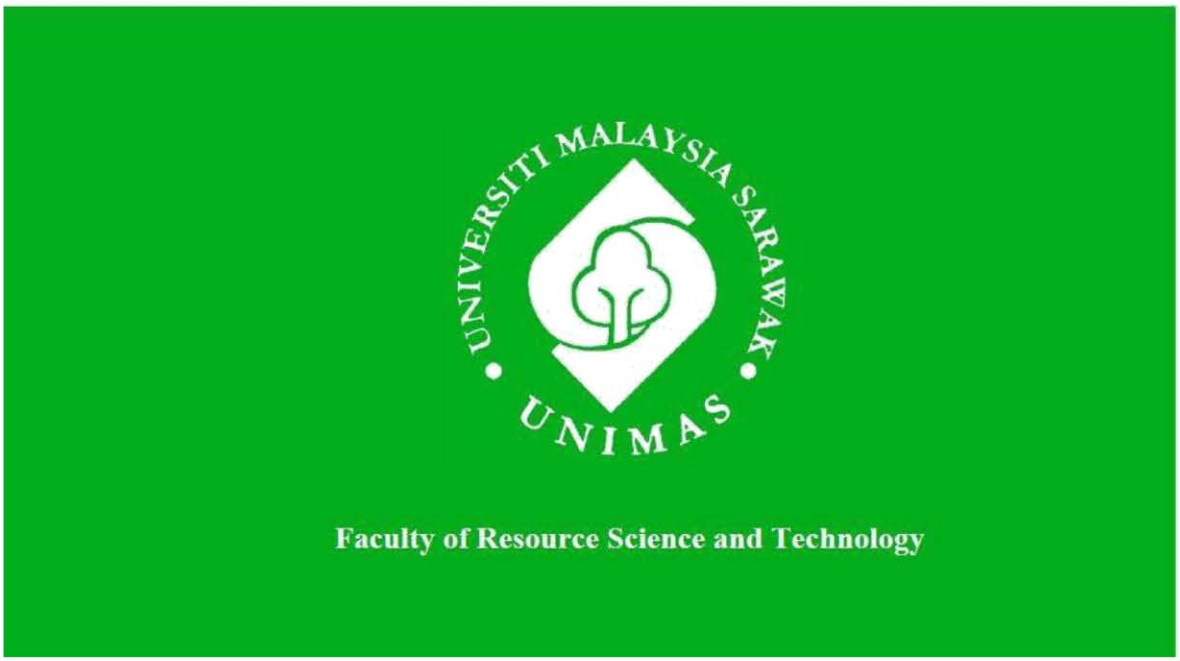

GROWTH OF *Rhizopus oligosporus* IN SAGO EFFLUENT AT DIFFERENT PH

Nur Aqlily Riana binti Nasar

Bachelor of Science with Honours
(Resource Biotechnology)
2015

Growth of *Rhizopus oligosporus* in Sago Effluent at Different pH

Nur Aqlily Riana binti Nasar

(37883)

A report submitted in partial fulfillment of the Final Year Project 2 (STF3015)

Supervisor: Dr. Micky Vincent

Co-supervisor: Dr. Devagi Kanakaraju

Resource Biotechnology
Department of Molecular Biology
Faculty of Resource Science and Technology
University Malaysia Sarawak
2015

ACKNOWLEDGEMENT

Assalamualaikum, first and foremost, I would like to bring all the glory to Allah S.W.T. for His abundant love and grace in the process of completing my final year project. I also would like to thank my supervisor, Dr Micky Vincent and co-supervisor, Dr Devagi Kanakaraju for giving me this opportunity to carry out this Final Year Project under his supervision. He always assisted and gives opinion to all of us during our Final Year Project. The help and guidance given by him time to time shall carry me long way in the journey of life on which I am about to embark.

My deepest gratitude goes to my parents, Mr Nasar Ibrahim and Mrs Laila Deris and also my brothers, Mr Qayyum, Mr Qawiem and Mr Iyad for their love, prayer, encouragement and support from beginning till the end. The constant encouragement they give to keep me going and their love to empower me that never fails all the time without which this project would not be possible. Thank you for supporting me.

Besides that, I would like to express my appreciation to postgraduate student, Ms Fazidah for her patient guardian and opinion throughout my research period. She never fails to support us during the Final Year Project. I also would like to express my thanks to laboratory mates, Ms Nadia Shuhada, Ms Shahirah Safin, Ms Khairunnisa, Ms Fatiyah, Ms Ummi Syahida, Ms Lailatul Munirah for their support and opinion when I faced problem in my experiments. Not to forget my friends Ms Suhailah, Ms Aisyah, Ms Amira Shafiza and Ms Qeela for their emotional support, useful opinion throughout the experiment. I gratefully acknowledge UNIMAS for the complete and convenient laboratories facilities for all of the final year students to carry out experiment and completed this project.

Lastly, I dedicate my utmost gratitude to all individuals that have been indirectly contributed in this research. Your kindness means a lot to me. Thank you very much.

DECLARATION

I hereby declare this Final Year Project entitled **"Growth of *Rhizopus oligosporus* in Sago Effluent at Different pH"** is based on my original work except for quotations and citation, which have been duly acknowledged. I also declare that it has not been previously submitted for any other degree at UNIMAS or other institutions.

_____

(Nur Aqlily Riana binti Nasar)

JUNE 2015

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

R. oligosporus	*Rhizopus oligosporus*
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
g	Gram
h	Hour
L	Liter
min	Minute
s	Second
mL	Milliliter
NSP	Non-starch Polysaccharide
pH	Power of Hydrogen
rpm	Revolution per minute
SmF	Submerged Fermentation
SSF	Solid State Fermentation
TSS	Total Suspended Solid
YMB	Yeast Malt Broth
µm	Micrometre
˚C	Degree Celsius
%	Percentage

LIST OF TABLES

Page

LIST OF FIGURES

LIST OF APPENDICES

Growth of *Rhizopus oligosporus* in Sago Effluent at Different pH

Nur Aqlily Riana binti Nasar

Resource Biotechnology
Faculty of Resource Science and Technology
University Malaysia Sarawak

ABSTRACT

The discharge of large amounts sago effluent into the river leads to severe water pollution. A study on the growth of *R. oligosporus* in the sago effluent at different pH was carried out. *R. oligosporus* was grown for the treatment of sago effluent with the production of high protein fungal biomass (HPFB) via submerged fermentation (SmF). The objectives of this study were to investigate the optimum pH for the growth of *R. oligosporus* in sago effluent and the effectiveness of SmF by *R. oligosporus* for water treatment. Sago effluent was adjusted to pH 4, 5, 6 and one replicate with no adjustment of pH. The optimum pH for *R. oligosporus* was notable at pH 4. The highest wet and dry biomass production of pH 4 were 98.67 g/L and 3.80 g/L respectively. There were three analysis that were successfully carried out which were phenol sulphuric test, nitrate and nitrite analysis. There was 98% reduction of carbohydrate concentration in sago effluent. Besides, the nitrate concentration was reduced from 0.266 to 0.257 g/L in pH 4. Concentration of nitrite was decreased from 0.040 to 0.029 g/L in pH 4. In addition to the production of HPFB, the decreasing of starch, nitrate and nitrite concentration would lead to a potential benefits to the environment.

Keywords: *Rhizopus oligosporus*, sago effluent, submerged fermentation (SmF), high protein fungal biomass (HPFB), water treatment

ABSTRAK

Pembuangan efluen sagu yang tinggi ke dalam sungai membawa kepada pencemaran air yang membimbangkan. Satu kajian mengenai pertumbuhan R. oligosporus di dalam efluen sagu pada pH yang berbeza telah dijalankan. R. oligosporus ditumbuhkan untuk merawat efluen sagu bersama penghasilan biomas kulat tinggi protin melalui fermentasi substrat cecair. Objektif kajian ini adalah untuk mengenalpasti pH yang optimum untuk pertumbuhan R. oligosporus di dalam efluen sagu dan keberkesanan fermentasi substrat cecair oleh R. oligosporus untuk rawatan air. Efluen sagu ditukarkan kepada pH 4, 5, 6 dan terdapat satu replika efluen sagu yang mana pH tidak diubah. pH yang optimum dikesan pada pH 4. Terdapat tiga analisis yang berjaya dijalankan iaitu ujian fenol sulfurik, nitrat dan nitrit analisis. Terdapat pengurangan sebanyak 98% kepekatan karbohidrat di dalam efluen sagu. Selain itu, kepekatan nitrat berkurang dari 0.266 kepada 0.257 g/L dalam pH 4. Kepekatan nitrit berkurang dari 0.040 kepada 0.029 g/L dalam pH 4. Berikutan penghasilan biomas kulat tinggi protin, pengurangan kepekatan kanji, nitrat dan nitrit, akan membawa kepada keuntungan kepada alam sekitar.

Kata Kunci: Rhizopus oligosporus, efluen sagu, fermentasi substrat cecair, biomas kulat tinggi protin, rawatan air

CHAPTER 1

INTRODUCTION

1.1 Introduction

The main exporter for sago is Sarawak, which is located in east Malaysia. The largest plantation area for sago is in Mukah, Sarawak (Awg-Adeni *et al*., 2010). Sago palm is easily to plant because it can resist stress condition such as strong wind, fire, floods and drought (Singhal *et al*., 2008). Sago palm consist of large fibrous root system that function to remove pollutant, heavy metal, faecal toxin and trap slit loads (Singhal *et al*., 2008). According to Singhal *et al*. (2008), sago starch is widely used compared to other starches because it has low production cost and high yields. There are 73% of amylopectin and 27% of amylose in sago starch (Singhal *et al*., 2008). Sago effluent from factories is usually disposed into nearby rivers, which can lead to river water pollution (Awg-Adeni *et al*., 2010). Based on Awg-Adeni *et al.* (2010), sago effluent contains high Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Total Suspended Solid (TSS).

Submerged fermentation (SmF) can be defined as the process of microorganism's cultivation in nutrient broth in the form of liquid (Subramaniyam & Vimala, 2012). Based on Subramaniyam and Vimala (2012), SmF is different from solid state fermentation (SSF) because SSF is using nutrient broth that is in solid state. Many Asian countries use the SmF process because it is the best alternative that gives efficient production of metabolite such as ganoderic acid and polysaccharide (Fang & Zhong, 2002). In this study, *Rhizopus oligosporus* was used to treat sago effluent. *R. oligosporus* was grown in sago effluent at different pH of 4, 5, and 6 to find the optimum pH by using SmF. *R oligosporus* is one of the fungi that provide high protein fungal biomass (Femi-Ola *et al*., 2013). Besides,

biomass of fungus usually considered as potential source of single cell protein (Ferreira *et al*., 2014). In addition to water treatment, biomass of *R. oligosporus* was converted into value-added product such as aquaculture feed as supplementary protein source (Vikineswary *et al*., 1997). For the water treatment, phenol sulphuric test, nitrate and nitrite analysis were conducted in order to identify concentration of nitrate, nitrite and total carbohydrate in the water.

The purpose of this study is to observe the growth of *R. oligosporus* in sago effluent at different pH.

The specific objectives of this study are:

1. To observe the growth of *R. oligosporus* at different pH in sago effluent.
2. To identify the optimum pH for *R. oligosporus* in sago effluent.
3. To determine the effectiveness of submerged fermentation (SmF) process by *R. oligosporus* to treat sago effluent.

1.2 Problem Statement

Sago effluent is abundant since it occurs naturally in large amount. Sago effluent is usually disposed into the river and causes water pollution since it has high organic material, causing COD, BOD and TSS of the water resources to be increased. By growing *R. oligosporus* in sago effluent, total organic load may be reduced. Besides, SmF can be used to provide high value product such as enzyme and animal feeds (Awg-Adeni *et al*., 2010). SmF process is widely used since it is easy to set up and monitor.

CHAPTER 2

LITERATURE REVIEW

2.1 Sago effluent

Sago is extracted from *Metroxylan sagu* (Figure 1) or also known as sago palm (Awg-Adeni *et al*., 2010). According to Wee *et al.* (2011), Malaysia which is the biggest exporter of sago, exports 25,000 to 40,000 tons of sago yearly. It is estimated that 7 tons of the sago effluent is produced daily (Awg-Adeni *et al*., 2010). Vikineswary *et al*. (1997) stated that sago effluent which consists of high organic load is drained into the river and it will cause increasing of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Total Suspended Solid (TSS). Based on Awg-Adeni *et al*. (2010), other than starch, sago effluent consists of hemicellulose, lignin and cellulose that are non-starch polysaccharide (NSP) pollutants. Sago effluent has horrible odour and irritating colour because it contains high organic material (Ruban, 2013).

Figure 1 Sago palm (*Metroxylan sagu*) in Sarawak, Malaysia (Retrieved from www.craunresearch.com).

According to Vikineswary *et al*. (1997), sago effluent can be divided into three parts, which are, wastewater, sago hampas and bark. Wastewater is known as liquid residue while sago hampas and barks are solid residue (Awg-Adeni *et al*., 2010). There are some treatments that can be used to treat wastewater using fungi and bacteria (Vikineswary *et al*., 1997). Based on Singhal *et al*. (2008), *Rhodopseudomonas palustris* which is an indigenous strain isolated from the wastewater of rice-noodle factory was grown in sago effluent in order to produce biomass. Currently, fungus such as *Rhizopus oligosporus* is used because it consumes low cost and its biomass can be converted into value-added product such as aquaculture feed. *R. oligosporus* is grown using submerged fermentation (SmF) process to reduce COD, BOD and TSS. Sago effluent will act as substrate for the fermentation process to grow *R. oligosporus*. Thus, river water pollution can be reduced.

2.2 Submerged Fermentation Process

Submerged fermentation (SmF) can be defined as microorganism cultivation in liquid substrate (Subramaniyam & Vimala, 2012). SmF usually occurs in the present of water. Example of the liquid substrate is sago effluent. According to Subramaniyam and Vimala (2012), utilization of substrate is rapid, therefore nutrient need to replace constantly. This fermentation technique is the best for microorganism that need a special condition such as high content of moisture (Subramaniyam & Vimala, 2012). In this process, there are several factors that can influence fermentation process for examples, pH, aeration, nitrogen concentration, temperature and this entire factor can give higher impact to the rate of fermentation and yield of the product (Chisti *et al*., 1999). Based on Chisti *et al.* (1999), substrate such as liquid media, wastewater and soluble sugar are usually used in the SmF process. If solid substrate is used in SmF, it needs to be in the slurry form by suspending it in large amount of water.

There are five types of submerged fermentation bioreactor which are bubble column, airlift fermenter, stirred-tank fermenter, trickle-bed fermenter and fluidized-bed fermenter (Chisti *et al*., 1999). These types of bioreactor are the major bioreactors that are used in SmF. SmF are usually used in the production of yoghurt, wine, soy sauce and beer. The extraction of the secondary metabolites mainly will be performed by SmF (Subramaniyam & Vimala, 2012). SmF is usually used because it is easy to handle and easy to monitor. According to Sandhya *et al*. (2005), there is advantage in SmF which is easy recovery of mycelia, spores and extracellular enzymes but the extraction of enzyme is less stable and product is diluted compared to SSF. Based on Subramaniyam and Vimala (2012), the other advantage is, the purification technique of SmF is easier compared to SSF.

2.3 *Rhizopus oligosporus*

R. oligosporus is a fungus that belongs to the family *Mucoraceae*. It is a filamentous fungus (Takaya *et al*., 1998). Figure 2 shows the morphology of *R. oligosporus* under microscope. Most of the other *Rhizopus* taxa consist of 5% of irregular spores compared to *R. oligosporus* that consists of larger irregular spores in the range of 10 to 31% (Jennessen *et al*., 2008). This fungus can be used in the production of metabolite, fermentation of food and pathogenesis (Jennessen *et al*., 2008). *R. oligosporus* is used in the production of tempeh, which is a well-known fermented food made from soybean (Jennessen *et al*., 2008). *R. oligosporus* produce fluffy white mycelia that able to bind the bean together. According to Jennessen *et al*. (2008), enzyme that is released by this fungus is important to human because it is protein-rich product and consumable. Based on Kovaĉ and Raspor (1997), filamentous fungi are widely used in the production of food since it able to improve taste of food, nutritional value and protect food from spoilage. Fermentation by *R. oligosporus* is classified into two types of process which is industrial fermentation and traditional fermentation (Kovaĉ & Raspor, 1997).

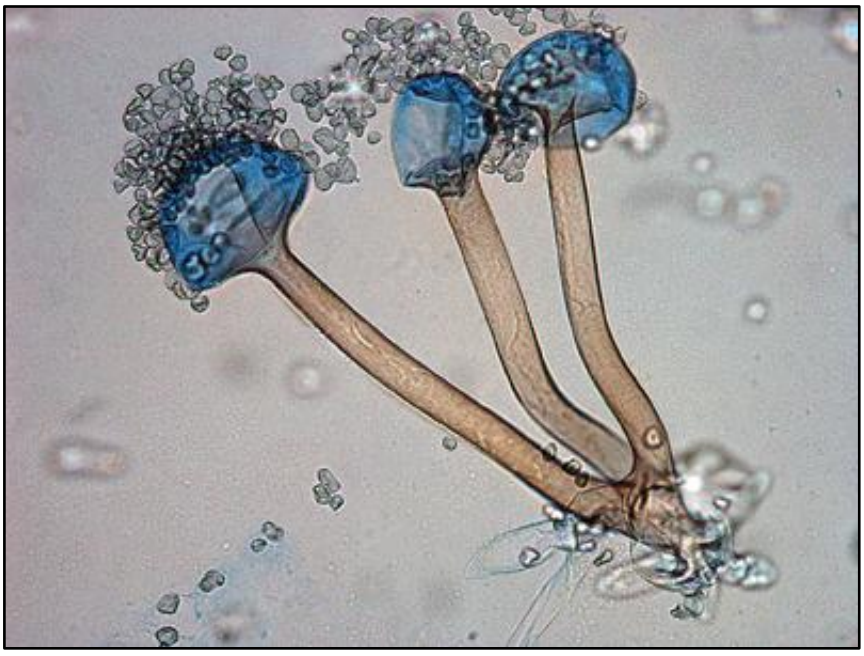

Figure 2 Morphology of *R. oligosporus* under microscope (Retrieved from www.mycology.adelaide.edu).

R. oligosporus is commonly used because it is low cost and also offer other advantages such as constraining growth of tumor, anaemia diseases, hypertension, lowering cholesterol level, and reducing diarrhea infection (Patrick *et al*., 2007). Instead of involve in food fermentation, *R. oligosporus* also helps in producing enzyme and treating wastewater (Jennessen *et al*., 2008). Filamentous fungi are used to treat wastewater and it can convert the wastewater into useful biomass that can be used as animal feed especially aquaculture life and source for human diet (Kovaĉ & Raspor, 1997). Compared to bacteria, fungi are more effective in metabolizing complex carbohydrate for example starch (Kovaĉ & Raspor, 1997).

2.4 pH

pH is known as logarithmic measure of the hydrogen ion concentration. pH is stands for power of Hydrogen. pH is one of the parameter that is widely used in the growth rate of microorganism. It is usually used as a parameter to determine the optimum pH for the highest growth rate of the microorganism. The usual pH value is in the range of 0 to 14 (Figure 3). Neutral pH can be shown in the solution of pH 7. Solutions that are above pH 7 are alkaline and less than 7 is considered acidic.

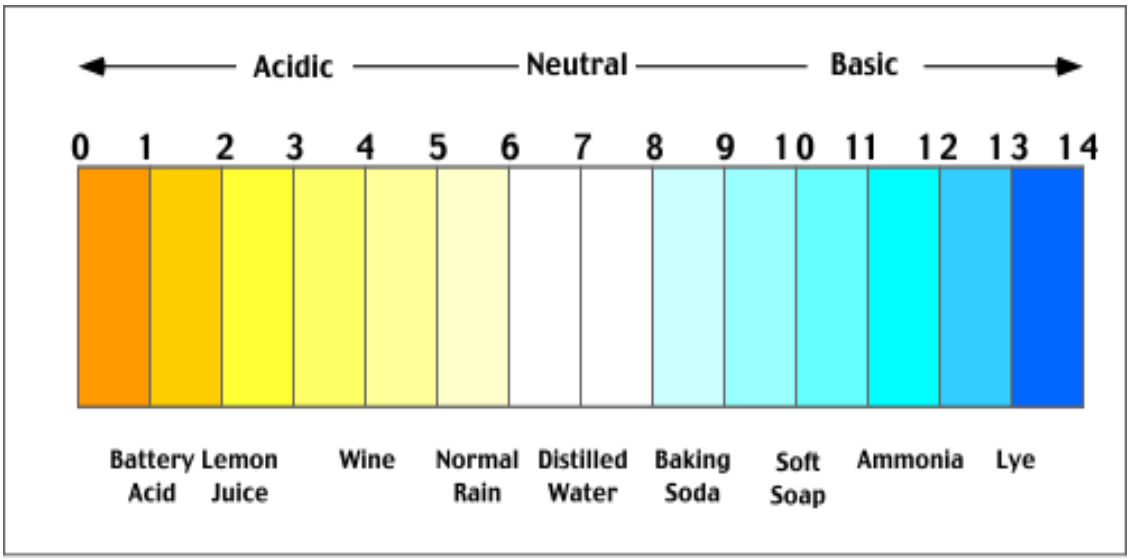

Figure 3 Range of pH value from pH 0 to 14 (Retrieved from www.eoeartht.com)

Buffer is used to resist changes in pH through the addition of small quantity of strong acid or strong base (Russo & Hanania, 1987). Therefore, buffer is added to maintain the pH. Buffer that is suitable for pH 4 and 5 is Citrate buffer, while for pH 7 is Tris buffer (Kulevich *et al*., 2014). Buffer for pH 6 is Acetate buffer. Example of function of buffer is buffer allowing living organism to live, for example *R. oligosporus*.

2.5 High Protein Fungal Biomass (HPFB)

High protein fungal biomass (HPFB) can be retrieve from the starch processing wastewater (Jin *et al*., 2002). Fungus such as *R. oligosporus* and *Aspergillus oryzae* can produce HPFB from starch processing water even though without nutrient supplementation (Jin *et al*., 2002). Figure 4 shows fungal biomass of *R. oligosporus* in Yeast Malt Broth (YMB) solution. The fungal biomass that is produced by this fungus contains more than 45% protein which is suitable for the animal consumption (Jin *et al*., 2002). pH can influence the biomass production of the fungus. The biomass product that has been produced from the submerged fermentation process is easily separated and efficiently harvested (Jin *et al*., 2002).

Figure 4 Biomass of *R. oligosporus* in Yeast Malt Broth (YMB) solution (Retrieved from www2.hawaii.edu).

The valuable product such as microbial biomass protein (MBP) is produced from the biotechnological treatment which can purify the effluent for example sago effluent (Jin *et al*., 2002). The effluent that has been purified can be used for agricultural irrigation or industrial use (Jin *et al*., 2002).

CHAPTER 3

METHODOLOGY

3.1 Pre-treatment of Substrate

Sago effluent was collected from Herdsen Sago Mill, Pusa Sarawak. Firstly, a 710 µm mesh size filter was used to filter sago effluent to isolate it from sago hampas. Suspended solid was removed because only supernatant are required. The most suitable sago effluent medium for strain collection is 50% (v/v) of supernatant. Sago effluent was transferred into 2000 ml Schott bottle (SCHOTT Duran Laboratory glass bottle, SCHOTT, Germany). The bottle was autoclaved first at 121 ^{o}C for 15 min.

3.2 Microorganism

R. oligosporus was obtained from Microbiology Laboratory from Department of Molecular Biology, UNIMAS. From isolation process in tempeh, the strain of *R .oligosporus* was obtained and it was cultured on Sabouraud agar and was incubated for 72 hours at ambient temperature. Then, from each plate, colonies were picked and subculture on Yeast Malt Broth (YMB) (Himeidia, India). The agar containing colonies were incubated for 72 hr at ambient temperature.