OPTIMIZATION OF PIGMENT PRODUCTION BY *Monascus purpureus* MTCC 1090

SUBMITTEDIN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE AWARD OF THE DEGREE OF

MASTER OF TECHNOLOGY

IN
BIOTECHNOLOGY

SUBMITTED BY:

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UNDER THE SUPERVISION OF

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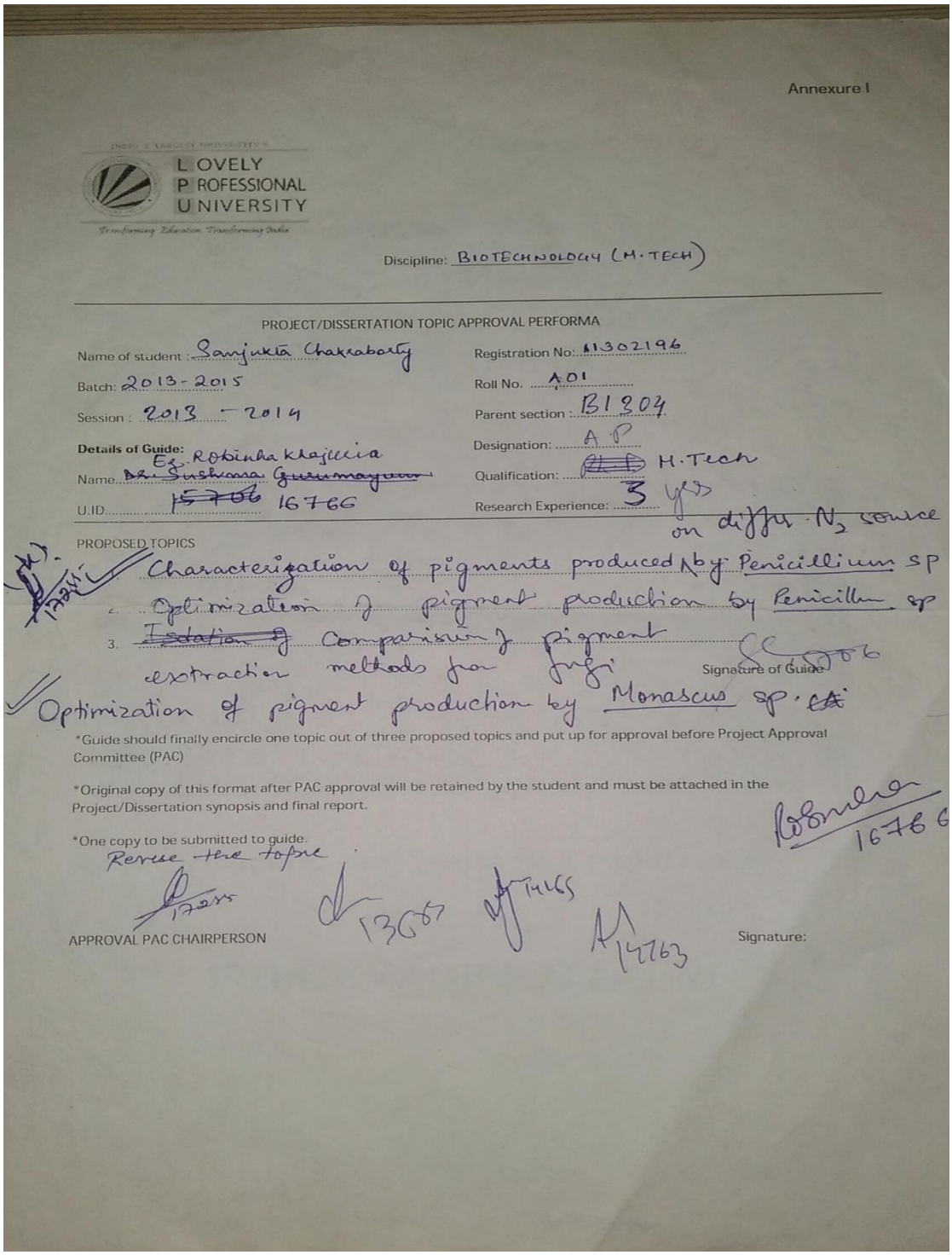
APPROVAL



Annexure I

LOVELY PROFESSIONAL UNIVERSITY

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Discipline: BIOTECHNOLOGY (M.TECH)

PROJECT/DISSERTATION TOPIC APPROVAL PERFORMA

Name of student: Sanjukta Chakraborty
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Session: 2013 - 2014
Parent section: B1304
Details of Guide:
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Qualification: ~~M.Tech~~ M.Tech
U.ID: ~~15706~~ 16766
Research Experience: 3 yrs

PROPOSED TOPICS

1. Characterization of pigments produced by Penicillium sp on diff. N_2 source
2. Optimization of pigment production by Penicillium sp
3. ~~Isolation of~~ Comparision of pigment extraction methods from fungi

Signature of Guide

Optimization of pigment production by Monascus sp. ~~CA~~

*Guide should finally encircle one topic out of three proposed topics and put up for approval before Project Approval Committee (PAC)

*Original copy of this format after PAC approval will be retained by the student and must be attached in the Project/Dissertation synopsis and final report.

*One copy to be submitted to guide.

Revise the topic

APPROVAL PAC CHAIRPERSON

Signature:

ABSTRACT

Pigments from *Monascus* are known to produce natural colors which are speculated to have great industrial and medical applications. Due to the extensive use of synthetic dyes, there is a growing public concern regarding their detrimental health and environmental impacts .This has increased an interest in natural pigments which are less harmful and also have many health benefits. The aim of this study was to optimize conditions for the synthesis of pigments by *Monascus purpureus* MTCC 1090 by solid state fermentation using agro industrial residues such as rice straw, wheat straw, okara, sugarcane bagasse, broken rice, broken wheat and sweet potato. This work also aimed at evaluating the stability of the pigments produced. Among all the agro industrial residues, it was found that broken rice gave the best pigment production of 22.2 CVU/gds after 15 days of incubation and least yield was given by wheat straw (1.32 CVU/gds). Addition of organic and inorganic nitrogen sources increased the pigment yield manifold with sodium nitrate giving the highest yield (195.07 CVU/gds) and yeast extracts the lowest (5.53 CVU/gds). Further pigment stability studies revealed that the pigment was stable in between 5-6 pH and temperature 40-90ºC.The pigments underwent degradation on increased exposure to visible light. Acetone was found to be the best solvent for the process of extraction (363.36CVU/gds) followed by methanol (313.93CVU/gds).

Keywords: Agro- industrial residues, Color Value, Methanol, *Monascus purpureus,* Solid state fermentation

ACKNOWLEDGEMENT

I Sanjukta Chakraborty, student of Masters of technology in Biotechnology, want to express my sincere gratitude towards Lovely Professional University to allow me and to provide various facilities to carry out my project work. Access to various journals, library access and all the relevant study material was provided on time.

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Lastly, I would like to thank the almighty and my parents for their moral support and my friends with whom I have shared my day-to-day experience and received lots of suggestions that improved my quality of work.

Sanjukta Chakraborty.

CERTIFICATE

This is to certify that **Sanjukta Chakraborty (11302196)** has completed Dissertation project report (BTY 731), entitled) "**Optimization of pigment production by *Monascus purpureus* MTCC 1090**" under my guidance and supervision. To the best of my knowledge, the present work is the result of their original investigation and study. No part of the report has ever been submitted for any other degree at any university.

This report is fit for submission and the partial fulfillment of the conditions for the award of M. Tech. in Biotechnology**.**

Date: **Supervisor Signature**

Er. Robinka Khajuria
Assistant Professor
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Phagwara, Punjab (India)

DECLARATION

I hereby declare that this thesis entitled "**Optimization of pigments produced by *Monascus purpureus* MTCC 1090**"is an authentic record of my own work carried out at School of Biotechnology and Biosciences, **Lovely Professional University, Phagwara,** for the partial fulfillment of the award of Master of Technology in Biotechnology under the guidance of Er. Robinka Khajuria, School of Biotechnology and Biosciences, Lovely Professional University, Phagwara.

This work is my original and has not been submitted for any degree/diploma in this or any other University. The information furnished in this dissertation is genuine to the best of my knowledge and belief.

Place:

Date :

Sanjukta Chakraborty

M.Tech (Biotechnology)

Reg. No: 11302196

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCION

Known by names of ang-khak rice mold, corn silage mold, maize silage mold, and rice kernel discoloration, *Monascus* is a genus of mold which includes four species: *M. pilosus, M. purpureus, M. ruber* and *M. floridans,* belonging to class Ascomycetes and the family Monascaceae. Most importantly known for its use in fermented food specially rice. At present many species of this mold are being explored for its possible use in medicines. It is also known to produce natural dyes which serve as an alternative to synthetic dyes that are very harmful to the environment (Velmurugan *et al.,* 2011). Synthetic dyes have been reported to have detrimental effect on environment and human health. Synthetic pigments such as azorubin and tartrazin are reported to cause harmful allergic reactions (Fabre *et al.,* 1993) while the C-red pigment has carcinogenic and teratogenic effects (Merlin *et al.,* 1987). Therefore, production and optimization of natural pigments have gained a lot of importance in the past few decades. Monascus is one such strain whose property has been used since a long time of almost 100 years in the orient as a food coloring agent and also has been reported to have antimicrobial properties. As a result now this particular microorganism is being explored as a potential source of pigment production.

Besides *Monascus* an array of fungi belonging to *Penicillium* and *Aspergillus* have the capacity to produce pigments. *Aspergillus* which is a member of the deuteromycetes fungi, are highly aerobic in nature and are found in highly rich oxygen environment. They too have the capacity to be grown in controlled environment to produce pigments. Several classes of *Penicillium* the filamentous fungi also has been reported to produce pigments of different colours. Other than just pigments some other pigments are produced in the form of PP-R (7-(2-Hidroxyethyl)-monascorubramine (Mapari *et al.,* 2006).Fungal pigments have gained immense importance in today's world as they are easier to produce and are environment friendly. They are easily degradable in nature and don't have any climatic constraints as they can be produced in labs. Furthermore, pigments produced by microorganisms are of industrial interest because of the fact that microorganism can grow rapidly under controlled

conditions, thereby leading to maximum productivity and availability throughout the year (Mapari *et al*. 2005, 2008; Mendez *et al.*, 2011). Many fungi are reported to produce a wide variety of pigments with high yield, stability, and less light sensitivity (Mapari et al., 2005; Babitha *et al.*, 2007)

Monascus is a native organism of China and Thailand and can be easily grown in several ecosystems and finds several uses, from conferring color to food products to medicinal uses and as meat preservative. *Monascus* is known to produce strong yellow, orange or red pigments as secondary metabolites (Juszlova *et al.,* 1996; Pitt and Hocking., 1997). It has been shown to lower blood cholesterol, prevent cancer, stroke, osteoporosis, Alzheimer's disease and other dementias and muscular degeneration (Cesar *et al.*, 2005).

Monascus, comprises of nine species, and can reproduce either vegetatively with filaments and conidia or sexually by the formation of ascospores. The most well-known species of genus *Monascus*, namely, *M. purpureus*, *M. ruber* and *M. pilosus*, are often used for rice fermentation to produce red yeast rice, a special product used either for food coloring or as a food supplement. This has shown to have many positive health benefits in human health. The colored appearance (red, orange or yellow) of *Monascus* fermented substrates is produced by a mixture of different pigments (Cesar *et al.,* 2005).It has been studied over the years that the above mentioned three species of monascus are most effective in producing secondary metabolites specially orange, yellow and red pigmentation. And among all the pigments the red pigments are the most important ones because they can be used as substitutes for nitrites in meat production and for synthetic colors. Besides this, biopigments from monascus also have antibiotic properties (Ajay *et al., 2012)* thereby increasing the interest of scientific community in pigment production from it.

There are different fermentation strategies, viz. solid state fermentation and submerged fermentation that are used for cultivation of fungal biomass and subsequently pigment production. Solid state fermentation is a biomolecule manufacturing process where the metabolites are generated from fungi or other microbes on a solid surface. Solid state fermentation process generates the maximum yield as compared to liquid or submerged

fermentation. In nature, growth of filamentous fungi is best on the ground, decomposing vegetable compounds under naturally aeration (Babitha *et al.,* 2006). Therefore, solid state fermentation enables the optimal development of filamentous fungi, allowing the mycelium to spread on the surface of solid compounds among which air can flow and moreover this process includes usage of very little water which is particularly favorable for the growth of mold. Solid state fermentation (SSF) hence has emerged as an effective alternative for submerged fermentation and culture based technologies which uses liquid culture media. The substrates used in SSF supply the basic nutrients to the microorganisms and serve as an anchor for the cells (Velmurugan *et al., 2011).* Interestingly, recent studies have reported that SSF provides a more optimum environment for fungi, which results in enhanced pigment production in a relatively low-cost process. Owing to very high cost of currently used technology of pigment production on industrial scale cheap substrates like agro industrial residues like wheat straw, rice bran, wheat bran, okara, and other cheap raw materials like broken rice, sweet potato can be evaluated as potential substrates for pigment production(Babitha *et al.,* 2006).

The aim of this study was to evaluate the pigment production potential of *monascus purpureus* by solid state fermentation (SSF) using agro industrial residues as substrates. The study also aimed at evaluating the stability of pigments produced.

CHAPTER 2

TERMINOLOGY

Fungal Pigments: pigments are substances which absorb lights of different frequency and transmit them. Fungal pigments are secondary metabolites produced by different classes of fungi in their log phase.

Solid state fermentation: solid state fermentation is a process by which microbes are grown on a solid media. It can be termed as a biomolecule manufacturing process and the biomolecules produced are generally microbial metabolites.

Submerged fermentation: submerged fermentation is also a biomolecule manufacturing process in which the microbes are grown in a liquid media instead of a solid one.

***Monascus purpureus*:** It is a species of mold and is reddish in color. It is known by the names of ang-khak mold, corn silage mold, rice silage mold, rice kernel discoloration. This *Monascus* produces pigments as their secondary metabolite. It has been recently discovered that this mold produces cholesterol-lowering statins and hence it has prompted for further medical research.

Solvent extraction: It is a process of separating a substance from one or more others using a solvent, here the two immiscible liquids are agitated vigorously so that they disperse one in another so that the solutes move from one solvent to other.

Agro industrial residue: Agro industrial residues are the organic materials that are produced as byproducts in agro industries.

CHAPTER 3

REVIEW OF LITERATURE

3.1 PIGMENTS FROM FUNGI: A NATURAL COLORING ALTERNATIVE

The topic of coloring agents used in our everyday lives and specially the use of synthetic dyes has become a topic of controversy. Going back to the days when dyes where being produced naturally by the early people for staining, painting, or as food additives the world has come a long way and the production of synthetic dyes have ever since increased many folds. At present synthetic colorants are largely being used in cosmetics, as food colors, dye stuffs and as pharmaceutical products. But as the fact is the synthetic colorants are posing a great threat to the environment as they have become more of an environmental hazard. Fabre *et al.*, 1993 reported that synthetic red pigments such as azorubin or tartrazin cause allergic reactions while C-red has carcinogenic and teratogenic effects (Merlin *et al.*, 1987). Besides this they are difficult to degrade or decompose and hence persist in the environment for long periods. Nature is a rich reservoir of colours (minerals, plants, microalgae, etc.), and pigment-producing microorganisms (fungi, yeasts, bacteria) are most abundant in nature. Among the molecules produced by microorganisms are melanins, carotenoids, flavins, quinones, and more specifically monascins, violacein or indigo (Laurent Dufosse, 2005).

Among microorganisms, fungi have proven to be the most efficient producer of pigments. Many species of the fungi like *Penicillium*, *Monascus* and *Aspergillus* have been reported to produce pigments. Espinoza-Hernandez *et al*, 2013 reported pigment production from *Penicillium purpurogenum* (GH2) and *Penicillium pinophilum* (EH2 and EH3). Similarly many works have been done on the *Aspergillus* species of fungi and the *Monascus* species which have equal or more potential than many other strains of bacteria to produce pigments (Carvalho *et al.*, 2005). These properties are being exploited extensively to render fungi as a potent source of pigment production and so that this concept becomes industrially as well as environmentally significant. The table under shows various pigments produced by different classes of microbes and also the color of their pigments produced. (Table 1)

Table: Production of microbial pigments. (Already in use as natural food colorants)(Duffose, 2006)

Molecule	Colour	Microorganism	Status*
Ankaflavin	yellow	*Monascus* sp. (fungus)	IP
Anthraquinone	red	*Penicillium oxalicum* (fungus)	IP
Astaxanthin	pink-red	*Xanthophyllomyces dendrorhous* (yeast), formerly *Phaffia rhodozyma*	DS
Astaxanthin	pink-red	*Agrobacterium aurantiacum* (bacteria)	RP
Astaxanthin	pink-red	*Paracoccus carotinifaciens* (bacteria)	RP
Canthaxanthin	dark red	*Bradyrhizobium* sp. (bacteria)	RP
Lycopene	red	*Blakeslea trispora* (fungus)	DS
Lycopene	red	*Fusarium sporotrichioides* (fungus)	RP
Melanin	black	*Saccharomyces neoformans* var. *nigricans* (yeast)	RP
Monascorubramin	red	*Monascus* sp. (fungus)	IP
Naphtoquinone	deep blood-red	*Cordyceps unilateralis* (fungus)	RP
Riboflavin	yellow	*Ashbya gossypi* (fungus)	IP
Rubrolone	red	*Streptomyces echinoruber* (bacteria)	DS
Rubropunctatin	orange	*Monascus* sp. (fungus)	IP
Torularhodin	orange-red	*Rhodotorula* sp. (yeast)	DS
Zeaxanthin	yellow	*Flavobacterium* sp. (bacteria)	DS
Zeaxanthin	yellow	*Paracoccus zeaxanthinifaciens* (bacteria)	RP
β-carotene	yellow-orange	*Blakeslea trispora* (fungus)	IP
β-carotene	yellow-orange	*Fusarium sporotrichioides* (fungus)	RP
β-carotene	yellow-orange	*Mucor circinelloides* (fungus)	DS
β-carotene	yellow-orange	*Neurospora crassa* (fungus)	RP
β-carotene	yellow-orange	*Phycomyces blakesleeanus* (fungus)	RP
Unknown	red	*Penicillium purpurogenum* (fungus)	DS
Unknown	red	*Paecilomyces sinclairii* (fungus)	RP

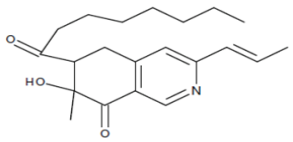
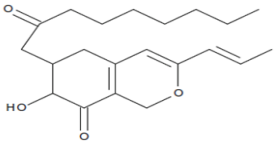
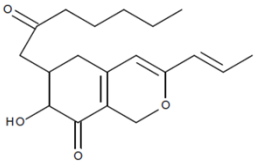
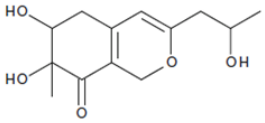
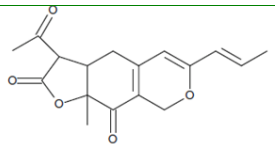
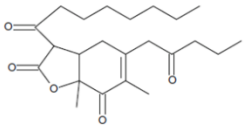
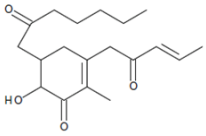
3.2 *MONASCUS* AS A PIGMENT PRODUCING MOLD.

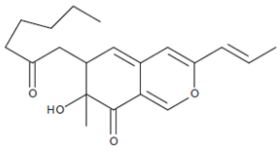
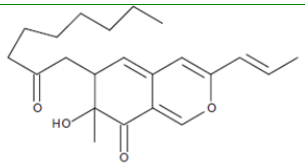
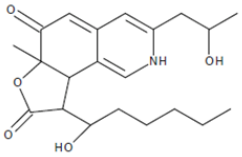
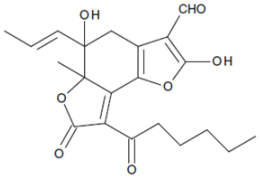
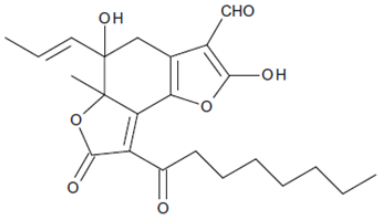
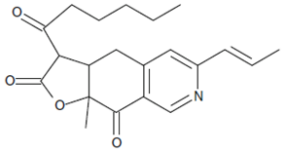
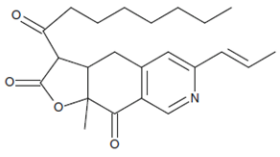
In the earliest of the literature as many as 20 species of *Monascus* has been reported since the genus *Monascus* was proposed in 1884 (Shao Y *et al.*, 2010). *Monascus* is a class of mold or fungi belonging to the family *Monascaceae* of the phylum *Ascomycecota.* This particular species have been used since the early times in Asian countries as food coloring agents.

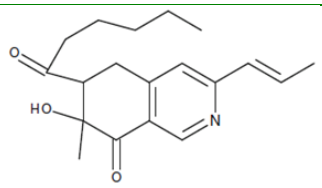
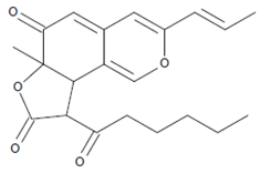
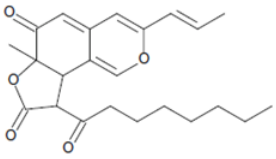
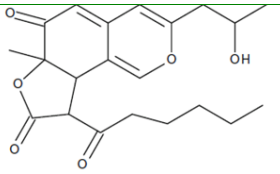
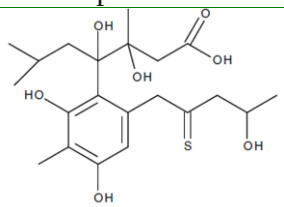
Monascus sp. has been used primarily in Southern China, Japan, and Southeast Asia for making red soybean cheese, red rice wine, and Anka (red rice) (Lin YL *et al.*, 2008). Till today this strain is under continuous research and works are being done to improve the pigments produced from this group of microorganism.

Monascus can be grouped into four species namely *M. pilosus, M. purpureus, M. ruber* and *M. floridans* that account for the majority of strains isolated from traditional oriental foods. *M. purpureus* is spherical in shape with an approximate diameter of 5 microns and the mycelium is white in the early stages rapidly changing to a rich pink and subsequently to a distinctive yellow orange color (Pattanagul *et al*., 2007). As noted *Monascus* has at least six molecular structures of pigment which is classified into three groups based on their color and each has two components of polyketide origin. Polyketides are secondary metabolites possessing a common azaphilone skeleton (Zhou *et al*., 2008).Most pigments are aromatic polyketides (Patakova, 2012). Table 2 shows the various *Monascus* polyketide pigments .the pigments are yellow pigments of monascin ($C_{21}H_{26}O_5$) and ankaflavin ($C_{23}H_{30}O_5$), the orange pigments of monascorubrin ($C_{23}H_{26}O_5$) and rubropunctatin ($C_{21}H_{22}O_5$) which possess the oxo-lactone ring and the red pigments of monascorubramine ($C_{23}H_27NO_4$) and rubropunctamine ($C_{21}H_{23}NO_4$) which are the nitrogen analogues of the orange pigments (Pattanagul et al. 2007; Nimnoi and Lumyong, 2009).

Table 2: *Monascus* polyketide pigments (adapted from Patakova, 2012)

 Monascopyridine D	*M. purpureus grown on rice*
 Monaphilone A	Mutant of *M. purpureus* grown on rice
 Monaphilone B	Mutant of *M. purpureus* grown on rice
 Monascusone A	*M. kaoliang* grown on rice
 Monascusone B	*M. kaoliang* grown on rice
 Purpureusone	*M.purpureus* mutant grown on rice
 Monashexenone	*M.purpureus* mutant grown on rice

 Monarubrin	*M. ruber* culture medium
 Rubropunctin (Y,BF)	*M. ruber* culture medium
 New red pigment	*M. purpureus* culture medium
 Xanthomonasin A	*M.pilosus* grown on rice
 Xanthomonasin B	*M.pilosus* grown on rice
 Monascopyridine A	*M.purpureus* grown on rice
 Monascopyridine B	*M. purpureus* grown on rice

 Monascopyridine C	*M. purpureus* grown on rice
 Monasfluor A	*M.sp* grown on rice
 Monasfluor B	*M.sp* grown on rice
 Compound R3	*M. purpureus* in culture medium
 Compound Y3	*M. purpureus* in culture medium

The variation in color depends on the associated amino acid or protein (Lian *et al*., 2007; Nimnoi and Lumyong, 2009). The pigments that have high protein adhesion, thermal stability and wide-range-pH are safe to be used because of their special characteristics. Dikshit and Tallapragada, 2005 reported pigment production from the *Monascus sanguineus* isolated from pomegranate. Production of the red pigment was found to be maximum on the 16th day of incubation with an optimal temperature for pigment production of 30°C and the optimum pigmentation was observed at pH 6.5. One drawback in extraction of pigments

from this species is the production of mycotoxins like citrinin which can be genetically modified to produce pigments without any toxins. (Dikshit and Tallapragada, 2013)

Certain species and strains of the fungus *Monascus* can produce pigments, lovastatin (monacolin K), citrinin, dimerumic acid and c-amino butyric acid, usually in stationary growth phase (Patakova, 2012). The major pigments produced by *Monascus* species are Monascin and ankaflavin (yellow), rubropunctatin and monascorubrin (orange) and rubropunctamine and monascorubramine (red). In addition to these major pigments that have been produced from this species there are many other minor pigments that have also been produced. It has been noted that the major pigment produced by *Monascus* is the red pigment (monascorubramine and rubropunctamine) is in great demand (Mukherjee and Singh, 2010).

3.3 SOLID STATE FERMENTATION

Inspite of the fact that fungi could be a potent source of natural pigment production the main problems faced by the industries producing the same are production cost and the difficulty in pigment production as till now the production was being done only on submerged fermentation which could not produce high yield (Lee *et al*., 1995). The alternative to submerged fermentation is solid state fermentation as it provides more nourishing habitat for fungi, resulting in very high pigment production in a relatively low-cost process (Velmurugan *et al.*, 2011). Solid state fermentation provides the necessary environment for the optimum growth of the fungus. In this process, solid substrates are used and they act as a medium to supply nutrients to the microbial culture and according to Pandey, 2003 and Babitha *et al;* 2006, the solid substrates used also act as an anchorage to the growing cells. According to Vidyalakshmi *et al*, 2009, solid state fermentation gives rise to a greater pigment yield than in submerged fermentation because in submerged fermentation the pigments get trapped in between the mycelium but in SSF the pigments are released into grains. However, besides the form of fermentation, variety of parameters affects the overall performance of production.

Temperature variations have shown to have a great impact on the biomass and pigment production (Dikshit and Tallapragada, 2013). According to their observations, at absorbance

of 510nm, red pigment was obtained at 32-35º C and pigment yield degraded beyond 40ºC. A considerable change in the absorbance spectra was also noted at different incubation temperature. According to Carvalho *et al.*, 2005, the most favorable temperature for the best pigment yield was 32ºC for an incubation period of 3 days. The optimum temperature growth for *monascus* is 28-32ºC which is a generally known fact. *Monascus purpureus* is mesophillic in nature and therefore the best temperature for the growth of this particular strain is 30ºC.

Fermentation is known to be dependent on moisture content of the substrate and nutrition (carbon or nitrogen source) concentration. Various parameters like Carbon, nitrogen and pH conditions have been shown to greatly affect the pigment production in solid state fermentation. The effect of initial pH also has been shown to have a huge impact on the pigment production and also the type of pigment produced. According to Velmurugan *et al;* 2011 *Monascus purpureus* showed a varying degree of change in the pigment and biomass yield according to the initial pH of the substrates. The growth of fungus was nearly nill at pH of 1 and 2 and at pH values above this yield changed and there was a notable change in the absorbance spectra too. As reported by Babitha *et al.,* 2007 pH 3 and 4 showed best growth and a maximum absorbance spectra was also noted. A very interesting finding by Youngsmith *et al.,* 2000 showed that a lower substrate pH led to production of yellow pigment and a higher pH produced red pigment. Pigment stability also depends on the varying range of pH. Pigment generally starts decolorizing at pH above 7.

Some of the advantages of Solid state fermentation are listed as under (Pérez-Guerra *et al.*, 2003).

- Solid state fermentation provides higher aeration than submerged fermentation.
- Because of the thick nature of the substrates the reactor design becomes very simple.
- The most important advantage being similar growth conditions provided by solid state fermentation as the natural conditions of fungal growth.
- The inoculation with spores (in those processes that involve some classes of fungi) aids in their uniform dispersion through the medium provided.
- Culture media used are generally less complex.
- Substrates used also don’t need much preparation.

- A very low quantity of water is required for this type of fermentation thereby reducing chances of contamination by different microbes. This helps in maintenance of aseptic conditions
- Energy requirements are very low (in some cases autoclaving or steam treatment, mechanical agitation and aeration are not necessary)
- Effluents generated are very less
- Solvents that are easily available are used for the product extraction due to its high concentration. And mostly the pigments generated are water soluble by nature.
- Many other compounds like enzymes, organic acids, amino acids, flavouring agents and other important compounds may be produced other than just pigments.
- Cost of the experimental setup is very low as compared to submerged fermentation.

3.4 USE OF AGRO-INDUSTRIAL RESIDUES AS SUBSTRATES

Agro industrial wastes are substances which are produced as waste products after industrial processing of plant products. Agro industrial are the most abundant renewable sources. Even though they are considered as waste by industries but they are also a very rich source of lignocellulosic materials which can be of a great use for production of industrially important substances using microbes. A very important factor considering the use of agro industrial wastes is that they are very cheap and can be used in a very large amount without bothering about the cost of raw materials. Another reason being their relatively less harmful properties meaning they are environment friendly and don't produce toxins and also the fact that agro industrial wastes serve as great substrates for solid state fermentation. Almost all groups of fungi are able to grow on agro-industrial residues and produce effective pigments. (Lopes *et al.*, 2013)

Carvalho *et al.*, 2006 reported rice as the best substrate for growth of the fungi in solid state fermentation, but some of the other substrates used also produce good quantity of pigment. Several agro crops such as cassava, barley, and agro-industrial residues such as wheat bran, rice bran, sugarcane bagasse, cassava bagasse, various oil cakes like coconut oil cake, palm kernel cake, soybean cake, fruit pulps like apple pomace, corn cobs, saw dust, seeds like

tamarind and jack fruit, coffee husk and coffee pulp, tea waste, and spent brewing grains are the most often and commonly used substrates for SSF processes (Pandey, 2008). There are many other natural substrates which act as very good sources of carbon and nitrogen source for fungal growth and pigment production. Solid state fermentation of bagasse has proven to give a higher yield of pigments (Couto & Sanromàn, 2006). Babitha *et al.*, 2006 reported various agro-industrial residues such as rice bran, wheat bran, cassava, etc as cheap and suitable substrates used for pigment production. Table no 3 represents the substrates and the average specific absorbance (AU/g dry substrates) based on the type of substrate used.

Table3: Pigment production as per substrate composition (adapted from Babitha *et al.*, 2006)

SUBSTRATES	AVERAGE SPECIFIC ABSORBANCE(AU/g dry substrates)
Rice	216
Wheat	79
corn	60
soy	13
Soy bran	22
cassava	119.6
Cassava starch	38.5
Cassava flour	98.1
Cassava bagasse	15.7
potato	4.7

However, in some cases, the supplementation with nutrients such as vitamins and inorganic and organic nitrogen supplements for these substrates is required (Carvalho *et al.*, 2006).

3.5 PIGMENT PRODUCTION AND EXTRACTION

In the work done by Velmurugan *et al.*, 2011 pigment from *Monascus* was produced by using corn cob as substrate. The work stated that corn cob waste as a substrate for pigment production was better than many other forms of substrates. This was evaluated using *M. purpureus* KACC 42430 by soaking the corn cob powder for 48 hrs at 80°C resulted in greater pigment production. Fermentation yielded a highest red pigment concentration (25.42 OD Units/gdfs) using corn cob compared to other agro industrial wastes such as coconut oil cake (0.118 OD Units/gdfs), groundnut oil cake (0.150 OD Units/gdfs), sesame oil cake (0.375 OD Units/gdfs), tamarind seed powder (1.146 OD Units/gdfs), cassava flour (1.458 OD Units/gdfs), wheat bran (3.525 OD Units/gdfs), spent brewing grain (4.356 OD Units/gdfs), palm kernel cake (7.650 OD Units/gdfs), and jackfruit seed powder (12.113 OD Units/gdfs) (6). Spectral analysis indicated maximum absorbance at 490 nm.

In another work done by Babitha *et al*., 2006 jackfruit seed powder was used as a substrate for pigment production from *Monascus purpureus*. Solid-state fermentation was carried out using jackfruit seed powder. The jackfruit seed is known to have a buffering nature, due to which color of pigments produced was stable over a wide range of initial pH of the substrate. Jackfruit seed powder with a particle size between 0.4 and 0.6 mm without any additional carbon source was found to be the best for pigment production. It was also found that when the jackfruit seed powder was infused with soybean meal, peptone, chitin powder or monosodium glutamate the particular species of *Monascus* gave water soluble pigments. In a similar manner many works have been done to establish a suitable substrate for efficient pigment production from monascus species. In the case of *Penicillium* while using submerged fermentation culture media like potato dextrose agar (PDA) was used to yield pigments.

As cited in the research work of Babitha *et al.*, 2005, extraction was done by mixing the dried product after fermentation in 90% ethanol and kept on a rotary shaker for a particular period of time. This was then rested for nearly 15 minutes which finally gave pure pigments. The type of solvent plays a major role in the extraction process as different solvents dissolve pigments at different levels. Various solvents were used for the extraction of pigments from

the powdered substrates (Carvalho *et al.,* 2006). The work included comparison of various solvents for the process of extraction of pigments. Methanol proved to be the best solvent for the extraction process followed by DMSO and ethanol. The work also stated that most of the pigments are insoluble in water and they change color when dissolved in water. The chemical structures of pigments vary from one another and this might also be a cause for the difference in solubility in different solvents. Another important factor is the amount of solvent used per gram of the substrate. More the amount of solvent used more will be the amount of pigment extracted

3.6 FACTORS INFLUENCING PIGMENT PRODUCTION

Several parameters like proper gaseous ambience, agitation, aeration and sources of carbon and nitrogen affect the pigment production by *Monascus*. In order to sustain the productivity of *Monascus* pigments, optimization of the culture conditions is critical. The effects of various environmental and nutritional factors need to be evaluated in order to determine their influence on pigment production in solid-state cultures. These including the initial moisture content, pH, inoculums size, and nutrient supplements (Lee *et al*., 2002).

3.7 EFFECT OF NITROGEN SOURCES

According to Dikshit and Tallapragada, 2013 the production of pigments was highly dependent on the percentage of nitrogen sources implemented and also on the types of substrates used. Rice gave a very high yield of pigment with implementation of 2% peptone (35.4 CVU/gds). The possible explanation for this fact was rice being a natural source of carbohydrates gave higher yield and also the ratio of peptone and carbohydrate fit perfectly. According to Shepherd and Carels, 1983, nitrogen sources tremendously affect the pigment production. According to Babitha *et al.*, (2006), monosodium glutamate was found to be the best for red pigment production, followed by peptone, soybean meal and chitin powder. Jackfruit seed powder without any addition of nitrogen source was not able to produce any water-soluble pigment which was showed in the study conducted by Babitha *et al*., (2006), in spectral analysis of its water extract. Organic and inorganic nitrogen sources affect the production of pigments differently. In the work done by Vidyalakshmi *et al.,* 2009, pigment

yield was higher when supplemented with inorganic nitrogen sources than when supplemented with organic sources. They also concluded that some pigments are formed only due to chemical transformations caused by additional nitrogen sources. Monosodium glutamate was shown to increase the pigment production by 56% in case of fermentation by *M. ruber.*

3.8 THE FUTURE OF NATURAL PIGMENTS

With the increase in pollution and accumulation of recalcitrant compounds in the environment and growing awareness about the harmful impact of these chemicals, it's the call of the hour to opt for something which is natural and harmless especially in the context of pigment production. Also the dye stuff industry has to incur high cost of feed stock and energy for dye synthesis, and is under a constant scanner from governments and social activists to decrease the damage caused to the environment by these dyes (Kumar *et al*., 2012). According to Velmurugan *et al*., (2009), a large amount of dyestuff is directly lost into water bodies due to the increased dyes or colorants applications in dyeing industries. In the case of reactive dyes, 50% of the initial dye load is present in the dye bath effluent and during the dyeing process, it is estimated that 10-35% of the dyes lost in the effluent. These dyes or colorants are cancerous to human health. Hence, the growing concern on the health and environment has lowering the demand on the use of synthetic coloring. Thus, leading to the demand for eco-friendly colorants to increase. Hence the safest option that is left out is the use of various classes of fungi and other microbes to produce pigments that will be natural.

The greatest advantage of natural pigment production is that they can be produced all around the year with equal efficiency, and they won't pose any climatic or production constraints. The various techniques for the same are being studied and though there have been many reports on the usability of *M. purpureus*, the isolated *M. sanguineus* strain also needs attention and exploration from researchers to establish itself as potential natural source for pigment production.

The natural pigment production from fungi is a very novel topic of research and there are many constraints still which are faced by scientists all over the world. Many factors and conditions need to be modified to establish its large scale industrial use. Over the period of time fungal strains of different species will definitely prove to be potent source of pigments for the food, dye and other pharmaceutical industries. If this is done then the losses and the harmful effects of the synthetic dyes and other coloring agents can be stopped and also the numerous ill effects that come along with it.

CHAPTER 4

RATIONALE AND SCOPE OF STUDY

Microbial pigments are secondary metabolites which are produced during the stationary or the log phase of the microbial growth kinetics. In this dissertation work *Monascus purpureus* MTCC 1090 was used to produce pigments by solid state fermentation. The main aim was to check the optimal condition of pigment production in solid state fermentation by *M. purpureus* MTCC 1090 using local agro industrial residues such as broken rice, broken wheat, sweet potato, wheat straw, rice straw, sugarcane bagasse and okara.

The novelty in the work was to test the best conditions for the production of pigments and to explore new beneficial sources of substrates from local agro industrial residues so that the cost of growth factor can be lowered down and fungi can be established as a natural source of pigment production for the dye and the food industries and also for the pharmaceutical industries other than the conventional synthetic methods to produce the same which are increasingly becoming threats to the environment and to the human population.

CHAPTER 5

OBJECTIVES OF THE STUDY

Monascus purpureus MTCC 1090 was procured from IMTECH Chandigarh to evaluate its pigment production potential under solid state fermentation (SSF) using agro industrial residues. The following objectives were met to carry out this work.

1) To evaluate the potential of *Monascus purpureus* MTCC 1090 for pigment production using agro industrial wastes as carbon sources.
2) To study the effect of nitrogen supplementation on pigment production.
3) To study the effect of temperature, pH, visible light and solvent on pigment stability.

Chapter 6

EQUIPMENTS, MATERIALS AND EXPERIMENTAL SETUP

6.1 EQUIPMENTS

6.1.1 INSTRUMENTS AND EQUIPMENTS

Table 4: Instruments and Equipments

S.No.	Materials	Company
1.	Autoclave	NSW Pvt. Ltd.,India
2.	Face Mask	Smart Care
3.	Glass wares	Borosil Glass
4.	Hot air oven	NSW Pvt. Ltd., India
5.	Incubator	Yorco Incubator Bacteriological
6.	Laminar air flow	Rescholar Equipment
7.	Microwave	INALSA
8.	Microscope	Magnus
9.	Micropipette	P'Fact A
10.	Microtips	TARSONS
11.	Orbital shaker	REMI
12.	Centrifuge	REMI
13.	Plastic wares	Poly lab
14.	Refrigerator	LG
15.	Weighing balance	Adventurer, DHAVS
16	UV VIS spectrophotometer	"ELICO" double beam

6.2 MATERIALS

6.2.1 Glass wares

Conical flasks (250 and 100ml), Petri plates, test tubes, Streaking rod, glass rod, glass beakers and inoculation loop.

6.2.2 Chemicals

Potato dextrose agar(PDA), tween 80, ethanol, methanol, acetone, diethyl ether, chloroform, yeast extract, monosodium glutamate(MSG), peptone, ammonium nitrate, ammonium sulphate, sodium nitrate, antibiotic (streptomycin sulphate)

The above chemicals used were procured from “LobaChemi” and “Himedia”.

6.2.3 Miscellaneous

Cotton, muslin cloth, butter paper, brown paper, thread, micro tips, burner, scissor, test tube stands, aluminium foil and distilled water.

6.3 EXPERIMENTAL SETUP

All the experimental works were carried out in the project lab of Department of Biotechnology and Biosciences (room no 28-301) at Lovely Professional University, Punjab under controlled aseptic conditions.

CHAPTER 7

RESEARCH METHODOLOGY

7.1 MAINTENANCE OF THE STRAIN.

Monascus purpureus MTCC 1090 obtained from Institute Of Microbial Technology, Chandigarh, was aseptically transferred to fresh Potato Dextrose Agar medium and then incubated at28±2^{o}C until confluent growth was achieved. These plates were stored at 4^{0}C and maintained in the active stage by transferring Mycelial plugs aseptically on fresh plates of PDA from time to time.

7.2 PREPARATION OF INOCULUM

M. purpureus MTCC 1090 inoculated on PDA slants was left to sporulate for 6-8 days at 28±2ºC .The spores were scrapped off using an inoculation loop under aseptic conditions and suspended in 8-10 ml of sterile distilled water mixed with tween 80 (100 ml sterile distilled water plus 0.5 ml tween 80). The obtained spore suspension was used as inoculum.

7.3 PREPARATION OF SUBSTRATES

Different carbon sources, viz. broken rice, broken wheat, sweet potato, sugarcane bagasse, wheat straw, rice straw, okara were prepared to be used as substrates for solid state fermentation. Sweet potatoes were washed properly to remove any dirt and then shredded using a shredder. Wheat straw and rice straw was cut to reduce the size. Rests of the substrates were used in their original form. All the substrates were first weighed and then soaked in distilled water overnight such that the moisture content was maintained at 60% .The moisture content of the substrates was calculated using the formula (Dikshit and Tallapragada, 2013)

Moisture content of substrate (%) = 100× (wet weight – dry weight) / wet weight

7.4 SOLID STATE FERMENTATION

10 g each of the dried substrates in the powder form was taken and 10 ml of distilled water was added to each. The substrates were allowed to soak well and then followed by autoclaving at 121°C, 15 psi. The autoclaved samples were then inoculated with the prepared spore suspension and incubated at 30°C for 5, 10, 15, 20 days respectively in the BOD incubator.

7.5 SUPPLEMENTATION OF NITROGEN SOURCES

The best carbon source was selected and various nitrogen sources were supplemented, viz. monosodium glutamate, yeast extract, peptone, which are organic sources and inorganic sources like ammonium nitrate, ammonium sulphate and sodium nitrate at 0.5% . A similar process of fermentation was done at 28±2ºC for 15 days followed by drying the samples and then grinding them to form powder.

7.6 EXTRACTION

Extraction was done according to the extraction process described by Babitha *et al.,* 2006. The fermented solids were dried in tray dryer for 24hrs at 55 to 60°C, and grinded using pestle and mortar to form powder. 0.1g of the powdered sample was taken and mixed with10ml 80% methanol. The setup was kept in rotary shaker at 200rpm for one hour, followed by centrifugation for 30 mins at 200rpm.

7.7 SPECTRAL ANALYSIS

Pigment estimation was done by using spectral analysis by recording the maximum absorbance of pigment extract using a double beam spectrophotometer taking in to consideration the dilution factor of the sample (Babitha *et al*., 2006). Double beam spectrophotometer was used to measure the pigment concentration at 410nm and 500nm (Carvalho *et al.,* 2006). In the work done by Velmurugan *et al*., 2011 absorbance at 410 and

500nm gave best results of color value. Methanol extracts of substrates was used as blank for the analysis.

7.8 STABILITY STUDIES

7.8.1EFFECT OF TEMPERATURE

5ml of the extract and blanks containing 80% methanol was taken and incubated in the water bath at different temperature, viz. 40º, 50º, 60º, 70º, 80º and 90º C respectively after which the OD was measured at 410 and 500 nm and the color value was calculated

7.8.2 EFFECT OF pH

The pH stability study was done on the substrate which gave the highest yield when supplemented with nitrogen source. Five aliquots each having 5 ml of the extract was taken and pH set within a range of 4-9. Stability of pigment was evaluated by spectral analysis at 410 nm and 500nm respectively.

7.8.3EFFECT OF VISIBLE LIGHT

5ml each of the extracted pigment was transferred to test tubes and after covering them properly with cotton plug they were kept exposed to visible light to check the stability of the pigments. Extracted pigments kept in dark served as the control. The color value was calculated using the formula (Dikshit and Tallapragada, 2013):

Color value= (O.D × dilution × volume of extracts) ÷ amount of sample (g).

7.8.4 EFFECT OF DIFFERENT SOLVENTS

Extraction of the pigment as done in various solvents, viz. methanol, ethanol, distilled water, acetone, diethyl ether and chloroform and then transferring them to centrifuge tubes. Two sets were made and the O.D of the first set was measured on that very day post extraction and

the next set was kept for 7 days and O.D was measured post 7 days. The color values of both the sets were compared to check the amount of extraction and the stability of pigments in different solvents.

CHAPTER 8

RESULTS AND DISCUSSIONS

8.1 MAINTENANCE OF CULTURE

Monascus purpureus MTCC 1090 was procured from Institute of microbial technology (IMTECH), Chandigarh. A portion of its mycelium was carefully extracted and inoculated in PDA media infused with antibiotic (0.01g in 50ml distilled water) and allowed to grow at 28±2ºC for 6-7 days until confluent was observed. The organism was sub cultured once every week. Mycelium was initially white, turning to the characteristic reddish color with time, with diffusion of the pigments through the agar (Fig.1)

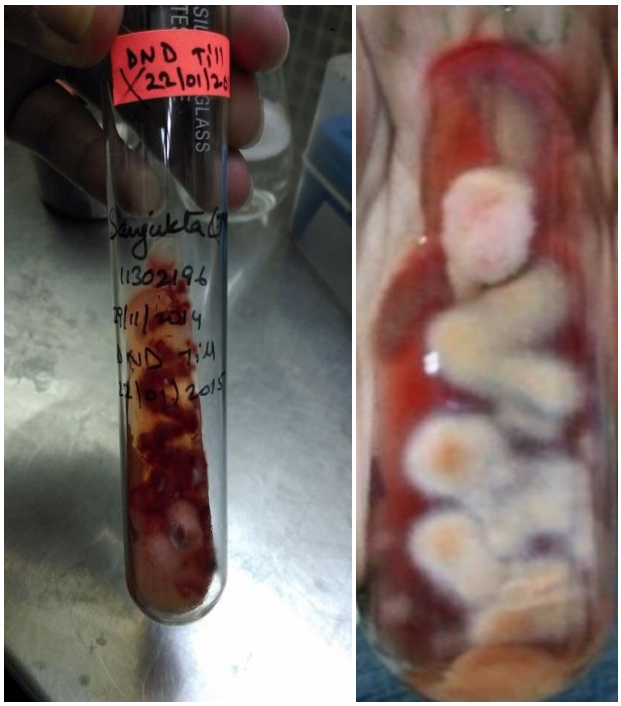


Fig 1: *Monascus purpureus* (MTCC 1090) sub cultured in slants.

8.2 SCREENING OF AGRO-INDUSTRIAL RESIDUES FOR PIGMENT PRODUCTION

Different substrates, viz. okara, rice straw, wheat straw, sugarcane bagasse, broken rice, sweet potato, broken wheat (Fig 2) were inoculated with *M. purpureus* MTCC 1090 and incubated at 28±2ºC for 20 days. Broken rice gave the maximum pigment (22.2 CVU/gds) followed by sweet potato (21.5 CVU/gds) and broken wheat (5.9 CVU/gds).Other substrates like wheat straw, sugarcane bagasse, okara showed very less color values with rice straw giving nil pigments(table no 5 and 6). Based upon the color values broken rice supported optimum pigment production and was used as the carbon source for further optimization studies. In addition, it was also found that maximum pigment production was obtained after 15 days of incubation at 410 nm even though the pigments were scanned in both 410nm and 500nm as both yellow and red pigments were produced. These results are in consistency with the studies reported by Carvalho *et al.,* 2006, where they showed that rice gave the best result in SSF whereas other substrates like cassava bagasse showed very less pigment yield. According to the works of Velmurugan and Hur, 2011, corn cob gave the best pigment yield with *M. purpureus.* Red pigment was maximum on the 16^{th} day of incubation (21.9 CVU/gds) after cultivation on *Oryza sp* proving the fact that rice is the best substrate for production of fungal pigments (Dikshit and Tallapragada, 2013). Other substrates, even though they have given very less pigment yield, but their low cost can compensate for low pigment yield.

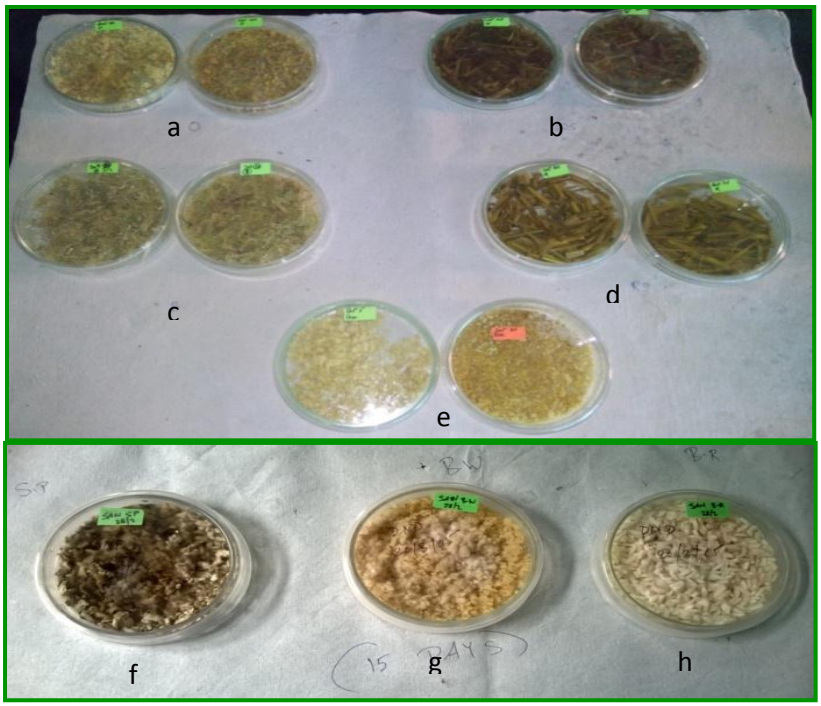


Fig 2: Carbon sources used as substrates for SSF, a)okara, b) wheat straw, c)sugarcane bagasse, d) rice straw, e) okara control, f) sweet potato, g)broken wheat, h)broken rice

Table 5: color values of different carbon substrates in 410 nm

Substrates / Incubation (in days)	COLOR Value(CVU/gds)			
	5	10	15	20
Okara	NIL	1.34±0.007	2.08±0.035	1.62±0.014
Rice straw	NIL	NA	NA	NA
Sugar cane bagasse	NIL	0.32±0.02	4.62±0.01	0.26±0.014
Wheat straw	NIL	1.32±0.01	1.32±0.01	0.73±0.007
Sweet potato	NIL	1.06±0.28	21.5±0.1	5.78±0.5
Broken rice	NIL	2.16±0.007	22.2±0.3	6.44±0.15
Broken wheat	NIL	2.07±0.02	5.9±0.07	3.67±0.01

Table 6: color value of different carbon substrates at 500 nm.

Substrates / Incubation (in days)	COLOR Value(CVU/gds)			
	5	10	15	20
Okara	NIL	0.33±0.02	1.14±0.08	0.87±0.02
Rice straw	NIL	NA	NA	0.25±0.007
Sugar cane bagasse	NIL	0.135±0.007	2.205±0.1	1.09±0.02
Wheat straw	NIL	1.49±0.007	1.15±0.01	1.80±0.001
Sweet potato	NIL	1.06±0.007	10.25±0.01	1.89±0.007
Broken rice	NIL	1.59±0.01	18.05±0.7	2.38±0.02
Broken wheat	NIL	0.88±0.1	1.8±0.2	1.88±0.01

8.3 EFFECT OF NITROGEN SOURCES

The substrate giving the best yield was chosen, i.e., broken rice (Fig 4) and it was supplemented with various nitrogen sources, both organic and inorganic. Supplementation with 0.5% sodium nitrate gave the highest pigment yield at 410 nm (195.07 CVU/gds) followed by ammonium sulphate (182.17 CVU/gds). Monosodium glutamate also yielded fairly good pigments (63.37 CVU/gds) at 410 nm (Fig 3 and Fig 5). These results can be compared to the work of Dikshit and Tallapragada, 2013. Their work explored the potency of *M. sanguineus* as a potential natural source of pigment production. According to their work *Oryza sp* showed a manifold increase in pigment production with 2% peptone (35.4 CVU/gds). According to Vidyalakshmi *et al.*, 2009, monosodium glutamate yielded maximum pigment 0.464(510nm) and 1.314(410nm) U/g of *Monascus* fermented rice (MFR) and Ammonium nitrate yielded 0.402 and 0.890 U/g of MFR production. It can be concluded that supplementation with nitrogen sources gave better results as compared to just carbon

sources. Supplementation with inorganic nitrogen sources as compared to organic ones gave better pigment yield (Table 7).

Table 7: supplementation of nitrogen sources

Wavelength	Organic nitrogen sources			Inorganic nitrogen sources		
	Peptone	Monosodium glutamate	Yeast extract	Ammonium sulphate	Sodium nitrate	Ammonium nitrate
410 nm	51.35±0.2	63.37±0.7	23.97±0.7	182.17±0.6	195.07±0.74	37.25±0.5
500 nm	12.1±0.07	13.72±0.02	5.53±0.007	93.85±0.02	141.22±0.002	10.98±0.03

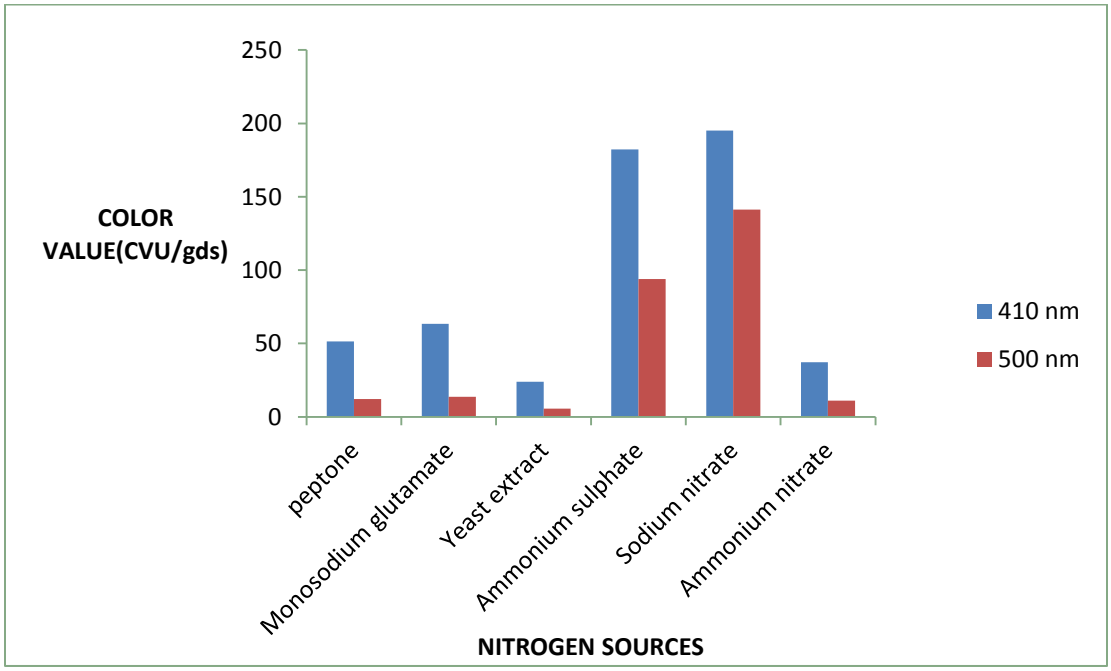


Fig3: Effect of various nitrogen sources

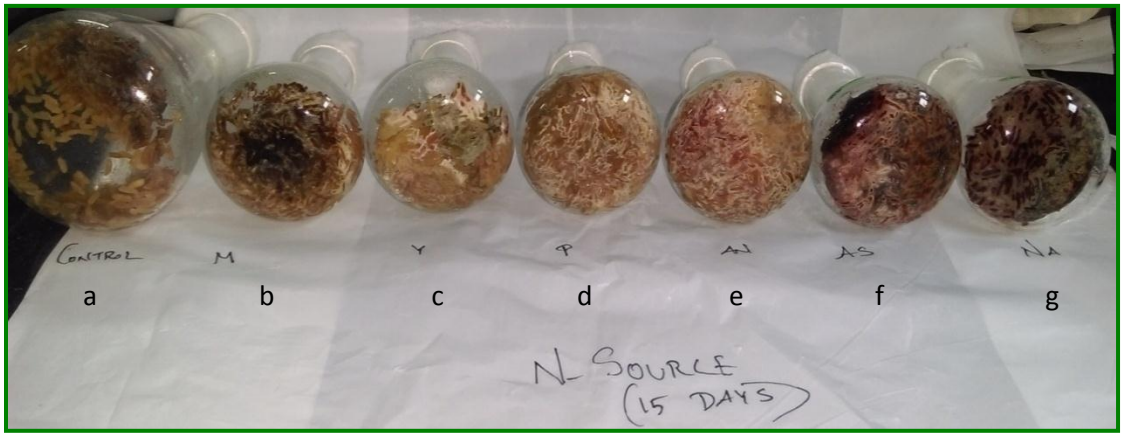


Fig 4: Supplementation of nitrogen sources, a) control b) monosodium glutamate c)yeast extract d) peptone e)ammonium nitrate f)ammonium sulphate g)sodium nitrate.

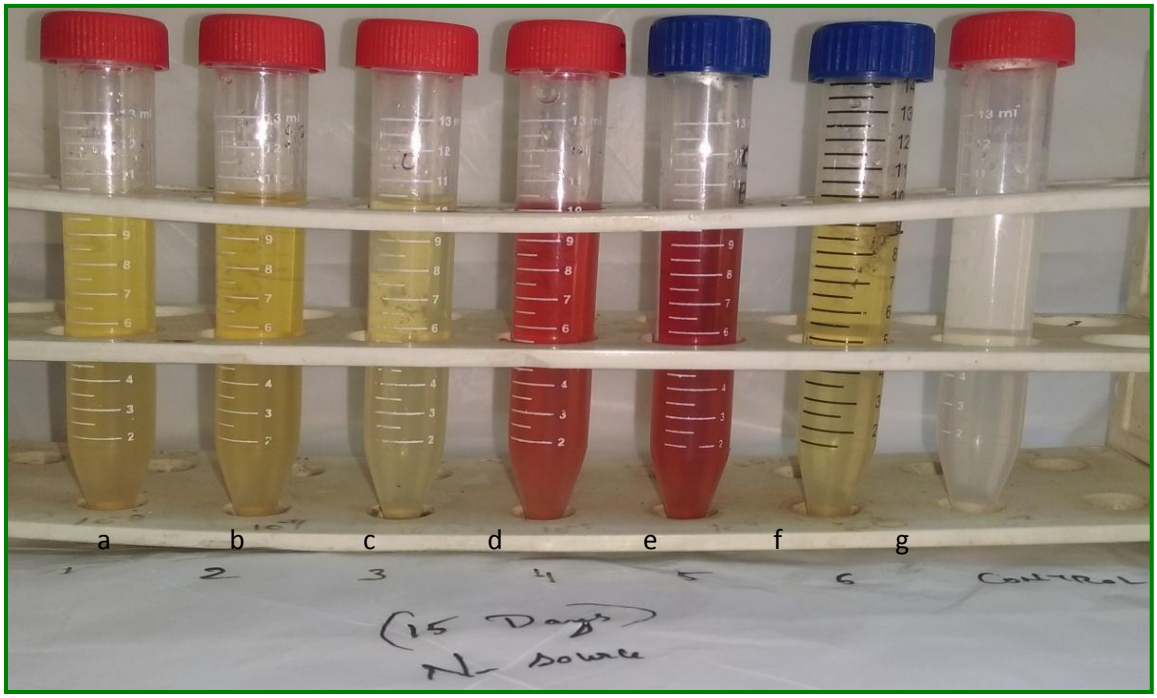


Fig 5: Extracts of nitrogen supplemented substrates: a) peptone b)monosodium glutamate c)yeast extract d)ammonium sulphate e)sodium nitrate f)ammonium nitrate.

8.4 PIGMENT STABILITY STUDIES

8.4.1 EFFECT OF TEMPERATURE

Results indicated that the pigments remained fairly stable from 40ºC-70ºC but a rapid degradation was seen above 70ºC (Table 8). This finding can prove to be really good for pigments to be used for food coloring and also in textile dyeing as there is not much degradation of the colors within a specific range.

Pigments produced by *Monascus* are generally stable till 100ºC (Socaciu, 2007), but few of the *Monascus* pigments have been reported to be unstable in higher temperatures and they generally fade away. (Dufosse, 2006). Velmurugan *et al*, 2011 also stated in their work that an exposure to constant high temperature degraded the pigments.

Table 8: Effect of temperature on pigment stability

Wavelength / Temperature	**Color value (CVU/gds)**					
	40ºC	50ºC	60ºC	70ºC	80ºC	90ºC
410 nm	399.2± 0.01	398.8± 0.001	384.71±0. 02	384.34± 0.07	204.23±0. 7	168.37 ±0.007
500 nm	260.6± 0.01	243.3± 0.001	233.45±0. 01	219.47± 0.7	160±0.7	85.03± 0.07

8.4.2 EFFECT OF pH

It can be seen that the pigment retains an overall stability over a wide range of pH (Table 9). But maximum stability is retained between pH range of 5-6 hence proving that the monascus pigments are more stable at acidic pH (Dikshit and Tallapragada, 2011). This result can also be compared to the results of Wongjewboot and Kongruang, 2011, where pigment extracted from *Monascus* remained more or less stable in varying pH and also the color spectrum did not change (Fig 6).

Table 9: Effect of pH on pigment stability

pH / Wave length	Color value (CVU/gds)					
	4	5	6	7	8	9
410 nm	88.35±0.01	92.30±0.7	96.68±0.7	89.01±0.7	84.00±0.2	82.82±0.02
500 nm	75.01±0.7	78.79±0.001	79.48±0.01	77.71±0.3	84.96±0.2	84.80±0.1

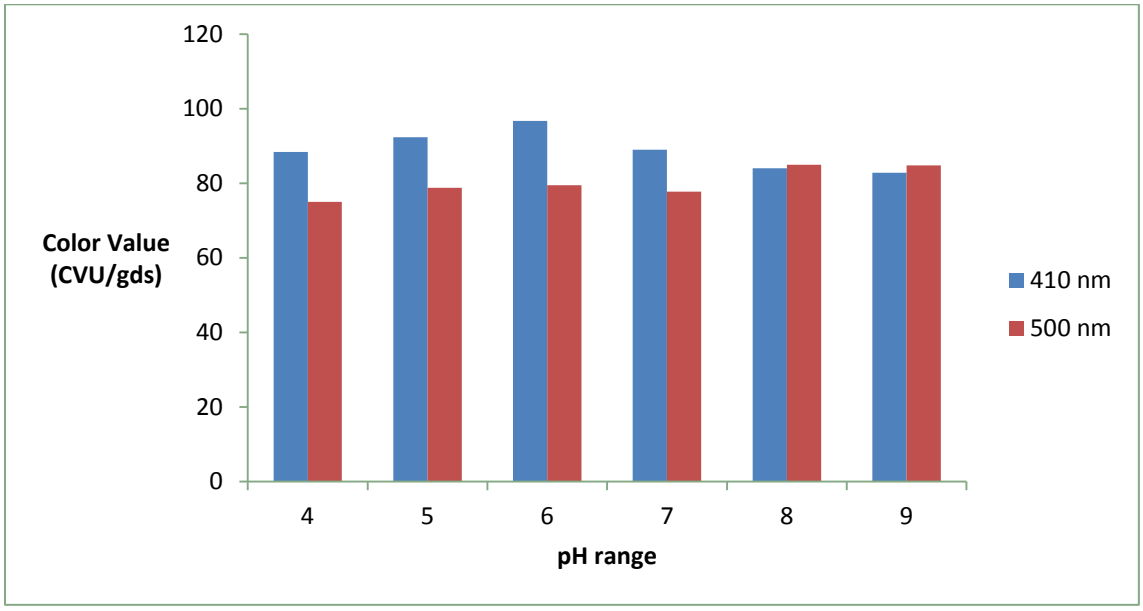


Fig6: Graph showing effect of varying range of pH on pigment production.

8.4.3STABILITY IN VISIBLE LIGHT

The pigments produced by *M. purpureus* MTCC 1090 were shown to be degrading under visible light (Table 10) *Monascus* pigments are lipophilic in nature and they are mostly insoluble in water. Due to this reason they show more solubility in organic and inorganic solvents and gradually fade away when exposed to visible light. (Socaciu, 2007). This result

can be further compared to the works of Velmurugan *et al.*, 2010, where extracellular pigments produced by *Monascus* resulted in maximum color value in darkness and the values degraded when exposed to visible light showing minimum value in unscreened light. Joshi *et al.*, 2003 also reported that microbial pigments are sensitive to acidity, air and light specifically visible light. Patakova, 2012 also reported that the pigment production from *M. purpureus* was greatly stimulated under total darkness and equally inhibited under light.

Table 10: Effect of visible light on pigment stability

Wavelength / Time	Color value (CVU/gds)					
	24hrs	48hrs	72hrs	96hrs	120hrs	144hrs
410	113.3±0.7	87.92±0.02	85.2±0.01	84.03±0.02	84.34±0.7	82.5±0.7
500	111.54±0.02	80.86±0.001	51.56±0.07	39.02±0.7	34.24±0.1	32.06±0.01

8.4.4 EFFECT OF DIFFERENT SOLVENTS

Extraction of sodium nitrate supplemented MFR was done with different solvents. From the table below it can be concluded that Acetone is the best solvent for extraction of pigments (394.91 CVU/gds) at 500nm closely followed by methanol (362.22 CVU/gds) at 500 nm(Table 7). This can be compared to the work done by Carvalho *et al.*, 2006 where rice was used as a substrate for production of pigment from a particular strain of *Monascus* and methanol was found to be the best solvent for the extraction process followed by DMSO.

Hence it can be concluded that extraction with acetone and all other solvents produces red pigments except diethyl ether where the pigment was poorly soluble. Ethanol also gave good color value (312.94 CVU/gds) (Fig 7) and since methanol is slightly toxic in nature, ethanol can be used, for large scale industrial production as it is non toxic and cheaper as compared to methanol.

Table 11: Effect of different solvents on pigment stability

Wavelength (nm)	Color value In Different Solvents(CVU/gds)					
	Ethanol	Chloroform	Diethyl ether	Distilled water	Methanol	Acetone
410 nm	291.08±0.7	327.87±0.7	66.26±0.1	331.48±0.2	313.93±0.7	363.36±0.01
500 nm	312.94±0.01	261.65±0.2	7.45±0.1	204.71±0.01	362.22±0.07	394.91±0.07

Fig 7: Effect of different solvents, E-ethanol, C- chloroform, M-methanol, A- acetone, DE- diethyl ether, DW-distilled water.

CHAPTER 9

CONCLUSION AND FUTURE SCOPE

The studies conducted on pigment production and their optimization by *Monascus purpureus* MTCC 1090 revealed that among all the agro industrial residues, broken rice is the best with 22.2 CVU/gds of pigment production after 15 days of incubation and when supplemented with sodium nitrate, it gave the maximum yield (195.07 CVU/gds) and yeast extract the lowest (5.53 CVU/gds).Other substrates like broken wheat, sweet potato, wheat straw, rice straw, okara and sugar cane bagasse yielded comparatively lesser amounts of pigments. Pigment stability studies revealed that the pigment was stable in between 5-6 pH and temperature (40-90ºC). It was also found that pigment was stable under dark and degraded gradually when kept in visible light. Acetone was found to be the best solvent for the process of extraction (363.36CVU/gds) followed by methanol (313.93CVU/gds).

Natural pigments from *Monascus* are of practical interest especially in the food and technology industry as they are capable of producing a wide range of edible pigments which are safe to be consumed and they have been shown to have anticancer and cholesterol lowering properties. Further work needs to be carried out to characterize these pigments before they can be evaluated for commercial applications.

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