Response surface optimization of process parameters for the production of microbial biomass and single cell protein from *Aspergillus niger* using banana peel as substrate

A THESIS

BY

MD. MOSTAFA KAMAL
Registration No. 1605213
Semester: January-June/2017
Session: 2016-2017

MASTER OF SCIENCE (MS)

IN

FOOD PROCESSING AND PRESERVATION

DEPARTMENT OF FOOD PROCESSING AND PRESERVATION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2017

Response surface optimization of process parameters for the production of microbial biomass and single cell protein from *Aspergillus niger* using banana peel as substrate

A THESIS

BY

MD. MOSTAFA KAMAL
Registration No. 1605213
Semester: January-June/2017
Session: 2016-2017

Submitted to the Department of Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur

In partial fulfillment of the requirements for the degree of

Master of Science (MS) in Food Processing and Preservation

DEPARTMENT OF FOOD PROCESSING AND PRESERVATION

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2017

Response surface optimization of process parameters for the production of microbial biomass and single cell protein from *Aspergillus niger* using banana peel as substrate

A THESIS

BY

MD. MOSTAFA KAMAL
Registration No. 1605213
Semester: January-June/2017
Session: 2016-2017

Approved as to the Style and Content By

……	……
Supervisor	Co-Supervisor
(Shakti Chandra Mondal)	(Md. Raihanul Haque)
Assistant Professor	Assistant Professor
Department of Food Processing and Preservation	Department of Food Engineering and Technology

Examined By

……	……
Member	Member

……
Chairman of the Examination Committee

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2017

ACKNOWLEDGEMENT

All praises are due to Allah, the creator of the universe and source of all knowledge who has enabled me to complete this research work and to prepare this thesis in partial fulfillment of the requirements for the degree of Master of Science in Food Processing and Preservation.

I sincerely wish to express my deepest sense of honor and gratitude, indebtedness to my respected supervisor Shakti Chandra Mondal, Assistant Professor and Chairman, Department of Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur; for his tremendous help in planning the research work, scholastic guidance, encouragement, untiring supervision, kind co-operation, meaningful suggestions and constructive criticism throughout the period of the research work as well as in the preparation of the thesis. I am thankful to him for being there anytime and for giving me continuous inspirations.

I would also like to express my sincere gratitude to my co-supervisor Md. Raihanul Haque, Assistant Professor, Department of Food Engineering and Technology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, whose constant encouragement, directions and instructions has made it possible for the thesis to come to recognition.

I extremely glad to take opportunity to express my heartfelt thanks and gratitude to all the teachers, Faculty of Engineering, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their valuable teaching, good advices, encouragement and co-operation during academic study period.

I am very grateful to the authority of Ministry of Science and Technology for financial support through the "National Science and Technology (NST) Research Fellowship" program for carrying out this research work.

A special note of thanks to senior brother Md. Rezaul Islam Shishir, PhD Fellow, Department of Food Science and Nutrition, Zhejiang University, China for his valuable information and support in collection of related documents regarding this thesis work.

Cordial thanks to the Lab Assistants of the Department of Food Processing and Preservation and Department of Plant Pathology for their excellent service during the research period.

Finally, I would like to acknowledge the whole-hearted indebtedness to my beloved Parents, sister and brother, my devoted friends and all well -wishers for their continuous inspiration, sacrifices and continuous blessings that opened the gate and paved the way for my higher studies.

Date: June, 2017 *Md. Mostafa Kamal*

ABSTRACT

Response surface methodology (RSM)-based central composite rotatable design (CCRD) on four variables viz. temperature (20-40^{o}C), pH (4-8), substrate concentration (5-25 %), and fermentation period (1-5 days) were employed for optimizing the production of biomass and protein from *Aspergillus niger* using banana fruit peel. The experimental results were significantly ($P<0.05$) fitted into second-order polynomial models to describe and predict the response quality in terms of the biomass and protein with R^2 of 0.9669 and 0.9521, respectively. The linear, quadratic and interactions effects of the four variables on the yield of biomass and protein were investigated. It was found that the effects of process variables on production of biomass and protein were significant ($P<0.05$) and the strongest effect was given by the fermentation period. After numerical optimization, the predicted optimum conditions were obtained as temperature of 34.27^{o}C, pH of 6.32, substrate concentration of 10% and fermentation period of 4 days which were verified through confirmatory experiments. Under these optimal conditions, the experimental values (biomass: 18.56 ± 0.16 g/L and protein: 59.06 ± 0.13 %) agreed well with the predicted values (biomass: 18.1105 g/L and protein: 58.7177 %) indicating the suitability of developed models within the acceptable range of the responses.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

1.1 Background Information

Single cell protein (SCP) refers to edible unicellular microorganisms. It is the dead, dry microbial cells or total proteins extracted from pure microbial cell culture and is produced using a number of different microorganisms including yeast, bacteria, fungi and algae (Anupama & Ravindra, 2000) which grow on different carbon sources. It is also known as biomass, bioprotein or microbial protein. Single cell protein is considered to be suitable since most of the microorganisms grow as single or filamentous individuals (Mondal et al., 2012). Besides high protein content (about 60-82% of dry cell weight), SCP also contains fats, carbohydrates, nucleic acids, vitamins and minerals (Jamal et al., 2008). Moreover, SCP is rich in certain essential amino acids like lysine, methionine which are limiting in most plant and animal foods. This protein can be used as an additive, added to the main diet instead of sources known very expensive such as soybean and fish (Ghasem, 2007; Gad et al., 2010).

1.2 Problem Statement and Justification

A growing concern for the acute food shortages for the world ẟ expanding population generates the challenge of providing necessary food sources. A large population of the world, especially those who are living below poverty line is suffering from malnutrition. Protein supply poses a problem since essential amino acids cannot be replaced. Proteins are present in all living cells as building block components of the body and key dietary component for the supply of nitrogen as well as sulfur (Yousufi, 2012).

There is a large gap between the demand for protein-rich food and its supply to the ever increasing world population. About 25% of the world population has the protein deficiency, which is a glaring example of protein gap (Azam et al., 2014). Therefore, it is essential to search for unconventional or novel proteins to supplement the available sources and in order to bridge this gap, single cell protein (SCP) is an innovative and an alternative approach to this way.

Production of bioprotein by fermentation of agricultural waste is one of the most promising approaches for increasing the availability of proteins in the world (Saheed et al., 2016;

Jamal et al., 2009). Currently, microbial protein is produced from different microorganisms including fungi, bacteria and algae. The enhancement of yield for bioprotein production can be achieved by selection of potential strains, substrates and optimum conditions (Jamal et al., 2008). Fungi especially *Aspergillus niger* is the most common due to their capability to propagate on agricultural wastes within a short period and ability to produce high protein content in their biomass (Anupama & Ravindra, 2000).

A significant quantity of wastes generates during agricultural activities and in food processing industry, which is rich in organic matter and could constitute new materials for value-added products (Bacha et al., 2011). Almost 50 % of the agricultural biomass produced is not used as a food or feed (Foyle et al., 2007), representing lignocellulosic residues. The majority of these waste materials are often improperly disposed, hence constitute huge environmental disorders (Essien et al., 2005; Lim et al., 2010). These wastes are the most abundant raw material consisting of cellulose as the major component, which is suited for the growth of microorganisms (Yunus et al., 2015), and the production of microbial proteins to enhances the nutritional supplements and achieve food stability (Yassine et al., 2013). Moreover, the utilization of fruits and vegetables wastages in the production of single cell protein will help in controlling pollution and also in solving waste disposable problem to some extent in addition to satisfy the world shortage of protein-rich food (Mondal et al., 2012; Najafpour, 2007; Khan et al., 2009). Among various fruit waste, banana peel constituted a considerable amount of lignocelluloses (Reddy et al., 2010) and other nutrients (Anhwange et al., 2009), which can be used to support microbial growth and single cell protein production (Yabaya & Ado, 2008; Adoki, 2008; Enwefa, 1991). Therefore, banana peel extract can be taken into consideration as an alternative fermentation medium for the production of *Aspergillus niger* biomass (Sankar et al., 2011).

Optimization of a fermentation medium is an important step during development of economically feasible bioprocesses. Optimization of process conditions could increase the amount of protein having high nutritional value and will have no competition with food for human consumption (Jamal et al., 2009). The classical optimization method for the medium and culture condition involves varying one parameter at a time while keeping the others at a constant level. This method is inappropriate for optimization and has various disadvantages since the effects of interaction among variables are neglected and does not guarantee the determination of optimal conditions (Luo et al., 2009; Survase et al., 2006).

In addition, as the process is time-consuming, determination of the optimum levels would be expensive and necessitates a number of experiments (A - ē k e l e T eng & X u, , 2 0 1 0 2008; He & Tan, 2006; Kaushik et al., 2006). Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest (output variable) is influenced by several independent variables (input variable) and the objective is to optimize this response (Khuri & Mukhopadhyay, 2010; Rezaei et al., 2010; Montgomery, 1997). Furthermore, the application of RSM to design optimization is aimed at reducing the cost of experiments by only a few experimental trials (Malinowska et al., 2009). Nowadays, RSM has been successfully used in the optimization of bioprocesses where the target production could be enhanced (Hao et al., 2010; Luo et al., 2009; Kumari et al., 2008; Kalil et al., 2000).

In most cases, single cell protein was produced from the waste of feed industries and few studies related to single cell protein production from *Aspergillus niger* using banana waste as the substrate.

1.3 Research Objectives

On the basis of the information so far accumulated, the main objectives of this research work is to produce microbial protein i.e. single cell protein from *Aspergillus niger* using banana peel as substrate. Therefore, the specific objectives are:

I. To investigate the effects of culture conditions on the production of microbial biomass and single cell protein.
II. To optimize the culturing conditions for the production of microbial biomass and single cell protein.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

2.1 General Introduction

The continued population growth especially in developing and third-world countries is resulting in increased food demand in parallel and is posing serious threats to food security due to yawning gap in demand and supply (Anupama & Ravindra, 2000). Chronic malnutrition and hunger are typically most prevalent in developing countries. Malnutrition is a consequence of not taking appropriate amount or quality of nutrients comprising diet. The gap between demand and supply is expected to grow unless planned actions are taken to improve the situation. Therefore, it is essential to search for un-conventional or novel proteins to supplement the available sources.

2.2 Single Cell Protein

The term single cell protein (SCP) was used for the first time in the 1960´s in aim to embrace microbial biomass products which were produced by fermentation, in order to solve the problem of worldwide protein shortage. The production methods of SCP evolved as bioconversion processes which turned low value by-products, often wastes, into products with added nutritional and market value (Ugalde & Castrillo, 2002). During the last decades the focus has shifted to exploit microbes as food sources for fortification of the food supply or for consumption as single cell protein.

Single cell protein is defined as the dead, dry microbial cells or total proteins extracted from pure microbial cell culture (Anupama & Ravindra, 2000) and for use as a protein source for human and animal consumption (Jamal et al., 2007; Yabaya & Ado, 2008). This microbial protein is referred to as a whole microbial biomass or bioprotein which can be derived from a variety of microorganisms both unicellular and multicellular namely bacteria, yeast, fungi and microscopic algae (Dunlop, 1975). It is convenient to use fungi and bacteria for production of SCP when grown on inexpensive waste material.

2.2.1 History of Single Cell Protein Production

The production of single-cell protein is not a recent development. Since 2500 B.C. a number of microorganisms (*Saccharomyces* sp.) have been used as a part of the diet in the form of fermented food (Frey, 1930). Cultured dairy products contain 107 to 1010 lactic acid bacteria per gram of product. During the first century B.C., edible mushrooms were extensively consumed in Rome. In the 16th century blue green algae (Spirulina) was consumed as a major source of protein (Nangul & Bhatia, 2013). The mass production of micro-organisms as a direct source of microbial protein was realized during World War-I in Germany, and consequently, *Saccharomyces cerevisiae* was produced (Ugalde & Castrillo, 2002). In post war period, the Food and Agriculture Organization of the United Nations (FAO) emphasized on hunger and malnutrition problems of the world in 1960 and introduced the concept of protein gap, showing that 25% of the world population had a deficiency of protein intake in their diet ((Ugalde & Castrillo, 2002)). In the 1960s, researchers at British Petroleum developed a "proteins-from-oil process", a technology for producing microbial protein using yeast fed with waxy n-paraffin, a byproduct of oil refineries (Bamberg, 2000). The method had a capacity to produce 10,000 tons of microbial protein p replaced by a new term ñSingle Cell Protei (Dolle, 1994; Bud, 1989).

2.2.2 Properties of Single Cell Protein

Single-cell organisms comprise proteins, fats, carbohydrates, ash ingredients, water and other elements such as phosphorus and potassium. The composition depends upon the organism and the substrate on which it grows. There are some typical compositions which are compared with soya meal and fish meal (Nangul & Bhatia, 2013). If SCP is to be used successfully, there are five main properties that must be satisfied. It must be safe to eat. It must have a high nutritional value, particularly of amino acid (Bhalla et al., 2007). It must be acceptable to the general public. It must have the functionality, i.e. characteristics, which are found in common staple foods. It must be economically viable to produce.

2.2.3 Main Criteria to be Single Cell Protein

In order to produce the single cell protein, some key factors must be keep in mind so that a good amount of pure, stable, and highly nutritious SCP can be produced (Nangul & Bhatia,

2013). The key criteria used in selecting suitable SCP include that any microorganism which is used as SCP must be easy to modify genetically so that any desired improvement can be made. Protein content should be 45-85% of the dry cell weight. The microorganism must have a short life cycle so that a large amount of SCP can be produced in a short time. The microorganism must be pH and temperature tolerant. It must be easy to grow in a number of cheap substrates, especially waste products, so that a cost-effective SCP can be produced. Microorganisms should be genetically stable so that the strain with optimal biochemical and physiological characteristics can be maintained easily.

The microorganism must have high specific growth rate, productivity and yields on a given substrate. It must be resistant to change in environmental conditions so that small variations in the environmental conditions do not decrease the production of SCP. Aeration requirements and foaming characteristics should be well studied before the production of SCP at industrial scale. Protein, RNA and nutritional composition of the product should have adhered to recommended parameters (Nangul & Bhatia, 2013).

2.2.4 Importance of Single Cell Protein

There have been studies as well as efforts to improve the protein quantity and quality of the finished food products by augmenting protein-rich cheaper ingredients in food formulations (Nasir & Butt, 2011; Hussain et al., 2007). Although animal proteins are considered to be best quality proteins (Saima et al., 2008), however microbial protein also known as single cell protein grown on agricultural wastes is one of the important optional proteins because of higher protein content and very short growth cycle of microorganisms, thereby, leading to rapid biomass production (Bekatorou et al., 2006). Moreover, microbes are also able to grow on cheap nutrient sources resulting in economical, potentially supplemental protein biomass for balanced nutrition. Yeasts and molds are the most promising source to produce single cell protein using cheap raw materials. It is also easy to harvest due to bigger cell size and flocculation ability with lower amount of nucleic acids compared to bacteria (Wolf et al., 2003). The microbial protein has also been reported to contain better percentage of essential amino acids and better chemical score than soya protein (Lyutskanov et al., 1990).

2.2.4.1 Advantages of Single Cell Protein

First of all, it is a promising industry, because the raw materials are almost free, at least can buy at a very cheap price but the product to sell are relatively expensive. Besides this, it

helps to reduce the environmental pollution and promote recycling. Microorganisms can grow faster and produce large quantities of single cell protein from relatively small area of land and time. Single cell protein have a much higher protein content of 30 i70% in the dry mass than vegetables or grains (Nasseri et al., 2011). The amino acid profiles of many SCP microorganisms often have excellent nutritional quality, comparable to a hen's egg. The scientist can alter the component of amino acids from single cell protein by genetic engineering. In many countries, use of microorganisms or biomass of microorganisms as a diet supplement has met with skepticism because of certain psychological barriers. But in the future even in these countries microbial biomass will play a major role via single cell protein feeds to the animals which will in turn be consumed by humans.

2.2.4.2 Disadvantages of Single Cell Protein

Although SCP shows very attractive features as a nutrient for humans, however there are some problems that deter its adoption on global basis. Fast growing microorganisms such as bacteria and yeast tend to have a high concentration of nucleic acid, notably RNA. Levels of must be limited in the diets of monogastric animals to <50 g per day. Ingestion of purine compounds arising from RNA breakdown leads to increased plasma levels of uric acid, which can cause gout and kidney stones. Uric acid can be converted to allantoin, which is excreted in urine. Nucleic acid removal is not necessary from animal feeds but is from human foods. A temperature hold at 64 °C inactivates fungal proteases and allows . However, this problem can be remediated (Nasseri et al., 2011). One common method consists in a heat treatment which kills the cells, inactivates proteases and allows endogenous RNases to hydrolyse RNA with release of nucleotides from cell to culture broth (Halasz & Lasztity, 1990). Similar to plant cells, the cell walls of some microorganisms such as algae and yeast contain non-digestible components, such as cellulose. The cells of some kind of SCP should be broken up in order to liberate the cell interior and allow complete digestion (Nasseri et al., 2011). Some kind of SCP exhibits unpleasant color and flavors. Depending on the kind of SCP and the cultivation conditions, care must be taken to prevent and control contamination by other microorganisms because contaminants may produce toxins such as mycotoxins or cyanotoxins (Ivarson & Morita, 1982). Many types of microorganisms produce some substances which are toxic to the humans and also to the animals. Therefore, it has to be made sure that the produced microbial biomass does not contain any of these toxic substances. Some microbial biomass when taken as diet supplements may lead to

indigestion and allergic reaction in humans. The high nucleic acid content of many types of microbial biomass may lead to poor digestibility, gastrointestinal problem and also some skin reactions in humans. Single cell protein production is a very expensive procedure as it needs high level of sterility control in the production unit or in the laboratory.

2.2.5 Sources of Single Cell Protein

2.2.5.1 Bacterial sources

Bacteria have short generation time, the cell mass of bacteria multiply within 20min-2hrs and they can grow rapidly, due to these characteristics bacteria are suitable in the production of SCP. They also have the ability to grow on different types of raw material ranging from liquid hydrocarbons such as fractions of petroleum and methane to gases and carbohydrates like sugars and starches (Saeed et al., 2016) to wastes of organic nitrogen and petrochemicals which include nitrogen, ethanol and methanol. It is also suggested to add mineral nutrient supplement that help the bacterial culture to fulfill deficiency of nutrients which is required in sufficient concentration for the growth in natural water. Potential phototrophic bacterial strains are recommended for single cell protein production.

Some researchers also suggest use of methanotrophic and other bacterial species for single cell protein production. The Methylophilusis generation time almost 2 hrs is useful for animal feed but generally they can produce favorable composition of protein than fungi or yeast. Therefore animal feed can produce a large quantity of single cell protein by using bacteria like Brevibacterium (Adedayo et al., 2011), *Acinetobacter calcoacenticus, Methylophilus methylitropous*, Bacillus megaterium, *Acromobacter delvaevate*, *Bacillus subtilis* (Gomashe et al., 2014), *Aeromonas hydrophilla*, *Cellulomonas* species, *Methylomonas methylotrophus*, *Lactobacillus species*, (Piper, 2004), *Thermomonospora fusca*, *Flavobacterium* species, *Pseudomonas fluorescens, Rhodopseudomonas capsulate* (Dhanasekaran et al., 2011).

2.2.5.2 Algae

Since ancient times, Spirulina was cultivated by people in Africa near Lakechad and in Mexico near Texcoco. They used it as a food after drying it. Spirulina is the most widely used algae so much that even astronauts during their space travel take it to space. Similarly, biomass obtained from Senedessmus and Chlorella has been harvested and used as source of food in many parts of world. Alga is used as a food in many different ways and its

advantages include high content of protein, simple cultivation, rapid growth and beneficial use of solar energy. The algae Spirulina has been considered for use as a supplementary protein (Raja et al., 2008). It is a green alga that excite the free radical in enzyme system and exhibits antioxidant activity. Healthy diet containing nutraceuticals and Spirulina maxima have the ability to protect progenitor/stem cells. This can also prevent the development of fatty liver due to carbon tetrachloride (CCl_4). It is concluded that the use of Spirulina should be encouraged in patients suffering from malnutrition, immune suppression, hepatic, neural compromise and etc. In a study, the production of SCP from five different strains of Chlorella (M150, M122, M121, M109 and M138) was isolated from a variety of habitats and also studied the effects of eight environmental factors (Saeed et al., 2016). Although, there is a need of further investigations on the antiviral effects of this alga and its clinical implications.

2.2.5.3 Fungal Sources

As a source of protein rich food many fungal species are used (Bhalla et al., 2007). Many other filamentous species are also used as source of single cell protein. In 1973 during second international conference convened held at MIT, it was reported that *Actinomycetes* and filamentous fungi produced protein from various substrates. For the period of the world war II, trials were made to utilize the cultures of *Rhizopus* and *Fusarium* (Yousufi, 2012) as a source of protein food are grown in fermentation. The inoculums of *Rhizopus arrhizus* (Anupama & Ravindra, 2000) or Aspergillus oryzae were selected due to their nontoxic nature. On complex organic compounds saprophytic fungi are grow and convert them into simple structures. High amount of fungal biomass is produced as a result of growth. Mycelia yield vary greatly which depends upon substrates and organisms.

There are some species of moulds such as *Aspergillus niger* (Yabaya & Ado, 2008), *A. fumigates*, *Fusarium graminearum* which are very dangerous for human, that the reason, such fungi, must not be used or before recommending to use as SCP toxicological evaluations should be done. Very recently, SCP technology is using fungal species for bioconversion of lignocellulosic wastes (Lenihan et al., 2010). The type of filamentous fungi that have been used are *Fusarium graminearum*, *Chaetomium celluloliticum* (Saeed et al., 2016), *Aspergillus fumigates*, *A. oryzae*, *A. niger*, *Cephalosporium cichorniae*, *Rhizopuschinensis*, *Scytalidum aciduphlium*, *Penicillium cyclopium*, , *Tricoderma alba Paecilomyces varioti* and *Tricoderma viridae* (Jaganmohan et al., 2013).

2.2.5.4 Yeast

Yeast single-cell protein (SCP) is a high nutrient feed substitute (Burgents et al., 2004). Among these, most popular are yeast species Candida (Saeed et al., 2016), *Hansenula*, *Pitchia*, *Saccharomyces* and *Torulopsis*. The production of SCP by using Saccharomyces cerevisiae grown on various fruit waste (Khan et al., 2010). The usual oily yeasts genera contain *Yarrowia*, *Candida*, *Cryptococcus*, *Rhodotorula*, *Rhodosporidium*, *Lipomyces* and *Trichosporon*. Orange peels and cucumber were evaluated for the production of SCP by using Saccharomyces cerevisiae by submerged fermentation (Saeed et al., 2016).

2.2.6 Production of Single Cell Protein by Fermentation

Precisely, SCP is the manufacture of cell mass by using different microorganisms that culturing on profusely available industrial and agriculture wastes. The production of microbial biomass is done either by a solid state or submerged fermentation process. Biomass is harvested after fermentation and it may be subjected towards different downstream processing steps such as washing, protein extraction, cell disruption and purification. The fermentation process requires a pure culture of specified microorganisms which was grown on suitable raw materials and then it is separated by screening in correct physiological state. This process contains a fermenter for the process to be carried out. A fermenter is provided with all the equipment needed to run the process smoothly. It is included a thermostat for the temperature regulation, pH detector for the measurement of pH, aerator for continuous supply of oxygen and a stirrer. Culture medium is placed in fermenter and the process is carried out leading to the cell separation and the supernatant of the cell is collected. The product is then obtained by protein extraction and purification and the effluent is treated (Nasseri et al., 2011; Anupama & Ravindra, 2000).

2.2.6.1 Submerged Fermentation

Submerged fermentation is one in which the liquid form of substrate is used to provide all the nutrients required by the microorganism for their growth. Operational conditions are applied continuously during fermentation process and the product is harvested after regular intervals. The harvested biomass is filtered and the centrifuged. Single cell proteins are then obtained after the process of drying. (Nasseri et al., 2011).

2.2.6.2 Semisolid Fermentation

The substrates preparations in solid state fermentations is less clear and are used more in the solid form rather than liquid. The process of cultivation is carried out by stirring of multiphase. The oxygen is transferred to the microorganisms in the form of bubbles through liquid phase. This liquid phase also regulates the temperature of the process. A special bioreactor called u-loop fermenter is used in solid state fermentation. Process is carried out by sterilization of the fermenter and growth medium, growth medium with suitable carbon source, production of specific microorganisms, harvesting of product biomass, its processing and purification (Nasseri et al., 2011).

2.2.6.3 Solid State Fermentation

Solid state fermentation is being extensively used for the production of solid cell proteins, enzymes, organic acids, pigments and flavor. Solid state fermentation is carried out in solid substrates with no free water and does not require pre arrangements of preparation of growth media. Fungi show good growth in low water activity and yields high product biomass as compared to submerged fermentation. Solid state fermentation involves efficient utilization of waste which act as solid substrate and produce commercial viable cells. The process mainly involves seeding of the rice or bran substrate with microbial cells. Then the substrate is left for several days in the form of flat beds. Then harvesting of cells, further processing and finally drying of the cells is carried out (Nasseri et al., 2011).

2.2.7 Nutritional Significance of Single Cell Proteins

Nutritional assessment of single cell proteins includes the content and composition of nutrients, amino acids and vitamins. Hence it can be used as an alternative food by the living microorganisms. Moreover, the nutritional benefits depend on its digestibility and allergic reactions when used for human consumption. Single cell proteins include not only proteins but also characterized as an important source of essential amino acids, carbohydrates, lipids and nucleic acids. The composition of single cell proteins depends on the substrate used for the production and suitable microorganism for high yield (Bogdahn, 2015; Zepka et al., 2010). Yeast is the most suitable microorganism for single cell protein production and produces the products with 50-55% protein. Yeasts are used as animal feed and are characterized by high contents of amino acids, B-group vitamins and lysine. Yeasts contain fewer amounts of methionine and cysteine and hence limit their use as single source of proteins. Yeast contains 2-6% fats and 6-12% nucleic acids. *Pitchia, Candida,*

Hansenula, *Saccharomyces* and *Torulopsis* are the important species used in the production of single cell proteins. Saccharomyces cerevisiae is the probiotic strain of yeast utilizes fruit waste as substrates (Ferreira et al., 2010; Gao et al., 2007). A number of fungal species are being consumed as rich protein sources. It was reported that *Fusarium* and *Rhizopus* are tried to be used as alternative protein sources during World War II (Yousufi, 2012). Algae contain large amounts of proteins, vitamins and fats. Alga is used as food for its high protein content. An Alga Spirulina is blue green algae is used as supplementary proteins and have strong antioxidant activity (Anupama & Ravindra, 2000).

2.3 *Aspergillus niger*

Aspergillus niger is one of the known filamentous fungus that give an important role in biotechnology. It is a fungus, but it is specified as a mold (Wadman et al., 2009). This eukaryotic organism belongs to the Fungi kingdom and the *Aspergillus* genus. The use of microorganisms in biotechnology is common, however *Aspergillus niger* is considered to be one of the most essential of those microorganisms (Schuster et al., 2002). The significance of *Aspergillus niger* is the industrial role that it plays in the production of proteins, enzymes and fermentation. It is capable of producing heterologous proteins (Semova et al., 2006), This very useful microbe is even referred to as an "industrial workhorse" because of the frequent use in many applications (Andersen et al., 2008; Pel et al., 2007). *Aspergillus niger* does have spores and reproduces asexually meaning that it can produce its offspring individually (Daud, 2012). Purwanto et al. (2009) discovered the morphology of Aspergillus niger using Scanning Electron Microscope (SEM).

2.3.1 Ecology

Aspergillus niger grows aerobically on organic matter, therefore it can be found almost everywhere in environments that contain soil. Also, it is found in waste, decaying plant material and compost in outdoor environments (Deepake, 2008; Schuster et al., 2002). *Aspergillus niger* has been exhibited to sustain growth in freezing temperatures, which indicates it as a thermotolerant that can also survive at very hig thermotolerant abilities that enable growth in a wide temperature range from 6 to 47°C with a preferred optimum temperature at 35-37°C. The fungus is capable of growing over a very wide pH range, from 1.4 to 9.8 pH. The growth ability in various temperature ranges, pH

ranges as well as the abundant amount of conidiospores allow the species to be continuously widespread. Conidiospores are distributed by air (Schuster et al., 2002).

2.3.2 Industrial use

Aspergillus niger is one of the most important microorganisms used in biotechnology. It has been in use already for many decades to produce extracellular (food) enzymes and citric acid. In fact, citric acid and many *Aspergillus niger* enzymes are considered GRAS by the United States Food and Drug Administration (Schuster et al., 2002). In addition, A. niger is used for bio-transformations and waste treatment. In the last two decades, *Aspergillus niger* has been developed as an important transformation host to over-express food enzymes. In addition to citric acid, *Aspergillus niger* is a rich source of enzymes. Pectinase, protease and amyloglucosidase were the first to be exploited, and were originally produced in surface culture (Schuster et al., 2002). *Aspergillus niger* in submerged culture, the technology was first applied to the production process of penicillin G by *Penicillium chrysogenum* in 1942. After 1950, production technology for fungal products gradually changed from surface culture to stirred-tank processes, but up until the mid1960s companies used surface culture processes (Barbesgaard et al., 1992). Several additional enzymes like cellulase and hemicellulase were manufactured using black *Aspergillus* strains in stirred tank processes. The glucose syrup and the alcohol industries are the principal users of amyloglucosidase produced by *Aspergillus niger* (Schuster et al., 2002). It is established practice to improve the baking process by adding hemicellulases from *Aspergillus niger* when mixing the dough. The enzymes modify the rheological properties of the dough and give higher loaf volume and better crumb structure of bread and pastry (Schuster et al., 2002). With the development of fermentation technology, species of *Aspergillus niger* has been used for the production of single cell protein using fruits and vegetable wastes as substrate (Anupama and Ravindra, 2001; Omwango et al., 2013; Madhumithah et al., 2011; Oshoma et al., 2005; Jaganmohan et al., 2013).

2.4 Factors Influence the Growth of Microorganisms

In the production of single cell protein from microorganisms, various factors such as carbon and nitrogen sources, temperature, pH of growth medium, phosphorus and potassium primarily influenced the fermentation process (Irfan et al., 2011; Tsao, 1976). To make the

production process cost effective prior to commercial scale production optimization of these parameters are essential (Irfan et al., 2011).

2.5 Agricultural Waste as Substrate for Microbial Growth and SCP Production

Agricultural wastes are basically the most useful substance for the production of single cell protein. In recent years, wastes such as p and other industrial wastes has been used as substrates for growing single cell protein (SCP) and its production (Nwufo et al., 2014). This is because proteins from other sources are costly and are not much affordable for the poor masses, but the use of agricultural wastes has been found to be very cheap in the production of high quality protein. Agricultural wastes are the most abundant raw material consisting of cellulose as the major component, which is suited for the growth of microorganisms and the production of single cell protein (SCP) biomass (Yunus et al., 2015). Mondal et al. (2012) used cucumber and orange peels to evaluate the production of single cell protein using Saccharomyces cerevisiae by submerged fermentation. The authors state that the bioconversion of fruit wastes into single cell protein production has the potential to solve the worldwide food protein deficiency by obtaining an economical product for food and feed. Fruit wastes rich in carbohydrate content and other basic nutrients could support microbial growth. Apple, turnip, papaya and banana peels were used for alcohol fermentation and biomass production by Kandari & Gupta (2012). The use of legume seeds as alternative nutrient media for bacteria and fungi has been reported (Shipra & Dikshit, 2017; Arulanantham et al., 2012; Tharmila et al., 2011).

2.5.1 Banana Peel as Substrate

Improved fruit and vegetable production th huge investments in fruit and vegetable processing across the world. Waste generation through these fruits is on the increase due to sustained surge in world population, improved economic growth in developing nations and improved access to nutrition education in high fruit producing countries (Saheed et al., 2016). Banana (Genus: Musa) plant is commonly cultivated all over the world. It is grown extensively in tropical and subtropical countries (Gabhane et al., 2014). Wastes emanating from fruits include peels, pulp and seeds that constitute about 40% of the total mass (Saheed et al., 2016). Most of these residual waste

produced due to banana cultivation is discarded by farmers into nearby rivers, lakes and on roads, which causes a serious environmental concern (Lim et al., 2010; Shah et al., 2005; Essien et al., 2005). However, banana waste contains good amount of lignocelluloses which can be converted into biofuels and other chemicals. These wastes contain simple and complex sugars that are metabolizable by microorganisms through secretion of extracellular products (Saheed et al., 2013). The cellulose, hemicellulose and lignin content of banana waste is reported as 28.92% 25.23% and 10.56%, respectively (Reddy et al., 2010), although minor variation could be possible with the change in species of banana, growth and other environmental conditions. Anhwange et al. (2009) studied chemical component of banana peels. It was found 0.90% protein, 1.70% crude fat, 59.00% carbohydrate, 31.70% fiber and 6.70% moisture. The high amount of carbohydrate and other basic nutrients content in banana fruit peel could support microbial growth and single cell protein production. In tropical climates, such as in Bangladesh, the banana trees continue bearing fruit throughout the year. Sugar represents that part of the fruits which is used by microorganisms for single cell protein production for both food and feed applications (Yabaya & Ado, 2008; Adoki, 2008; Enwefa, 1991). Previous studies reported that banana waste has been utilized for the production of biogas (Del Rosario & Pamatong, 1985), starch (Sharma et al., 1988), biomass (Enwefa, 1991), Lactic acid (López-Baca & Gómez, 1992) and lignolytic enzymes (Robinson et al., 2001). Shah et al., 2005; Sharma et al. (2007) and Bello et al., (2014) used banana waste for the production of ethanol. The use of such a cheap and readily available substrate is desirable to lower the cost of production, reduce waste disposal and management problems, converse natural resources and provide feed for livestock purposes.

2.5.2 Others Agricultural Waste as Substrate

Production of single cell protein from orange peels using *Aspergillus niger* and *Saccharomyces cerevisiae* was conducted by (Azam et al., 2014) and it was found that *Aspergillus niger* had shown a tremendous increase in its protein content (27.15 to 29.75 %).

Growth of *Aspergillus niger* on rice bran medium in submerged fermentation was studied by Oshoma and Ikenebomeh (2005). They found that *Aspergillus niger* biomass cropped on rice bran medium supplemented with glucose, $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4.7H_2O$ and $ZnSO_4.7H_2O$ designated as Glucose supplemented rice bran (GRB) medium was highest at

2.03±0.02g/l while the un-supplemented rice bran medium (RBM) has the lowest value of 0.97±0.00g/l.

A studies on production of single cell protein by *Aspergillus niger* in solid state fermentation of rice bran was investigated by Anupama and Ravindra (2001). They reported that the biomass yield was best with rice bran based medium with sodium nitrate as the nitrogen source for single cell protein production.

Jamal et al. (2008) conducted a study on media optimization for bioproteins production from cheaper carbon source and observed that maximum biomass produced was 21.89 g/L with optimum fermentation conditions of wheat flour (4 g/L), nitrogen concentration (0.5 g/L), nutrient concentration (0.1 g/L), and four days of fermentation.

The effect of temperature and substrate moisture content on the growth and production of amylase, protease and phytase by *Aspergillus niger* during solid-state fermentation was investigated by Saithi et al. (2016).

Khan et al. (2010) investigated the growth performance of *Rhizopus oligosporus* on various fruit wastes such as papaya waste, cucumber peelings, pomegranate fruit rind, pineapple fruit skin and watermelon skin for the production of single cell protein. The study revealed that papaya fruit waste generates highest amount of protein per 100 g of substrate used, followed by cucumber peelings, pomegranate rind, pineapple fruit skin and water melon skin respectively with 59.5 mg, 57.3 mg, 51.6 mg, 48.0 mg and 43.2 mg crude protein respectively.

A study aimed to assess the feasibility of using beles fruit peel for single cell protein production by Haddish (2015) and showed that beles fruit peels generates about 63.5 % of single cell protein when fermented with yeast cell *Saccharomyces cerevisiae*.

Bioconversion of various fruit wastes into single cell protein production was conducted by Mondal et al. (2012). The result revealed that cucumber peel generates higher amount of protein followed by that of orange with 53.4% and 30.5% crude protein respectively per 100 gm of substrate used.

Saheed et al. (2016) conducted a study by utilizing different fruit peels (banana peel, pineapple peel and papaya peel) as carbon source for white rot fungi biomass production

under submerged state bioconversion and observed that all the sole and composite substrates supported fungal growth.

Production of microbial biomass protein by sequential culture fermentation of *Arachniotus* sp. and *Candida utilis* was done by Ahmed et al. (2010) and reported 23.51% crude protein.

A research was conducted by Khan et al. (2010) on single cell protein production from *Saccharomyces cerevisiae* by utilizing fruit wastes and reported that banana skin generates highest amount of protein, followed by that of rind of pomegranate, apple waste, mango waste and sweet orange peel respectively with 58.62%, 54.28%, 50.86%, 39.98% and 26.26% crude protein per 100 g of substrate used. *Saccharomyces cerevisiae* growth was also evaluated by Hezarjaribi et al. (2016) to specify an optimum culture medium to reach the highest protein production. They reported 44.6 % protein content.

A study was taken up by Madhumithah et al. (2011) to utilize different vegetable wastes as substrate for protease production using *Aspergillus niger*.

Sankar et al. (2011) conducted a study on single cell protein production by *Trichoderma harzianum* using waste banana peel as substrate and reported that the maximum sporulation and biomass was observed when medium supplemented with sucrose as carbon source.

Gomashe et al. (2014) utilized liquid whey as potential substrate for single cell protein production from *Bacillus subtilis*. A research on utilization of *Opuntia ficus indica* waste for production of *Phanerochaete chrysosporium* bioprotein was carried out by Gad et al. (2010).

Optimization of process parameters for the production of single cell biomass of *Candida utilis* in solid state fermentation was conducted by Irfan et al. (2011). They utilized wheat bran as substrate and reported that incubation period of 4 days was suitable for maximum cell biomass of *Candida utilis* with 10% (v/v) inoculum size at P^H 6.5 and also obtained 48% crude protein.

Jamal et al. (2009) optimized the media composition for the production of bioprotein from pineapple skins by liquid state bioconversion and stated 514.2 g/kg bioprotein after 5 days of fermentation.

A study was planned by Bacha et al. (2011) to assess the feasibility of using agro-industrial wastes for *Saccharomyces cerevisiae* production and to evaluate protein quality of

produced single cell protein (SCP) biomass. On the basis of higher SCP biomass production, potato peels were selected for further biomass production and the SCP biomass contained 49.29±1.126% crude protein.

A study conducted by Babu et al. (2014) on the optimization of biomass production using *Kluyveromyces marxianus* isolated from paneer whey. Various parameters like pH, temperature and nitrogen source were optimized for maximum biomass yield. Babu et al. (2014) also produced single cell protein using *Kluveromyces marxianus* isolated from paneer whey.

Protein enrichment of pineapple waste with *Saccharomyces cerevisiae* by solid state bioprocessing was investigated by Correia et al. (2007) and reported that optimum protein content (22% dry basis) was reached at 48 h of incubation when 0.25% $(NH_4)_2SO_4$ was added to pineapple waste powder (10 g).

Jaganmohan et al. (2013) produced single cell protein (SCP) with *Aspergillus terreus* using solid state fermentation and the results have shown that the *A. terreus* possesses a high protein value and can be used as a better choice for SCP production using cheap energy sources like eichornia and banana peel. Four types of agro-industrial waste products were tested for their suitability as substrates for *Chlorella* single-cell protein production (Zafaralla et al., 1990).

Amande & Itah (2011) conducted a study on single cell protein (SCP) production using banana peels as mono ï substrate. The results showed that the percentage crude protein doubled within the fermentation period ranging from 5.00 ï 10.94% with reduction in the total carbohydrate content.

Extracts of papaya fruit were used as substrate for single cell protein (SCP) production using Saccharomyces cerevisiae by Ojokoh & Uzeh (2005) and reported that the dry biomass contained 35.5% protein.

A study conducted by Omwango et al. (2013) on nutrient enrichment of pineapple waste using *Aspergillus niger* and *Trichoderma viride* by solid state fermentation. Results showed that fermentation of pineapple waste by solid state fermentation using the fungi *A. niger* and *T. viride* significantly ($P < 0.05$) enriches the nutrient content of the waste, particularly increasing the crude protein and ash content while lowering the crude fiber content.

Rudravaram et al. (2006) conducted an optimization study for protein enrichment of deoiled rice bran by solid state fermentation using Aspergillus oryzae and various process parameters effect such as moisture, pH of the substrate, inoculum size, temperature and nitrogen source for maximum protein enrichment were studied.

Dhanasekaran et al. (2011) accompanied a study on production of single cell protein from pineapple waste using yeast. In this study, pineapple waste was used as sole carbon source in five concentrations for preparation of fermentation media on which two strains of yeasts, *Saccharomyces cerevisiae* and *Candida tropicalis* were grown.

In a research study on production of single cell protein from fruits waste by using *Saccharomyces cerevisiae* was carried out by Uchakalwar and Chandak (2014). In this study pomegranate waste, Orange waste, Banana waste, Watermelon waste was used as sole carbon source for preparation of fermentation media.

Ogbonda et al. (2007) investigated the influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina* sp. and the results showed that the combination of 30 °C and pH 9 gave the highest values of 4.9 mg/ml and 48.2 g/100 g for biomass and total crude protein, respectively. The effect of pH was modulated by temperature and vice versa during biomass production.

Rajoka et al. (2006) conducted a study on production of single cell protein from rice polishings using Candida utilis and the results showed that the dried biomass showed crude protein content of 27.8% and a gross metabolizable energy value of 2678 kcal/kg and contained all essential and non- essential amino acids. Single cell protein was produced from the defatted rice polishings using *Candida utilis* in aerated by fermentor to optimize bioprocess variables (Rajoka et al., 2004).

Paraskevopoulou et al. (2003) studied the functional properties of single cell protein produced by keýr microþora. The experiment and temperature (30ºC) conditions. The protein obtained from the biomass was 53.9%.

Optimization of fermentation technology for producing single cell protein from yam starch by orthogonal test was performed by Chen et al. (2016). The production of SCP reached 241.54±0.15 g wet weight/100 g dry starch under optimal conditions were showed as followed: 30 mL the medium volume in 250 mL flask, inoculum size 17%, fermentation time 69 h, pH 5.0.

2.6 Optimization of Fermentation Process

The optimization of a fermentation medium is an important step in the development of economically feasible bioprocesses. The successful design of a fermentation process involves optimizing the media composition, fermentation conditions, and fermenter design as well as developing superior strains by mutation (Singh et al., 2017). Medium optimization by employing the one-factor-at-a-time method involves changing one independent variable while keeping the other variables constant. This single-dimensional approach is laborious and time-consuming, especially for a large number of variables, and frequently does not guarantee the determination of optimal conditions (Luo et al., 2009; Survase et al., 2006). Such drawback of the one-factor-at-a-time method can be overcome by statistical optimization techniques (Chen et al., 2008). Thus, Statistical optimization, allows rapid screening of a number of factors and factor interactions, and reflects the role of each component (Vimalashanmugam & Viruthagiri, 2012).

2.7 Response Surface Methodology (RSM)

Response surface methodology (RSM) is a three-factorial design method, which provides the relationship between one or more measured dependent responses and a number of input (independent) factors (Coman et al., 2012). RSM is a statistical modeling technique used for multiple regression analysis of quantitative data obtained from rationally designed experiments to solve multivariable equations simultaneously (Kumari et al., 2008; Rao et al., 2000). The response surface method is advantageous because it requires a small numbers of experiments, it is suitable for multiple factor experiments, it seeks relativity between multiple factor experiments, and it finds the most suitable correlation and forecast. Therefore, it finds the optimum values of the factors under investigation, and it predicts the response to the optimum conditions (Popa et al., 2007). Limitations of the single factor optimization can be eliminated by employing response surface methodology, which is used to explain the combined effects of all factors in a fermentation process (Montgomery, 2013; Zheng et al., 2008; Popa et al., 2007).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present study was conducted in the Laboratory of Plant Pathology and Food Processing and Preservation, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of September, 2016 to June, 2017. This chapter describes the methodology used during the production of *Aspergillus niger* biomass and protein.

3.1 Collection of Culture Strain

Culture *Aspergillus niger* was collected from the Laboratory of Plant Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200.

3.2 Inoculum Preparation

Inoculum preparation (spore suspension) was carried out according to the method suggested by Jamal et al., (2005) with little modification. The fungal strain was cultured on 3.9% potato dextrose agar (PDA) at 32°C for 7 days and then transferred into Erlenmeyer flask (250ml) using 100 ml of sterile distilled water. It was shaken in a rotary shaker at 150 rpm for 24 hours and then filtered. The filtrate was used as inoculum after measuring its concentration (spore ml^{-1}). The spore suspension obtained was counted by a haemacytometer (Labtronics, Model No. 37, Korea) and spore concentration was adjusted to 2×10^7 spores/ml. The suspension inoculums were subcultured every 2 weeks and kept in chiller at 4°C until further use. All flasks, funnels, filter papers, and distilled water were sterilized in a sterilizer (LS-2D, Rexall Industries Co. Ltd., Taiwan) prior to use.

3.3 Collection and Preparation of Substrate

Fresh banana (*Musa sapientum*) peels was collected from fruits vendors near to Hajee Mohammad Danesh Science and Technology University, Dinajpur. Peels were thoroughly washed with tap water to remove attached foreign materials. Fruit peels were dehydrated in a cabinet dryer (Model- 136-120, Seoul, Korea) at 60°C for two days immediately after cleaning to stop destructive activity of microorganism. The dried peels were ground to prepare powder by a laboratory grinder (Jaipan CM/L-7360065, Japan) and the prepared powder was passed through a sieve (Sieve no.MIC-300) to achieved 2 mm particle size.

The screened powder was packed in high density polyethylene (HDPE) pouches and stored in an airtight container for subsequent use while ungrounded ones were kept at room temperature in airtight plastic bags.

3.4 Preparation of Fermentation Media

Banana peel powders were degraded to convert cellulose content into more available sugars by chemical treatments with little modification to the procedure of Lenihan et al. (2010). For this, 10 % (w/v) HCl was added to the banana peel powder in conical flask maintaining liquid to solid ratio of 10:1. The mixture/solution was then placed in water bath (VS-1205SW1, Vision Scientific Company Ltd.) at 100^0C for 1.5 h. After being allowed to cool, it was filtered through Whatman No. 1 filter paper. The residue was washed with 10 % NaOH until neutralization (confirmed by litmus paper). The filtrates were diluted with sterile distilled water at varying concentrations and autoclaved at 121^0C for 15 minutes. The sterile solution/broth thus prepared was used as carbon and nitrogen source for biomass production.

3.5 Fermentation Process

Submerged fermentations were carried out in 250 ml Erlenmeyer flask containing 100 ml media as per experimental design. The process conditions used in all experiments were based on the central composite design (CCD) from Design Expert (Version 7.0.0, Stat - Ease Corp. Minneapolis, MN, USA) software. In all experiment, the media was initially adjusted to different P^H level according to the design using 1N H_2SO_4 and/or 1N NaOH to obtain the maximum biomass. Appropriate amounts of each medium was transferred into 250 ml Erlenmeyer flasks and sterilized at 121^oC for 15 minutes. Inoculum (2×10^7cfu/ml) from suspension of culture strain (microbes) was aseptically transferred into each medium. However, fermentation was carried out in shaking incubator (VS-8480SN, Vision Scientific Co. LTD.) at different conditions as per the design of the experiments for optimization of the process parameters intended for biomass and single cell protein production.

3.6 Harvesting of Biomass

After fermentation, the biomass was collected by centrifugation in a laboratory centrifuge (Korea MF-300, Human Lab Instrument Co.) at 3500 rpm. The biomass was filtered by

vacuum filtration using Whatman No. 1 filter paper and washed three times with 20 ml of distilled water. Before taking the weight of the biomass, it was transferred into an aluminum disk and dried in a hot air oven (101C-3B, Shanghai Experimental Apparatus Co. Ltd.) at 103ºC-105ºC for one hour followed by cooling in desiccators to balance the temperature and weight (Jamal et al., 2005). The collected biomass was analyzed for biomass protein.

3.7 Estimation of Biomass Protein

Preparation of Standard Curve: Stock solution of Bovine Serum Albumin **(**BSA) was prepared by adding 5 mg BSA to 1 ml distilled water. 500, 1000, 1500, 2000 and 4000 µl of stock solution was taken in 6 different falcon tubes to which distilled water was added to make the final volume of 5000 µl. Five (5) millilitre of Bradford reagent was then added to each of the 6 falcon tubes and mix by vortexing (KMC-1300V, Korea) for 4-5 minutes. In another falcon tube, 5000 µl of distilled water was taken for use as blank. The absorbance of the prepared solution was measured at 595 nm using spectrometer (T80 UV/VIS Spectrometer, PG Instruments LTD.) against the blank. The absorbance of the standards solution was plotted against their concentration. The best fit of the data to a straight line in the form of the equation "y = mx + c" was determined; where, y = absorbance at 595 nm and x = protein concentration (mg/ml).

Estimation of Protein

Protein content of the biomass was measured spectrophotometrically according to Bradford method (Bradford, 1976) with little modification. 0.5g of sample was taken in a beaker and then 10 ml of distilled water was added to it. Then the sample was stirred with magnetic stirrer and filtered with a filter paper. Then 500 µl filtered samples was taken into a falcon tube and diluted to 4500µL with distilled water. Then 5 ml of Bradford reagent was added and mixed by vortex (KMC-1300V, Korea) for few minutes. The concentration of protein in the solution was determined from the absorbance at 595 nm (T60 U, PG instrument, United Kingdom) against the blank (containing same reagents without sample). Protein content was determined using the following formula by a comparison of the values obtained with the standard curve of BSA and the results were expressed as percentage.

$$\% \text{ Protein} = \frac{\text{X } (mg/ml) \times \text{Voume made (ml)}}{\text{Weight of sample (g)}} \times 100$$

3.8 Experimental Design

In this study, four factors and five levels Central Composite Rotatable Design (CCRD) was employed to investigate and optimize the effect of process variable on the maximum yield of biomass and single cell protein. Temperature (20-40^{o}C), pH (4-8), substrate concentration (5-25%), and fermentation period (1-5 days) were selected as independent variables (Table 3.1), whereas biomass and protein was selected as the response. A total number of thirty (30) experiments with 6 center points (used to estimate experimental error) were ascertained. Experimental data were fitted to second order polynomial mathematical equation in order to express the relationship between independent variables and responses. The generalized form of second order polynomial equation was given by (Moorthy et al., 2015) as follows:

$$Y = \beta_o + \sum_{j=1}^{K} \beta_j X_j + \sum_{j=1}^{K} \beta_{jj} X_j^2 + \sum_{i} \sum_{<j=2}^{K} \beta_{ij} X_i X_j \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

where, Y is the response; X_i and X_j are variables (i_o is the model intercept, β_j the linear coefficient, β_{jj} the quadratic coefficient and β_{ij} the interaction coefficients of the second-order terms, respectively; k is the number of independent parameters (k = 4 in this study). Statistical analysis of the experimental data was performed using the Design Expert 7.0.0 statistical software (Stat-Ease Inc., Minneapolis, USA).

Table 3.1. Range and levels of independent variables.

Independent Variables	**Symbol**	**Level**				
		- 2	**- 1**	**0**	**+ 1**	**+ 2**
Temperature (^{o}C)	A	20	25	30	35	40
pH	B	4	5	6	7	8
Substrate Concentration (% w/v)	C	5	10	15	20	25
Fermentation Period (day)	D	1	2	3	4	5

3.9 Statistical Analysis

Statistical software package Design Expert 7.0.0 was used for regression analysis of the data obtained and to estimate the coefficient of the regression equation (Eq. 3.1). The equations were validated by the statistical tests called the ANOVA analysis. Design-based

experimental data were matched according to the second order polynomial equation. The independent variables were fitted to the second order model equation and examined for the goodness of fit. The quality of fit of the second order equation was expressed by the coefficient of determination R^2, and its statistical significance was determined by F-test (Balasubramani et al., 2015; Maran et al., 2015; Lee et al., 2000). To establish the individual and interactive effects of the test variable on the biomass and single cell protein production response surfaces were drawn.

3.10 Optimization and Validation of Optimized Condition

Numerical optimization technique was adapted in this study to optimize the process conditions. For optimization of process variables, the regression model developed in this study was used to determine the optimal condition which could provide maximum biomass and single cell protein yield. The nature of the optimal condition (point of maximum or minimum or a saddle point response) was also evaluated by transforming the developed regression model into conical form and the Eigen values were computed using the Design Expert 7.01 statistical software (Stat-Ease Inc., Minneapolis, USA).

For finding a solution, the goals were combined into an overall composite function called desirability function (Giri & Prasad, 2007), which is defined as:

$$D\ (X) = (d_1 \times d_2 \ldots\ldots\ldots\ldots\ldots \times d_n)^{1/n} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.2)$$

Where, d_1, d_2 é é $_n$dre responses and n expressed as the total number of responses in the measure.

The function D(X) considers as the desirable ranges for each response (d_i), desirability is an objective function that ranges from zero to one. The maximum point of desirability function considers as optimize point. The goal-seeking starts at a random starting point and keep forward the steepest slope to a maximum point. Because of curvature nature in the response surface, there may be two or more m a x i m a and their combination into the desirability function.

To determine the validity of optimized condition, additional duplicate experiments were performed under optimal conditions and average values of the experiments were compared w i t h t h e p r e d i c t e d v a l u e s o f t h e o p t i m i z e d suitability of the optimized conditions.

CHAPTER IV

RESULTS AND DISCUSSION

CHAPTER IV

RESULTS AND DISCUSSION

The present study was conducted to improve the production process for microbial biomass and single cell protein from *Aspergillus niger* using banana peel extracts as substrates and to optimize the production process using response surface methodology (RSM). The response data (Table 4.1) were analyzed by using multiple regression techniques to develop response surface models. Moreover, the results of the experiments were handled based on the experimental design. The statistical significance of linear, quadratic, and interaction effects was calculated using ANOVA for each response. The multiple regression coefficients for each response were obtained by employing a least square technique to predict the polynomial models. To examine the interactive effect of four process parameters (independent variables) on the biomass and single cell protein production, a central composite rotatable design (CCRD) of response surface methodology (RSM), a total number of thirty (30) experiments (with 6 center points) with different combinations of the factors were performed (Table 4.1).

4.1 Model Fitting and Statistical Analysis

The experimental data in terms of the biomass and protein yield are recorded in Table 4.1. These tabulated values were used as a raw data in response surface methodology (RSM) program to generate the best predicted model and its statistical analysis. By applying multiple regression analysis on the experimental data, second-order polynomial equations including linear, quadratic and interactive terms was developed which can express the relationship among process variables and the response terms of coded factors is given below:

$$\textbf{Biomass Yield} = 19.77 + 1.17A + 0.57B + 1.66C + 3.23D + 0.38AB - 0.29AC + 0.50AD + 0.55BC + 0.18BD + 1.36CD - 2.86A^2 - 1.63B^2 - 0.57C^2 - 0.90D^2 \quad (4.1)$$

$$\textbf{Protein Yield} = +50.71 + 7.32A + 2.11B + 3.11C + 10.95D + 1.73AB - 0.38AC + 3.10AD + 0.38BC - 0.24BD + 0.63CD - 5.65A^2 - 3.17B^2 - 0.88C^2 - 4.06D^2 \quad (4.2)$$

Where, Y is the biomass yield, A, B, C and D are temperature, pH, substrate concentration and fermentation time, respectively.

Table 4.1. Central composite rotatable design (CCRD) matrix of factors and real values along with biomass and protein as response.

Run Order	Uncoded process variables				Responses	
	Temperature (°C)	pH	Substrate Concentration (%)	Fermentation Period (Day)	Biomass (g/L)	Protein (%)
1*	30	6	15	3	19.90	47.46
2	25	5	10	2	9.13	20.89
3*	30	6	15	3	20.77	52.87
4	30	6	15	1	9.12	10.26
5	20	6	15	3	7.84	15.49
6	30	6	15	5	24.68	65.11
7*	30	6	15	3	19.23	51.01
8	25	7	10	2	9.39	18.50
9	25	7	20	4	18.44	38.91
10	25	5	10	4	11.63	31.16
11	30	6	5	3	14.19	40.95
12	35	7	10	2	11.33	33.39
13*	30	6	15	3	19.47	52.86
14	35	5	10	2	11.87	23.64
15	30	6	25	3	22.28	59.87
16	25	5	20	2	9.72	22.50
17	30	8	15	3	15.09	45.28
18	35	7	20	4	22.73	61.02
19*	30	6	15	3	20.77	53.24
20	35	5	20	4	18.83	54.38
21	30	4	15	3	12.84	37.21
22	25	5	20	4	16.79	37.05
23*	30	6	15	3	18.49	46.84
24	25	7	20	2	10.13	25.54
25	35	7	10	4	16.47	55.34
26	35	7	20	2	12.87	35.24
27	35	5	10	4	15.19	49.43
28	25	7	10	4	10.76	32.06
29	35	5	20	2	9.91	26.49
30	40	6	15	3	10.32	47.14
*Center Point						

The statistical significance of the developed models was based on the results of analysis of variance (ANOVA). The regression coefficient values for the second-order polynomial equations are presented in Table (4.2), and it indicates that the equation adequately represents the actual relationship between the response and their significant variables.

As shown in Table (4.2), ANOVA of regression model demonstrates that the models are highly significant, as it is evident from the high F-values such as 31.25 and F 21.28 for biomass and protein, respectively with a very low probability value ($p < 0.0001$). These indicate that most of the variation in the response could be explained by the developed models (Prakash Maran et al., 2017). R^2 describe the degree of fitness of the models (Prakash Maran et al., 2013). The high values of R^2 (0.9669 and 0.9521 for biomass and protein, respectively) clearly demonstrated that the developed models are precise in exhibiting the relationship between the response and independent variables. The value of adjusted R^2 for biomass (0.9359) and protein (0.9073) is also high and advocates a high correlation between the observed and predicted values. In this study, the predicted R^2 values for biomass (0.8343) and protein (0.7522) are in reasonable agreement with the adjusted R^2. Moreover, the low CV values for biomass (8.25%) and protein (11.31%) clearly indicated that the deviations between experimental and predicted values are low and also showed a high degree of precision and a good deal of reliability in conducted experiments (Prakash Maran et al., 2013).

Adequate precision measures the signal to noise ratio. A ratio of greater than four is desirable. In this study, the adequate precision (signal to noise ratio) was found to be greater than four for all responses, which indicat that these models can be used to navigate the design space (Manivannan & Rajasimman, 2011). The óóýtnessôô of the models were eva indicated the adequacy of models to accurately predict the variation as it was not significant ($P > 0.05$) relative to the pure error.

Table 4.2. Analysis of Variance (ANOVA) for response surface quadratic model for the production of biomass and single cell protein.

Source	**df**	**Biomass**				**Single Cell Protein**			
		β	**Sum of square**	**F- value**	**P level**	**β**	**Sum of square**	**F- value**	**P level**
Model	14	19.77	669.78	31.25	0.0001	50.54	6005.26	21.28	0.0001
Temperature (A)	1	1.17	33.06	21.59	0.0003	7.48	1285.02	63.76	0.0001
pH (B)	1	0.57	7.67	5.01	0.0408	1.94	106.76	5.30	0.0361
Substrate Conc. (C)	1	1.66	66.15	43.21	0.0001	2.77	231.73	11.50	0.0040
Fermentation period (D)	1	3.23	250.99	163.94	0.0001	10.62	2878.42	142.83	0.0001
AB	1	0.38	2.36	1.54	0.2330	1.73	47.68	2.37	0.1448
AC	1	-0.29	1.38	0.90	0.3567	-0.13	2.30	0.11	0.7404
AD	1	0.50	3.98	2.60	0.1279	3.35	154.17	7.65	0.0144
BC	1	0.55	4.83	3.16	0.0958	0.13	2.34	0.12	0.7380
BD	1	0.18	0.52	0.34	0.5703	-0.49	0.92	0.045	0.8341
CD	1	1.36	29.76	19.44	0.0005	0.13	6.28	0.31	0.5850
A^2	1	-2.86	223.62	146.07	0.0001	-5.69	875.96	43.47	0.0001
B^2	1	-1.63	73.27	47.86	0.0001	-3.21	275.59	13.67	0.0021
C^2	1	-0.57	8.82	5.76	0.0298	-0.92	21.17	1.05	0.3217
D^2	1	-0.90	22.31	14.57	0.0017	-4.10	451.93	22.43	0.0003
Lack of fit	10		18.92	**2.34**	**0.1806**		261.0	**3.16**	**0.1080**
R^2			**0.9669**				**0.9521**		
Adjusted- R^2			**0.9359**				**0.9073**		
Predicted- R^2			**0.8343**				**0.7522**		
Adequate Precision			**19.573**				**16.724**		
C.V. %			**8.25**				**11.31**		

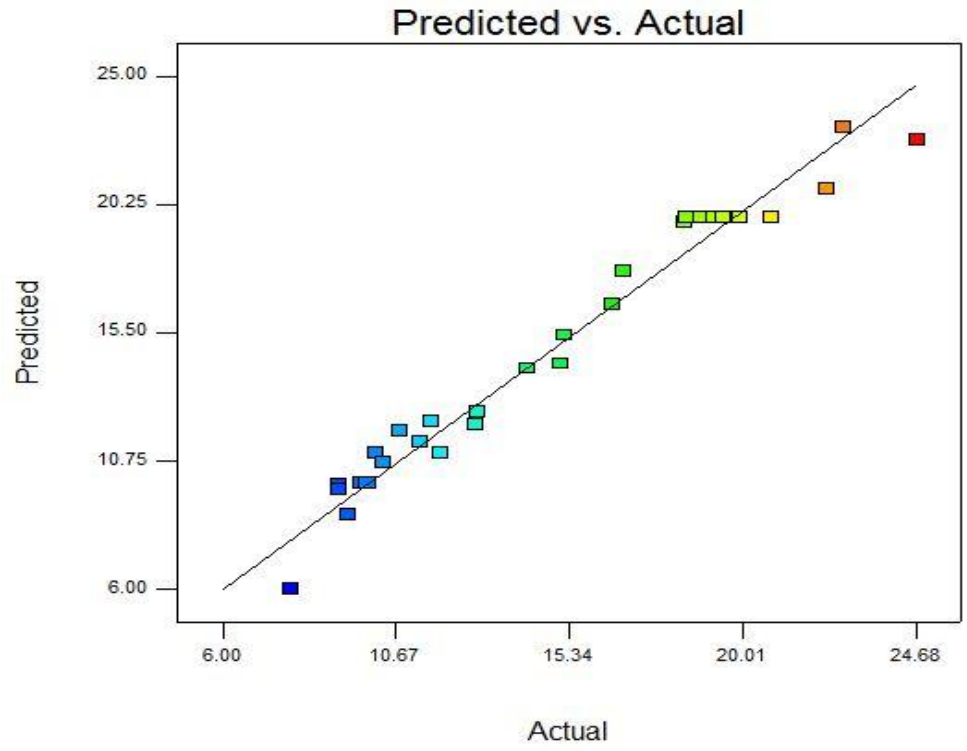

Figure 4.1a. Diagnostic plots showing the closeness between experimental and predicted values for biomass.

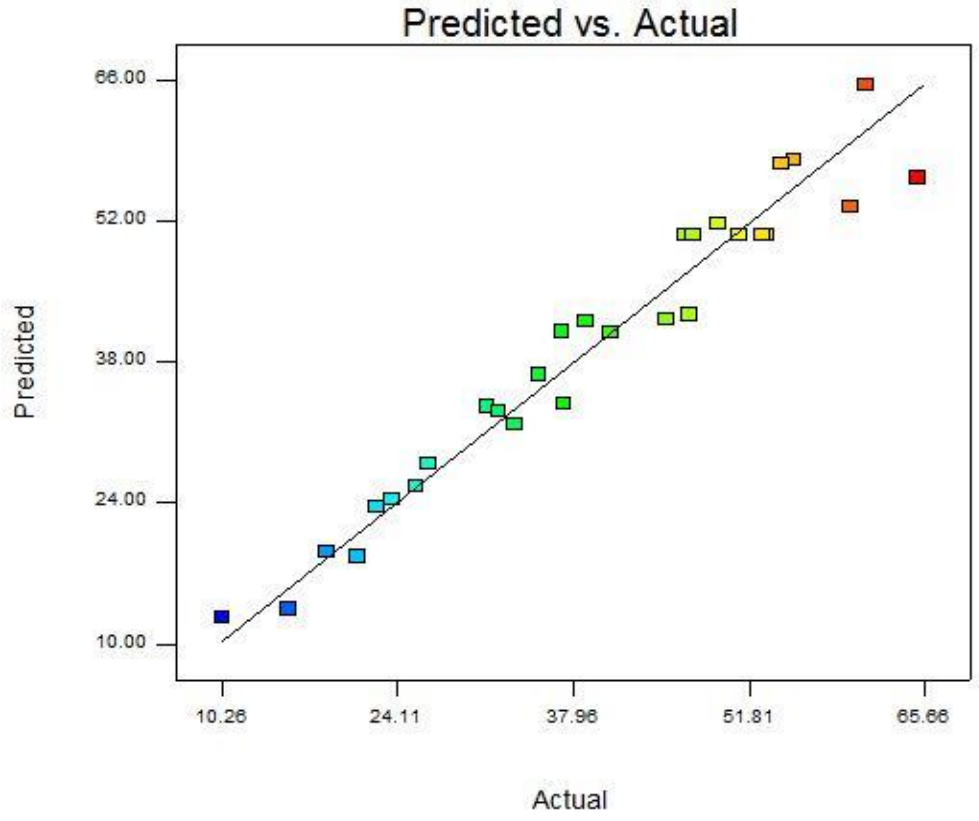

Figure 4.1b. Diagnostic plots showing the closeness between experimental and predicted values for protein.

In general, the exploration of fitted response surface models may lead to misleading results, unless the model exhibits adequacy essential (Prakash Maran et al., 2015). The diagnostic plot such as predicted versus actual values (Fig. 4.1 a & b) is used to evaluate the relationship and model satisfactoriness between experimental data and predicted data obtained from the developed models. The data points lie very closely to the straight line (Fig. 4.1a & Fig. 4.1b), which exhibited high correlation between the experimental data and predicted data obtained from the models.

4.2 Influence of Process Variables

In the present study, four factors at five level central composite rotatable design (CCRD) was used to study the influences of temperature, pH, substrate concentration, and fermentation were investigated on the two response, such as *Aspergillus niger* biomass and protein. Response surface and contour plots are graphical representations of a regression equation that illustrate the main and interactive effects of independent variables on a response variable. The response variables (substrate concentration-fermentation time on biomass and temperature-fermentation time on protein) are represented in three dimensional (3D) plots. The 3D contour plots of the quadratic model with one parameter maintained constant at the midpoint and the other parameters changed within the experimental limits are presented in Fig. 4.2 and Fig. 4.3.

4.2.1 Effects of Process Parameters on Biomass Production

Table 4.2 showed that all the process parameters such as temperature, pH, substrate concentration and fermentation period have a positive and significant ($P < 0.05$) effect on the biomass yield. For biomass response, the model ($R^2 = 0.99$) indicates the maximum positive effect of fermentation period ($\beta = 1.66$), substrate concentration ($\beta = 1.66$), temperature (β = positive signs of linear terms revealed that with the increase of linear independent variables, there will be an increase in biomass production. However, all the quadratic terms (A^2, B^2, C^2 and D^2) have a negative impact on biomass yield which is significant at 5% level of significance.

The response surface and contour plot (Fig. 4.2) was generated for the fitted model to visualize the combined effect of different variables on the yield of biomass. Moreover, the interaction term ósubstrate concentration influence on the biomass production ($P < 0.05$). From Fig. 4.2, it is inferred that an increase in fermentation period (2 to 4 days) with combination of substrate concentration (10 to 20%) resulted in an increase in biomass production. An incubation time of 4 days was also reported by various researchers for enzyme production using microbial strains (Managamuri et al., 2016; Mohan et al., 2014). Dasari et al. (2011) also reported that *Amycolatopsis alba* var. nov. DVR D4 strain produced maximum bioactive compound after 4 days of incubation.

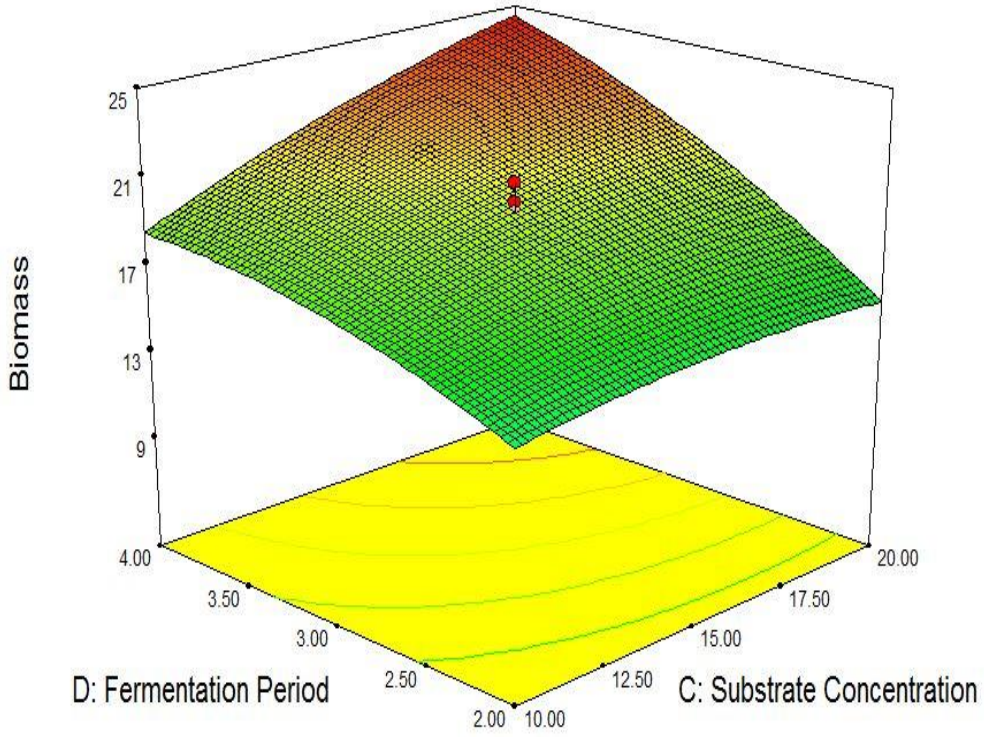

Figure 4.2. Three dimensional (3D) plot showing the effect of fermentation period and substrate concentration on biomass production.

In addition, carbon and energy sources are the most important nutrient required for the growth of the microorganism and biomass formation (Nasseri et al., 2011; Suman et al., 2015). Since banana peel contains a huge amount of lignocellulosic compounds, which during fermentation converted into the reducing sugar by microorganisms. Therefore, sugar components of the substrate are metabolized by fungal strains resulting in an enhanced production of biomass (Saheed et al., 2015; Yabaya & Ado, 2008; Adoki, 2008). Similar outcomes was also found by Jamal et al. (2009) and Essien et al. (2005).

4.2.2 Effects of Process Parameters on Protein Content

The F and P values in Table 4.2 reveals that all the process variables have a positive and significant effect on the protein yield from biomass. The β value clearly illustrates that fermentation period is the main factor on the protein yield followed by temperature (β = 7.48), pH (β = 1.94). This outcome revealed that as the fermentation period increases protein production also increased. This is possibly due to the high content of lignocellulosic compounds in banana peel, which during fermentation converted to sugars that helped to proper distribution of nutritional contents required for the growth of microorganisms at different fermentation period (Isaac & Chiedu, 2016). It is clearly identified in Table 4.2 that all the quadratic terms of temperature, pH, substrate concentration and fermentation period have negative and significant ($P < 0.05$) effect on protein content with the exception of fermentation period.

To obtain a better understanding of the parameters effect, response surface 3D contour plots are drawn (Fig. 4.3.) from the developed mathematical model (Eq. 4.2).

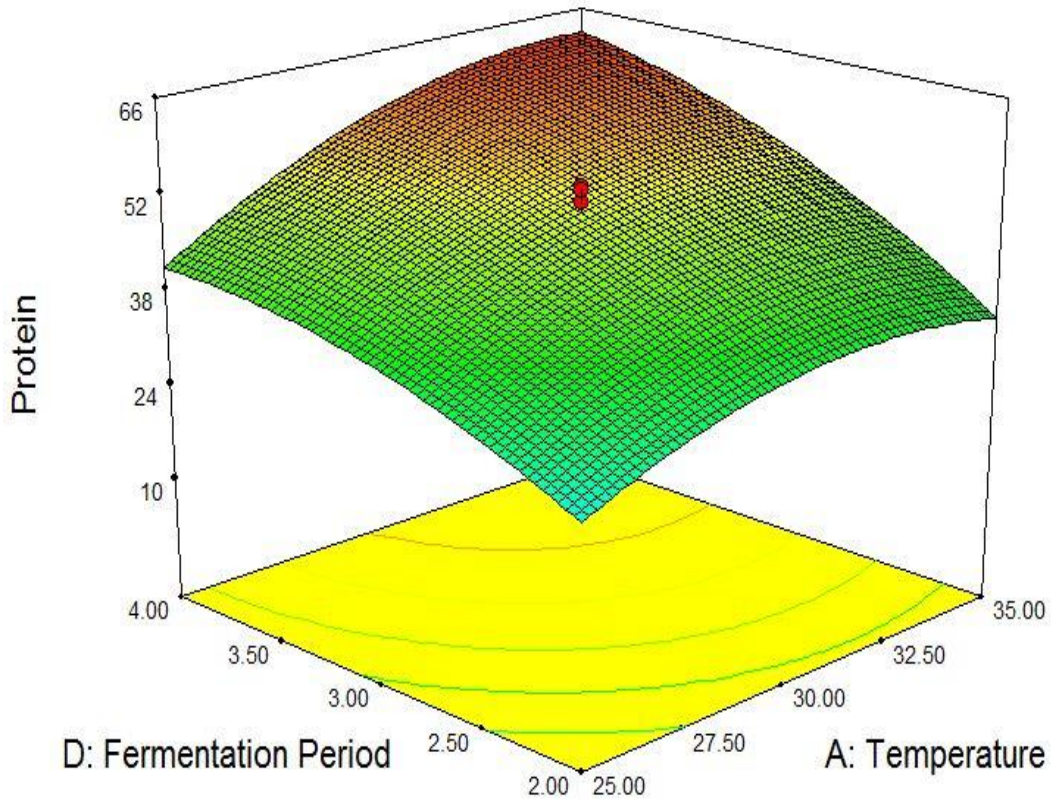

Figure 4.3. Three dimensional (3D) plot showing the effect of temperature and fermentation period on protein content.

The interaction term ó temperature and ferm statistically significant effect on the protein content at 95% confidence level. The results showed that the protein content increased with the increasing temperature from 25 to 35ºC and fermentation period from 2 to 4 days. Generally, the temperature of the cultivation medium is one of the most important variables for the growth of microbes. The range of temperature affects growth rate, nutritional requirement, chemical and enzymatic composition of the cells (Chi & Zhao, 2003; Umesh et al., 2017). Shehu & Bello (2011) and Dinarvand et al. (2017) reported that the optimum temperature for the growth of *Aspergillus* species ranged from 30-35ºC which supports the present findings.

4.3 Optimization of the Process Parameters

Numerical optimization was performed using the developed models for the biomass and protein yield. The temperature, pH, substrate concentration and fermentation period were set in the range. Moreover, the response for biomass and protein were set at maximum. The Design Expert program was run for the optimum conditions and the solutions. The best solution was found with a maximum desirability value selected as the optimum conditions for biomass and protein production. Therefore, the predicted optimum conditions were obtained as temperature of 34.27ºC, pH of 6.32, substrate concentration of 10% and fermentation period of 4 days.

4.4 Validation of the Models

In terms of model verification, duplicate experiments were carried out under the recommended optimum condition with a slight modification in temperature by 34ºC and pH by 6 in exchange of 34.27ºC and 6.32, respectively. The experimental values (means of two replications) and predicted values of various responses are presented in Table 4.3. It is clearly shown in Table 4.3 that the obtained experimental values were adequate with the predicted values of the response surface model because the experimental values were very close to the predicted values, which satisfy the predicted model.

Table 4.3. Comparison of experimental values with predicted values.

Response	Predicted Value	Experimental Value (Mean ± SEM)
Biomass (g/L)	18.1105	18.56 ± 0.16
Protein (%)	58.7177	59.06 ± 0.13

CHAPTER V

SUMMARY AND CONCLUSION

CHAPTER V

SUMMARY AND CONCLUSION

Central composite rotatable design coupled with response surface methodology (RSM) was successfully employed to optimize and study the individual and interactive effect of process variables such as temperature (20-40^{o}C), pH (4-8), substrate concentration (5-25%) and fermentation period (1-5days) on the production of biomass and protein from *Aspergillus niger* using banana fruit peel. From this study, second-order polynomial models were developed to describe the relationship between the independent variables and the responses which was significant at 95 % confidence level ($P < 0.05$). The developed models could have the ability to predict the biomass and protein and was found to be comparable with that of the experimental values. Analysis of determination values (R^2) of 0.9669 for biomass and 0.9521 for protein, ensuring a satisfactory fit of the second-order polynomial models with the experimental data. Results also revealed a significant positive effect of the process variables (e.g. temperature, pH, substrate concentration and fermentation period) on the biomass and protein yield. Furthermore, the substrate concentration and fermentation period were found to have the most significant effect on the production of biomass and protein, respectively. After numerical optimization, temperature of 34.27^{o}C, pH of 6.32, substrate concentration of 10% and the fermentation period of 4 days was found as the optimum conditions for biomass and protein production from *Aspergillus niger* using banana peel as the substrate, which predicted 18.1105 g/L biomass and 58.7177% protein, respectively. Under the optimized conditions, the experimental values (biomass: 18.56 g/L and protein: 59.06%) closely agreed with the predicted values and indicated the suitability of the developed models. Overall, the results of the present study suggest that the model obtained through response surface methodology is adequate for the improved production of biomass and protein from *Aspergillus niger* using banana peel as substrate. However, further study is recommended to confirm the toxicity levels of the produced biomass before using as protein.

REFERENCES

REFERENCES

Adedayo, M. R., Ajiboye, E. A., Akintunde, J. K. & Odaibo, A. (2011). Single Cell Proteins As Nutritional Enhancer. *Advances in Applied Science Research*, *2*(5), 396 ï409.

Ahmed, S., Ahmad, F., Hashmi, A. B. U. S., Ahmed, S. & Al, E. T. (2010). Production of Microbial Biomass Protein By Sequential Culture Fermentation of *Arachniotus* Sp., and *Candida Utilis*. *Pakistan Journal of Botany*, *42*(2), 1225 ï1234.

Amande T. J. & Itah, A. (2011). Single Cell Protein (SCP) Production Using Banana Peels As Mono - Substrate. *Nigerian Journal of Microbiology*, *25*, 2332 ï2338.

Andersen, M. R., Nielsen, M. L. & Nielsen, J. (2008). Metabolic model integration of the bibliome, genome, metabolome and reactome of *Aspergillus niger*. *Molecular Systems Biology*, *4*(178), 1 ï13.

Anhwange, B. A., Ugye, T. J. & Nyiaatagher, T. D. (2009). Chemical Composition of Musa sepientum (Banana) Peels . *Electronic Journal of Environmental, Agricultural and Food Chemistry*, *8*(6), 437 ï442.

Anupama & Ravindra, P. (2001). Studies on Production of Single Cell Protein by *Aspergillus niger* in Solid State Fermentation of Rice Bran. *Brazilian Archives of Biology and Technology*, *44*(1), 79 ï88.

Anupama & Ravindra, P. (2000). Value-added food: Single cell protein. *Biotechnology Advances*, *18*(6), 459 ï479.

Azam, S., Khan, Z., Ahmad, B., Khan, I. & Ali, J. (2014). Production of Single Cell Protein from Orange Peels Using *Aspergillus niger* and *Saccharomyces cerevisiae* Pakistan Council of Scientific and Industrial Research Laboratories Complex ,. *Global Journal of Biotechnology & Biochemistry*, *9*(1), 14 ï18.

Babu, M., Raj, S. P., Nirmala, C. B. & Deccaraman, M. (2014). Studies on the Optimiization of Biomass Production Using *Kluyveromyces marxianus* Isolated From Paneer Whey. *International Journal of Pharma and Bio Sciences*, *5*(4), 1030 ï1036.

Bacha, U., Nasir, M., Khalique, A., Anjum, A. A. & Jabbar, M. A. (2011). Comparative

Assessment of Various Agro-Industrial Wastes for *Saccharomyces Cerevisiae* Biomass Production and Its Quality Evaluation as Single Cell Protein. *The Journal of Animal & Plant Sciences*, *21*(4), 844–849.

Balasubramani, P., Palaniswamy, P. T., Visvanathan, R., Thirupathi, V., Subbarayan, A. & Prakash Maran, J. (2015). Microencapsulation of garlic oleoresin using maltodextrin as wall material by spray drying technology. *International Journal of Biological Macromolecules*, *72*, 210–217.

Bamberg, J. (2000). *British Petroleum and Global Oil , 1950-1975 : The Challenge of Nationalism*. *British Petroleum series*. Cambridge University Press.

Bar-Even, A., Noor, E., Lewis, N. E. & Milo, R. (2010). Design and analysis of synthetic carbon fixation pathways . *Proceedings of the National Academy of Sciences of the United States of America*, *107*(19), 8889–8894.

Barbesgaard, P., Heldt-hansen, H. P. & Diderichsen, B. (1992). On the safety of *Asperyillus oryzae*: a review. *Applied Microbiology Biotechnology*, *36*, 569–572.

Bekatorou, A., Psarianos, C. & Koutinas, A. A. (2006). Production of food grade yeasts. *Food Technology and Biotechnology*, *44*(3), 407–415.

Bhalla, T. C., Sharma, N. N. & Sharma, M. (2007). Production of Metabolites, Industrial enzymes, Amino acid, Organic acids, Antibiotics, Vitamins and Single Cell Proteins. *National Science Digital Library, India*, 1–47.

Bogdahn, I. (2015). Agriculture-independent, sustainable, fail-safe and efficient food production by autotrophic single-cell protein. *PeerJ PrePrints*, 2–20.

Boyle, N. R. & Morgan, J. A. (2011). Computation of metabolic fluxes and efficiencies for biological carbon dioxide fixation. *Metabolic Engineering*, *13*(2), 150–158. https://doi.org/10.1016/j.ymben.2011.01.005

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254.

Burgents, J. E., Burnett, K. G. & Burnett, L. E. (2004). Disease resistance of Pacific white

shrimp, *Litopenaeus vannamei*, following the dietary administration of a yeast culture food supplement. *Aquaculture*, *231*(1–4), 1–8.

Chen, H., Zhang, Q., Shu, G. & Li, Q. (2016). Optimization of Fermentation Technology for Producing Single Cell Protein from Yam Starch by Orthogonal Test. *Advance Journal of Food Science and Technology*, *10*(11), 833–837.

Chen, W., Zhao, Z., Chen, S. F. & Li, Y. Q. (2008). Optimization for the production of exopolysaccharide from *Fomes fomentarius* in submerged culture and its antitumor effect in vitro. *Bioresource Technology*, *99*(8), 3187–3194.

Chi, Z. & Zhao, S. (2003). Optimization of medium and cultivation conditions for pullulan production by a new pullulan-producing yeast strain. *Enzyme and Microbial Technology*, *33*(2–3), 206–211.

Contesini, F. J., da Silva, V. C. F., Maciel, R. F., de Lima, R. J., Barros, F. F. C. & de Oliveira Carvalho, P. (2009). Response surface analysis for the production of an enantioselective lipase from *Aspergillus niger* by solid-state fermentation. *Journal of Microbiology (Seoul, Korea)*, *47*(5), 563–71.

Correia, R., Magalhães, M. & Macêdo, G. (2007). Protein enrichment of pineapple waste with *Saccharomyces cerevisiae* by solid state bioprocessing. *Journal of Scientific and Industrial Research*, *66*(3), 259–262.

Dasari, V. R. R. K., Nikku, M. Y. & Donthireddy, S. R. R. (2011). Screening of antagonistic marine actinomycetes: optimization of process parameters for the production of novel antibiotic by *Amycolatopsis alba* var. Nov. DVR D4. *Journal of Microbial and Biochemical Technology*, *3*(5), 92–98.

Daud, S. K. B. (2012). Production of Extracellular Protease Enzyme by *Aspergillus niger*. Universiti Malaysia Pahang.

Deepake, U. (2008). Aero-microbiological studies of Moisture Affected Buildings in the Indoor Environment. *Journal of Young Investigatos*, *19*(11).

Del Rosario, E. J. & Pamatong, F. V. (1985). *Continuous flow fermentation of banana fruit pulp sugar into ethanol by carrageenan-immobilized yeast. Biotechnology letters*.

Kluwer/Plenum Publishers.

Dhanasekaran, D., Lawanya, S., Saha, S., Thajuddin, N. & Panneerselvam, A. (2011). Production of Single Cell Protein from Pineapple Waste using Yeast. *Innovative Romanian Food Biotechnology*, *8*, 26 i32.

Doelle, H. W. (1994). *Microbial Process Development*. World Scientific Publishing Co Inc. https://doi.org/10.1142/2129

Dunlop, C. E. (1975). Production of single-cell protein from insoluble agricultural wastes by mesophiles. *Single Cell Protein II, International Conference on Single Cell Protein*. Retrieved from http://agris.fao.org/agris-search/search.do?recordID=US201303074915#.WVokjjo24ME.mendeley

Enwefa, C. (1991). Biomass production from banana skins. *Applied Microbiology and Biotechnology*, *36*, 283 i284.

Essien, J. P., Akpan, E. J. & Essien, E. P. (2005). Studies on mould growth and biomass production using waste banana peel. *Bioresource Technology*, *96*(13), 1451 i1456.

Omwango, E. O., Njagi, E. N. M., Orinda, G. O. & Wanjau, R. N. (2013). Nutrient Enrichment of Pineapple Waste using *Aspergillus niger* and *Trichoderma viride* by Solid State Fermentation. *African Journal of Biotechnology*, *12*(43), 6193 i6196.

Ferreira, I. M. P. L. V. O., Pinho, O., V

Saccharomyces yeast biomass: characteristics and potential applications. *Trends in Food Science & Technology*, *21*(2), 77 i84.

Foyle, T., Jennings, L. & Mulcahy, P. (2007). Compositional analysis of lignocellulosic materials: Evaluation of methods used for sugar analysis of waste paper and straw. *Bioresource Technology*, *98*(16), 3026 i3036.

Frey, C. N. (1930). History and Development of the Mordern Yeast Industry. *Industrial & Engineering Chemistry*, *22*(11), 1154 i1162.

Gabhane, J., Prince William, S. P. M., Gadhe, A., Rath, R., Vaidya, A. N. & Wate, S. (2014). Pretreatment of banana agricultural waste for bio-ethanol production: Individual and interactive effects of acid and alkali pretreatments with autoclaving,

microwave heating and ultrasonication. *Waste Management*, *34*(2), 498 ï503.

Gad, A. S., Hasan E. A., Aziz, A.Abd. E. (2010). Utilization of *Opuntia ficus indica* waste for production of *Phanerochaete chrysosporium* bioprotein. *Journal of American Science*, *6*(8), 208 ï216.

Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B. & Cosby, B. J. (2003). The Nitrogen Cascade. *American Institute of Biological Sciences*, *53*(4), 341.

Gao, L., Chi, Z., Sheng, J., Ni, X. & Wang, L. (2007). Single-cell protein production from *Jerusalem artichoke* extract by a recently isolated marine yeast *Cryptococcus aureus* G7a and its nutritive analysis. *Applied Microbiology and Biotechnology*, *77*(4), 825 ï 832.

Ghasem D. Najafpour. (2007). CHAPTER 14 ï Single-Cell Protein. In *Biochemical Engineering and Biotechnology*, 332 ï341.

Coman, G. , L e ,uI.x Geeorgesru, L. & Bahrim, G. (2012). Optimization of Protein Production by *Geotrichum candidum* MIUG 2.15 by Cultivation on Paper Residues using Response Surface Methodology. *BioResources*, *7*(4), 5290 ï5303.

Giri, S. K. & Prasad, S. (2007). Optimization of Microwave-Vacuum Drying of Button Mushrooms Using Response-Surface Methodology. *Drying Technology*, *25*(5), 901 ï 911.

Gomashe, A. V, Pounikar, M. A. & Gulhane, Substrate for Single Cell Protein Production from *Bacillus subtilis* NCIM 2010. *International Journal of Life Sciences*, *2*(2), 119 ï123.

Haddish, K. (2015). Production of Single Cell Protein from Fruit of Beles (*Opuntia Ficus-Indica* L.) Peels Using *Saccharomyces cerevisiae*. *Journal of Microbiology & Experimentation Production*, *3*(1), 1 ï4.

Halasz, A. & Lasztity, R. (1990). *Use of Yeast Biomass in Food Production*. Taylor & Francis. Retrieved from https://books.google.com.bd/books?id=xbZRt2pKwqUC

Hao, L. M., Xing, X. H., Li, Z., Zhang, J

Optimization of effect factors for mycelial growth and exopolysaccharide production by *Schizophyllum commune*. *Applied Biochemistry and Biotechnology*, *160*(2), 621 ï 631.

Hezarjaribi, M., Ardestani, F. & Ghorbani, H. R. (2016). Single Cell Protein Production by *Saccharomyces cerevisiae* Using an Optimized Culture Medium Composition in a Batch Submerged Bioprocess. *Applied Biochemistry and Biotechnology*, 1 ï10.

Isaac, G. & Chiedu, C. (2016). Effect of Fermentation Periods on the Physicochemical and Sensory Properties of Gari. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 10*(1), 37 ï42.

Ivarson, K. C. & Morita, H. (1982). Single-Cell Protein Production by the Acid-Tolerant Fungus *Scytalidium acidophilum* from Acid Hydrolysates of Waste Paper. *Applied and Environmental Microbiology, 43*(3), 643 ï647.

Jaganmohan, P., Daas, B. P. & Prasad, S. V. (2013). Production of Single Cell Protein (SCP) with *Aspergillus terreus* Using Solid State Fermentation. *European Journal of Biological Sciences, 5*(2), 38 ï43.

Jamal, P., Alam, M. Z., Ramlan, M., Salleh, M. & Nadzir, M. M. (2005). Screening of *Aspergillus* for citric acid production from palm oil mill effluent. *Biotechnology*, *4*(4), 275 ï278.

Jamal, P., Alam, M. Z. & Salleh, N. U. (2008). Media Optimization for Bioproteins Production from Cheaper Carbon Source. *Journal of Engineering Science and Technology*, *3*(2), 124 ï130.

Jamal, P., Alam, M. Z. & Umi, N. (2007). Potential strain to produce bioprotein from cheaper carbon source: Hope for millions. *American Journal of Biochemistry and Biotechnology*, *3*(2), 42 ï46.

Jamal, P., Fahrurrazi, T. M. & Alam, M. Z. (2009). Optimization of Media Composition for the Production of Biomass From Pineaapple Skin By Liquid State Bioconversion. *Journal of Applied Sciences, 9(17),* 3104-3109.

Kandari, V. & Gupta, S. (2012). Bioconversion of Vegetable and Fruit Peel Wastes in

viable product. *Journal of Microbiology and Biotechnology Research*, *2*(2), 308 ï312.

Ogbonda, K. H., Aminigo, R. E. & Abu, G. O. (2007). Influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina* sp. *Bioresource Technology*, *98*(11), 2207 ï2211.

Khan, M., Khan, S. S., Ahmed, Z. & Tanveer, A. (2009). Production of fungal single cell protein using *Rhizopus oligosporus* grown on fruit wastes. *Iological Forum – An International Journal*, *1*(2), 26 ï28.

Khan, M., Shaukat Saeed Khan, Ahmed, Z. & Tanveer, A. (2010). Production of Single Cell Protein from *Saccharomyces cerevisiae* by utilizing Fruit Wastes. *Nanobiotechnica Universale*, *1*(2), 127 ï132.

Kumari, M., Survase, S. A. & Singhal, R. S. (2008). Production of schizophyllan using *Schizophyllum commune* NRCM. *Bioresource Technology*, *99*(5), 1036 ï1043.

Lee, J., Ye, L., Landen, W. O. & Eitenmiller, R. R. (2000). Optimization of an Extraction Procedure for the Quantification of Vitamin E in Tomato and Broccoli using Response Surface Methodology. *Journal of Food Composition and Analysis*, *13*(1), 45 ï57.

Lenihan, P., Orozco, A., O-Neill, E., Ahmad, M. N. M., Rooney, D. W. & Walker, G. M. (2010). Dilute acid hydrolysis of lignocellulosic biomass. *Chemical Engineering Journal*, *156*(2), 395 ï403.

Lim, J. Y., Yoon, H. S., Kim, K. Y., Kim, K. S., Noh, J. G. & Song, I. G. (2010). Optimum conditions for the enzymatic hydrolysis of citron waste juice using response surface methodology (RSM). *Food Science and Biotechnology*, *19*(5), 1135 ï1142.

Liu, C. H., Lu, W. B. & Chang, J. S. (2006). Optimizing lipase production of *Burkholderia* sp. by response surface methodology. *Process Biochemistry*, *41*(9), 1940 ï1944.

López-Baca, A. & Gómez, J. (1992). Fermentation patterns of whole banana waste liquor with four inocula. *Journal of the Science of Food and Agriculture*, *60*(1), 85 ï89.

Luo, J., Liu, J., Ke, C., Qiao, D., Ye, H., Sun, Y. & Zeng, X. (2009). Optimization of medium composition for the production of exopolysaccharides from *Phellinus baumii* Pilát in submerged culture and the immuno-stimulating activity of

exopolysaccharides. *Carbohydrate Polymers*, *78*(3), 409 ï415.

Lyutskanov, N., Koleva, L., Stateva, L., Venkov, P. & Hadjiolov, A. (1990). Protein extracts for nutritional purposes from fragile strains of *Saccharomyces cerevisiae*: Reduction of the nucleic acid content and applicability of the protein extracts. *Journal of Basic Microbiology*, *30*(7), 523 ï528.

Madhumithah, C. G., Krithiga, R. & Sundaram, S. (2011). Utilization of Vegetable Wastes for Production of Protease by Solid State Fermentation Using *Aspergillus niger*. *World Journal of Agricultural Sciences 7*, *7*(5), 550 ï555.

Yousufi, M. K. (2012). Utilization of various fruit wastes as substrates for the production of Single Cell protein using *Aspergillus oryzae* and *Rhizopus oligosporus*. *International Journal of Advanced Scientific and Technical Research*, *5*(2), 92 ï95.

Malinowska, E., Krzyczkowski, W., Herold, F. & (2009). Improved G. simultaneous production of mycelial biomass and polysaccharides by submerged culture of *Hericium erinaceum*: Optimization using a central composite rotatable design (CCRD). *Journal of Industrial Microbiology and Biotechnology*, *36*(12), 1513 ï1527.

Managamuri, U., Vijayalakshmi, M., Poda, S., Ganduri, V. S. R. K. & Babu, R. S. (2016). Optimization of culture conditions by response surface methodology and unstructured kinetic modeling for bioactive metabolite production by *Nocardiopsis litoralis* VSM-8. *3 Biotech*, *6*(229), 1 ï19.

Manivannan, P. & Rajasimman, M. (2011). Optimization of Process Parameters for the Osmotic Dehydration of Beetroot in Sugar Solution. *Journal of Food Process Engineering*, *34*(3), 804 ï825.

Prokash Maran, J., Priya, B. & Nivetha, C. V. (2015). Optimization of ultrasound-assisted extraction of natural pigments from *Bougainvillea glabra* flowers. *Industrial Crops and Products*, *63*, 182 ï189.

Babu, M., Raj, S. P., Nirmala, C. B., Deccaraman, M. & Sagadevan, E. (2014). Production of Single Cell Protein using *Kluveromyces marxianus* isolated from paneer whey. *International Journal of Biomedical And Advance Research*, *5*(5), 255 ï257.

Mekonnen, M. M. & Hoekstra, A. Y. (2014). Water footprint benchmarks for crop production: A first global assessment. *Ecological Indicators*, *46*, 214 ï223.

Mohan, S. K., Viruthagiri, T., & Arunkumar, C. (2014). Statistical optimization of process parameters for the production of tannase by *Aspergillus flavus* under submerged fermentation. *3 Biotech*, *4*, 159 ï166.

Mondal, A. K., Sengupta, S., Bhowal, J. & Bhattacharya, D. K. (2012). Utilization of Fruit Wastes in Producing Single Cell Protein. *International Journal of Science, Environment and Technology*, *1*(5), 430 ï438.

Montgomery, D. C. (2013). *Design and analysis of experiments*. John Wiley & Sons, Inc.

Moorthy, I. G., Maran, J. P., Surya, S. M., Naganyashree, S. & Shivamathi, C. S. (2015). Response surface optimization of ultrasound assisted extraction of pectin from pomegranate peel. *International Journal of Biological Macromolecules*, *72*, 1323 ï 1328.

Irfan, M., Nazir, M. I., Nadeem, M., Gulsher, M., Quratualain S. & Shahjahan, B. (2011). Optimization of Process Parameters for the Production of Single Cell Biomass of *Candida utilis* in Solid State Fermentation. *American-Eurasian Journal of Agricultural and Environmental Science*, *10*(2), 264 ï270.

Nangul, A. & Bhatia, A. (2013). Marvelous Source of Single Cell Proteins. *Journal of Microbiology, Biotechnology and Food Sciences*, *3*(1), 15 ï18.

Nasir, M. & Butt, M. S. (2011). Maize Germ: A Nutrient Dense Substance for Food Value-Addition: Potential of Maize Germs and Its Fractions for Value Addition to Improve Food Security. *LAP Lambert Academic Publishing, Germany*. LAP Lambert Academic Publishing, Germany.

Nasseri, A. T., Rasoul-Amini, S., Morowvat, M. H. & Ghasemi, Y. (2011). Single Cell Protein: Production and Process. *American Journal of Food Technology*, *6*(2), 103 ï 116.

Nwufo, M. I., Amadi, M. C. & Ihejirika, G. O. (2014). Production of Single Cell Protein Using Agricultural Wastes. *International Journal of Agriculture and Rural*

Development, *17*(3), 1942 ï1946.

Ojokoh, a. O. & Uzeh, R. E. (2005). Production of *Saccharomyces cerevisiae* biomass in papaya extract medium. *African Journal of BiotechnologyJournal of Biotechnology*, *4*, 1281 ï1284.

Saheed, O. K., Jamal, P., Karim, M. I. A., Alam, M. Z. & Muyibi, S. A. (2013). Cellulytic Fruits Wastes: A Potential Support for Enzyme Assisted Protein Poduction. *Journal of Biological Sciences*, *13*(5), 379 ï385.

Saheed, O. K., Parveen Jamal, P., Karim, M. I. A., Alam, M. Z. & Muyibi, S. A. (2015). Utilization of fruit peels as carbon source for white rot fungi biomass production under submerged state bioconversion. *Journal of King Saud University – Science*, *28*(2), 143 ï151.

Oshoma, C.E. & Ikenebomeh, M. J. (2005). Production of *Aspergillus niger* Biomass from Rice Bran. *Pakistan Journal of Nutrition*, *4*(1), 32 ï36.

Paraskevopoulou, A., Athanasiadis, I., Kanellaki, M., Bekatorou, A., Blekas, G. & Kiosseoglou, V. (2003). Functional properties of single cell protein produced by kefir microflora. *Food Research International*, *36*(5), 431 ï438.

Pel, H. J., de Winde, J. H., Archer, D. B. H. (2007). Genome sequencing and analysis of the versatile cell factory *Aspergillus niger* CBS 513.88. *Nature Biotechnology*, *25*(2), 221 ï231.

Popa, O., Babeanu, N., Vamanu, A. & Vamanu, E. (2007). The utilization of the response surface methodology for the optimization of cultivation medium and growth parameters in the cultivation of the yeast strain *S. cerevisiae* 3.20 on ethanol. *African Journal of Biotechnology*, *6*(23), 2700 ï2707.

Prakash Maran, J., Manikandan, S., Priya, B. & Gurumoorthi, P. (2015). Box-Behnken design based multi-response analysis and optimization of supercritical carbon dioxide extraction of bioactive flavonoid compounds from tea (*Camellia sinensis* L.) leaves. *Journal of Food Science and Technology*, *52*(1), 92 ï104.

Prakash Maran, J., Manikandan, S., Vigna Nivetha, C. & Dinesh, R. (2017). Ultrasound

assisted extraction of bioactive compounds from *Nephelium lappaceum* L. fruit peel using central composite face centered response surface design. *Arabian Journal of Chemistry*, *10*, S1145 ïS1157.

Prakash Maran, J., Mekala, V. & Manikandan, S. (2013). Modeling and optimization of ultrasound-assisted extraction of polysaccharide from *Cucurbita moschata*. *Carbohydrate Polymers*, *92*(2), 2018 ï2026.

Prakash Maran, J., Sivakumar, V., Thirugnanasambandham, K. & Sridhar, R. (2013). Artificial neural network and response surface methodology modeling in mass transfer parameters predictions during osmotic dehydration of *Carica papaya* L. *Alexandria Engineering Journal*, *52*(3), 507 ï516.

Uchakalwar, P. R. & Chandak, A. M. (2014). Production of Single Cell Protein from Fruits waste by using S*accharomyce scerevisiae*. *International Journal of Advanced Biotechnology and Research(IJBR)*, *5*(4), 770 ï776.

Purwanto, L. A., Ibrahim, D. & Sudrajat, H. (2009). Effect of Agitation Speed on Morphological Changes in *Aspergillus niger* Hyphae During Production of Tannase. *World Journal of Chemistry*, *4*(1), 34 ï38.

Raja, R., Hemaiswarya, S., Kumar, N. A., Sridhar, S. & Rengasamy, R. (2008). A Perspective on the Biotechnological Potential of Microalgae. *Critical Reviews in Microbiology*, *34*(2), 77 ï88.

Rajoka, M. I., Khan, S. H., Jabbar, M. A., Awan, M. S. & Hashmi, A. S. (2006). Kinetics of batch single cell protein production from rice polishings with *Candida utilis* in continuously aerated tank reactors. *Bioresource Technology*, *97*(15), 1934 ï1941.

Rajoka, M. I., Kiani, M. A. T., Khan, S., Awan, M. S. & Hashmi, A. S. (2004). Production of single cell protein from rice polishings using *Candida utilis*. *World Journal of Microbiology and Biotechnology*, *20*(3), 297 ï301.

Rao, K. J., Kim, C. H. & Rhee, S.-K. (2000). Statistical optimization of medium for the production of recombinant hirudin from *Saccharomyces cerevisiae* using response surface methodology. *Process Biochemistry*, *35*(7), 639 ï647.

Reddy, Y. H. K., Srijana, M., Reddy, D. M. & Reddy, G. (2010). Coculture fermentation of banana agro-waste to ethanol by cellulolytic thermophilic *Clostridium thermocellum* CT2. *African Journal of Biotechnology*, *9*(13), 1926 ï1934.

Rezaei, P. S., Darzi, G. N. & Shafaghat, H. (2010). Optimization of the fermentation conditions and partial characterization for acido- t h e r m e amylase from ز *Aspergillus niger* NCIM 548. *Korean Journal of Chemical Engineering*, *27*(3), 919 ï 924.

Robinson, T., Chandran, B. & Nigam, P. (2001). Studies on the production of enzymes by white-rot fungi for the decolourisation of textile dyes. *Enzyme and Microbial Technology*, *29*(8 ï9), 575 ï579.

Rudravaram, R., Chandel, A. K., Linga, V. R. & Pogaku, R. (2006). Optimization of Protein Enrichment of Deoiled Rice Bran by Solid State Fermentation Using *Aspergillus oryzae* MTCC 1846. *International Journal of Food Engineering*, *2*(4), 1-14.

Saeed, M., Yasmin, I., Murtaza, M. A., Fatima, I. & Saeed, S . (2 0 1 6) . S i n g l e A novel value added food product. *Pakistan Journal of Food Sciences*, *26*(4), 211 ï 217.

Saheed, O. K., Jamal, P., Karim, M. I. A., Alam, M. Z. & Muyibi, S. A. (2016). Utilization of fruit peels as carbon source for white rot fungi biomass production under submerged state bioconversion. *Journal of King Saud University - Science*, *28*, 143 ï151.

Saima, M. A., Khan, M. Z. U., Anjum, M. I., Ahmed, S., Rizwan, M. & Ijaz, M. (2008). Investigation on the Availability of Amino Acids From Different Animal Protein Sources in Golden Cockerels. *Journal of Animal and Plant Science*, *18*(2 ï3), 53 ï56.

Saithi, S., Jorgen, B., Montira, N. & Anan, T. (2016). Mathematical Modeling of Biomass and Enzyme Production Kinetics by *Aspergillus niger* in Solid-State Fermentation at Various Temperatures and Moisture Contents. *Journal of Microbial & Biochemical Technology*, *8*(2), 123 ï130.

Sankar, N. R., Kumar, V. K., Shailaja, R., Saritha, K. & Naidu, N. V. (2011). Single Cell Protein Production by *Trichoderma harzianum* Using Waste Banana Peel. *Inernational Journal of Microbiological Research*, *2*, 78 ï81.

Schuster, E., Dunn-Coleman, N., Frisvad, J. & Van Dijck, P. (2002). On the safety of *Aspergillus niger* - A review. *Applied Microbiology and Biotechnology*, *59*(4 ï5), 426 ï 435.

S e m o v a , N . , S t o r m s , R . , J o h n , T . , G a u d e t , P Generation, annotation, and analysis of an extensive *Aspergillus niger* EST collection. *BMC Microbiology*, *6*(7), 1 ï10.

Shah, M. P., Reddy, G. V., Banerjee, R., Ravindra Babu, P. & Kothari, I. L. (2005). Microbial degradation of banana waste under solid state bioprocessing using two lignocellulolytic fungi (*Phylosticta* spp. MPS-001 and *Aspergillus* spp. MPS-002). *Process Biochemistry*, *40*(1), 445 ï451.

Sharma, N., Kalra, K. L., Oberoi, H. S. & Bansal, S. (2007). Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation. *Indian Journal of Microbiology*, *47*(4), 310 ï316.

Sharma, S. K., Mishra, I. M., Sharma, M. P. & Saini, J. S. (1988). Effect of particle size on biogas generation from biomass residues. *Biomass*, *17*(4), 251 ï263.

Shehu, K. & Bello, M. T. (2011). Effect of Environmental Factors on the Growth of *Aspergillus* S p e c i e s A s s o c i a t e d w i t h S t o r e d M i l l e constitute a major problem in the storage of agricultural products especially cereals . In the present study , the effe, *19*, 218 ï223.

Shipra, J. & Dikshit, S. . (2017). Alternative culture media for fungal growth using different formulation of protein sources. *International Journal of Pharma and Bio Sciences*, *8*(1), 445 ï452.

Singh, V., Haque, S., Niwas, R., Srivastava, A., Pasupuleti, M. & Tripathi, C. K. M. (2017). Strategies for fermentation medium optimization: An in-depth review. *Frontiers in Microbiology*, *7(2087)*.

Suman, G., Nupur, M., Anuradha, S. & Pradeep, B. (2015). S i n g l e C e l l P r o t e i n A Review. *InternatIonal Journal of Current Microbiology and Applied Sciences*, *4*(9), 251 ï262.

Survase, S., Saudagar, P. & Singhal, R. (2006). Production of scleroglucan from *Sclerotium rolfsii* MTCC 2156. *Bioresource Technology*, *97*(8), 989 ï993.

Teng, Y. & Xu, Y. (2008). Culture condition improvement for whole-cell lipase production in submerged fermentation by *Rhizopus chinensis* using statistical method. *Bioresource Technology*, *99*(9), 3900 ï3907.

Tsao, G. T. (1976). Principles of microbe and cell cultivation, S. John Pirt, Halsted Press, Division of John Wiley and Sons, New York. *AIChE Journal*, *22*(3), 621 ï621.

Ugalde, U. O. & Castrillo, J. I. (2002). Single cell proteins from fungi and yeasts. *Applied Mycology and Biotechnology*, *2*, 123 ï149.

Umesh, M., Priyanka, K., Thazeem, B. & Preethi, K. (2017). Production of Single Cell Protein and Polyhydroxyalkanoate from Carica papaya Waste. *Arabian Journal for Science and Engineering*, *42*(6), 2361 ï2369.

Vimalashanmugam, K. & Viruthagiri, T. (2012). Response Surface Methodology Optimization Of Process Parameters for Xylanase Production by *Aspergillus fumigatus* in SSF using Central Composite Design. *International Journal of Engineering Research and Applications*, *2*(6), 277 ï287.

Wadman, M. W., de Vries, R. P., Kalkhove, S. I., Veldink, G. A. & Vliegenthart, J. F. (2009). Characterization of oxylipins and dioxygenase genes in the asexual fungus *Aspergillus niger*. *BMC Microbiology*, *9*(1), 59.

Wolf, J., Bindraban, P., Luijten, J. & Vleeshouwers, L. (2003). Exploratory study on the land area required for global food supply and the potential global production of bioenergy. *Agricultural Systems*, *76*(3), 841 ï861.

Yabaya, A. & Ado, S. A. (2008). Mycelial Protein Production by *Aspergillus niger* using Banana Peels. *Science World Journal*, *3*(4), 9 ï12.

Yassine, M., Amar, I. & Raya, R. (2013). Enrichment of the Protein Content of Biomass Derived from Coastal Remains of Marine Organisms to be used as Poultry Fodder. *Journal of Entomology and Zoology Studies*, *1*(5), 43 ï47.

Yunus, F. N., Nadeem, M. & Rashid, F. (2015). Single-cell protein production through

microbial conversion of lignocellulosic residue (wheat bran) for animal feed. *Journal of the Institute of Brewing*, *121*(4), 553 i557.

Zafaralla, M. T., Vidal, L. R. & Travina, L. E. (1990). Agroindustrial waste products as sources of cheap substrates for algal single-cell protein production. In *Culture and use of algae in Southeast Asia*, 67 i76.

Zepka, L. Q., Jacob-Lopes, E., Goldbeck, R., Souza-Soares, L. A. & Queiroz, M. I. (2010). Nutritional evaluation of single-cell protein produced by *Aphanothece microscopica* Nigeli. *Bioresource Technology*, *101*(18), 7107 i7111.

Zheng, Z., Hu, Q., Hao, J., Xu, F., Guo, N., Sun, Y. & Liu, D. (2008). Statistical optimization of culture conditions for 1,3-propanediol by *Klebsiella pneumoniae* AC 15 via central composite design. *Bioresource Technology*, *99*(5), 1052 i1056.

APPENDICES

APPENDICES

Appendix I. Bovine Serum Albumin (BSA) standard curve for estimation of protein.