OPTIMIZATION of YELLOW PIGMENT PRODUCTION by *Monascus purpureus* from BANANA PEEL

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Thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor in Chemical Engineering (Biotechnology)

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STUDENTS DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Dedicated to my parents: Abdul Satapa bin Zakaria and Zizah binti Abdul Rashid

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

For several decades, both natural dyes and synthetic dyes were widely used in various fields of everyday life such as food production (Pandey *at el*., 2001), textile industries, paper production, agricultural practices and studies, water science and technology (Tibor, 2007).

In 2007, the value of the international food colorant market was estimated at around $1.15 billion USD, up 2.5% from $1.07 billion USD in 2004 (Mapari *at el*., 2010). The use of food dyes as supplement material in food industries is extremely beneficial for both the manufacturers and users in deciding the admissibility of processed food (Spears *at el*., 1988; Griffiths, 2005). Possible reasons for consumption coloring in food substances is to preserve the original meals appearance even after processing and storage, to make sure color uniformity to prevent a seasonal variation in tone color, to intensify the regular color of the food and to maintain quality, enhancing color ordinary food and therefore to maintain quality, taste and glow to safeguard vitamin even when exposed to the and boost acceptance food as tempting item (FNB Food Colors, 1971).

Predominantly natural coloring are environmentally friendly, biodegradable, much less toxic and fewer allergenic than synthetic coloring agents. However, research has shown that certain of natural dyes might have a mutagenic impact detected like elderberry color, safflower yellow and carmine that can causing asthma through continues inhalation, but it arguably that most of the natural dyes secure and some have healing impact for example curcumin in turmeric has antibacterial properties (Ali *et al*., 2007). Gardenia yellow (Sato *et al*., 2007) and safflower yellow is natural color. Both of them are low in toxicity, but are subject to the season change, resulting in an unstable industrial production. Although natural dyes have several advantages but there are some limitations as well such tedious extraction of colorings component from the raw materials with low colorings component. For example, in order to obtain 14 g of the dye about 1200 molluskes are needed. Besides that, it also limited number of suitable dyes because it allows only dyeing wool, natural silk, linen and cotton (Sato *et al*., 2007).

According to green technology curriculum, less toxic products and more natural starting material is favorable for today's production lines .In case of dyes, it is well known that synthetic dyes that derived from minerals likes lead chromate and copper sulphate may cause serious health problem (FDA/IFIC, 1993) and environment hazardous effect (Francis, 1987) such as can prevent sunlight penetration decreasing photosynthetic activity in aquatic environment (Yogendra, 2008). Therefore, within the past several decades, synthetic addictives was criticized and consumer refusal shown towards this product (Francis, 1987). During the late 1960s in the United States, environmentalists makes several demonstrating against

the use of synthetic coloring agents and these behavior has dispersed widely (Carvalho *et al*., 2003). Therefore, an extensive research was carried out to look for an alternative in order to make sure dyes output and consumption will meet the requirements of the environment and the highly secured (Georgeta *et al*., 2004).

To overcome this limitation, other biological sources such as fungi which includes moulds and yeast, bacteria, algae and plant cell cultures have been used (Saintis *at el*., 2005). There are a number of microorganisms declared have the ability to producing pigment in a high yield, includes Monascus species, Paecilomyces, Serratia, Cordyceps, Streptomyces and Penicillium (Hajjaj *et al.,* 2000). Among them, various species *Monascus* has drawn special attention as they have the capabilities producing vary colored pigment and indicate high chemical stability (Mak *et al*., 1990;. Yongsmith *et al*., 1994; Hamdi *et al*., 1997; Hajjaj *et al*., 2000). *Monascus* pigments were reported for producing non-toxic and may enhance the appearance of food coloring (Vidyalakshmi *et al*., 2009).

In order to produce pigments, *Monascus purpureus* have to be cultured under specific conditions. Two types of culture techniques are used which are solid state fermentation (SSF) and submerged fermentation (SmF). *Monascus* pigment production by submerged and solid-state cultures in complex media has been thoroughly studied (Mak *et al*., 1990; Johns and Stuart, 1991; Yongsmith *et al*., 1994). Biopigments produced by fermentation present a great potential for food applications (Carvalho *et al*., 2005) and have the capacity to increase the marketability of products (Tibor, 2007) because they are natural, produced quickly

when compared with vegetable or animal pigments and can be produce at any time of the year (Carvalho *et al*., 2005). They also display advantageous biological activities as antioxidants and anticancer agents.

1.2 IDENTIFICATION OF PROBLEM

The most important issue regarding natural pigment is the price of final product which is more expensive than cheap synthesized dye. According to Diana et al., (2005), the main disadvantages of natural dyes are their extraction yield factors (a few gram of pigment per kg of dried raw material). This makes their current market price about US$1/g, thus limiting their application.

Meanwhile, the use of synthetic dye has several disadvantages which have a potential of carcinogenicity (Fabre *et al*., 1993; Tibor, 2007), ambient pollution possibility and increase of the cutaneous allergies for the user of the product even it is cheaper

Green technology is leading all producers to go towards ecological and less polluted products with fewer by-products; in the case of synthesized dye, natural pigments can be considered as an ideal alternative. Beside that, less toxic products and more natural starting material is favorable for today's production lines (Francis, 1987; Yogendra, 2008).

In this research possibility of using cheap growth media (agricultural wastes) such as banana peel which leads to inexpensive and competitive product, will be been studied with *Monascus purpureus* as original stain.

1.3 STATEMENT OF OBJECTIVES

To studying the effect of different parameters which are initial substrate moisture content, pH and temperature in order to optimize yellow pigment production by *Monascus purpureus* from banana peel.

1.4 SCOPE OF STUDY

In order to achieve the objective, 4 parameter are studies:

i. Effect of different substrate pH on yellow pigment production. The pH range is 4.5, 5.0, 5.5, 6.0 and 6.5.

ii. Effect of different temperature on pigment production. The temperature range is 30°C, 35°C, 40°C, and 45°C, 50 ^{0}C.

iii. Effect of different initial substrate moisture content on pigment production. The range is 45% 50%, 55%, 60% and 65% w/w.

iv. Study the relation of pH, temperature and initial moisture content on yellow pigment of banana peel by optimization method using design expert.

1.5 RATIONAL AND SIGNIFICANCE

This study is believed to provide an optimization method of yellow pigment production by using *Monascus purpureus.* The substrates used in solid state fermentation supply the basic nutrients to the microorganisms and serve as an anchor for the cells. Interestingly, recent studies report that solid state fermentation provides a more adequate habitat for fungi, resulting in high pigment production in a relatively low-cost process when agro-industrial wastes are used as substrate.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The Chinese medical book published in the first century described Anka (also called as anka-kak) or red mold rice as a health benefits. Later, literature documented the use of red color obtained from anka for colouring foods (Khairak *et al*., 2000). In 1884 a French Botanist Philippe van Thieghem isolated the fungus, a red mold growing on potato and and called it as *Monascus ruber*. According to Went (1895), Monascus purpureus from anka obtained from the market of Java and Indonesia. The interest for the color resulted in the characterization of several *Monascus* species isolated from different foods around the world. Presently, there are over 50 patens describing the use of *Monascus* pigments for food. The annual consumption of pigment in Japan approximately 100 tons valued at $1.5 million (Laurent Duffose *et al*., 2005).

Monascus moulds have been commercially employed for the production of major red pigment (Yongsmith *et al*., 1993).However, in recent years; two novel yellow pigments produced *by Monascus sp*. KB20M10.2 (Sato *et al.*, 1992) have been discovered. It was found that *Monascus sp.* KB11304 fermentation produced yellow and red pigments respectively. When strain KB11304 was UV-irradiated to improve its pigment production, the mutant KBM10M16 produces 1.5 times more

red pigments than KB11304. When *Monascus* KBM10M16 was itself exposed to UV radiation, Monascus strain KB20M10.2 was obtained which produce yellow pigments (Yongsmith *et al*., 1993). In this paper, yellow pigment production using *Monascus purpureus* strain 5356 was studied.

2.2 DEFINITION OF PIGMENT

The word pigment has a Latin origin and initially denoted a color (in the sense of colored matter), but it was later extended to indicate the colored objects such as makeup. Pigment is defined as the coloring agent in substances which can be produced either by living organisms or chemical reagents. The history of pigment application dates back to prehistoric cave painting, which gives evidence of the use of ocher, hematite, brown iron ore and other mineral-based pigments more than 30,000 years ago (Daniel, 1986).

The most satisfactory way to classify pigment is according to its source, because most of the significant properties which any pigment groups may have in common can be attributed to their composition. They are divided into two major groups which are natural and synthetic pigments. Synthetic pigment is divided into two main groups which are known as organic and inorganic pigments (Daniel, 1986).

2.2.1 Natural Pigment

Naturally pigments are substances generated by living organisms that having color resulting from selected color absorption. A biology pigment includes plant pigments and pigmented animals. A lot of biological structures, like skin, eyes, fur and hair contain pigments such as melanin within specialized cells called chromatophores (Ball, 2002).

2.2.1.1 Importance of Using Natural Pigments

Environmental anxiety regarding synthetic dyes saw resurgence in requests for naturally dyes as naturally dyes are more environmentally friendly than their synthetic counterparts. Natural coloring may showcases better biodegradability and generally having high compatibility with the surroundings (Kamel *et al*., 2005). Lately, the potential for obtaining natural color from the pigmented microbes to be used as naturally dyes are being investigated (Nagia and EL-Mohamedy, 2007). Table 2.1 shows the microbial production of pigments and their status of development (Liu and Nizet, 2009).

It is interesting to note from Table 1.1 that a lot of pigmented production of the bacterial resources is still classified as a research project or in the development stage. Therefore, employed on production of pigment from bacteria should be enhanced particularly in search of affordable and appropriate growth medium which may cut costs and applicable for industrial production.

Table 2.1: Microbial production of pigments (already in use as natural food colorants or with high potential in this field) (Liu and Nizet, 2009).

Microorganism	Pigment	Colour	Status*
	Bacteria		
Agrobacterium aurantiacum	Astaxhantin	Pink-red	RP
Paracoccus carotinifaciens	Astaxhantin	Pink-red	RP
Bradyrhizobium sp.	Canthaxhantin	Dark-red	RP
Streptomyces echinoruber	Rubrolone	Red	DS
Flavobacterium sp.	Zeaxanthin	Yellow	DS
Paracoccus zeaxanthinifaciens	Zeaxanthin	Yellow	RP
	Fungus		
Monascus sp.	Ankaflavin	Yellow	IP
Monascus sp.	Monascorubramin	Red	IP
Penicillium oxalicum	Anthraquinone	Red	IP
Blakeslea trispora	Lycopene	Red	DS
Fusarium sporotrichioides	Lycopene	Red	RP
Cordyceps unilateralis	Naphtoquinone	Deep blood-red	RP
Ashbya gossypi	Riboflavin	Yellow	IP
Monascus sp.	Rubropunctatin	Orange	IP
Blakeslea trispora	ß-carotene	Yellow-orange	IP
Fusarium sporotrichioides	ß-carotene	Yellow-orange	RP

Neurospora crassa	ß-carotene	Yellow-orange	RP
Phycomyces blakesleeanus	ß-carotene	Yellow-orange	RP
Penicillium purpurogenum	Unknown	Red	DS
	Yeast		
Saccharomyces neoformans var. nigricans	Black	Melanin	RP
Xanthophyllomyces dendrorhous	Astaxanthin	Pink-red	DS
Rhodotorula sp.	Torularhodin	Orange-red	DS

*Industrial production (IP), research project (RP)

2.2.2 Synthetic Pigments

A huge number of dyes have been synthesized and used mainly for dying textiles. According to their chemical structure they are generally classified into six classes: Azo, indigoid, anthracene, azobenzene, phtalocyanine, triphenylmethan (trityl).

However, the structural features of dyes sometimes overlapping united in the molecule greater than one element and making it impossible in classification. In addition, besides their use in the textile industry, various dyes have found application in a variety other areas of research up-to-date and industrial activity (Heinrich, 2003).

Due to the German ban on azo dyes, now there are is one step to search a renewable resource to augment requirements for secure dye industry and this trend

has led to investigation into the output of natural dyes on a commercial scale (Vankar *et al*., 2007).

2.2.2.1 Effects of Using Synthetic Dyes

a) Effects on Health

In pharmaceutical industry, the synthetic food dye is added in order to add color to many medicinal products, as well as to ensure the same color for all the batches of a given product. Adding a color makes the medicinal product more attractive, easier to recognize, and in some cases, by forming an opaque layer, it stabilizes the ingredients of the medicine which are light sensitive (Jaworska *et al*., 2005).

Although synthetic food dyes are greater long lasting and often cheaper compared the original, however now, using lot of this dye gave rise to the serious bookings regarding health. Some of them, such as tartrazine (E 102),cochineal red (E 124), and sunset yellow (E 110), belonging to the group of azo dyes, can themselves, or in combination with other colorants, provoke allergic or pseudo-allergic reactions (PARs), particularly in people allergic to aspirin and other non-steroidal anti-inflammatory agents, or those suffering from urticaria or asthma (Rowe and Rowe, 1994).

Even supposing the synthetic colorants approved by the Food and Drug Administration (FDA) for use in foods, pharmaceuticals and cosmetic preparations have undergone rigorous scrutiny for their toxicity, surprisingly, a study on the examination of cancer chemo preventive effect of synthetic colorants revealed that, a number of these products were evaluated for their in vitro antitumor promoting effect on Epstein–Barr virus (EBV) antigen induced by tumor promoter 12-*O*-tetradecanoylphorbol-13- actate (TPA). Among these are azo dyes, tartrazine (FD & C Yellow # 5), and derivatives of indigo, indigo carmine (FD & C Blue # 2) (Kapadia *et al*., 1998). For external use synthetic dyes, dye several were withdrawn caused to their clearly hazards. For instance, benzidine dyes may lead bowel cancer, while carbon black, pigment printing ink, regarded as a potential carcinogen.

b) Effects on environment

From the environmental point of view, a great variety of synthetic dyes used for textile dyeing and other industrial applications causes serious pollution when part of these dyes penetrates into waste water during these processes. Most of these compounds are toxic, carcinogenic and highly resistant to degradation (Chung *et al*., 1992).

Azo dye, which is largest group of synthetic dyes, is used extensively in various industries. Although textile mills mostly use them, azo dyes can also be found in food, pharmaceutical, paper and printing, leather, and cosmetics

industries. It is not surprising that this compound has become a main environmental anxiety. A lot of these dyes locate their way into environment through the wastewater accessibility. Due to these compounds maintain their color and structural integrity under the disclosures to sunlight, soil, bacteria and sweat, they showcases high obstacles to microbial degradation in wastewater treatment systems (Eichlerová *et al*., 2006).

The synthetic dyes led to the collapse of a huge industry and gave rise to a redistribution of wealth, to the small companies now providing a large proportion

2.2.3 Short History of Yellow Pigments

The oldest yellow pigment is yellow ochre, which was among the first pigments used by humans. Egyptians and the primordial world made wide use of the mineral orpiment for a more brilliant yellow than yellow ochre. In the Middle Ages, Europeans manufactured lead tin yellow. They later imported Indian yellow and rediscovered the method for the production of Naples yellow which was used by the Egyptians. Modern chemistry led to the creation of many other yellows, including chrome yellow, cadmium yellow, lemon yellow and cobalt yellow.

2.2.3.1 Napple Yellow

Naples Yellow**,** or lead antimoniate, has been extremely popular among painters for its pale warmth. Its use as a colorant can be traced back to about 1400

B.C. In its native state, Naples Yellow is a lead-based pigment, and therefore highly toxic.

2.2.3.2 Cobalt Yellow

Cobalt Yellow or Aureolin replaced an earlier pigment called Gamboge, an Asian yellow gum used until the 19th century. Discovered in 1848 by N.W. Fischer in Germany, Aureolin remained popular until the late 19th century, when less expensive, cleaner and more lightfast pigments like the Cadmiums were introduced.

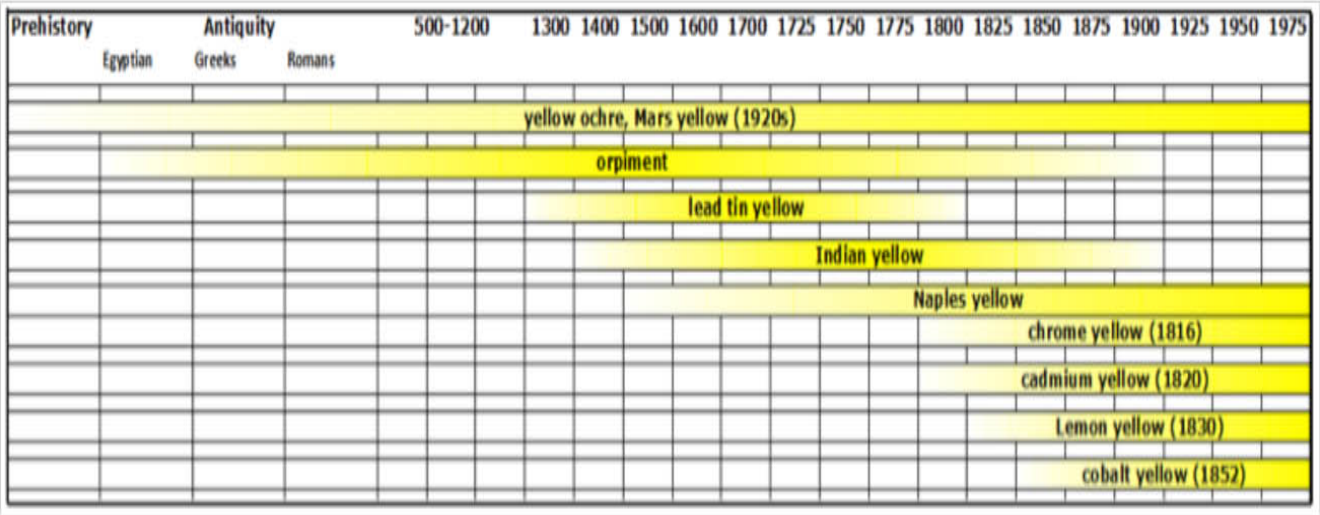


Figure 2.1: Timeline of yellow pigment

2.3 SPECIES AND THE USE OF *Monascus* pigments

The genus *Monascus* can be divided into four species: which are *M.pilosus, M.purpureus, M.ruber and M.froridanus*. *Monascus* belongs to the kingdom of fungi, class of Ascomycetes and family *Monascaceae* in which there are more than thirty kinds now recognized in the world (Juzlova et al., 1999: Chung *et al.* 2006).

Many recent studies show that *Monascus* can produce several fungal metabolic derivatives, such as ethanol, monascus pigments, g-aminobutyric (GABA), monacolins (including monacolin K, dehydromonacolin K, methyl ester of monacolin K hydroxyl-acid from, hydroxyl-acid form of monacolin K, monacolin L, and methyl ester of monacolin L hydroxyl acid form) (Chung *et al.* 2006).

The main uses of *Monascus* in Asian countries especially Japan and China for many centuries are to colour and flavour food such as rice wine, soy bean and beverages. In addition, the interest in pigments produced by *Monascus* sp. in the food industry has been growing because of their wide application (meat, fish, and ketchup) and also due to the carcinogenic and teratogenic effects of some synthetic colorants, like nitrosamines formed from nitrites and nitrates in cured meats (Chen and Johns, 1993: Hamano and Kikilian, 2006). *Monascus* is used in the production of monacolin K which provides the ability to lower blood-lipid levels in animal models and in humans (Endo, 1979).In addition, there is a scientific report on the production of antibiotic activity of *M. purpureus*, on *Bacillus*, *Streptoccus* and *Pseudomonas* (Juzlova *et al*., 1999). *Monascus* sp., a filamentous fungus has been used to make rice wine, soy bean cheese and anka (red rice) in many Asian countries (especially Japan and China) for centuries. *Monascus* spp. is stained easily, and need rigorous culture conditions in fermentation (Chung *et al.* 2006).

2.4 *Monascus purpureus*

Monascus purpureus is a red mold species which may be cultivated on starch containing substrate. The solid state fermentation of rice by Monascus purpureus has a long tradition in East Asian countries (Erdogrul and Azirak, 2004). This mold belongs to the polycetides and has slight bactericidal effects. The production of pigments by this mold was studied and the mixture of pigments are stable from the chemical point if view. As reported, the group includes the orange, yellow and red pigment (Margalith, 1992). *Monascus purpureus* has been well known for red *pigment* production but less study was investigated for yellow pigment production. A monascus purpureus mutant strain-YLCI was obtained for yellow pigment production (Evans and Wang, 1987).

Among the various pigment-producing microorganisms, *Monascus* is reported to produce non-toxic pigments, which can be used as a food colorant. The pigment of *Monascus* improves the coloring appearance of foods (Blanc *et al*., 1995). The optimal cultivation temeperature for this fungal to grow varies from 25 ˚ C to 37 ˚C (Juzlovo *et al*., 1996). Neverthless, the most frequently cited temperature is 30 ˚C.

2.5 PIGMENT FROM *Monascus purpureus*

During growth, *Monascus* spp. breaks down the starch substrate into several metabolites. The structure of pigments as secondary metabolites depends on substrate types and other factor during cultivation such as pH, temperature and moisture

content. Carbon (glucose, maltose and ethanol) and nitrogen sources (peptone and ammonium nitrate) may be used to induce pigment production in *Monascus purpureus.*

Monascus purpureus produce a complex mixture of three categories of pigments which are orange, red, and yellow. Each two have components of polyketide origin. These secondary metabolites are having a common azaphilone skeleton (Campoy *et al.,* 2006, Carvalho *et al.,* 2003). The orange pigment includes monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$). The red pigment includes monascorubramine ($C_{23}H_{27}NO_4$) and rubropunctamine ($C_{21}H_{23}NO_4$) that are the nitrogen analogues of the orange pigment. The yellow pigment includes monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$) (Zhou *et al.,* 2009). The chemical structures of the pigments are shown in Figure 2.1.

Monascus pigments have been used in food industries and have the potential for therapeutic use (Lin *et al*., 2008). The antimicrobial effect of *Monascus* culture, due of monascidin A, confirmed by scientific investigations, was proved against some bacterial and fungal strains. The brute pigment obtained by growing the *Monascus* strain in surface culture had an antifungal action against some species of *Aspergillus*, *Mucor*, *Penicillium* and *Fusarium* genus. The yellow pigment isolated from red yeast rice also inhibits bacteria of the genera of *Bacillus*, *Pseudomonas* and *Escherichia*. *M. purpureus*, cultured in a shrimp and crab shell powder (SCSP)

medium, displayed protease activities and the ability of enhancing the growth of rape (Wang *et al.,* 2002, Liang *et al.,* 2006).

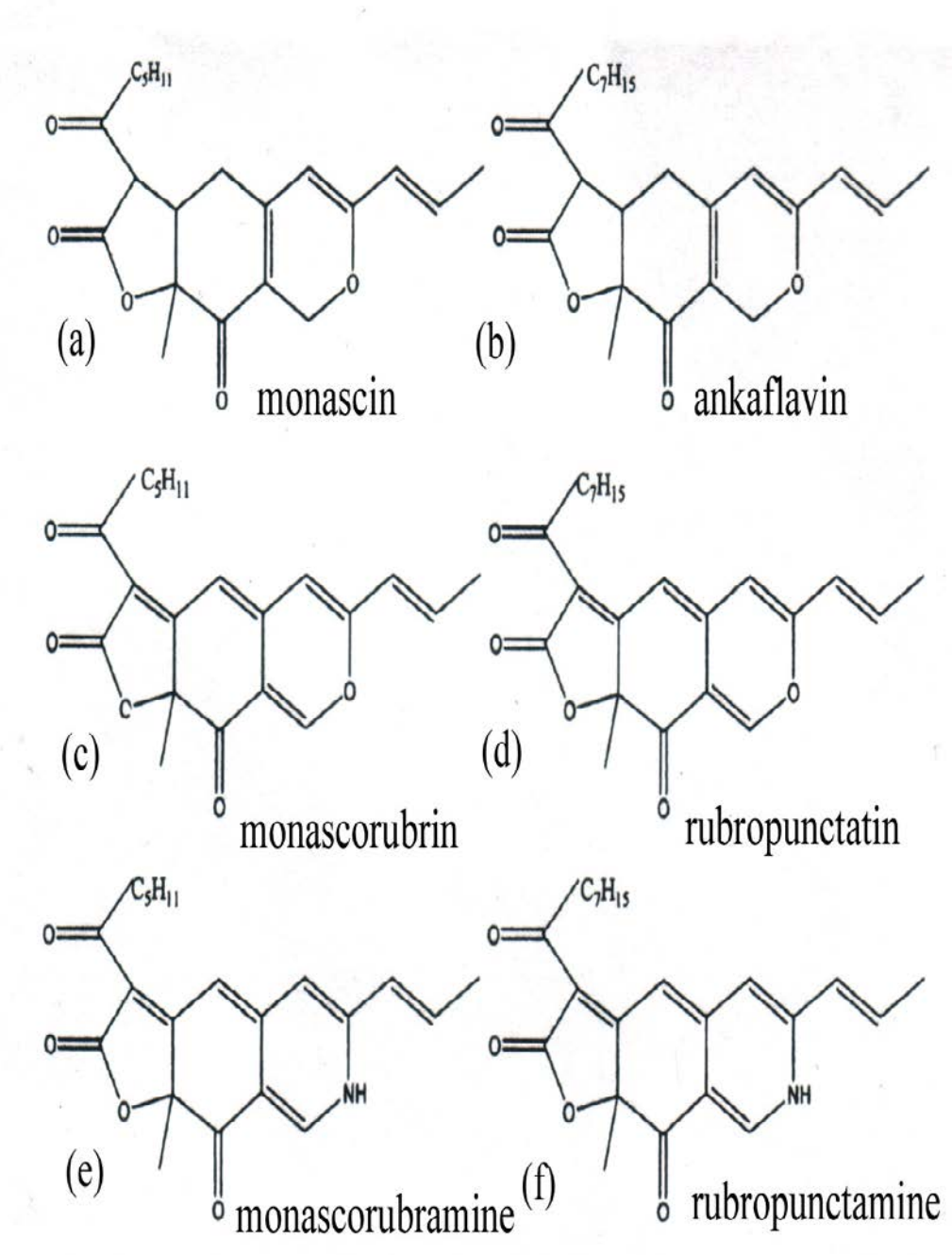


Figure 2.2: Chemical structure of *Monascus* pigments, (a) and (b) yellow pigments,(c) and (d) orange pigments, (e) and (f) red pigments (Adapted from Jung *et al.,* (2003))

2.6 FERMENTATION CONDITIONS

The important growth factor and pigment production for *Monascus purpureus* are effect of light, inoculums size, pH, and temperature and moisture contents. The explanations about this factor are explained above.

2.6.1 Effect of light

Effect of light on mycelium development and spore formation of *Monascus* in shaking flasks has been reported by Miyake et al. (2005).

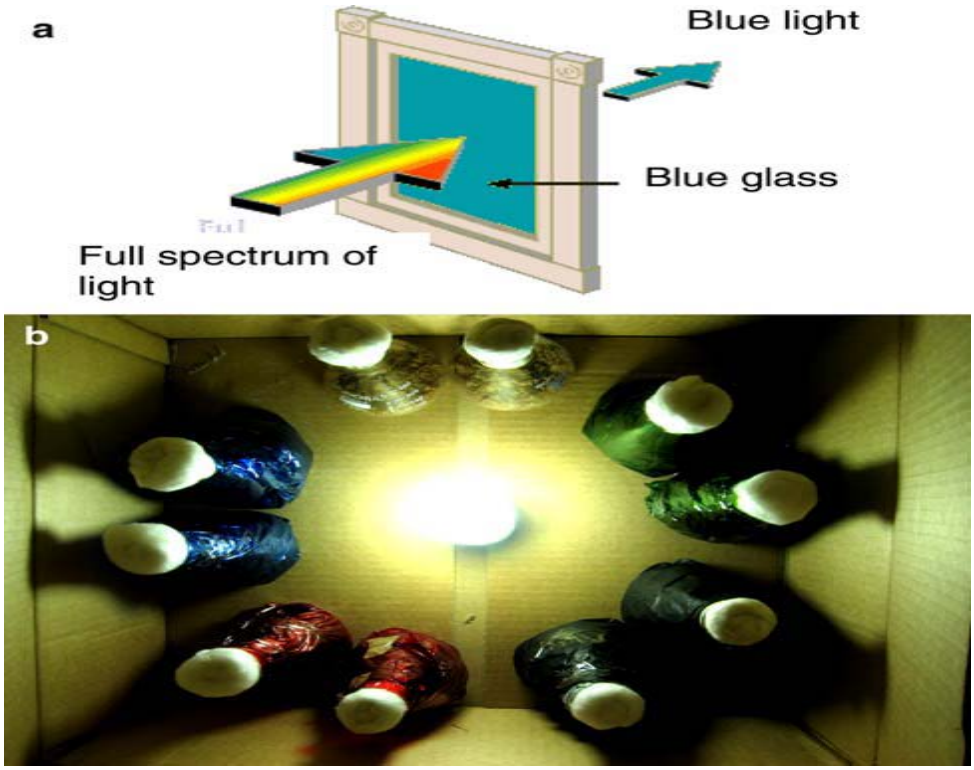


Figure 2.3: (a) Principle, (b) Experimental set up to study the effect of light on pigment production and growth in *Monascus purpureus* (Adapted from Babhita *et al*., (2008))

Incubation in total darkness resulted in increased pigment production (about 2-fold). This observation finds significance as it is against the postulated photo-protective role of biopigments (Yong and Lee 1991; Salih *et al*., 2000; Seagle *et al*., 2005). The studies on effect of the light revealed that the incubation in total darkness was most effective in inducing the pigment production. The colonies grown under the direct illumination resulted in no pigment production; thus postulating the existence of photoreceptors responsive to dark and light in this fungus.

2.6.2 Inoculums size

Determining the optimum inoculums size is another factor crucial to pigment production. If the inoculums size is too small, this can slow growth of the microorganism.

2.6.3 pH

pH is a scale of 1-14 in which it contains chemicals which are acids and alkalis. It is scale which determines the strength of the acid ar alkali (pH 1 being strongest alkali and pH 7 being neautral). Acids are chemicals between pH 1-6 which donate H+ ions in aqueous solution and alkalis take away OH- ions away in aqueous solution.

Substrate pH is one of the most important factors determining microbial growth and metabolic activity. Yongsmith *et al*., (2000) reported that a suitable pH

for pigment production is from pH 4 to pH 7. However pH used for growth is from 2.5 until pH 8. Lower substrate pH (pH 4) is for synthesis of yellow pigments, whereas a higher pH results is for red pigments. The recent study has reported by Mak *et al*., (1990) that the production of yellow pigment can be increased by using pH until pH 5.5.

2.6.4 Temperature

Because of the mesophilic nature of *Monascus purpureus*, maximum growth and pigment production was suggested at 30^0C. These temperatures are in agreement with Babita *et al*., (2009) who reported an optimum temperature of 30^0C to 37^0C for *Monascus purpureus*.

2.6.5 Moisture contents

Moisture contents of the substrate are an important consideration in solid state fermentation. Much solid state fermentation is done with substrate moisture content between 30-80 w/w %. According to Tengerdy *at el*., (1985) high moisture levels leads to aggregation of substrate particles, poor aerations and possible anaerobic conditions.

2.7 FERMENTATION TECHNIQUES FOR PIGMENT PRODUCTION

2.7.1. Submerged fermentation (SmF)

Submerged fermentation is defined as the cultivation of microorganisms in liquid nutrient broth. The growth of the microorganism at a large scale involve use of closed large vessel (up to 100 cubic meters in volume) containing a rich nutrient broth and enough amount of oxygen (Enshasy *et al.,* 2008). Fermentation parameters such as medium composition, pH, and aeration are important variables that affect the production of enzyme in SmF (Maurer, 2004).

SmF has its own advantages and drawbacks. Serious pollution problems, low product concentration and high production cost are the major draw backs associated with the use of SmF. However, presence of high water content that is favorable for most bacterial growth, homogeneity of the fermentation system, ease in process parameter measurement and presence of well developed industrial equipment are some of the advantages of submerged fermentation (Holker & Lenz, 2005). At present, more than 90 % of the commercial microbial enzymes, including alkaline proteases, are produced using submerged fermentation (Underkofler *et al.,* 1958; Aguilar *et al.,* 2008).

2.7.2 Solid state Fermentation

Solid state fermentation is defined as the growth of microorganisms on moist solid substrates in the absence of free flowing water. The microorganism obtains water, carbon, nitrogen, minerals, and other nutrients from the solid substrate. The substrate

also provides anchorage for the micro organism, thus stimulate the growth condition occurring in nature (Pérez-Guerra *et al.*, 2003). Control of fermentation parameters such as pH, moisture content of the solid substrate, and temperature during fermentation are important variables that affect enzyme yield in SSF (Pandey *et al*., 1999).

Recently, production of industrial enzymes using solid state fermentation gaining more attention. This is because, compared to SmF, SSF has the following advantages (Holker & Lenz, 2005). First, cheap agricultural residues can be used as substrates, thus greatly reducing the cost of enzyme production.

Secondly, solid state fermentation requires easier operation. This includes requirement for a smaller space, simple aeration system, and less pollution. In addition, lower energy requirement for operation, possibility to use semi-sterilized condition, and reduced risk of contaminations are also important features of SSF that make the operation system simple (Pérez-Guerra *et al.*, 2003).

Thirdly, solid state fermentation significantly minimizes catabolic repression in production of hydrolytic enzymes in nutrient rich solid state medium. This is because of limited exposure of nutrients to microorganism, and decline in mass transfer that favour slower but constant microbial growth rate. In addition, the presence of active regulatory mechanisms in SSF is also believed to play important role in inducing high volumetric product biosynthesis (Barrios-Gonzalez *et al.*, 2005). More detail descriptions for some of the important biological, ecological and engineering aspects of SSF are shown in Table 1 below.

Table 2.2: Differences between Solid-state and Submerged Fermentation (Adapted from Jung *et al*., (2003))

No.	Factor	Submerged fermentation	Solid state fermentation
1	Substrate	Soluble substrates (usually sugars)	Insoluble polymeric substrates (starch, cellulose, pectin, lignin)
2	Aseptic condition	Heat sterilization and aseptic control	Vapor treatment, even non-sterile conditions can be used
3	Water	High volumes of water consumed and effluents discarded	Limited consumption of water; low amount or, no effluent produced and discarded
4	Metabolic heating	Easy control of temperature	Low heat transfer capacity, difficulty in control of temperature
5	Aeration	Limitation by soluble oxygen, high level of air required	Easy aeration and high surface exchange air/substrate
6	pH control	Easy pH control	Buffered solid substrates
7	Mechanical agitation	Good homogenization	Static conditions preferred
8	Scale up	Industrial equipments available	Need for engineering and new design equipment
9	Inoculation	Easy inoculation	Spore inoculation (fungi), batch
10	Contamination	Risks of contamination for single strain bacteria	Risk of contamination for low rate growth fungi
11	Energetic considerations	High energy consuming	Low energy consuming
12	Volume of equipment	High volumes and high cost technology	Low volumes and low costs of Equipments
13	Effluent and pollution	High volumes of polluting effluents generated	No effluents, less pollution generated
14	Concentration of products	Low yield and diluted product	Highly concentrated product

2.8 SUBSTRATE IN PIGMENT PRODUCTION

Solid state fermentation should be used to define only those processes in which the substrate itself acts as carbon or energy source and occurring in the absence of free water (Pandey *et al.*, 2000).

2.8.1 Agriculture Waste as a Substrate

The selection of a substrate for solid state fermentation depends upon several factors mainly related with cost and availability and thus may involve screening of several agro-industrial residues. In the solid state fermentation process, the solid substrate not only supplies the nutrients to the micro-bial culture growing on it, but also serves as an anchorage for the cells. The substrate that provides all the needed nutrients to the micro-organism growing in it should be considered as the ideal substrate.

The search for inexpensive substrates is necessary to reduce the production cost of yellow pigment. Considerable interest has been shown in using agricultural wastes for pigment production because of the problems in waste management faced by agro-based industries, particularly in developing countries. The current emphasis is on biological conversion of agricultural wastes into value-added products. For this purpose, different agro-industrial residues such as grape pomace, apple pomace, banana, sugar beet, okara, and jack fruit carpel and kiwi fruit peel were investigated as possible substrates (Angumeenal and Venkappayya, 2005: Khare *et al*., 1995).

Banana peels is selected as a substrate in this study because of its high content in carbohydrates, which due to their organic nature are easily metabolized by the fungus (Ready *et al*., 2003). It was estimated that the banana peel contains 14.6% glucose and 56% sucrose (Goewert and Nicholas, 1980). Banana is one of the most abundantly available fruits in tropical countries.

The addition of various nutrients to the solid medium may improve the growth of organism and pigment production (Pandey *et al*., 2004). The result from previous study indicated that among various carbon source tested had a little effect or negative effect on pigment production. This means that, natural banana peel provided all the nutrients needed by the organism for cell growth and pigment production. The exogenous addition of various nutrients is needless. This is of great interest for industrial production of pigment, for the cost of the addition of nutrients would be saved (Sun *et al*., 2011).

World production of banana is estimated at 48.9 million tones out of which 10.4 million tones, is contributed by India. India is the leading country in the production of banana followed by Brazil, Indonesia, Philippines, China and Australia. In India, peels are available at a cheap price, in all seasons, throughout the country. All parts of banana tree are useful to human beings except the peel. In some villages in India, banana peel is seldom used as cattle feed and it is discarded simply as garbage (Karthikeyan and Sivakumar, 2010). Hence the banana peel was tried in this study as a cheap substrate for yellow pigment production.

2.8.2 Musa sapientum (Banana Peels) nutritional properties

Banana peel was purchased from a local market in Gambang's area and was used as the substrate for solid state fermentation. Banana peel has been widely used as a substrate for wine, ethanol and biogas production because of high nutrients (Mohapatra *et al*., 2010). Table 4.1 shows the nutritional information of the banana peels.

Table 2.3: Nutritional of banana peel (Mohapatra *et al*., 2010)

Compositions	**Content (%)**
Starch	3
Crude fat	3.8-11
Total dietary fiber	43.2-49.7
Lignin	6-12
Pectin	10-21
Cellulose	7.6-9.6
Hemicellulose	6.4-9.4

CHAPTER 3

RESEARCH METHODOLOGY

3.1 IINTRODUCTION

This chapter describes the materials and methods used in the experimental work. Experimental work focused on solid state fermentation. Effects of the initial moisture content of the substrate, pH and temperature were examined on the pigment production. Extensive experimental which is Design Expert were used to asses the combined effects of the parameter on the pigment production.

3.2 MATERIALS PREPARATION

All the glassware used during this experiment were washed with distilled water and sealed and sterilized by autoclaving at 121°C for 15 minutes. All reagents and chemicals were of analytical grade.

3.2.1 Microorganism

A culture of *Monascus purpureus* strain 5356 .is used for the experiment. The microorganism was maintened on Potato Dextrose Agar (PDA) plates and slants at 4°C and sub cultured once in every three weeks (Velmuragan *et al*., 2011, Babitha *et al*., 2006, 2007).

3.2.2 Chemicals

Table 3.1: Chemicals used

Name	Formula
Acetylacetone	$C_5H_8O_2$
Ammonium nitrate	$(NH_4)NO_3$
Ethanol	C_2H_5OH
Hydrochloric acid	HCL
Magnesium sulphate-7-hydrate	$MgSO_4.7H_2O$
N-acetyl-D-glucosamine	$C_8H_{15}NO_6$
p- Dimethylaminobenzaldehyde	$C_9H_{11}NO$
Potassium di-hydrogen phosphate	KH_2PO_4
Potato Dextrose Agar	
Sodium chloride	NaCl
Sodium hydroxide	NaOH

3.3 Fermentation preparation

3.3.1 Preparation of Potato Dextrose Agar (PDA) medium

First, the powder of PDA is weighed (19.5 g) and dissolved in a Schott bottle with boiling distilled water (500 ml) while stirring with a magnetic stirrer until fully dissolved. Second, the PDA solution is put in an autoclaved at 121°C for 15 minutes (ATCC, 2007).

Third, the medium is cooled at 50^0C and then transfer to the laminar flow Medium that is still in liquid phases is poured into agar plate/petri dish (triplicate) and into universal bottle (triplicate) at the rate of 12-15 ml per dish (half of petri dish). Medium spread evenly and make sure there is no air bubbles. Medium is cooled in laminar chamber before stored at 4^0C until needed. The plates in chiller are rearranged upside down to prevent any condensation from dripping onto the agar surface.

3.3.2 Substrate Preparation

Banana peel was obtained from local market. The collected fresh peels wash twice with distilled water to remove adhering dust. Then, their finger stalk and black regions is removed (Karthikeyan and Sivakumar, 2010).

3.3.2.1 Solid-State Fermentation

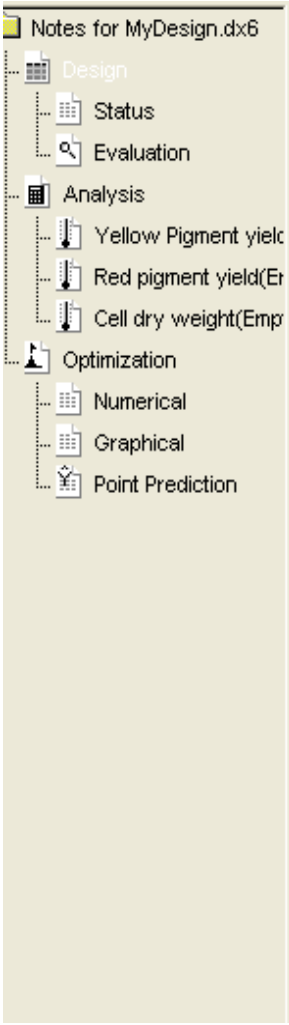
10 g of banana peel was placed in a 250 mL Erlenmeyer flask and distilled water (10.5 ml, 11.2 ml, 11.5 ml, 12.6 ml and 13.1 ml) was added to achieve initial moisture content (45% 50%, 55%, 60% and 65%) in different flask. A nutrient salt solution (4 mL) containing (g/L): KH_2PO_4, 2 g; NH_4NO_3, 5 g; NaCl, 1 g; and $MgSO_4 \cdot 7H_2O$, 1g was added (Sumathy et al., 2006: Babitha et al., 2006) to each flask. The banana peel then allowed soaking at room temperature for different time. Flask contents were mixed thoroughly, autoclaved at 121°C for 20 min, and cooled to room temperature. The flask was inoculated with the 2ml of *M. purpureus* spore suspension and incubated at 30°C for 10 day. Unless otherwise mentioned, these conditions were maintained throughout (Dikshit and Tallapragada, 2011). At every 24 h, pigments were extracted from the samples and UV-VIS analyses of the samples are taken.

3.3.2.2 Design of Experiments

In order to identify the optimum conditions, a Central Composite Design (Wang *et al*., 2003) was selected. The crucial factors involved are initial moisture

content (A), pH (B), and temperature(C). These factors, and the level at which the experiments were carried out are given in Tables 4.1 and 4.2. A total of 20 runs with centre points were generated. The central point of the design arrangement decided on was: initial moisture content of 65 %; ph of 5.5 and temperature 30 ˚C.

Table 3.2: Process variable and levels in the three-factor, three-level response surface design.



	Std	Run	Block	Factor 1 A:Initial substrt %	Factor 2 B:pH -	Factor 3 C:Temperature
	2	1	Block 1	65.00	4.50	30.00
	4	2	Block 1	65.00	6.50	30.00
	19	3	Block 1	57.50	5.50	40.00
	10	4	Block 1	70.11	5.50	40.00
	8	5	Block 1	65.00	6.50	50.00
	17	6	Block 1	57.50	5.50	40.00
	11	7	Block 1	57.50	3.82	40.00
	5	8	Block 1	50.00	4.50	50.00
	20	9	Block 1	57.50	5.50	40.00
	14	10	Block 1	57.50	5.50	56.82
	9	11	Block 1	44.89	5.50	40.00
	15	12	Block 1	57.50	5.50	40.00
	18	13	Block 1	57.50	5.50	40.00
	6	14	Block 1	65.00	4.50	50.00
	13	15	Block 1	57.50	5.50	23.18
	1	16	Block 1	50.00	4.50	30.00
	3	17	Block 1	50.00	6.50	30.00
	7	18	Block 1	50.00	6.50	50.00
	12	19	Block 1	57.50	7.18	40.00
	16	20	Block 1	57.50	5.50	40.00

3.3.2.3 *Monascus purpureus* CULTIVATION METHOD

3.3.2.3.1 Inoculumn preparation

The aseptic stock culture of *Monascus purpureus* strain 5356 were maintened on Potato Dextrose Agar (PDA). Spores of strain were growing on PDA at 30 ˚C for 14 days (Julio *et al.*, 2007) under static conditions. After that, spores was scrapped off from the agar slant and then diluted with10 ml sterile distilled water under aseptic technique in order to get approximately 10^7 spores' per mL (Babitha *et al*., 2006, 2007). The spores' number was counted using a hemocytometer slide (Bibhu *et al*., 2010).

3.3.2.3.2 Cultivation using inoculmn preparation

The sterile banana peel was inoculated under aseptic technique with 2 mL spore suspension per 10 g of banana peel. The flask were covered with aluminum foil and incubated at 30˚C for 10 days.

3.4 ASSAY METHODS FOR PARAMETER'S STUDY

3.4.1. Moisture content determination

To study effect of moisture content on yellow pigment production, 10 g of banana peel was placed in 250 mL Erlenmeyer flask and distilled water (10.5 ml, 11.2 ml, 11.5 ml, 12.6 ml and 13.1 ml) was added for different periods to get different moisture content (Ellaiah *et al*., 2002) at 45% 50%, 55%, 60% and 65%

w/w. A solution of 4 ml salt solution was added to each flask. The flask containing soaked banana peel were then covered with two layers of aluminium foil to prevent moisture loss. Then, the wet substrates were autoclaved at 121^0C for 20 min and cooled to room temperature. It was inoculated with 2 ml spore suspension (Babhita *et al*., 2007) *Monascus purpureus* and incubated under static conditions at 30^0C for 10 days.

$$\text{Moisture content (\%)} = 100\% \times \frac{(\text{wet weigh of the solids} - \text{dry weight})(\text{g})}{\text{dry weigh of the solids (g)}} \qquad (3.1)$$

At every 24 h, pigments were extracted from the samples and UV-VIS analyses of the samples are taken.

3.4.2 Temperature

The effect of temperature on growth of *Monascus purpureus* is also studied. The mixture of 10 g banana peel was incubated at five different temperatures (30°C, 35°C, 40°C, and 45°C, 50 ^{0}C) in oven for 10 days in static conditions.

3.4.3 pH

The effect of pH on growth of *Monascus purpureus* is also studied. 10 gram of the substrate and distilled water was soaking for a period and the pH was measured by using calibrated pH meter. pH was adjusted to 4.5, 5.0, 5.5, 6.0 and 6.5.with 0.5 N HC1 or 0.5 N NaOH before autoclave (Babhita *et al*., 2007).

3.4.4 Extraction and analysis of Pigment

Every 24 h during 10 days, 0.5 g of fermented solids (Dikshit and Tallapragada, 2011) was extracted with 10 ml of 95 % (v/v) ethanol in a rotary shaker for 1 h at 200 rpm in an Erlenmeyer flask. The extract was then filtered through Whatman #1 filter paper (Babitha *et al*., 2007).

Pigment estimation was done as described by Tseng *et al*., (2000) in which the optical density at its absorbance maxima were expressed as the concentration of pigment produced. The filtrated whose concentration will be determine by measuring the optical density of the supernatant using a UV-VIS spectrophotometer at 400 nm and 500 nm for yellow and red pigments (Zhou *et al*., and 2009). Ethanol extracts of unfermented substrate will keep as blanks (Dikshit and Tallapgrada, 2011). The results were expressed as optical density (OD) per gram of dried solid.

$$\text{Optical density (OD g}^{-1}) = \frac{\text{Optical density} \times \text{df}}{0.5} \qquad (3.2)$$

Figure 3.1: Extraction of banana peel with ethanol

3.4.5 Biomass

Total fungal biomass was determined by measuring the N-acetylglucosamine released by acid hydrolysis of the chitin present in the fungal cell walls (Nimmoi and Lumyong, 2009; Sakurai *et al*., 1977).

In brief, 0.5 g of dried fermented banana peel powder was mixed with 2 mL of concentrated 60% (vol/vol) sulfuric acid (H_2SO_4) and the mixture was kept at 30˚ C for 24 h (Roopesh *et al*., 2006). After that, this mixture was diluted with distilled water to make a 1 N solution of sulfuric acid, autoclaved for 1 hr and neutralized with 1N NaOH and the final volume was made up to 100 ml with distilled water (Roopesh *et al*., 2006).

Acetyl acetone reagent (1 mL) was added to the 1 ml solution, which was then placed in a boiling water bath for 20 min. After cooling to room temperature, 6

mL of 95% ethanol was added, followed by 1 mL of Ehrlich reagent (Sigma-Aldrich, Milwaukee, WI, USA) and incubated at 60°C for 10 min. After cooling to room temperature, optical density (OD) was measured at 530 nm against the reagent blank using using UV visible spectrophotometer. N-acetylglucosamine (Sigma-Aldrich) as the external standard. The result obtained is expressed as mg glucosamine per gram dry solid (gds).

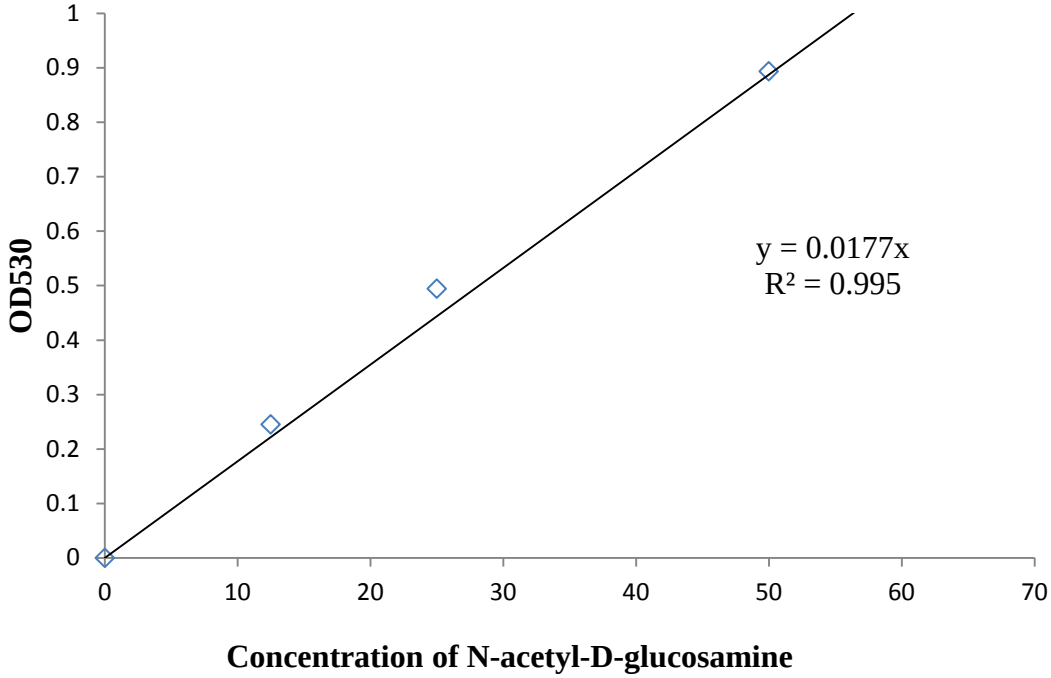


Figure 3.2: Standard biomass concentration

Figure 3.3: Banana peel is dried for biomass analysis.

CHAPTER 4

RESULT AND DISCUSSION

4.1 PRELIMINARY STUDIES

4.1.1 Introduction

The applicability of banana peel as a substrate for pigment production was evaluated using *M.purpureus* FTC 5356. First, the suitable conditions for producing a maximum yellow pigment are identified. For this purpose, preliminary studies are done in Erlenmeyer flask where the result from the parameter at pH: 5.5, Temperature: 30˚C and initial moisture content: 60% are used as references. The influence of temperature on fungal growth and pigment production was determined. Effects of pH were examined and the effect of initial moisture content on pigment and biomass production was described.

4.1.2 Incubation Time

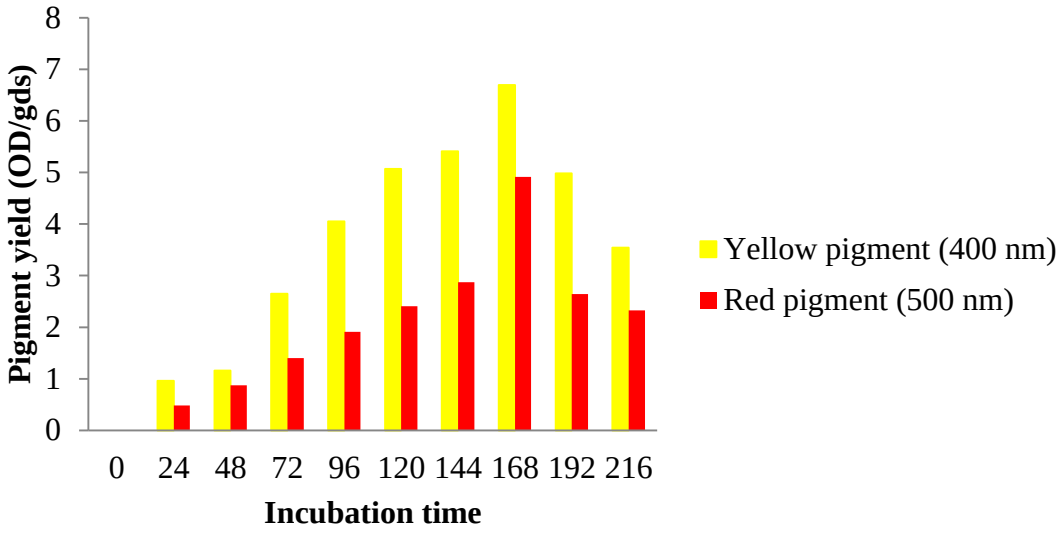


Figure 4.1: Pigment yield (400 and 500 nm) of *M.purpureus* in solid state fermentation in response to incubation period.

SSF were in assessed in Erlenmeyer flask. 10 g banana peel with 60% (w/w) initial moisture content and 4ml of slat solution was sterilized in each 250 ml of Erlenmeyer flask. After that, the substrate was cooled to room temperature and then it was inoculated with 10^7 spores per mL and incubated at 30˚C, as described in Chapter 3.3.2.1. The cultures then were grown for 10 days. During this experiment, three Erlenmeyer flask were used for triplicate analyses as described in Chapter 3.7

Figure 4.1 reflect the fermentation data in solid state fermentation. Production of pigment began on day 1 and pigment concentration increased until day 7. The concentration of the yellow and red pigments was 6.6960 and 4.9176 OD/gram dry solid. After which, there was a decrease in pigment production on day 8.

4.1.3 Effect of initial moisture content

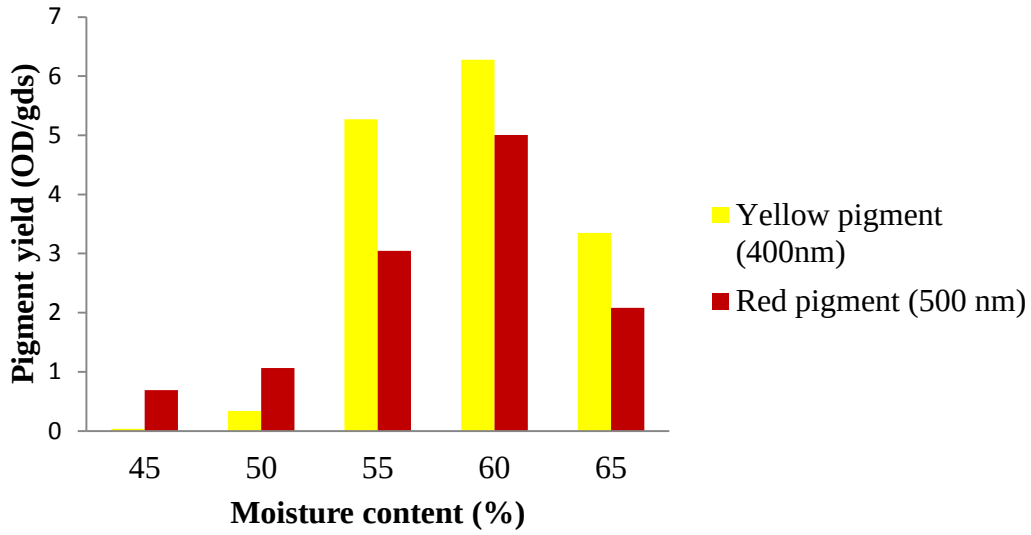


Figure 4.2: Pigment yield (400 and 500 nm) of *M.purpureus* in solid state fermentation in response to initial substrate moisture content

For solid state fermentation, moisture is a key parameter to control the growth of microorganism and metabolic production (Pandey *et al*., 2003). Sufficient water is necessary to ensure fungal development is good, but too much water dropped the porosity and oxygen diffusion in mass and favors contamination of fungal (Raimbult, 1998).

The influence of the initial moisture content of banana peel on pigment production was examined by inoculating triplicate, 10 g of banana peel in Erlenmeyer flask and distilled water (10.5 ml, 11.2 ml, 11.5 ml, 12.6 ml and 13.1 ml) was added for initial moisture content value of approximately 45, 50, 55, 60 and 65% were investigated as described in the chapter 3.6. The fermentation was incubated in

the incubator for 10 days at 30˚C. The maximum pigment yield production occurred after 8 days incubation and the analysis of pigment production as explained in chapter 3.4.4.

The effect of initial substrate moisture content on the pattern of yellow (A_{400nm}) and red (A_{500nm}) pigment production was presented in Figure 4.2. From the figure 4.2, it clearer showed that production of pigments by *M.purpureus* displayed a strong correlation with initial moisture content. The mycelium *of Monascus purpureus* should permeate inside the banana peel and then use the substrates to manufacture enzymes and pigments (Lee *et al.*, 2002). The data plotted in Figure 4.2 are the maximum pigment concentration (mean of three sample) observed. The maximum pigment was obtained at 60% initial moisture content. The maximum values were 6.2784 and 5.004 OD/gds, for yellow and red respectively. This can be attributed to effective utilization of sugars in the substrate. Result is consistent with Yongsmith *et al*., (2000), who reported maximum pigment production by *Monascus* sp.KB9 at 60% moisture. It can be concluded that the initial moisture content of 60% are the most conducive to the development yellow and red pigments in solid state fermentation. The top or bottom initial moisture content of 60%, pigment output was reduced.

Figure 4.3 shows clearer visual pigment production during growth. The entire banana peel surface had been covered with the fungus at day 7 meanwhile figure 4.4; shows the flask become misty and the banana peel looked wet. Beside that, the color had changed from black-brown to black-red.

Figure 4.3: Pigment production during fungal growth on day 7

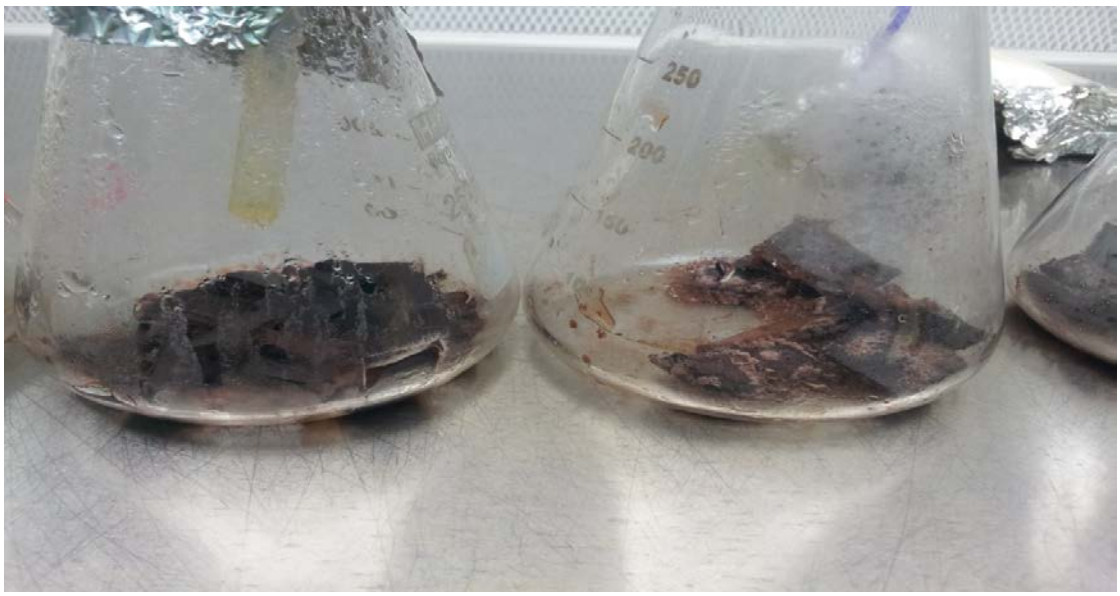


Figure 4.4: Flask is misty and banana peel looked wet

A decrease in pigment yield was observed when the moisture level was higher or lower than optimum. This result was similar to the findings of Johns and Stuart (1991) who reported that initial moisture content less than 50% gave less

pigmentation, but that 55-60% could give the highest pigmentation. Highest initial moisture in solid state fermentation leads to suboptimal product formation due to reduced mass transfer process and decrease in initial moisture level results in reduced solubility minimizes heat exchange, oxygen transfer and low availability of nutrients to the culture (Carrizales and Rodriquez, 1981). It has been reported that each *Monascus* strain has its own individual optimum initial moisture content for pigment production. Lotong and Suwanarit (1990) reported that at high initial moisture content, *Monascus* sp. NPI could liberate high glucose that can inhibit pigment production. Previous studies have shown that initial moisture content in the solid substrate is one key factor affecting glucoamylase activity as well as pigmentation of the *Monascus* (Yongsmith *et al*., 2000).

In SSF, the intensity of microbial of microbial growth generally depends on the initial moisture level an it indirectly affects the production. In general, fungal culture requires low moisture level (20-70%) compared to the requirement of a bacterial culture, which is higher than 70%. Higher moisture content resulted in agglomeration of the substrate, subsequently restricting the supply of oxygen for microorganism growth and also be a possible explanation for reduction in pigment production at high initial moisture (Gautam *et al*., 2002). Meanwhile, the decrease of pigment production at low moisture content is a result of low nutrient availability due to reduced nutrient salt solution, as well as less efficient heat exchange and oxygen transfer (Babitha *et al*., 2006).

4.1.2 Effect of substrate pH

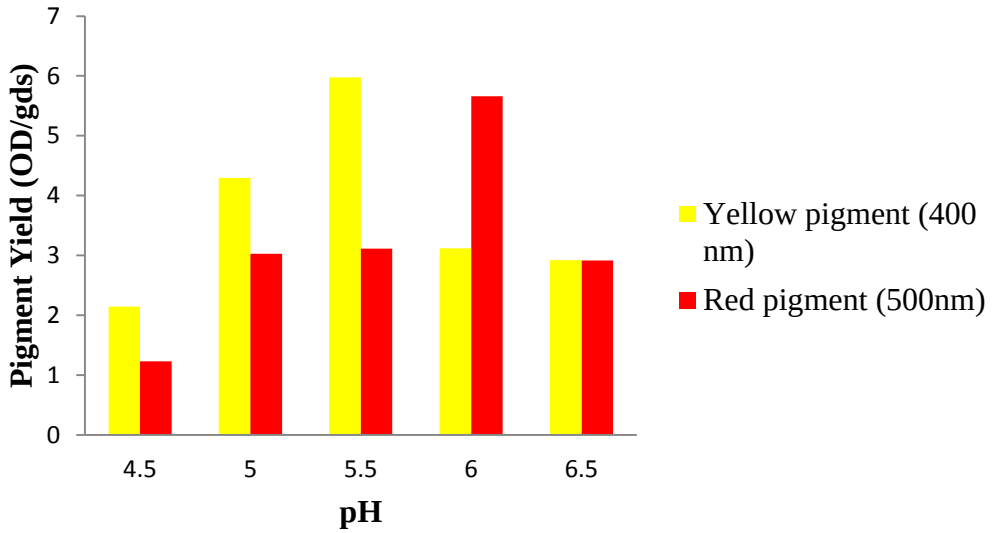


Figure 4.5: Pigment yield (400 and 500 nm) of *M.purpureus* in solid state fermentation in response pH substrate

Substrate pH is one of the most important factors determining metabolic activity in solid state fermentation. Therefore, in order to investigate the significance effect of pH on pigment production, the banana peel was inoculated with *Monascus purpureus* at different initial pH values (4.5-6.5) in static shake flask cultures. In this work, pH was adjusted only before fermentation. From Figure 4.5, the following conclusions can be deduced. First, profile of yellow pigment production reach their maximum at the same pH point 5.5. Meanwhile for red pigment production, the highest are at the pH 6.0. This result are supported by Yongsmith *et al*., (1991), where it has reported that a lower substrate pH predominance to synthesis of yellow pigments, whereas a higher pH results in red pigments.

It has been reported that many kinds of fungi usually grow best at slightly acidic pH of around 5-7 during culture for highest pigment production (Bae *et al.*, 2000). This statement have been supported by Lee *et al*., (1999) where it has been reported that many kinds of ascomycetes have more acidic pH optima during culture. Pigment yield increase from pH 4.5 until 6 but then decrease when the pH used is 6.5. The optimum pH is the maximum rate of reaction that occurs in the range. When the pH is altered below or above the optimum the pigment production is decreased or become denatured.

4.1.3 Effect of Temperature

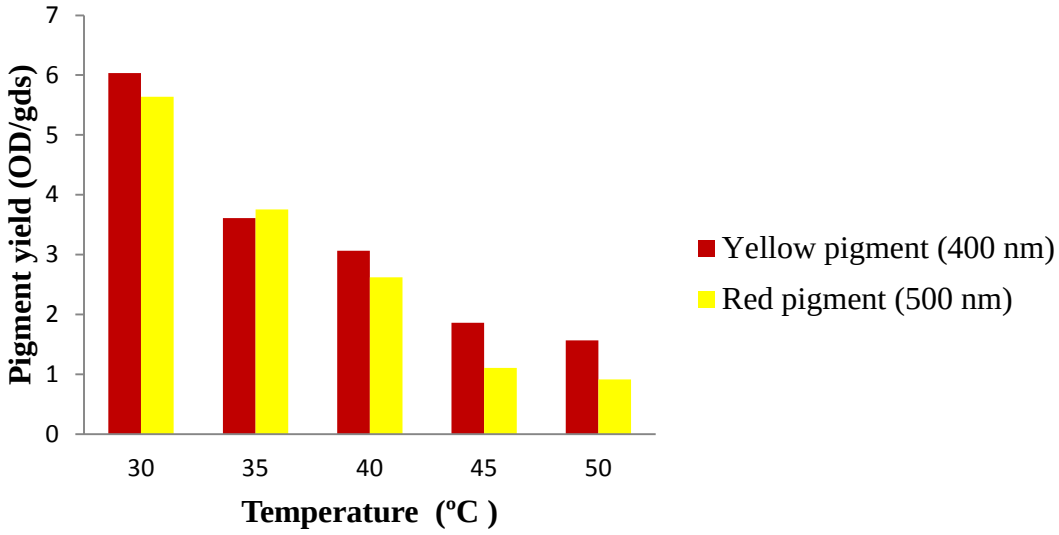


Figure 4.6: Pigment yield (400 and 500 nm) of *M.purpureus* in solid state fermentation at different incubation temperature**.**

Temperature is one of the most critical factors in solid-state fermentation based on fungal cultures because it influences metabolic activities and microbial growth. To find the optimal temperature for pigment production, Monascus purpureus was

cultivated under various temperatures (30- 50˚C). Consequently, from the result the optimal temperature it was evident that maximum pigment production was obtained at 30˚C. This observation was in agreement with Domsch *et al*., (1980) who reported an optimum temperature between 30 and 37˚ C for different isolates of *Monascus* sp.

4.2 Optimum culture conditions based on Central Composite Design and Response Surface Design (RSM)

4.2.1 Introduction

Pigment production in solid state fermentation significantly influenced by temperature, initial moisture content and pH as viewed in chapter 4.1.Fermentation time has been fixed at 168 hr as the maximum total pigment productivity has been achieved at this time as described in. Thus, the overall productivity of statistical optimization pigment has study using initial moisture content, pH and temperature as factors.

This research used RSM to investigate optimum culture conditions taking account of the three factors. The study involved the production of yellow pigment, red pigment and cell dry weight. The three most important responses, designated by the letter "Y", are:

- Y_1 – Yellow pigment
- Y_2 – Red pigment
- Y_3 – Cell dry weight

Table 4.1: Factors for Response Surface Study

Factor	**Units**	**Low Level (-1)**	**High Level (+1)**
A-initial moisture content	**%**	50	65
B-temperature	(˚C)	30	50
C-pH		4.5	6.5

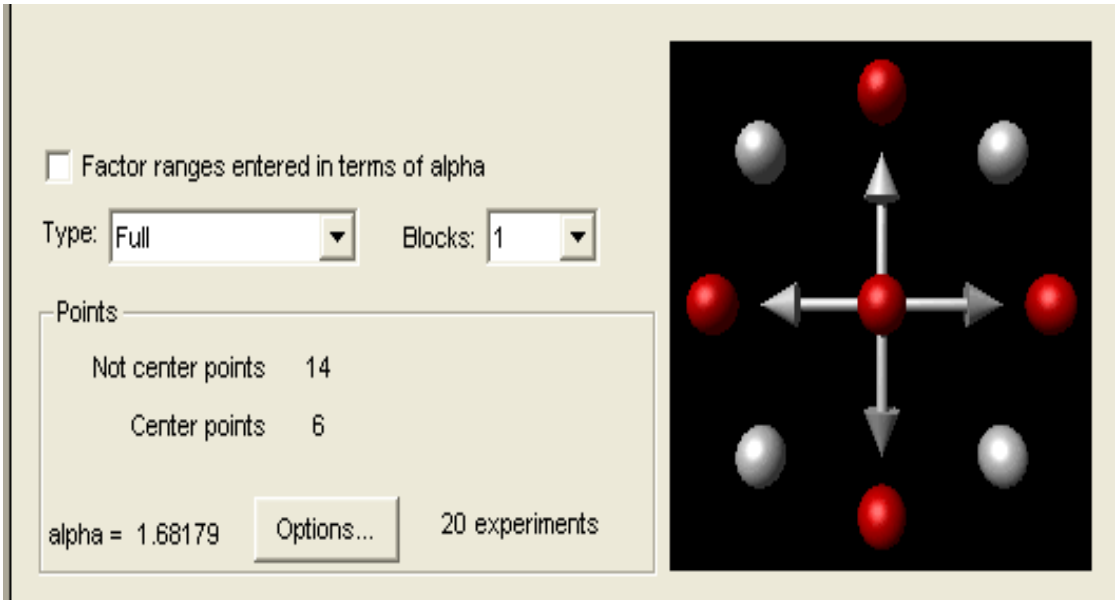


Figure 4.7: Central Composite Design Dialog Box-After Selecting 1 Blocks

The default selection of "Alpha," set at 1.68179 in coded units, is the axial distance from the center point and makes the design rotatable. A rotatable design provides equally good predictions at points equally distant from the centre, a very desirable property for RSM.

4.2.2 Central Composite Design (CCD) and response surface analysis

The central composite design (CCD) was conducted to evaluate the optimum initial moisture content, pH and temperature (independent variables) for yellow pigment, red pigment and cell dry weight (dependent variables). The levels of the variables for the CCD experiments were chosen based on the results of the previous experiments. The design matrix and the corresponding experimental data were shown in Table 4.2.

Table 4.2: Results and design of response surface experiment

Std	Run	Block	Factor 1 A:Initial substrt %	Factor 2 B:pH -	Factor 3 C:Temperature	Response 1 Yellow Pigmen OD/gds	Response 2 Red pigment yi OD/gds	Response 3 Cell dry weight
1	1	Block 1	50.00	4.50	30.00	3.514	3.067	47.9666
13	2	Block 1	57.50	5.50	23.18	0.734	0.619	5.4802
7	3	Block 1	50.00	6.50	50.00	2.527	1.663	5.9322
9	4	Block 1	44.89	5.50	40.00	2.326	2.037	37.4011
2	5	Block 1	65.00	4.50	30.00	3.938	3.189	50.5085
12	6	Block 1	57.50	7.18	40.00	2.614	1.936	35.6497
20	7	Block 1	57.50	5.50	40.00	2.405	2.527	37.5706
6	8	Block 1	65.00	4.50	50.00	3.161	1.526	7.9096
10	9	Block 1	70.11	5.50	40.00	2.495	2.253	36.6667
5	10	Block 1	50.00	4.50	50.00	2.066	1.49	30.5085
8	11	Block 1	65.00	6.50	50.00	2.902	1.728	5.8192
18	12	Block 1	57.50	5.50	40.00	2.398	2.527	37.4011
17	13	Block 1	57.50	5.50	40.00	2.434	2.527	37.4576
15	14	Block 1	57.50	5.50	40.00	2.448	2.527	37.6271
4	15	Block 1	65.00	6.50	30.00	3.398	3.261	49.096
11	16	Block 1	57.50	3.82	40.00	3.017	1.886	37.1751
3	17	Block 1	50.00	6.50	30.00	3.283	3.232	42.8249
16	18	Block 1	57.50	5.50	40.00	2.333	2.527	37.4011
19	19	Block 1	57.50	5.50	40.00	2.477	2.527	37.5706
14	20	Block 1	57.50	5.50	56.82	0.086	1.339	5.6517

Standard Order	Actual Value	Predicted Value
1	3.51	2.97
2	3.94	3.33
3	3.28	2.80
4	3.40	2.64
5	2.07	1.82
6	3.16	2.65
7	2.53	2.14
8	2.90	2.45
9	2.33	2.84
10	2.50	3.40
11	3.02	3.68
12	2.61	3.37
13	0.73	1.68
14	0.086	0.55
15	2.45	2.38
16	2.33	2.38
17	2.43	2.38
18	2.40	2.38
19	2.48	2.38
20	2.40	2.38

Figure 4.8: Experimental (actual value) and predicted value of yellow pigment

Standard Order	Actual Value	Predicted Value
1	3.07	2.37
2	3.19	2.49
3	3.23	2.45
4	3.26	2.54
5	1.49	1.60
6	1.53	1.69
7	1.66	1.75
8	1.73	1.81
9	2.04	2.50
10	2.25	2.65
11	1.89	2.26
12	1.94	2.43
13	0.62	2.04
14	1.34	0.78
15	2.53	2.50
16	2.53	2.50
17	2.53	2.50
18	2.53	2.50
19	2.53	2.50
20	2.53	2.50

Figure 4.9: Experimental (actual value) and predicted value of red pigment

Table 4.3 and 4.4 showed ANOVA results of response surface design for yellow pigment; red pigment and cell dry weight production. The following equations, where the factors take their code value, were obtained from regression analysis and final equation is in terms of coded factors:

$$\text{Yellow pigment yield} = 2.38 + (0.17 \times A) - (0.091 \times B) - (0.33 \times C) + (0.26 \times A^2) + (0.41 \times B^2) - (0.44 \times C^2) - (0.13 \times A \times B) + (0.12 \times A \times C) + (0.12 \times B \times C)$$

$$\text{Red pigment yield} = 2.50 + (0.045 \times A) + (0.051 \times B) - (0.38 \times C) + (0.027 \times A^2) - (0.056 \times B^2) - (0.39 \times C^2) - (8.000^{-003} \times A \times B) - (6.250^{\wedge 003} \times A \times C) + (0.017 \times B \times C)$$

$$\text{Cell dry weight} = 37.36 - (1.11 \times A) - (2.62 \times B) - (10.25 \times C) + (0.76 \times A^2) + (0.54 \times B^2) - (10.36 \times C^2) + (3.28 \times A \times B) - (3.94 \times A \times C) - (2.51 \times B \times C)$$

Table 4.3: Analysis of variance for the production of Yellow pigment, red pigment and cell dry weight .df (Degree of freedom).

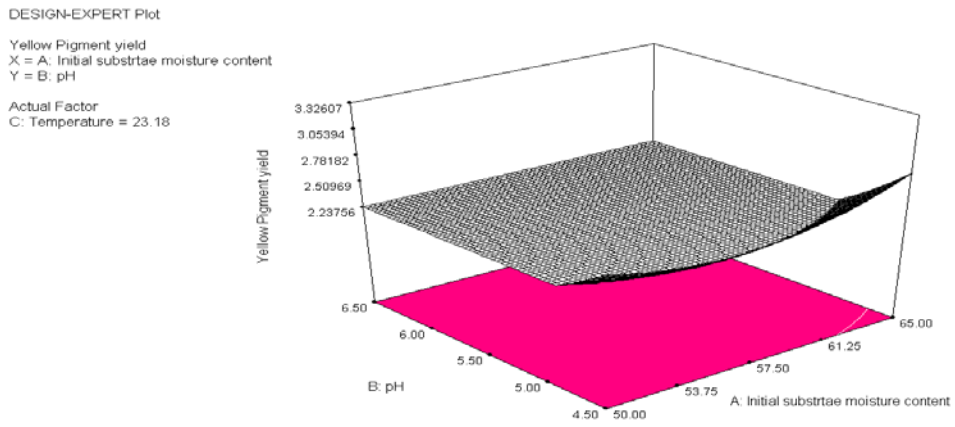
Source	Yellow pigment		Red Pigment		Cell dry weight	
	Df	**Sum of squares**	**Df**	**Sum of squares**	**Df**	**Sum of squares**
Residual	10	5.38	10	5.26	10	1183.19
Lack of fit	5	5.37	5	5.26	5	1183.14
Pure error	5	0.012	5	0.000	5	0.047

Table 4.4: ANOVA results of response surface design for yellow pigment production dependent variable: yellow pigment (OD units)

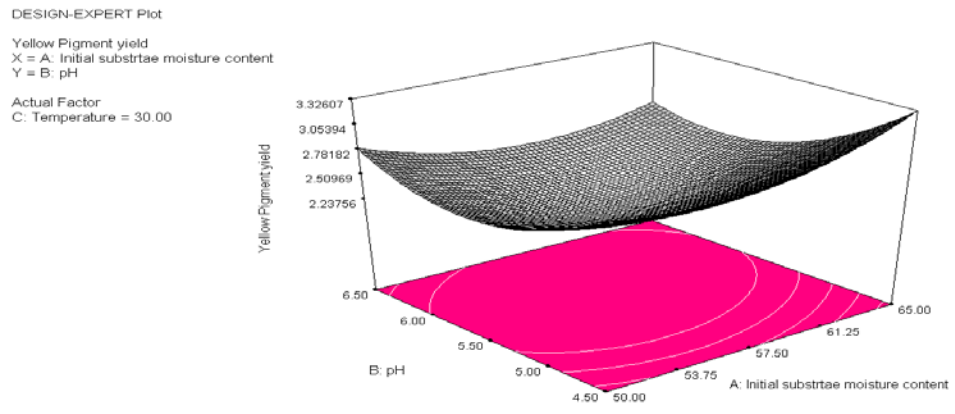
Source	df	Sum of Squares	Mean squares	F value	Prob>F
Model	9	9.22	1.02	1.90	0.1649
Pure Error	5	0.012	2.481^{-003}		
Corrected Total	19	14.6			
R^2	0.6315				
Coefficient var	29.02				

4.2.2.1 Analysis of yellow pigment

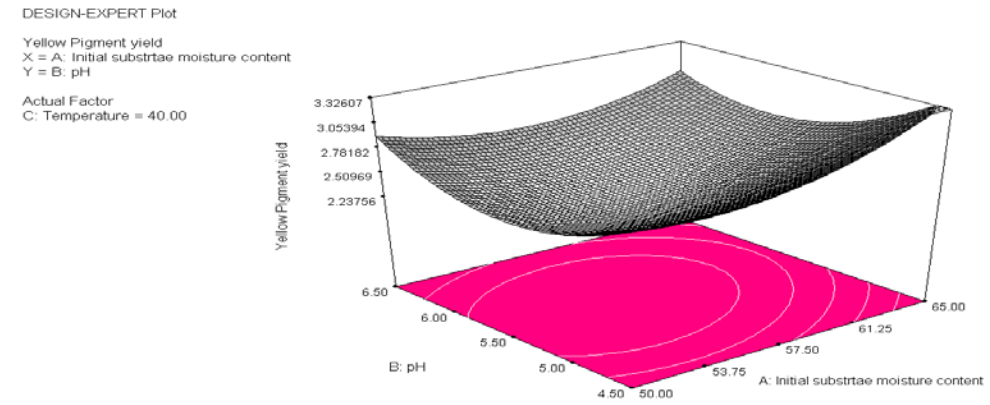
A. Temperature = 23.18˚C



B. Temperature = 30 ˚ C



C. Temperature = 40˚C



D. Temperature = 50˚C

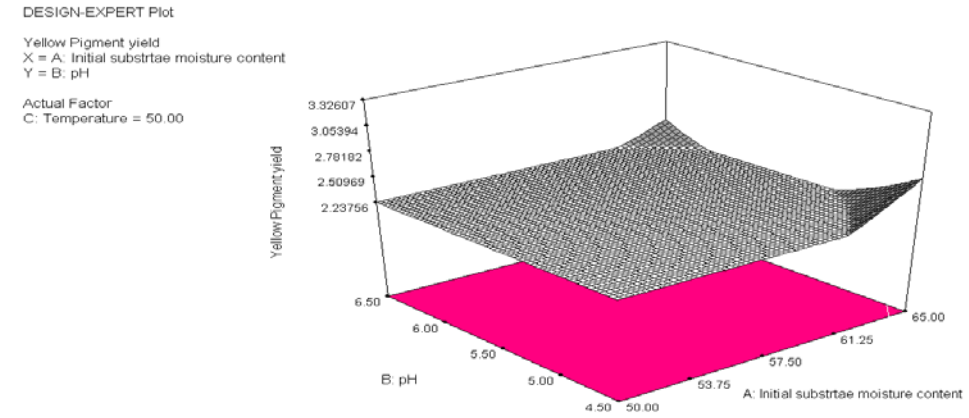


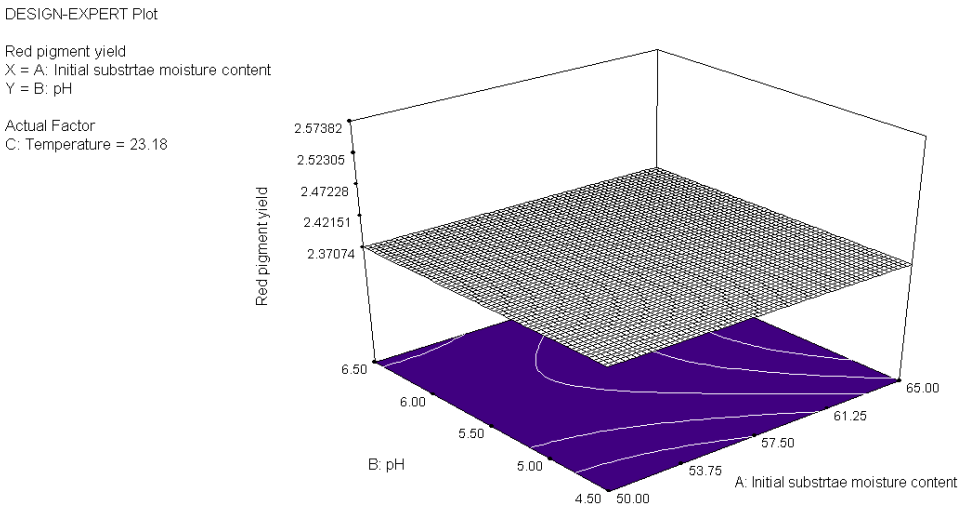
Figure 4.10: A-D: The response surface for the production of yellow pigment at various initial substrate moisture content and pH

Figure 4.10 shows three-dimensional (3-D) response surface plots of the effect of cultivation initial moisture content and pH on the production of yellow pigment. The best curve is at the temperature 30˚C where the highest production is at initial. Moisture content 65% and pH 4.5. The yellow pigment production at this parameter

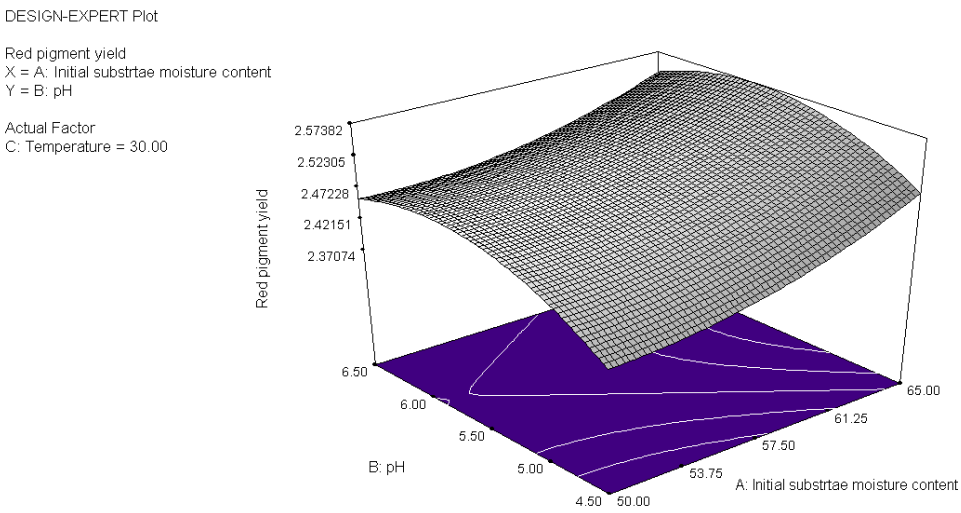
is 3.938 OD/gds. When the pH 4.5 and initial moisture content 50%, the pigment production is 3.514 OD/gds. Yellow pigment production give the value 3.398 OD/gds at the initial moisture content 65% and pH 6.5. Meanwhile, at the pH 6.5 and initial moisture content 50% and pH 5.50 give the production 3.238 OD/gds. However, all this value is much lower compare to the preliminary study where the highest yellow pigment production is 6.6960 OD/gds because of error during the fermentation process where limitations of aeration and surface area for cultivation were probably the reasons for lower pigment production and longer fermentation time to reach maximum value in the shake flask study. In solid state fermentation, aeration is used to supply oxygen, remove carbon dioxide that produce during the process of metabolism and also to control the temperature of substrate (Gervais and Molin, 2003). As state by Han and Mudgett (1992), fermentation that used *Monascus* is quite sensitive to aeration and can reduced pigment production because of the accumulation of excess carbon dioxide. According to the Lonsane *et al*., (1985) the optimum aeration in SSF processes depends on the nature of the microorganism used, the oxygen requirements for product synthesis, the amount of heat to be removed from the mass, the degree to which carbon dioxide and other volatile metabolites must be eliminated, the thickness and the available air spaces of the substrate. As for the cultural surface, Lee *et al.*, 2011 suggest that one of the possible solutions would consist to increase the cultural surface is by increasing the dimension of reaction surface in the reactor.

4.2.2.2 Analysis of red pigment

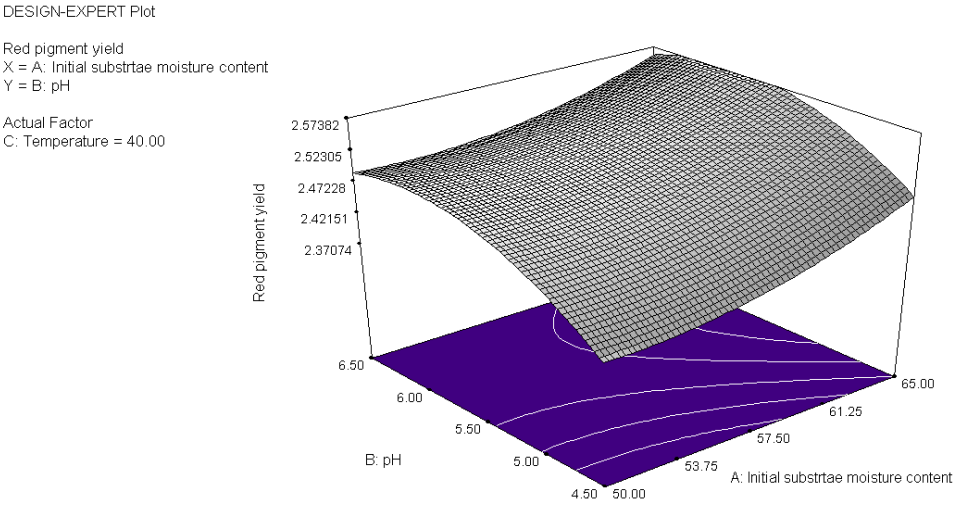
A. Temperature = 23.18˚C



B. Temperature = 30˚C



C. Temperature = 40 ˚C



D. Temperature = 50˚C

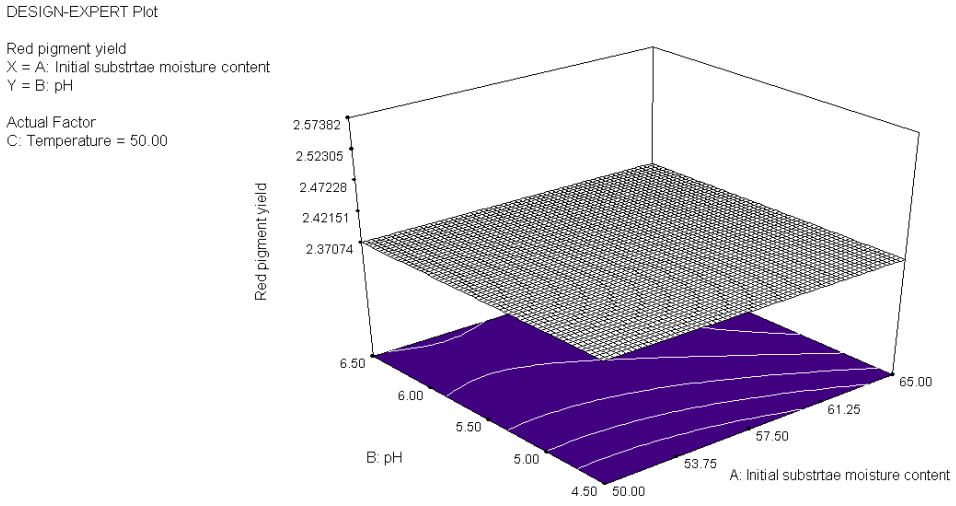
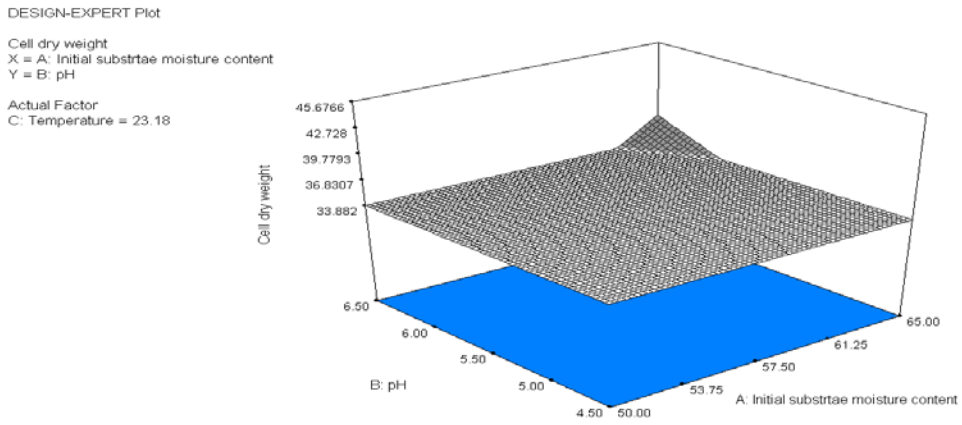


Figure 4.11: A-D: The response surface for the production of red pigment at various initial substrate moisture content and pH.

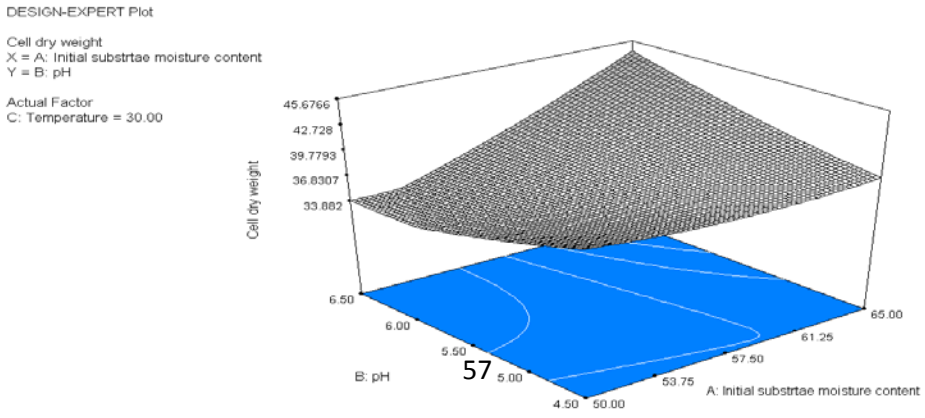
Figure 4.11 shows 3-D response surface plots of the effect relationship between red pigment, initial moisture content and pH. The addition of temperature lower the production of red pigment, red pigment went down when the temperature was above 30˚C. In addition, when comparing Figure 4.10 and 4.11, it is obvious that yellow and red pigment had similar trends under the same cultivation conditions; conditions that increase yellow pigment also increased the production of red pigment. Thus, production of yellow and red pigment is related.

4.2.2.3 Analysis of cell dry weight

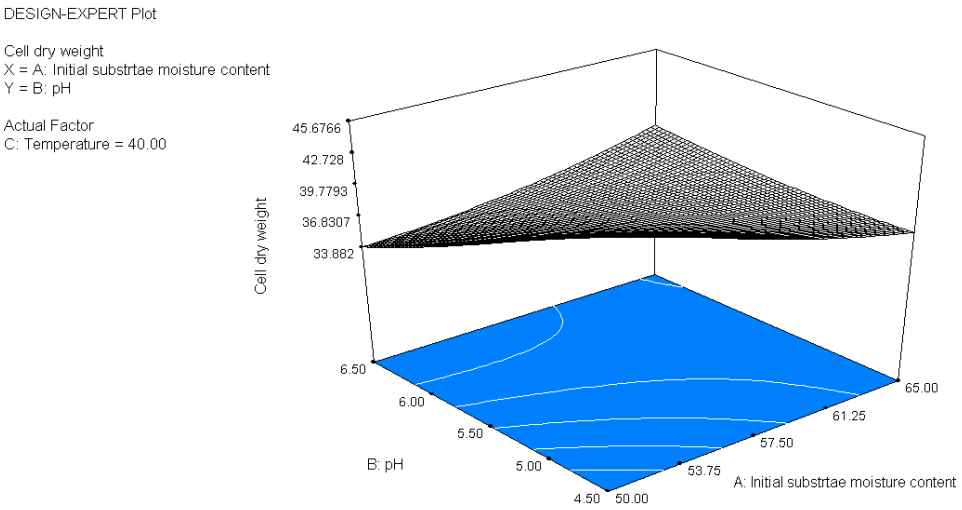
A. Temperature = 23.18˚C



B. Temperature = 30˚C



C. Temperature = 40˚C



D. Temperature = 50˚C

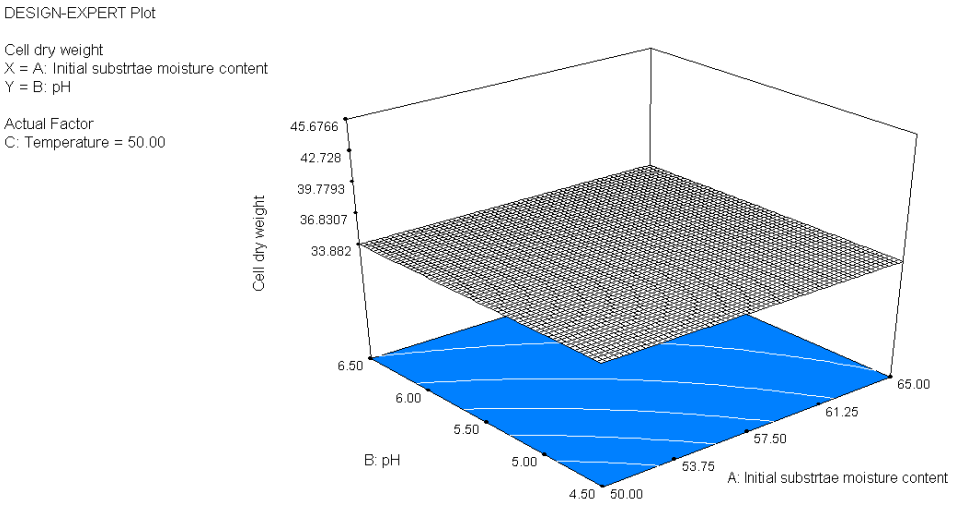


Figure 4.12: A-D: The response surface for the production of cell dry weight at various initial substrate moisture content and pH.

Figure 4.12 shows 3-D response surface plots of the effect of initial moisture content and pH on the cell dry weight. Increase of temperature brought the curve downward, so the cell dry weight decreased. Cell dry weight is optimum at a temperature of around 30˚C.

4.3 VERIFICATION OF OPTIMUM CONDITIONS

Design expert give the point prediction at**:**

Table 4.5: Point prediction by Design Expert

Initial substrate moisture content	57.5%
pH	5.5
Temperature	40˚C

From the point prediction, it is expected that yellow pigment production is 2.3753 OD/gram dry solids. To confirm the results, *Monascus* yellow pigment fermentation was conducted under the optimum conditions of culture medium calculated by response surface analysis. After the final fermentation, the average yield of *Monascus* yellow pigment could reach 2.2104 OD/gram dry solid. The results demonstrate that the good correlation between predicted and measured values of these experiments verified the validity of the response model and the existence of an optimum point.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In this project, the growth of *Monascus purpureus* FTC5356 and pigment production on agricultural wastes, banana peel was studied in solid satate fermentation. To achieve the target different growth factors (initial substrate moisture content, pH and temperature) were examined.

Effect of different temperatures (30°C, 35˚C, 40˚C, 45˚C and 50°C) and initial substrate moisture content (45%, 50%, 55%, 60%, 65%) has been studied on different growth cultures to show the optimum temperature suitable for growth of fungi and production of pigment. *Monascus purpureus* were capable of intensive growth and pigment production at 30°C and 60% initial moisture content.The pigment production has been tested towards pH. It showed that at lower pH is the best for the production of yellow pigment and at higher pH is for red pigment.

Characterization of pigment obtained from banana peel carried out with employing UV-VIS at 400 nm and the optimization using Design Expert 6.1 at temperature 40˚C, initial substrate moisture content 57% and pH 5.5.

For recommendation, it suggested to compare the pigment production from banana peel by using *Monascus purpureus* FTC 5356 in both solid and submerged culture and with the addition of nitrogen and carbon source.

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APPENDIX A

STANDARD CURVE OF N-ACETYL-D-GLUCOSAMINE

Standard curve of N-acetyl-D-glucosamine was measured by modified Elson Morgan described by Herbert et al., (1971). Prepare a dilution series of N-acetyl-D-glucosamine standards in four ten tubes according to the following scheme.

Dilution series of N-acetyl-D-glucosamine standards:

Stock tube	Dilution	N-acetyl-D-glucosamine concentration (μg/ml)
1	0 μL of stock (1mg/ml) + 125 μL of H_2O	0
2	12.5 μL of stock (1mg/ml) + 112.5 μL of H_2O	12.5
3	25 μL of stock (1mg/ml) + 100 μL of H_2O	25
4	50 μL of stock (1mg/ml) + 75 μL of H_2O	50
5	62.5 μL of stock (1mg/ml) + 62.5 μL of H_2O	62.5

Procedure:

1) Prepare 1.25 mg/mL of standard N-acetyl-D-glucosamine solution (0.125 g of N-acetyl-D-glucosamine in 125 ml distilled water.
2) Transfer (12.5, 25, 50, and 62.5 μL) to test tube.
3) Add distilled water (112.5, 100, 75 and 62.5 μL) to each test tube and mix it well.
4) Mixed with freshly prepared 0.125 mL (125 μL) acetyl acetone reagent.
5) Solutions were boiling for 20 min.

6) Acetylation was terminated and adds 0.625mL (625 μL) of 95% ethanol followed by 0.125 mL (125 μL) of Ehrlich reagent.
   Ehrlich reagent: 2.0 g of p–dimethylaminobenzaldehyde (DMAB) in 50 mL of 95% ethanol and 50 mL of concentrated hydrochloric acid
7) The tubes uncapped to release carbon dioxide build-up were placed at 65˚C for 10 min.
8) Allow the test tube to cool to room temperature
9) Read absorbance at 530 nm
10) For the blank, prepared exactly as the procedure one to nine but using distilled water in place of the N-acetyl-D-glucosamine solution.

Results:

N-acetyl-D-glucosamine concentration (μL/mL)	Absorbance value at 530 nm
0	0.000
12.5	0.245
25	0.494
50	0.893
62.5	1.080

APPENDIX B

DATA FOR FIGURES

Data for figure 4.1: Incubation time for yellow pigment

Time (hr)	OD before dilution			OD after dilution			Average	OD = $\frac{OD \times df}{0.5}$
0	0	0	0	0	0	0	0	0
24	0.296	0.185	0.274	0.148	0.111	0.140	0.133 ± 0.016	0.9576
48	0.395	0.304	0.316	0.180	0.150	0.154	0.161 ± 0.013	1.1592
72	0.987	0.998	0.909	0.375	0.379	0.349	0.368 ± 0.013	2.6496
96	1.579	1.589	1.509	0.570	0.573	0.547	0.563 ± 0.012	4.0536
120	1.973	1.984	1.995	0.700	0.704	0.708	0.704 ± 0.003	5.0688
144	2.122	2.131	2.140	0.749	0.752	0.755	0.752 ± 0.002	5.4144
168	2.572	2.616	2.823	0.898	0.912	0.981	0.930 ± 0.036	6.6960
192	1.973	1.984	1.995	0.700	0.677	0.699	0.692 ± 0.011	4.9824
216	1.332	1.343	1.354	0.489	0.493	0.496	0.492 ± 0.003	3.5424

Data for figure 4.2: Incubation time for red pigment

Time (hr)	OD before dilution			OD after dilution			Average	OD = $\frac{OD \times df}{0.5}$
0	0	0	0	0	0	0	0	0
24	0.049	0.050	0.061	0.066	0.066	0.070	0.067 ± 0.002	0.4824
48	0.207	0.216	0.225	0.118	0.121	0.124	0.121 ± 0.002	0.8712
72	0.493	0.412	0.421	0.212	0.185	0.188	0.195 ± 0.012	1.404
96	0.641	0.650	0.669	0.261	0.264	0.270	0.265 ± 0.004	1.908
120	0.888	0.897	0.806	0.342	0.345	0.315	0.334 ± 0.013	2.4048
144	1.085	1.094	1.003	0.407	0.410	0.380	0.399 ± 0.013	2.8728
168	1.773	1.811	1.981	0.700	0.647	0.703	0.683 ± 0.026	4.9176
192	0.987	0.996	0.905	0.375	0.378	0.348	0.367 ± 0.013	2.6424
216	0.839	0.840	0.851	0.326	0.314	0.330	0.323 ± 0.007	2.3256

Data for figure 4.2: Moisture content of Yellow pigment (A_{400nm})

Moisture content (%)	OD before dilution			OD after dilution			Average	OD = $\frac{OD \times df}{0.5}$
65	1.431	1.427	1.439	0.465	0.463	0.466	0.465 ± 0.001	3.3480
60	2.566	2.172	2.232	0.917	0.917	0.783	0.872 ± 0.063	6.2784
55	2.269	2.042	1.998	0.798	0.707	0.690	0.732 ± 0.047	5.2704
50	0.296	0.423	0.376	0.014	0.059	0.069	0.047 ± 0.024	0.3384
45	0.148	0.165	0.186	0.003	0.005	0.008	0.005 ± 0.002	0.036

Data for figure 4.2: Moisture content of Red pigment (A_{500nm})

Moisture content (%)	OD before dilution			OD after dilution			Average	OD = $\frac{OD \times df}{0.5}$
65	0.853	0.788	0.915	0.296	0.269	0.303	0.289 ± 0.015	2.081
60	1.973	1.731	2.170	0.700	0.621	0.765	0.695 ± 0.059	5.004
55	1.135	1.135	1.127	0.424	0.424	0.422	0.423 ± 0.001	3.046
50	0.296	0.300	0.297	0.148	0.149	0.148	0.148 ± 0.000	1.066
45	0.148	0.154	0.144	0.090	0.101	0.097	0.096 ± 0.004	0.691

Data for figure 4.5: Yellow pigment (A_{400nm})

pH (%)	OD before dilution			OD after dilution			Average	OD = $\frac{OD \times df}{0.5}$
4.5	0.937	0.788	0.915	0.395	0.269	0.303	0.322 ± 0.053	2.146
5.0	1.677	1.731	1.570	0.603	0.621	0.568	0.597 ± 0.022	4.298
5.5	2.368	2.501	2.231	0.831	0.874	0.785	0.830 ± 0.036	5.976
6.0	1.159	1.192	1.137	0.429	0.449	0.425	0.433 ± 0.010	3.118
6.5	1.135	0.976	1.132	0.424	0.372	0.423	0.406 ± 0.024	2.923

Data for figure 4.5: Red pigment (A_{500nm})

pH (%)	OD before dilution			OD after dilution			Average	OD = $\frac{OD \times df}{0.5}$
4.5	0.395	0.385	0.318	0.180	0.177	0.155	0.171 ± 0.011	1.231
5.0	1.135	1.131	1.110	0.424	0.423	0.416	0.421 ± 0.004	3.031
5.0	1.139	1.152	1.197	0.426	0.429	0.445	0.433 ± 0.008	3.112
6.0	2.358	2.112	2.234	0.827	0.746	0.786	0.786 ± 0.033	5.659
6.5	0.977	0.967	1.123	0.369	0.372	0.423	0.420 ± 0.025	2.916

Data for figure 4.6: Yellow pigment (A_{400nm})

Temperature (˚C)	OD before dilution			OD after dilution			Average	OD = $\frac{OD \times df}{0.5}$
30	2.566	2.188	2.415	0.896	0.771	0.846	0.838 ± 0.051	6.036
35	1.381	1.367	1.364	0.505	0.501	0.499	0.502 ± 0.002	3.614
40	1.184	1.130	1.171	0.440	0.422	0.436	0.426 ± 0.008	3.067
45	0.641	0.637	0.625	0.261	0.260	0.259	0.260 ± 0.001	1.865
50	0.612	0.600	0.617	0.252	0.254	0.148	0.218 ± 0.050	1.569

Data for figure 4.6: Red pigment (A_{500nm})

Temperature (˚C)	OD before dilution			OD after dilution			Average	OD = $\frac{OD \times df}{0.5}$
30	2.463	2.147	2.063	0.862	0.758	0.730	0.783 ± 0.057	5.638
35	1.578	1.585	1.518	0.570	0.573	0.419	0.522 ± 0.072	3.758
40	0.937	0.946	0.976	0.359	0.362	0.372	0.364 ± 0.006	2.621
45	0.320	0.309	0.318	0.156	0.152	0.155	0.154 ± 0.002	1.109
50	0.257	0.230	0.217	0.135	0.125	0.122	0.127 ± 0.006	0.914

Data for yellow pigment by optimization using design expert

Run	Factor 1: Initial substrate moisture content (%)	Factor 2 : pH	Factor 3 : Temperature (˚C)	Yellow pigment after dilution		Average	Pigment Yield (OD/gds)
1	50.00	4.50	30.00	0.497	0.478	0.488 ± 0.010	3.514
2	57.50	5.50	23.18	0.101	0.103	0.102 ± 0.001	0.734
3	50.00	6.50	50.00	0.358	0.343	0.351 ± 0.008	2.527
4	44.89	5.50	40.00	0.318	0.328	0.323 ± 0.005	2.326
5	65.00	4.50	30.00	0.537	0.557	0.547 ± 0.010	3.938
6	57.50	7.18	40.00	0.232	0.245	0.363 ± 0.007	2.614
7	57.50	5.50	40.00	0.322	0.345	0.334 ± 0.012	2.405
8	65.00	4.50	50.00	0.434	0.443	0.439 ± 0.005	3.161
9	70.11	5.50	40.00	0.354	0.339	0.347 ± 0.008	2.495
10	50.00	4.50	50.00	0.298	0.276	0.287 ± 0.011	2.066
11	65.00	6.50	50.00	0.408	0.398	0.403 ± 0.005	2.902
12	57.50	5.50	40.00	0.332	0.335	0.333 ± 0.002	2.398
13	57.50	5.50	40.00	0.328	0.338	0.333 ± 0.005	2.434
14	57.50	5.50	40.00	0.345	0.335	0.340 ± 0.005	2.448
15	65.00	6.50	30.00	0.476	0.467	0.472 ± 0.005	3.398
16	57.50	3.82	40.00	0.417	0.421	0.419 ± 0.002	3.017
17	50.00	6.50	30.00	0.466	0.445	0.456 ± 0.011	3.283
18	57.50	5.50	40.00	0.312	0.335	0.324 ± 0.012	2.333
19	57.50	5.50	40.00	0.323	0.365	0.344 ± 0.021	2.477
20	57.50	5.50	56.82	0.016	0.008	0.012 ± 0.004	0.086

Data for red pigment by optimization using design expert

Run	Factor 1: Initial substrate moisture content (%)	Factor 2 : pH	Factor 3 : Temperature (˚C)	Red pigment after dilution		Average	Pigment Yield (OD/gds)
1	50.00	4.50	30.00	0.428	0.423	0.426 ± 0.003	3.067
2	57.50	5.50	23.18	0.082	0.089	0.086 ± 0.004	0.619
3	50.00	6.50	50.00	0.225	0.237	0.231 ± 0.006	1.663
4	44.89	5.50	40.00	0.142	0.143	0.283 ± 0.001	2.037
5	65.00	4.50	30.00	0.453	0.433	0.443 ± 0.010	3.189
6	57.50	7.18	40.00	0.272	0.265	0.269 ± 0.003	1.936
7	57.50	5.50	40.00	0.354	0.348	0.351 ± 0.003	2.527
8	65.00	4.50	50.00	0.219	0.205	0.212 ± 0.007	1.526
9	70.11	5.50	40.00	0.317	0.309	0.313 ± 0.004	2.253
10	50.00	4.50	50.00	0.212	0.202	0.207 ± 0.005	1.490
11	65.00	6.50	50.00	0.242	0.238	0.240 ± 0.002	1.728
12	57.50	5.50	40.00	0.354	0.332	0.343 ± 0.011	2.469
13	57.50	5.50	40.00	0.324	0.338	0.331 ± 0.007	2.383
14	57.50	5.50	40.00	0.344	0.348	0.346 ± 0.002	2.491
15	65.00	6.50	30.00	0.461	0.445	0.453 ± 0.008	3.261
16	57.50	3.82	40.00	0.267	0.256	0.262 ± 0.006	1.886
17	50.00	6.50	30.00	0.455	0.444	0.449 ± 0.006	3.232
18	57.50	5.50	40.00	0.324	0.352	0.338 ± 0.014	2.434
19	57.50	5.50	40.00	0.334	0.324	0.329 ± 0.005	2.369
20	57.50	5.50	56.82	0.185	0.187	0.186 ± 0.001	1.339

Data for biomass concentration by optimization using design expert

Run	Factor 1: Initial substrate moisture content (%)	Factor 2 : pH	Factor 3 : Temperature (˚C)	Concentration of Biomass after dilution		Average	Biomass
1	50.00	4.50	30.00	0.818	0.879	0.849 ± 0.031	47.9666
2	57.50	5.50	23.18	0.096	0.098	0.097 ± 0.001	5.4802
3	50.00	6.50	50.00	0.107	0.102	0.105 ± 0.003	5.9322
4	44.89	5.50	40.00	0.666	0.657	0.662 ± 0.005	37.4011
5	65.00	4.50	30.00	0.889	0.898	0.894 ± 0.005	50.5085
6	57.50	7.18	40.00	0.605	0.657	0.631 ± 0.026	35.6497
7	57.50	5.50	40.00	0.673	0.657	0.665 ± 0.008	37.5706
8	65.00	4.50	50.00	0.178	0.102	0.140 ± 0.038	7.9096
9	70.11	5.50	40.00	0.641	0.657	0.649 ± 0.008	36.6667
10	50.00	4.50	50.00	0.534	0.546	0.540 ± 0.006	30.5085
11	65.00	6.50	50.00	0.104	0.102	0.103 ± 0.001	5.8192
12	57.50	5.50	40.00	0.673	0.651	0.662 ± 0.011	37.4011
13	57.50	5.50	40.00	0.669	0.657	0.663 ± 0.006	37.4576
14	57.50	5.50	40.00	0.663	0.669	0.666 ± 0.003	37.6271
15	65.00	6.50	30.00	0.854	0.884	0.869 ± 0.015	49.0960
16	57.50	3.82	40.00	0.658	0.657	0.658 ± 0.001	37.1751
17	50.00	6.50	30.00	0.747	0.768	0.758 ± 0.011	42.8249
18	57.50	5.50	40.00	0.667	0.657	0.662 ± 0.005	37.4011
19	57.50	5.50	40.00	0.673	0.657	0.665 ± 0.008	37.5706
20	57.50	5.50	56.82	0.097	0.098	0.098 ± 0.001	5.6517

LIST OF FIGURES

LIST OF SYMBOLS

$C_{23}H_{26}O_5$	monascorubrin
$C_{21}H_{22}O_5$	rubropunctatin
$C_{23}H_{27}NO_4$	monascorubramine
$C_{21}H_{23}NO_4$	rubropunctamine
$C_{21}H_{26}O_5$	monascin
$C_{23}H_{30}O_5$	ankaflavin
Df	Degree of freedom
g	Gram
gds	Gram dry solid
hr	hour
mg	miligram
min	Minutes
ml	Millimeter
nm	nanometer
rpm	Revolution per minute
SmF	Submerged fermentation
SSF	Solid state fermentation

OPTIMIZATION OF YELLOW PIGMENT PRODUCTION BY Monascus purpureus FROM BANANA PEEL

ABSTRACT

The purpose of this research is mainly to study the optimization of yellow pigment production by *Monascus purpureus* from banana peel. There has been an increasing trend towards replacement of synthetic colorants with natural pigments in last decades due to the high demand of natural products. Bacterial pigments are considered as an alternative to produce natural colour. Production of yellow pigment of *Monascus purpureus* grow on agricultural waste that is banana peels was studies. From the study, the optimum growth temperature of *Monascus purpureus* and pigment production is at 30°C, 60 % moisture content and optimum pH is 5.5. The pigment extracted was with ethanol as a solvent. Measurement of yellow pigment carried out using UV-VIS 1800 spectrophotometer at 400 nm. The relation of three parameters, were further studies on Response Surface Methodology. By the point prediction tool of Design-Expert 6.7, the optimum values of the factors for maximum pigment production were determined: initial substrate moisture content 57.5%, pH 5.5 and temperature 40˚C. With this point optimization, the pigment yield for yellow pigment is 2.2104 OD/gram dry solid was closely to 2.3754 OD/gram dry solid with is the predicted yield.

OPTIMUM PENGELUARAN PIGMEN KUNING DARIPADA Monascus purpureus OLEH KULIT PISANG

ABSTRAK

Tujuan kajian dilakukan adalah untuk mengkaji optimum pengeluaran pigmen kuning daripada *Monascus purpureus* oleh kulit pisang. Terdapat peningkatan hala tuju dalam beberapa dekad ini terhadap penggantian pewarna sintetik dengan pigmen asli disebabkan peningkatan permintaan pengguna terhadap produk-produk asli dan pigmen daripada bakteria dianggap sebagai alternatif kepada pewarna sintetik. Penghasilan dan pengekstrakan pigmen kuning oleh *Monascus purpureus* yang dikulturkan di atas sisa pertanian iaitu kulit pisang telah di kaji. Daripada kajian ini, suhu optimum untuk pertumbuhan dan penghasilan pigmen oleh *Monascus purpureus* ialah pada 30°C, kandungan kelembapan 60% dan pH optimum ialah 5.5. Pigmen tersebut diekstrak menggunakan etanol sebagai pelarut. Pencirian pigmen kuning ini dilakukan dengan menggunakan spektrofotometer UV-VIS 1800 pada 400 nm.Response Surface Methodhology telah dipilih untuk kajian selanjutnya. Dengan alat ramalan titik Pakar Rekabentuk-6.7 (Design- Expert 6.7), nilai factor optimum untuk pengeluaran pigmen maksimum telah ditentukan: kandungan lembapan 57.5%, pH 5.5 dan suhu 40 ˚ C. Dengan pengoptimuman ini, hasil pigmen pigmen kuning 2.2104 OD / gram pepejal kering (Od/gds) adalah dekat dengan 2.3754 OD / gram pepejal kering (OD/gds) hasil yang diramalkan.

LIST OF ABBREVIATIONS

DF	Dilution Factor
EBV	Epstein–Barr virus
FDA	Food and Drug Administration
GABA	g-aminobutyric
OD	Optical Density
PDA	Potato Dextrose Agar
SCSP	shrimp and crab shell powder
TPA	tumor promoter 12-*O*-tetradecanoylphorbol-13-actate
Uv-vis	Ultra-violet spectrophotometer