80 mg/L

Arsenate and hydrogen sulfide interfere.

Highly buffered samples or extreme sample pH may exceed the buffering capacity of the reagents and require sample pretreatment *(see pH Interference in Section I)*.

PRECISION

In a single laboratory, using a standard solution of 1.00 mg/L PO_4^{3-} and two lots of reagent with a DR/2000, a single operator obtained a standard deviation of ±0.01 mg/L PO_4^{3-}.

In a single laboratory, using a standard solution of 1.00 mg/L PO_4^{3-} and two representative lots of AccuVac ampuls with the DR/2000, a single operator obtained a standard deviation of ±0.02 mg/L PO_4^{3-}.

SUMMARY OF METHOD

Orthophosphate reacts with molybdate in an acid medium to produce a phosphomolybdate complex. Ascorbic acid then reduces the complex, giving an intense molybdenum blue color.

REQUIRED REAGENTS (Using Powder Pillows)

Description	Quantity Required Per Test	Units	Cat. No.
PhosVer 3 Phosphate Reagent Powder Pillows	1 Pillow	100/pkg	2125–99

REQUIRED REAGENTS (Using AccuVac Ampuls)

PhosVer 3 Phosphate Reagent AccuVac Ampuls	1 ampul	25/pkg	25080–25

REQUIRED APPARATUS (Using Powder Pillows)

Clippers, for opening Powder pillows	1	each	968–00

REQUIRED APPARATUS (Using Powder Pillows)

Adapter, AccuVac Vial	1	each	43784–00
Beaker, 50 mL	1	each	500–41
Cap, ampul, blue	1	6/pkg	1731–06
Vial, zeroing	1	each	21228–00

Appendix C-5: Analysis method for Phosphorus, Reactive test - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 511-522. (1996). (page 12 of 12)

PHOSPHORUS, REACTIVE, continued

OPTIONAL REAGENTS

Hydrochloric Acid Standard Solution, 6.0 N (1:1)	500 mL	884–49
Mercuric Chloride Solution, 10 g/L	100 mL	14994–42
Phosphate Pretreatment Powder Pillows	50/pkg	14501–66
Phosphate Standard Solution, 1 mg/L as PO_4^{3-}	500 mL	2569–42
Phosphate Standard Solution, Voluette ampul, 50 mg/L as PO_4, 10 mL	16/pkg	171–10
Sodium Chloride, ACS	454 g	182–01
Sodium Hydroxide Standard Solution, 5.0 N	100 mL* MDB	2450–32
Water, demineralized	4 L	272–56

OPTIONAL APPARATUS

AccuVac Snapper Kit	each	24052–00
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391–33
pH Meter, EC10, portable	each	50050–00
Pipet, 2 mL serological	each	532–36
Pipet, TenSette, 0.1 to 1.0 mL	each	19700–01
Pipet Tips, for 19700–01	50/pkg	21856–96
Pipet Filler, safety bulb	each	14651–00
Spoon, measuring, 0.1 g	each	511–00

For additional ordering information, see final section.
In the U.S.A. call 800–227–4224 to place an order.

*Larger sizes available.

APPENDIX D

Maintenance for Spectrophotometer

Appendix D-1: Maintenance – Cleaning for Spectrophotometer, Sample Cells, and Pour-Thru Sample Cell; Replacement Instructions: Battery Replacement, and Lamp Replacement; Lamp Calibration Adjustment - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 43-44. (1996). (page 1 of 2)

Source: HACH. (1996). *DR/2000 Spectrophotometer Instrument Manual* (7-21-95 3rd ed., Rev. 2, 01/96).U.S.A.: Hach Company.

SECTION 5 MAINTENANCE

5.1 Cleaning

5.1.1 Spectrophotometer

The spectrophotometer and sample cells should be kept clean at all times and spills should be wiped up promptly. The photocell window, located on the left-hand side of the cell holder, can be wiped with lens tissue or a soft, lint-free cloth that will not leave an oil film.

5.1.2 Sample Cells

Sample cells should be cleaned with detergent, rinsed several times with tap water and then rinsed thoroughly with demineralized water. Sample cells used with organic solvents (chloroform, benzene, toluene, etc.) should be rinsed with acetone before the detergent wash and again as a final rinse before drying. Polystyrene disposable sample cells are available. Refer to *Section 7 "Replacement Parts and Accessories."*

5.1.3 Pour-Thru Sample Cell

Remove the Pour-Thru cell occasionally to check for accumulation of film on the windows. If the windows appear dirty or hazy, soak in a detergent bath and then rinse thoroughly with demineralized water. Do not use solvents (e.g., acetone) to clean the Pour-Thru cell. The Pour-Thru cell can be disassembled for cleaning if necessary.

CAUTION

Do not use the Pour-Thru Cell in tests that call for the use of organic solvents such as toluene, chloroform, trichloroethane or cyclohexanone. These solvents may not be compatible with the plastic components of the Pour-Thru Cell and create the potential for equipment damage and chemical exposure for the analyst.

ADVERTENCIA

No utilice la Célula de Flujo Continuo para pruebas que requieran el uso deo solventies orgánicos tales como tolueno, cloroformo, tirelorotano o ciclohexanona. Es posible que estos solventes sean incompatibles con los componenetes de material plástico de la Célula de Flujo Continuo y existe el riesgo de daños al equipo y exposición del analista a las substancias químicas.

AVISO

Não use a Pilha de Vazamento em teste que exigem o uso de dissolventes orgânicos como tolueno, cloroformo, tricloroetano e ciclohexanona. Existe a possibilidade que estes dissolventes não dejam compatíveis com os componentes de plástico da Pilha de Vazamento, o que pode criar a possibilidade de estrago ao equipamento e exposição química para o analista.

ATTENTION

Ne pas utiliser la cuve à circulation dans les techniques d'analyses qui utilisent des solvants organiqus tels que le toluène, le chloroforme, le trichloroéthane ou la cyclohexanone. Les solvants organiques peuvent ne pas être compatibles avec les composants en plastique de la cuve à circulation et endommager l'équipement en creant un risque chimique pour l'opérateur.

WARNHINWEIS

Die "Pout-Thru-Zelle" darf nicht in Tests verwendet werden, die organische Lösungsmittel wie Toluol, Chloroform, Trichlorocthan oder Cyclohexanon erfordern. Die Möglichkeit besteht, daß diese Lösungsmittel nicht mit den Kunststoffkomponenten der "Pour-Thru-Zelle" kompatibel sind und somit Geräteschaden verursachen und eine Chemikaliengefahr für den Untersuchungschemiker darstellen konnen

5.2 Replacement Instructions

5.2.1 Battery Replacement

The battery power source must be replaced or recharged whenever a **LOW BATTERY** message appears in the display. If alkaline D-size dry cell batteries are being used, all six should be replaced, and if a rechargeable battery is installed, recharge as soon as possible. Refer to *Section 4.3* for battery installation instructions for D-cell replacement.

5.2.2 Lamp Replacement

If the lamp fails and must be replaced as determined by information in *Section 6.2.4*, proceed as follows:

1. Disconnect power to the instrument and empty the cell holder. Place the instrument upside down on a padded surface.

Appendix D-1: Maintenance – Cleaning for Spectrophotometer, Sample Cells, and Pour-Thru Sample Cell; Replacement Instructions: Battery Replacement, and Lamp Replacement; Lamp Calibration Adjustment - according to the DR/2000 Spectrophotometer Instrument Manual, pp. 43-44. (1996). (page 2 of 2)

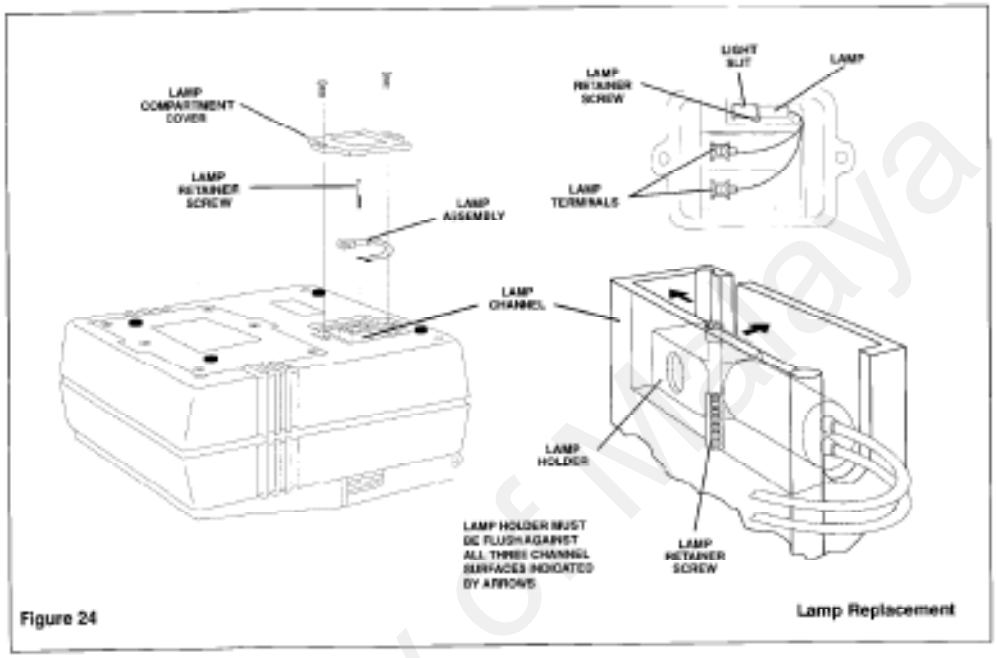


Figure 24 Lamp Replacement

2. Remove the two screws securing the lamp compartment cover and remove the cover (*see Figure 24 Lamp Replacement*).

3. Remove the lamp retainer screw and metal sleeve from the lamp channel. The sleeve is tapered at the bottom end and, because of its snug fit, probably will need to be loosened with a tool (needle-nose pliers recommended) for removal. Remove the lamp. Loosen the two terminal screws to free the lamp leads.

4. Place the new lamp in the lamp channel with the lamp light slit toward the light slit in the lamp channel. Push the lamp to the bottom of the lamp channel and install and firmly tighten the lamp retainer screw and sleeve in the channel to secure the lamp. The sleeve must be installed with the beveled end down. The lamp must be held tightly in the proper position, flush against the bottom, the end, and the light slit side of the lamp channel (*Figure 24 Lamp Replacement*). Connect the lamp leads of the new lamp assembly at the lamp terminals (lead orientation does not matter). Do not overtighten.

5. Install the lamp compartment cover. Return the instrument to the upright position and restore power.

6. Perform the Lamp Calibration Adjustment procedure described in *Section 5.3*.

5.3 Lamp Calibration Adjustment

1. Select the Constant On mode. Refer to *3.4.1 Constant On/Momentary Mode Selection*. Select the percent transmittance mode by pressing **SHIFT**, **%T**.

2. Empty the cell compartment and close the cover. Adjust the wavelength control to approximately 850 nm. Press the **ZERO** key. The display shows 100.0 %T.

3. Place the calibration filter assembly into the sample compartment with the orientation projection aligned with the notch in the instrument case. Close the cover.

4. Using the wavelength control on the side of the instrument, begin at 850 nm and slowly adjust the wavelength dial counterclockwise