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MICROBIAL PIGMENTS AS A NATURAL COLOR: A REVIEW

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ABSTRACT: Natural pigments have great interest with in the market now a day's particularly microbial pigments. The various microorganisms such as *Micrococcus, Bacillus, Rhodotorula, Monascus, Phaffia, Sarcina* and *Achromobacter* have the capability to produce different pigments. These colors have number of beneficial properties like anti-cancerous, immunosuppressive, antibiotic, anti-proliferative, biodegradability etc. Further, they have broad area of application mainly in food, dairy, printing, textile and pharmaceutical industries etc. Since, the microorganisms are a significant source of carotenoids and major source of naturally occurring pigments. These carotenoids basically protect cells against photo oxidative damage and hence have vital applications in environment, food and nutrition, disease control, and as potent antimicrobial agents. Use of microbial pigments in processed food is another promising area with large economic potential. However, microbial pigments provide challenges due to lower stability, high cost and variation in shades due to changes in pH. Further, it is also essential to have toxicology studies of these microbial pigments before their use as natural colorants in food products.

INTRODUCTION: Color is the most vital attribute of any article particularly food. Biocolour word consists of two words bio and colour that means something natural used for coloring purpose ¹. Hence, biocolorants can be one of the alternatives to artificial color for addition into any food material. These are basically those coloring agents, which are obtained from the biological sources such as plants, animals and microbes as sources of natural pigments. These natural colors are generally extracted from fruits, vegetables, seeds, roots and microorganisms and are often called as "biocolors" due to their biological origin ^{2, 3}. During recent years, pigments extracted from natural sources are highly in demand as natural food coloring agent.



Being natural they can replace the synthetic dyes. Since, an artificial color additive tends to impart undesirable taste, negative health issues related to the consumption such as allergenic and intolerance reactions. Food with good texture, nutrients and flavor should be of appealing color then only it can be desirable for human consumption. The demand for natural sources of such compounds is increasing day by day as a result of awareness of positive health benefits out of natural compounds ⁴. Currently, the search is also for pigments produced by microorganisms and used commercially as food colorants.

In recent years, search for microorganisms producing non toxic metabolites has been performed by several researchers ^{5, 6}. Further, the waste generated from food industry can also be one of the substrate for growth of these biocolors producing microorganisms. As the food industry normally, generates a large quantity of waste like peel, seed, pomace, rags kernels, etc. which are biodegradable in nature and can be used as a substrate for growth of these microorganisms.

The waste generated is a rich source of carbohydrates, minerals, proteins and dietary fibers and hence a good source of nutrients for microorganism growth ⁷. Usage of food industry waste can also help to deal with problems like environmental pollution. Hence, in the present review paper the findings of the various researchers worked on the utilization of waste for production of microbial color sources have been summarized.

History of bio-colorants: Trends of coloring the processed food is an old tradition, but isolation of pigment from micro-organisms is a recent approach. Man has always been interested in colors. The dyeing process is an old practice which was practiced in Europe during Bronze Age. According to the earliest records i.e. 2600 BC these natural dyes were used in china for the first time ⁸. In Indian Subcontinent, the use of natural dyes is reported during Indus Valley Civilization (2500 BC), which is proved by the presence of traces of madder dye in the colored garments of cloth found in the ruins of Mohenjo-Daro and Harappa civilization (3500 BC).

In Egypt, mummies wrapped with colored clothes and presence of Alizarin, a pigment isolated from madder in the tomb of King Tutankhamen also confirms the use of natural dyes. The Aztec and Maya culture period people of Central and North America used cochineal dye. The dyes such as woad, madder, weld, brazil wood, indigo and reddish-purple were recognized until 4th Century AD, even Brazil got its name from the dye woad found there ⁹. Bible has the mention of saffron, whereas the usage of Henna is dated even before 2500 BC ¹⁰. The previous record also states the consumption of colored processed food by people of some sections according to the text in shosoin of the Nara period of 8th century from Japan.

Source of Biocolors: There are different sources of natural pigments. Some of these have been summarized in later sections. These have been extracted from fruits, vegetables, seeds roots and even microorganisms¹¹.

• **Plants Pigments:** Any type of colored substance produced by the plants is called as "Plant Pigment". Photosynthesis is the primary function of pigments in plant, which uses the

green pigment chlorophyll. Carotenoids are also essential for their functions in photosynthesis ¹². There are different types of plant pigments, found in different classes of organic compounds. Plant pigments can give color to leaves, flowers and fruits. Some of the naturally derived pigments from plant sources have been summarized in **Table 1**.

- Animals Pigments: Even animals produce bichroms or biological pigments, as mentioned earlier. In most of the plants chlorophyll is the primary biological pigment, and in mammals melanin is the main biochrome that is found. However, melanin is responsible for the color of hair and fur of an animal. A small parasitic insect i.e Cochineal beetle, Latin name Dactylopius Confusus, that lives and dines on the prickly pear cactus, or *nopal*, in spanish. Cactus is a plant from which the insects or, grana are harvested and ground into a red pulp color. A brilliant red liquid pigment produced from their blood is used by the ancient Meso-Americans to make a beautiful steadfast dye. Through the world, Cochineal is still used in many products. It is commonly used in red lipsticks, and is one of the few red pigments allowed to be used in eye shadow. Further, the color additive used in Cherry Coke is also made from *cochineal* 13 .
- Microbial Pigments: The natural pigments extracted from microorganism are termed as "microbial pigments". Table 2 shows the naturally derived pigments from microorganism. Microorganism's bacteria. algae and fungi produce variety of pigments and therefore, are the promising source of food colorants^{14, 15}. These pigments from biological or microbial sources have desirable properties like stability to light heat and pH¹¹. Microbial pigments possesses anti-cancer properties and are a source of pro-Vitamin A. Microbial production has various benefits as their production is independent to weather condition, easy and fast with different colors uses. Hence, microbial production of colorant has many advantages over the other colorants as they can be produced under controlled condition in a very less time.

S. No.	Plant Sources	Pigments	Color/appearance
1	Turmeric	Cucumin	Bright lemon Yellow
2	Marigold and alfalfa	Lutein	Golden yellow
3	Palm oil	Natural mixed carotenes	Golden yellow to orange
4	Bixa orelana	Bixin/ nor-bixin	Orange
5	Paprika/capsicum annum	Capsanthin/ casorubin	Reddy Orange
6	Tomatoes	Lycopene	Orange red
7	Black grape skin, elderberries, black carrots, red cabbage	Anthocyanin	Pink/Red to mauve
8	Red table beet root	Betanin	Pink to red
9	Grass, lucern and nettle	Chlorophyll	Olive green

TABLE 1: NATURALLY DERIVED COLORS FROM PLANTS SOURCES ¹¹

TABLE 2: NATURALLY DERIVED COLORS FROM MICRO-ORGANISM ^{16, 17}

S. No.	Micro-organisms	Pigments	Color/appearance
1	Staphylococcus aureus	Zeaxanthin	Golden yellow
2	Serratia marcescens	Prodigiosin	Red
3	Phaffia rohodozyma	Astaxanthin	Red
4	Blakesela trispora	Lycopene β-carotene	Red Yellow-orange
5	Flavobacterium spp.	Zeaxanthin	Yellow
6	Pseudomonas aeruginosa	Pyocyanin Blue	Green
7	Dunaliella Salina	β-carotene	Cream
8	Monascus sp.	Monascorubramin, Rubropunctatin	Yellow, Orange, Red
9	Rhodotorula sp. Rhodotorula glutinis	Torularhodin	Orange-red
10	Monascus roseus	Canthaxanthin	Orange-Pink

Pigment producing micro-organisms: Recently, there has been a considerable interest worldwide in production of pigment from microbial sources, as their production is advantageous being independent of environmental conditions and can be on cheaper medium ¹⁸. A large number of different species of bacteria, yeast, mold and algae produce pigments. Some of the examples of microorganisms producing natural pigments are as follows:

The Monascus purpureus: cultivation of Monascus purpureus (Red Mold species) can be done on substrates rich in starch. During first century A.D there was a tradition in East Asian Countries of using Monascus for solid state fermentation of rice ¹⁹. Red yeast rice in china is called as angkak. Red yeast rice has been used as staple food and food additive for centuries in Asia and Indonesia. In Japan red rice is known as benikoji and its pigments are widely used for food coloring. Red yeast rice is also used as preservative for meat and fish, for adding color and flavor to food and for brewing wine and liquor in China, Taiwan, Okinawa, and Philippines²⁰. Interestingly, even the Ben Cao Gang Mu of Li Shi-Zhen, the ancient Chinese pharmacopoeia of medicinal foods and herbs have the mention of red yeast rice as a useful medication for improving digestion and revitalizing the blood ²¹.

Monascus species are known to produce wellknown azaphilone pigments like monascorubrin *rubropunctatin* 2^{22} and more recently, a yellow Monascus mutant has been identified ²³. Due to unique Monascorubrin structure of and rubropunctatin they have high affinity towards compounds having primary amino groups (aminophiles). water soluble pigments The monascorubramine and rubropunctamine are produced when reacted with amino acides ²⁴. In fact, various pigments derivatives with improved functional properties in the color range of orangered to violet-red can be produced by Monascus fermentations in the presence of different amino acid²⁵. However, *Monascus* fermented rice has also been found to contain the mycotoxin citrinin along with the pigments ²⁶. Therefore, the use of Monascus is limited for the production of natural food colorants²⁷.

Phaffia rhodozyma: In 1960's, Herman Phaff isolated *Phaffia rodozyma* during his studies on yeast ecology ²⁸. Isolation of yeast was initially limited to certain geographical regions; subsequently the isolates were obtained from Russia, Chile, Finland and United states. The yeast's biological diversity is vaster then studied by Phaff and his collaborates. As two more species, anamorph *Phaffia rhodozyma* and the teleomorph *xanthophyllomyces dendrorhous* are also appear to exist. The important carotenoid astaxanthin is synthesized by yeast, due to which yeast is under considerable biotechnological interests. The remarkable findings that astaxanthin isolated from P. rhodozyma had the 3R, 3¢R-configuration, which is opposite to that of astaxanthin from other sources that had been investigated ²⁹. The property of yeast to produce this economically important pigment has called for the research on biology of the yeast as well as its development as an industrial microorganism for production of astaxanthin by fermentation. Further, it has also been reported that nutritional and environmental factors affect the production of astaxanthin, whereas the astaxanthin protects the yeast from reactive oxygen species damage. In 1970's it was proposed that Phaffia rhodozyma can act as economically important pigment source for animal diets like salmonids, lobsters, and the egg yolks of chickens and quail, to give them desirable characteristics plus colors 30 .

Rhodotorula: A common environmental yeast Rhodotorula can be found in air, soil, lakes, ocean water, milk and fruit juice. These species belong to phylum basidomycota, colonise plants, humans, other mammals and produce pink to red colonies. Rhodotorula produce blastoconidia and have unicellular, lacking pseudohyphae and hyphae³¹. Rhodotorula was grown on different carbon sources which were glucose, sucrose, xylose, succinate, glycerol, mannitol, lactose and starch to see the effect on growth. It was reported that yeast can be grown on substrate having monosaccharides Tada and Shiroishi, 1982 ³² and disaccharides Polulyakh, 1991³³. The effect of nitrogen sources like NH₄Cl, NaNo₃ and urea on the production and growth of *Rhodotorula* has been studied 34 .

Sarcina: Spherical in shape and are found in cuboidal packets of eight or more, divides in three perpendicular planes. These are gram-positive, non-motile and produce yellow color pigment carotenoid by nature ³⁵. *Sarcina* flavour carotenoid has four fractions which are hydrocarbons, mono and dihydroxylated compounds and polar fractions. Fractionation of these pigments was done with chloroform and methanol solvents ³⁶.

Micrococcus: Before the discovery of Pencillin in 1928, Sir Alexander Fleming discovered *Micrococcus luteus* named it as *Micrococcus*

lvsodeikticus ³⁷. Micrococcus was used as primary experimental microbe in Fleming discovery of lysozyme, because of this reason it becomes useful in microbiology and medicine. It is also useful in bio-remidiation. biodegradation of manv environmental pollutants and biotechnology. The microorganism has also been over exploited for its proficiency in isoprene synthetic reactions in chemical and pharma industries ^{38, 39}. *Micrococcus* flora breakdowns some normal *luteus* on present in sweat that produce compounds unpleasant body odours. Micrococcus luteus has also been isolated from 120 million old block of amber ⁴⁰. Molecular and biological techniques show that *Micrococcus luteus* and related members of genus have multiple adaptations for it survival in extreme and nutrient poor environment. For the dispersal in the environmental, these adaptations for survival are very important for the bacteria. Further, use of succinic acid and processed terpene related compounds presents in natural amber shows bacteria's ability to use them 40 .

Chromobacter violaceum: Chromobacterium violaceum is a Gram-negative bacteria belonging to the Rhizobiaceae family, is a saprophyte found in soil and water in tropical and subtropical areas. In most cases, it is a minor component of the total microflora⁴¹. The colonies of *Chromobacter* violaceum are slightly convex, not gelatinous, regular, and violets, although irregular variants and non-pigmented colonies can also found ⁴². The pigment produced from the violet colony is violacein [3-(1, 2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1, 3-dihydro-2Hindol-2-one] which have a strong bactericidal, trypanocidal, tumoricidal, mycobactericidal and antioxidant activity ⁴³. Further, Violacein also antibacterial activities showed against Staphylococcus. Streptococcus, Bacillus. Mycobacterium, Neisseria and Pseudomonas⁴⁴.

Growth and Fermentation conditions: The growth and pigmentation of microorganism is greatly affected by two types of fermentation i.e. Solid state fermentation and submerged fermentation. The easy production and isolation of colour pigment have lead to advancements in fermentation techniques. Generally, submerged fermentation (SmF) is used for industrial scale pigment production.

However, solid-state fermentation (SSF) systems appear to be promising due to the natural potential and advantages ²⁰. Microbial pigments can be produced either by solid substrate fermentation or by submerged fermentation. In solid substrate fermentation (SSF), the microbial pigment biomass occurs on the surface of a solid substrate for the cultivation of microbial colour 45, 46. This SSF technique has many potential advantages including savings in waste water and higher yield of the metabolites. On the other hand, microorganisms are cultivated in liquid medium aerobically with proper agitation to get homogenous growth of cells and media components in submerged fermentation technique⁴⁷. Moreover, researchers investigated the influence of various process parameters such as carbon source, nitrogen source, temperature, pH, aeration rate for pigment production ⁴⁸. But, due to the high cost of using synthetic medium, there is a need to develop new low cost process and extraction procedure for the production of pigments. Efforts are on to utilize the agroindustrial waste for large scale production of microbial pigments. Some studies have focused on production of carotenoids from agro-industrial waste such as whey, apple pomace, spent grain and crushed pasta etc.⁴⁹. Therefore, such kind of agroindustrial waste utilization procedures not only lower down the production cost but also act as effective waste management tool as well.

Factor affecting Microbial pigment production:

- 1. Temperature: The production of microbial pigments is greatly depends on the type of microorganism and temperature is the main factor for microbial pigment production. *Monascus* sp. requires 25-28°C temperature for the growth and production of microbial pigment whereas *Pseudomonas sp.* requires 35-36°C for its growth and pigment production ⁵⁰.
- 2. pH: The pH is also another important factor for microbial pigment production. The pH of the medium is affected by the growth and type of pigment produced in which the microorganism are grown. The slightly change in pH may change the shade color of microbial pigment and it varies from one microorganism to another. The optimum pH for *Monascus* sp. is 5.5-6.5 and for *Rhodotorula* sp. is 4.0-4.5

respectively. The pH favours lycopene formation from neutral to slightly alkaline whereas β -carotene formation forms from neutral to acidic ¹¹.

- 3. Incubation time: The different incubation periods ranging from 24 to 96 hrs also effects growth and pigmentation the of the microorganism. While studying the parameters one factor at a time was considered while the other remained constant for the yield of biomass and pigmentation. The incubation period 24-96 hrs for the growth of Micrococcus sp. which showed the highest biomass production selected was for each microorganism ⁵¹.
- 4. Carbon Source: The pigment production through mycelial growth of microorganism is affected by the type of carbon sources like glucose, fructose, lactose, maltose, sucrose, glactose etc. Carbon source like glucose and its oligosaccharides are better for the growth and pigment production. The volumetric pigment formation by the Monascus sp. is best on starch and dextrin whereas moderate on glucose and maltose but poor on fructose. The cellobiose more pigmentation for Phaffia forms rohodozyma whereas glucose promoted both growth and pigmentation. The shade of the pigment is also influenced by the type of sugar used for production of microbial pigments¹¹.
- 5. Nitrogen Source: The nitrogen sources are also an important factor for microbial pigments depending upon the microorganism. The ammonium chloride has showed the highest production of *Monascus* pigment followed by the ammonium nitrate and then glutamate. Further, it has also been shown that peptone also plays an important role in pigment production as the addition of peptone 50 .

Chemical modification of microbial pigment: Microbial colours produced from microorganism with different shades like pink (*Rhodotorula*), red (*Chromobacterium*), yellow (*Sarcina*) and light yellow (*Micrococcus*) were produced using apple pomace as substrate by solid state fermentation were characterized and modified into water soluble pigments. β -carotene produced by the most of the colour producing microorganism are not water soluble where as several components of food are water soluble. So, if microorganism produced these pigments then their effectiveness as food colorants can be made when these biocolors becomes water soluble ⁵². Modified pigment produced by the *Rhodotorula, Chromobacterium, Sarcina, Micrococcus* can be chemically modified to make them water soluble and promising source of natural colorants to use in various food products. Wong and Koehler, 1983 ⁵³ modified the pigment from fungus *Monascus purpureus* using different solvents such as ethanol, amino acetic acid, aminobenzoic acid and gelatin. The complete flow sheet for the process is shown in **Fig. 1**. Later, Joshi and Attri, 2014 5^2 tried the different solvents for modification of pigment from (*Rhodotorula*, *Chromobacterium*, *Sarcina*, *Micrococcus*) and concluded that the *Rhodotorula* pigment in gelatin and amino acetic acid gave the best conversion at pH 9.2. With respect to *Chromobacterium* in amino acetic acid at pH 9.2 followed by aminobenzoic acid at pH 7, while those from *Sarcina* and *Micrococcus* have the highest conversion at pH 9.2 with amino acetic acid.



FIG. 1: FLOW SHEET FOR THE CHEMICAL MODIFICATION OF MICROBIAL PIGMENT

Application of biocolors: Biocolors have wide applications as colorants in pharma, food, textile, printing industries etc.

- Pharmaceutical industry: Investigation of most of the microorganisms has shown the efficiency in potential clinical applications of secondary metabolites (pigmented) for treating various diseases. These have many properties antibiotic, anticancer and immunelike suppressive properties ⁵⁴. The property of bacteria to produce biopigments, is used to produce medically important products. Adonirubin and astaxanthin are the xanthophylls, which also act as nutraceuticals. These xanthophylls by the process of antioxidation, anti free radical or other mechanisms help to prevent carcinogenesis ⁵⁵. nutraceuticals The functions of these xanthophylls and carotenes also help to prevent problems like heart attacks and strokes ⁵⁶. A red pigment, astaxanthin is important carotenoids which has great commercial value, and is also used as pharmaceuticals feed. Further, the Monascus purpureus produce pigments which help in the inhibition of hepatitis virus replication by interfering with viral RNA polymerase activity ⁵⁷. A strong therapeutics molecules prodigiosins are known for their immune suppressive anticancer properties ⁵⁸. Hahella chejuensis produces a pigment which is also known to have immune suppressant and antitumor properties ⁵⁹.
- **Dairy industry:** *Monascus species* are known to produce nontoxic pigments, which can be used as food colorants, flavour enhancers and as a food preservative. *Monascus ruber* is used to prepare flavoured milk by utilizing rice carbohydrate for its metabolism and produces pigment as a secondary metabolite ⁶⁰. The solid state fermentation of rice produces red, orange and yellow pigments ⁶¹.
- **Textile industry:** The textile produces large amount of waste which mainly consist of synthetic dyes. These synthetic dyes are used in industries, due to their easy and cheap synthesis, stability towards light, temperature and advanced colors covering whole color

spectrum. However, these synthetic dyes have many drawbacks like toxicity, mutagenicity and carcinogenicity properties leading to various health problems like skin cancer and allergies ⁶², ⁶³. Hence, consumers demand for dyes of natural origin as colorants.

- Nutritional supplements: Chemical compounds in the biocolor are produced by plant cells known as vegetal active principles. These compounds are the means for obtaining biological active substances and many other natural compounds which are used in food, pharmaceuticals and cosmetics industries with having important commercial value 64 . As β carotene is the precursor of Vitamin A, so carotenoids can be used as Vitamins supplements ⁶⁵. Rice is the main food in under developed countries, so there is possibility of deficiency of vitamin A which causes night blindness and in serious cases to xerophthalmia. Another example of natural food grade biocolorant is Riboflavin which is also a source of vitamins, available in milk, several leafy vegetables, meat and fish $^{66, 67}$. Yellow β xanthins are used as biocolorants and can also be used as biocolorant and for introducing essential dietary amino acids into food stuffs⁶⁸.
- **Printing industry:** To conserve the forest resources and reduction of wastes it's important to reuse and recycle the papers in offices etc. The reuse of papers is important but it's also important to disappear the prints from paper. Decolorable ink contains *Monascus* pigments used for inkjet printing. These pigments from *Monascus* when exposed to irradiation of visible or ultraviolet light get discolored ⁶⁹.
- Food colorants: An important goal of food industry is to produce food with an attractive appearance. Food producers are opting for natural food colors, as artificial ones shows many negative impacts on health when consumed. Demand for natural food colorants are more than its availability in food industry. Many natural colors are available, in which microbial colorants play important role as food coloring agent as its production and down streaming process is easy.

Penicillium oxalicum is a fungus which produces red color used in cosmetic and food industry ⁷⁰. These colors are useful in different products like baby foods, breakfast cereals, pastas, sauces, processed cheese, fruit drinks, vitamin-enriched milk products, and some energy drinks and so on. Therefore, natural colors are environment friendly and moreover serve as the dual need for visually attractive colors and health benefits in food colorants of ⁷¹. Therefore, pigments probiotic from microbial sources are good alternative. The addition of natural or microbial colors in food products can also help to overcome the growing public concern over the adverse effects. In addition, natural colorants will not only be beneficial to human health but will also be helpful for the maintenance of biodiversity.

CONCLUSION: Biocolors are natural, prepared from renewable sources that are easily degradable and without production recalcitrant of intermediates when they enter in the environment. Natural dyes and colours have growing importance not only in dyeing but also because of their medicinal properties. The awareness among people towards natural dyes and their therapeutic uses are increasing because of their nontoxic or less toxic properties, with fewer side effects. On the other hand, synthetic colours are based on toxic raw materials. The continuous use of synthetic colours not only causes considerably environmental pollution but also many health related problems in human beings i.e. carcinogenic effects etc. It is therefore, essential to explore various natural sources of food grade colorants and their potential uses. Hence, natural products produced by microorganism as pigments are safer and better than synthetic products.

Future Perspective: Microbial pigment production can be increased in huge amount through genetic engineering. Production of colours by fermentation has several advantages i.e. cheaper production, probably easier extraction, higher yields, and no seasonal variations with adequate raw material. Many microbial pigments not only used as colouring agents in food and cosmetic industry but also act as anticancer, anti-inflammatory, antimicrobial and anti-oxidant. Isolation of new microorganisms producing colouring pigment can be an alternate source of colorants used in foods, textile, pharma industry etc. In this way, biotechnology may play an essential role for large amount of production of biocolorants through fermentation.

Fermentation techniques have to be improved by genetic engineering and the advancements in the economics of microbial pigments. Further, the researches on nontoxic microbial pigments have to be increased. However, technological limitations are the most important bottleneck for the commercial utilization of the microbes for bicolour production and designing of proper bioreactors.

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CONFLICT OF INTEREST: No.

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