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Microbial pigment production for sustainable production: a review

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Abstract Microbial pigments have great potential and strength to produce natural colour on an industrial scale at an affordable cost. However, their development faces challenges due to the presence of inexpensive synthetic pigments dominating the market. These synthetic pigments often contain azo dyes, which are harmful to human health and the environment. Hence, there is an urgent need for regulations, prevention measures, and research to shift from synthetic to natural pigments. Despite this, microbial pigments are gaining attention as alternatives in various industries. Challenges in producing them on an industrial scale include pigment stability, product shelf life, and expensive raw materials. This review aims to provide a systematic and precise description of how microbial pigments are developed from raw materials, outlining specific phases for their production. Additionally, it will highlight technological challenges and limitations faced in their utilization.

Keywords: microbial pigment, natural pigment, fermentation, microorganism, colour

Classification numbers: 1.3.1, 1.3.2

1. INTRODUCTION

Colours are essential and significant visual properties in parts of our life. The colour determines the product's acceptance and has influenced human life. Colours provide attractive and specific appearances like a code for the people to recognize the flavour of the food's marketable products like sugar lumps, jelly beans, powdered drinks, and sweets. The growing interest around the world in colouring food with pigments made from natural sources is rising in stature. Pigments are compounds with characteristics of importance to many industries. The pigments contribute to the food industry as additives, antioxidants, intensifiers, etc. These pigments come in a wide range of colours; some are water-soluble [1]. Natural pigments are those that are obtained from the natural matter of plants, microorganisms, and animals, whereas synthetic pigments are those that are chemically synthesized in laboratories.

Synthetic pigments are man-made chemically synthesized in laboratory, used since 1500 BC, offer stability and cost-effectiveness but pose health risks such as toxicity problems, carcinogenic, hyperallergenicity and teratogenic properties [2 - 4]. As a result, many synthetic dyes are banned. Consequently, many of the synthetic colour pigments have been banned due to

those problems. With the increasing awareness of human safety and environment, there is increasing fresh enthusiasm for development of colorants from natural sources [5].

Natural pigments are generally derived from vegetables, fruits, flowers, roots, insects and microorganisms [5 - 6]. The demand of utilization of natural pigments in foodstuff, cosmetics, dyestuff and pharmaceutical manufacturing process have been increased in market due to believe to be safe [6]. Besides, their antioxidant, anticancer, anticarcinogenic, non-toxic and biodegradable properties further add to their positive effects [7]. Although there are number of natural pigments, only few are available in sufficient amounts to be useful for industries as usually these are derived from plants. Microorganisms are particularly attractive pigment sources due to their consistent production, higher yields, and scalability [8 - 9]. Unlike plants, microbial pigment production isn't season-dependent and can use low-cost substrates, including agro-industrial waste. Microbial pigments come from fungi, algae, yeast, and bacteria, producing melanin, flavonoids, carotenoids, and more. Examples include β-carotene (Blakeslea trispora), Arpink Red (Penicillium oxalicum), and astaxanthin [11 - 15]. In short, the microorganisms have immersed potential to produce diverse biopigments which have been investigated by several researchers.

Production cost remains high, but agro-industrial residues such as fruit peels and dairy by-products have proven to reduce expenses while aiding environmental sustainability [10]. Despite numerous studies on specific microbial pigments, a comprehensive overview is lacking. This review consolidates data on bacterial pigment isolation, optimization, characterization, and applications. This initiative has the potential to contribute to a more sustainable and environmentally friendly way in production.

2. ISOLATION OF BACTERIA

Pigment-producing bacteria are explored for natural colorant development [17]. Isolation identifies microbial species and their potential applications in food, pharmaceutical, and cosmetic industries [18 - 20]. Thus, isolation of microbial pigments become a priority procedure in order to market in various industries. Traditionally, isolation of pigment bacteria was achieved mainly by performing streak, spread and dilution-to-extinction (DTE) techniques to obtain desired bacteria, which are still very useful nowadays [21].

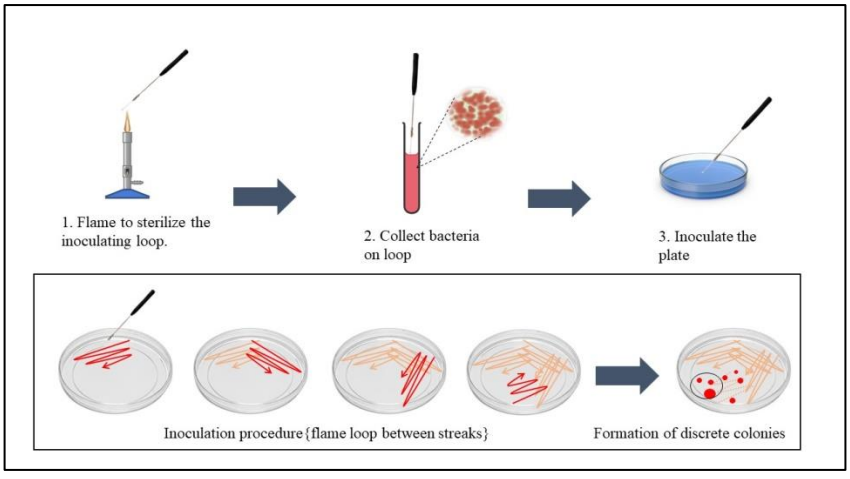

Figure 1. Streak plate method.

Streak technique is conventional, simple and easy method to obtain pure colonies of microbial pigment. This will produce only a single type of independent colony, the colonial type of which morphology is consistent with the initial isolation. Hence, as streaking the bacteria in such a way enable the colonies grow well separated from each other (Fig. 1) [22]. The spread plate technique, a conventional method requiring serial dilution, is commonly used for bacterial enumeration and isolation. Although time-consuming and procedurally complex, it is effective for isolating heat-resistant microbes and avoids subsurface colony formation, simplifying colony recovery (Fig. 2). Shah [23] used this method to isolate 36 soil and water samples, with three pigment-producing strains selected for further study. Jumare et al. [24] employed a similar approach in Sokoto State, Nigeria, collecting samples from various local sites. Likewise, Jeong et al. [25] isolated a xanthophyll-producing marine bacterium (Erythrobacter sp. strain SDW) using 1.5 % NaCl and marine agar.

However, some microorganisms, particularly marine oligotrophs like "SAR11" [26], are difficult to culture using standard plating methods. These approaches may disrupt essential host-microbe interactions or create toxic conditions due to nutrient concentration. To address this, researchers have developed the Dilution-to-Extinction (DTE) method, a top-down enrichment strategy based on actual viable cell numbers (Fig. 3). DTE enables the selective cultivation of low-diversity, stable microbial communities with targeted metabolic functions [28]. Microorganisms from diverse environments continue to be promising candidates for industrial pigment production due to their adaptive capabilities (Table 1).

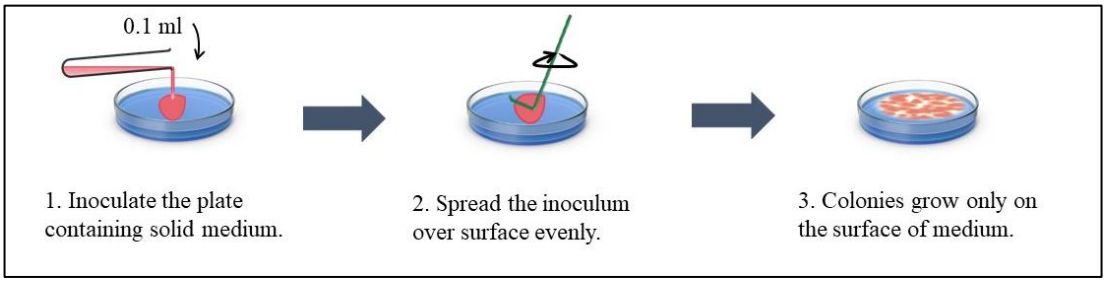

Figure 2. Spread plate method.

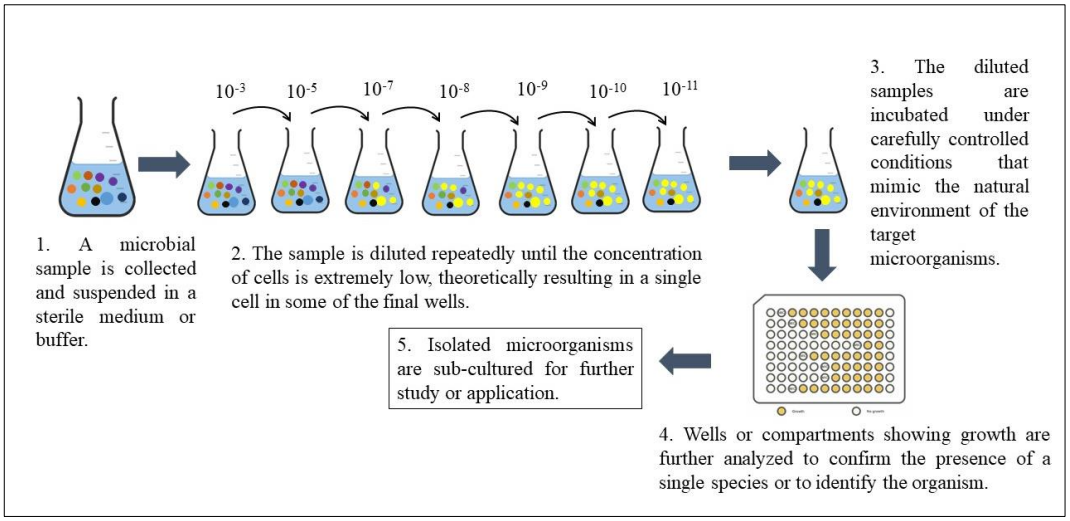

Figure 3. Dilution-to-extinction (DTE) method.

Table 1. Examples of selected pigments producing microbes isolated using different techniques in different environmental conditions

Bacteria/Microorganism	Sources of isolation	Isolation technique	Temperature	pH	Agitation	Pigment	Yield	References
Thermus scotoductus	Hot water pipeline	Filtration	65	7.5	-	Dark-brown melanin	-	[29]
Aeromonas sobria	Soil	Streak	37	4.71 and 5.48	-	Yellow, red, dark orange, pink	-	[30]
Erythrobacter sp.	Coastal seawater	Spread	37	7	-	Yellow	263 ± 12.9 mg/L	[25]
Bacillus haynesii	Hot spring	Spread	55	7	150 rpm	Brown	-	[31]
Streptomyces cavourensis	-	Filtration	22	-	-	Dark-brown	-	[32]
Hortaea werneckii	Marine habitat	Spread	37	5-5.5	180 rpm	Dark brown to black	0.420 g/L	[33]
Carotenoid *Kocuria rosea*, *Micrococcus luteus*	Rotten fruits and vegetables	Streak	37	7	-	Yellow, dark orange	-	[21]
	Soil	Streak	37	7	-	Yellow, dark orange		
Kocuria fava *Pontibacter korlensis* *Staphylococcus saprophyticus*	beach clam *Donax cuneatus*	Spread	Room temp	-	-	Yellow Orange Pale yellow	100 to 6.25 µg/ml	[34]
Sphingomonas paucimobilis *Microbacterium arborescens*	Fresh water sediment	Spread	30	11	-	Yellow Pink	-	[35]
Chromobacterium violaceum *Pseudomonas aeruginosa* *Salinococcus roseu*	Soil	Spread	35 37 40	8 7 7	Shake	Purple Blue-green Orange	-	[36]
Serratia	Water and soil	Spread	37	-	-	Red	-	[23]
Xanthomonas				-	-	Yellow	-	

Bacteria/Microorganism	Sources of isolation	Isolation technique	Temperature	pH	Agitation	Pigment	Yield	References
Erythobacter				-	-	Orange	-	
Vibrio alginolyticus *Vibrio harveyi*	Coral reef ecosystem	Spread	28	8.0 ± 0.2	-	Black	50 mg ml−1 40 mg ml−1 30 mg ml−1	[37]
Pseudoalteromonas amylolytica sp.	Surface seawater	Spread	30	7.0-7.2	-	Violet	-	[38]
Micrococcus flavus	Marine water and sediment	Spread	37	-	-	Yellow	-	[39]
Streptomyces glaucescens	Soil	Spread	30	7.2	100 – 200 rpm	Brown to black	-	[40]
Phaeocystis globosa	Coastal surface water	Spread	28	7.6 - 7.8	150rpm	Prodiogin	-	[41]
Streptomyces sp.	Soil	Spread	28	7 ± 0.2	-	Brown	300 mg/ 100 ml	[42]
Bacillus pumilus	Water	Spread	37	-	-	Black	239.44 ± 9.03 mg/L	[43]
Arthrobacter sp.	Seawater	Spread	20	7	-	Red	084 g l-1	[44]
P. aeruginosa	River; Swimming pool; drainage water	Spread	35	-	-	Blue-green	-	[45]
Pseudomonas guinea	Seawater	Spread	25	7	200rpm	Dark brown; black	5.35 ± 0.4; 2.79 ± 0.2 mg/mL p	[46]
Zooshikella ganghwensis sp	tidal flat	Spread	25	-	-	Red	-	[47]
proteobacterium Hahella sp.	Soil from seawater	Spread	28	-	-	Red	1.1 mg/ml	[48]
Serratia marcescens	Avocado peel waste	Spread	35	8	-	Red	5.41 ± 0.14 ml/min	[49]
Talaromyces purpureogenus	Soil	Spread	30	-	120	Red	138.3 U/mL	[50]

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3. SOURCES OF MICROBES PRODUCING PIGMENTS

Microorganisms adapt to diverse environments, including soil, where their growth and pigment production are influenced by factors like temperature, pH, and nutrient availability. Temperature plays a crucial role, with psychrophiles, mesophiles, thermophiles, and hyperthermophiles producing pigments suited to their thermal niches, such as heat-stable pigments in thermophiles. Soil pH also affects microbial growth and pigment synthesis, with acidophiles and alkaliphiles thriving in acidic or alkaline soils. Diverse environment will flourish microorganisms' growth in which enhance production of pigments. Pigments often serve as protective molecules against environmental stressors, such as UV radiation and desiccation, while also aiding in nutrient acquisition and microbial competition. These adaptations enable microorganisms to thrive and contribute to the soil's ecological functions.

3.1. Soil

Soil, particularly the rhizosphere, supports diverse microbial communities due to nutrient availability [51]. Rhizosphere is referring to soil compartment that found in narrow zone of soil that surrounds and influenced by filamentous network with roots, rock and soil particle [52]. It acts as microbial storehouse due to the biological, and chemical feature of soil as well as large amount of nutrient availability. The other reasons of increasing number of microbial and activities in the rhizosphere is because the high amount of organic carbon exhibit from the roots [53]. Furthermore, the interaction of microbial activities with soil is very crucial in the ecosystem especially for plant functioning. It anables to assist the plants in nutrients uptake and offers protection against pathogen attack. The soil inhibiting fungi is one of microbial species mostly be found in soil as it can secrete enzyme that can digest soil organic matter composed from lignin and celullose [52]. The known symbiotic nitrogen-fixing bacteria such as rhizobium species helps to detect flavonoid compounds which secreted from roots of living plants that leads to formation of root nodules [54]. Among them, the *Duganella* spp. has a great success in contributing for biotechnology due to its high potential in agronomic. This species produces purple-violet pigment which has significantly high yield without optimizing the conditions compared to previously reported for other violacein-producing bacteria [53]. Numerous Gram-negative bacteria, notably *Chromobacterium violaceum*, the first and most extensively researched violacein-producing bacterium, have been documented to make violacein in soil and oceans in tropical, subtropical, and glacial conditions [55 - 56].

Non-rhizosphere or bulk soil refers to the soil outside the influence of plant roots. As it lacks root exudates, it contains fewer natural organic compounds and supports lower microbial populations. Despite this, bulk soil offers unique conditions that favor the growth of specific pigment-producing microorganisms. These pigments function as adaptive traits for survival under stress and may also act as antimicrobial secondary metabolites, providing a competitive advantage in nutrient-poor environments. For example, *Streptomyces* spp. produce melanin to protect against UV radiation and oxidative stress [57]. *Pseudomonas* spp. synthesizes pyocyanin and pyoverdine to aid in iron chelation and stress protection [58]. *Micrococcus* spp. generates carotenoids to mitigate UV damage and desiccation [59]. Similarly, soil fungi like *Penicillium* and *Aspergillus* spp. produce polyketide pigments for survival under adverse conditions [60].

3.2. Extreme environment

Humans are accustomed to moderate surface temperatures on Earth, making extremely hot or cold conditions seem unusual. However, many microorganisms naturally thrive in such extreme environments, and these specialized organisms are collectively referred to as extremophiles. Remarkably, these organisms have adapted over time to thrive and achieve optimal growth at the specific temperatures of their natural environments.

3.2.1 Cold environment

Psychrophiles are organisms with optimal growth at ≤ 15 °C, a maximum below 20 °C, and a minimum at or below 0 °C. In contrast, psychrotolerant organisms can grow at 0 °C but have higher optimal temperatures (20 - 40 °C) and are more commonly encountered. They are frequently isolated from refrigerated foods and temperate environments such as soil and water. While less cold-adapted than psychrophiles, their versatility allows survival in cooler habitats.

In polar regions, psychrophilic microbes-such as algae and bacteria-form dense communities within and beneath sea ice and on glacier surfaces. Their presence often imparts visible coloration to ice and snow due to high pigment concentration [61]. Psychrophiles exhibit molecular adaptations-such as higher α-helix content in enzymes and membranes rich in unsaturated fatty acids-that support pigment production in cold environments. These structural features enhance pigment stability and functionality at low temperatures. For instance, *Janthinobacterium* spp. from Antarctica produce purple pigments with potential antioxidant properties, offering protection against cold stress and UV radiation [62]. Pigment synthesis thus serves as a key defense mechanism in psychrophilic microbes. Microbes use the creation of pigment as a survival strategy in situations with very low temperatures [63]. They devour pigment molecules as a source of energy, to fuel the process of photosynthesis, to combat stress, oxidants, severe heat, desiccation, and UV radiation, as well as for safety [64]. This interplay between pigment production and molecular adaptations allows psychrophiles to thrive in their extreme environments, offering insight into their potential biotechnological applications.

Thermophilic environments, such as hot springs, deserts, and hot water pipelines, support microbes that thrive at high temperatures. Thermophiles grow best around 60 °C, while hyperthermophiles tolerate up to 80 - 95 °C. *Bacillus* species are common in these settings and play a role in organic matter degradation due to their ability to produce heat-stable enzymes, many of which are commercially valuable. [65]. Besides, microorganisms in geothermal hot springs can be active and produce various enzymes under extreme conditions, depending on their environment. On the other hand, the *Bacillus* species also have discovered in hot spring like at Uttarakhand [66]; green and blue pigments, such as pyocyanin producing bacteria excreted from the *Pseudomonas aeruginosa* strain GIM 32 isolated from Bakreshwar Hot Spring [67]; the *Bacillus licheniformis* and the *Thermomonas hydrothermalis* isolated from Jordonian Hot Spring [68]; the *Bacillus haynesii* CamB6 excrete melanin pigment isolated from Chilean Hot Spring [31].

Thermophilic prokaryotes have also been found in man-made thermal environments such as hot water heaters, where temperatures typically range from 60 to 80 degrees Celsius. These conditions provide an ideal habitat for thermophilic organisms to thrive. Similar to those found in natural hot springs, such as the *Thermus aquaticus*, microorganisms resembling these thermal-loving species have been isolated from both residential and commercial hot water heaters [61, 69]. It produces yellow to orange pigments, contributing to its survival in high-temperature conditions. Additionally, thermophiles are present near power plants, hot water outflows, and

other artificial heat sources [61]. Thermophiles can be cultured using complex media at temperatures mimicking their natural habitats. Their pigments play key roles in survival under extreme heat by stabilizing cellular structures and offering protection against UV radiation and oxidative stress. These pigments also aid in light absorption for energy production and remain stable at high temperatures, supporting thermophiles' resilience in harsh environments. [61].

3.2.2 Alkalinity and acidity environment

The pH scale measures how acidic or alkaline a solution is. A pH of 7 is neutral. Values below 7 are acidic; values above 7 are alkaline. Each pH unit reflects a tenfold change in hydrogen ion concentration. For example, vinegar (pH 2) has far more hydrogen ions than ammonia (pH 11). Most microbes grow within a narrow pH range, usually between 4 and 9. Alkaliphiles are microbes that prefer high pH levels, often above 8. They are commonly found in soda lakes and carbonate-rich soils. [70]. Among them, halophilic microorganisms are able to grow with the presence of saline environment. It requires saturated salt concentration in which moderate halophilic organisms require salt concentration ranging 0.5 to 3.5M NaCl. Meanwhile, extreme halophilic organisms require salt concentration up to 3.0M NaCl. The *Desulfonatronospira thiodismutans*, *Deltaproteobacteria sp*., *halobacterium* and *Halomonas malpeensis* are haloalkaliphilic bacteria that can grow in high salt and alkaline pH environments [71]. Many microbes, including Halomonas species, have been isolated from saline and hypersaline environments such as oceans and salt lakes. Their survival in extreme conditions is supported by the production of protective metabolites, including osmolytes that help maintain cellular function. Halomonas also produces pigments and exopolysaccharides (EPS), which aid in stress tolerance, surface adhesion, and nutrient retention. EPS contributes to biofilm stability and forms a protective barrier against salinity and microbial threats.Halophilic bacteria *Bacillus altitudinis* producing bioactive melanin which confers black and brown colour pigments that is isolated from salt sediment. This melanin pigment producing bacteria offers a good anti-bacterial and radical scavenging activities [72]. Besides, extreme halophilic archaeon, *Halovenus aranensis* exhibits carotenoid extract which possess antioxidant properties that can further be studied for application in industry [73 - 74]. Yellow pigment from the *Halomonas antri sp*. have been isolated from surface seawater proved that seawater is the habitat for the halophilic microbes [75]. Various pigments that have been produced by halophilic bacteria work as protector in order to ensure the delicate and fragile cells not to be broken from external damages [76].

Acidophiles are microorganisms that thrive at pH values below 5.5. Some prefer mildly acidic conditions, while others can survive in extreme acidity, even below pH 3, like Acidithiobacillus species. Most cannot tolerate environments more than two pH units above their optimal range [77]. These microbes are found in natural acidic habitats such as sulfur springs, geysers, acid mines, and volcanic vents, as well as in human-influenced sites like coal mines and metal ore deposits [78-80]. Acidophiles adapt through proton-pumping membranes or acid-stable proteins. For example, *Acidiphilium aminolytica* grows at pH 3–6 and produces light brown pigment, while *Ocallabacter* (pH 5.5) produces yellow pigment in savannah-like soils. Industrially, acidophiles are valuable for biomining and enzyme production [81-83]. Pigment-producing acidophiles like *Alicyclobacillus* (carotenoids, pH 3.0), *Mucor pusillus* (pectinase, pH 5.0), and *Aspergillus* (xylanase) are used in food and feed industries [84]. Ferroplasma species, including F. acidiphilum, inhabit sulphidic ore environments like pyrite. They are chemoautotrophs that oxidize ferrous iron and fix carbon dioxide for energy and can survive at extremely low pH (as low as 1.7) [85-86]. These microbes have also been explored for

biological electricity generation. Studies show that extreme environments support pigment-producing microorganisms with valuable industrial applications. Exploring these habitats is crucial for identifying strains with high cultivation potential.

4. FERMENTATION

Biotechnological production of colourants using microorganisms is gaining increasing attention. Two main strategies are employed: identifying new pigment sources and enhancing their productivity, or optimizing known strains through fermentation and genetic approaches. However, large-scale production remains limited by infrastructure and R&D costs. Advances in fermentation physiology and gene technology, including heterologous expression of pigment pathways, offer promising solutions. Optimizing culture media is crucial for maximizing pigment yield. Key factors include nutrient composition, pH, temperature, aeration, and agitation. Tools like Response Surface Methodology (RSM) and Central Composite Design (CCD) allow efficient parameter optimization, unlike the traditional One Factor at a Time (OFAT) approach, which is time-consuming and fails to capture variable interactions. [87]. Meanwhile, the RSM is experimental design to build models, to evaluate the effect of factors and to predict optimum conditions for the factors whereas Plakett-Burman is screening and trace nutrients in a fermentation medium [88]. This process resulted in designing the process in large scale. In spite of optimization approach, there are other types of fermentation used, which are solid-state fermentation (SSF) and submerged fermentation (SF). Mishra *et al*. [89] stated that SF has been used for large-scale processes but SSF appears to be more promising to the industries demand. This is due to SSF technique saves wastewater and increases yield amount of metabolites. Solid-state fermentation process involves only surface of the solid substrate while submerged fermentation requires cultivation of microorganisms in liquid medium. SF process needs to be precise on the agitation in order to produce homogenous growth of cells and media components [90].

In this regard, Embaby *et al.* [91] studied the effective approach for efficient co-valorization by using raw agro-industrial waste which are corn cob and aid with glycerol for the production of bio pigments. The result indicates that for the optimum level of inoculum size (12 x 1011 spores/mL), and glycerol concentration (2.17M), maximal colour values of 33.77 colour value units/mL (orange pigment) and 108.02 colour value units/mL (red pigment) were achieved at 30 ^{o}C after 10 days; as deduced from central composite design (CCD) with an agitation speed of 150 rpm. This study validates co-solid state fermentation of the two agro-industrial wastes corn cob and glycerol successfully provoked high levels of orange and red monascus pigments production by *M. purpureus* strain ATCC 16436.

In another study, Aruldass *et al*. [92] investigated the ability of liquid pineapple waste as substrate for the production of yellowish-orange pigment from *Chryseobacterium artocarpi* CECT 8497. Akpinar *et al.* [93], studied the influence of substrate on fungal laccase production under solid-state fermentation. Due to high demand of laccase production, the cost of it determining the economy of process. Bioprocessing costs are heavily influenced by substrate choice, prompting the search for low-cost, high-efficiency alternatives. Agro-industrial waste is a cost-effective and sustainable option, offering essential nutrients for microbial growth and fermentation. Common fruit wastes like orange, papaya, and banana peels have been successfully used to co-produce enzymes such as pectinase and lipase. These wastes are also applied in solid-state fermentation to reduce enzyme purification costs [94]. For the production of microbial pigment, various main sources of agro-industrial waste were used. Besides, banana wastes also contribute high yield of the *Aspergillus niger* production which was 259.00 ± 1.23

U/mL. The wastes exhibit amylase enzymes which helps the growth of microorganisms [95]. Apple pomace, a waste product from the manufacture of apple juice pigments, is high in minerals, carbohydrates, and acids and was used to produce microbial pigments [96].

Natural materials and industrial by-products such as sugarcane molasses, corn steep liquor, and cheese whey are often used as low-cost media in fermentation, since media can make up 38–73 % of production costs. Corn steep liquor, a by-product from corn wet-milling, has long been used in penicillin production and is rich in nitrogen and minerals. It is especially useful in producing red pigments from Monascus ruber, which are gaining interest in the food industry as safer alternatives to synthetic colorants like nitrosamines, known for their harmful effects [97].

Whey is one of the most common waste items in the dairy industry. It's a by-product of the cheese-making process, obtained after the milk has been precipitated and removed. Whey is a lactose- and protein-rich by-product that is used as a growth substrate for lactose-consuming bacteria. There is an investigation of using whey as medium supplement for probiotic *Lactobacillus casei*. The optimization process has also been statistically analyzed using Response Surface Methodology (RSM) in order to study the main and interaction effects of distinct factors on bacteriocin production [98]. Table 2 indicates the diverse of types of wastes used as substrate using different types of fermentation techniques. The use of RSM and CCD for optimizing the fermentation conditions in combination with raw agro-industrial waste can lead to improved bio pigment production, reduced waste and cost, and increased sustainability. Overall, the biotechnological synthesis of colors using microorganisms has the potential to provide a sustainable and cost-effective alternative to traditional methods of colourant production.

Table 2. Different microorganisms exhibit various pigments in different conditions

Microorganism	Pigments	Substrate	Fermentation method	pH	Temperature (℃)	Incubation time	Agitation speed (rpm)	Carbon sources	References
Monascus purpureus	Red	Oil palm frond	SSF	8	30	8 days	18	4 % (w/w) of peptone	[99]
Monascus rubber	Red yellow	(MEA) (50 g L−1) with 5 g L−1 maltose syrup (containing 70.0 g L−1 maltose and 2.23 g L−1 glucose)	Liquid – State Fermentation	4 2 - 2.5	30	7 days	100	5 g glycine; 5 g dipotassium phosphate; 0.1 g calcium chloride; 0.5 g magnesium sulfate heptahydrate; 0.01 g ferrous sulfate heptahydrate; 0.01 g zinc sulfate heptahydrate and 0.03 g manganese(II) sulfate monohydrate	[100]
Rhodotorula sp *Wickerhamomyces onychis* *Sporidiobolus pararoseus* *Sporobolomyces japonicu*	-	Solid cassava bagasse(CB) or hydrolyzed cassava bagasse (HCB) (3 % or 5 %, w/v)	Submerged	-	28	96 hrs	180	0.3 % or 0.5 % (w/v) of corn steep liquor (CSL)	[101]
Streptomyces sp.	Brown	50 g of sterilized wheat bran	SSF	-	28	10 - 14 days	220	1 % starch; 1 % peptone	[102]
Monascus purpureus	Red	Bakery wastes	SSF	4	30	7 days	-	-	[103]
			Submerged				250	5 to 50 g/l glucose	
Monascus purpureus	Red and orange	Agro-industrial waste	Submerged	3.5	30	7 days	Static	g/L: potassium nitrate, 50; agnesium sulfate, 16.67 and sodium dihydrogen phosphate, 25 30 g/L rice grains	[91]
Pseudomonas guinea	Dark brown; black	Vegetable wastes	Submerged	7	25	72 hrs	200	30 % Marine broth	[104]
s Zooshikella ganghwensis sp	Red	Marine agar	Submerged	-	25	3 weeks	-	-	[47]
M. purpureus	Red	Jackfruit seed	SSF	-	30	7 days	-	Salt solution (2 ml) containing (g/l) potassium dihydrogen phosphate 2 g, ammonium nitrate 5 g, NaCl 1 g, magnesium sulfate heptahydrate1 g	[105]

Microorganism	Pigments	Substrate	Fermentation method	pH	Temperature (℃)	Incubation time	Agitation speed (rpm)	Carbon sources	References
								and distilled water	

5. EXTRACTION OF MICROBIAL PIGMENTS

The extraction of purified microbial pigments remains a major challenge in large-scale production. Intracellular pigments require cell disruption, increasing time and cost, while environmental factors such as UV light, high temperatures, and prolonged processing can cause degradation and isomerization. Organic solvents are commonly used polar solvents (e.g., ethanol, acetone) for polar pigments and non-polar solvents (e.g., hexane, THF) for non-polar compounds but they may pose safety and environmental risks. To overcome these limitations, five main extraction strategies have emerged: non-thermal energy methods, mechanical disruption, high-pressure/temperature techniques, enzyme and ultrasound-assisted extraction, and biological processes such as fermentation. These are summarized in Table 3.

Non-thermal methods, such as pulsed electric field (PEF) and microwave-assisted extraction (MAE), enhance membrane permeability to release pigments efficiently while reducing solvent use and time [106]. However, thermal drying methods like oven or microwave drying may degrade pigments, making freeze-drying preferable, albeit costly [107].

Mechanical methods including high-pressure homogenization (HPH) and bead milling (BM) will apply physical force to break cells but can be energy-intensive and risk pigment damage [108]. High-pressure techniques like supercritical fluid extraction (SFE) and CO_2 depressurization efficiently extract pigments with minimal solvent use, though they require specialized equipment. However, SFE may be less effective in aqueous matrices due to the hydrophobic nature of pigments and solvents [109 - 110]. Enzyme-assisted extraction (EAE) and ultrasonication (US) offer milder alternatives by degrading the cell wall through enzymatic hydrolysis or ultrasound-induced cavitation, improving yield and reducing processing time [111]. Fermentation enables pigment biosynthesis directly by microbes and can yield high-quality products, though it may require longer times and specific conditions [112].

Pre-treatment steps are often essential, especially for tough cell-walled organisms like *Haematococcus pluvialis* or diatoms. Methods include physical (e.g., freeze-thaw, grinding), chemical (e.g., acid/base), enzymatic, or biological treatments to enhance pigment recovery [113]. For example, Mezzomo *et al.* [114] extracted astaxanthin and carotenoids from prawn residues using various solvents and techniques.

Traditionally, solvents like acetone, methanol, chloroform, and hexane have been widely used, sometimes in combinations to enhance extraction efficiency. Solvent choice depends on pigment polarity, sample composition, and moisture content. Due to environmental and health concerns, ethanol and acetone are now preferred over more hazardous solvents such as chloroform and dichloromethane [115 - 116]. Recent advances have focused on sustainable alternatives, including ionic liquids and green solvents, to improve pigment extraction with reduced ecological impact.

Table 3. Table of green extraction methods on microbial pigments production adapted from Wani *et al*. [117] and Martinez *et al*. [118]

Green extraction method	Advantages	Limitation	Materials studied	Aim of study	Outcomes	References
Pulse electric field	non-thermal short process high-selectivity non-destructive	Dependence on medium composition (conductivity) High cost of equipment	Purple fleshed potato	Anthocyanin extraction	Pulsed electric field was possible with water, a more environmental-friendly solvent than ethanol, without decreasing the anthocyanin yield	[119]
			Microalgae *Tetrasel mis chuii* (*T. chuii*) and *Phaeodactylum tricornutum* (*P. tricornutum*)	Pigments and polyphenols extraction	The use of a low electric field strength to promote microalgae electroporation to improve extraction efficiency.	[120]
Microwave	Easily to scale up High operational speed	High energy consumption Degradation of thermo-labile compounds	Marine microalgae	Microalgal pigments extraction	Microwave-assisted extraction was identified well than conventional extraction as it combined rapidity, reproducibility, homogeneous heating and high extraction yields.	[113]
MEF	Increase permeability of cytoplasmic membrane	-	Rice bran	Anthocyanins extraction	Increased yield recovery of colourant (anthocyanin)	[121]
Supercritical fluid extraction	Low toxicity Low cost Ease of separation of the extracted product Simple purification process	Low yield of polar carotenoids	Fruit and vegetable waste	Carotenoids extraction	SFE is a viable method for the recovery of carotenoids from the fruit and vegetable waste, carotenoid-rich extracts obtained by SFE adds value to fruit and vegetable waste	[122]
			marine cyanobacterium *Synechococcus* sp.	Carotenoid and chlorophyll extraction with supercritical CO_2	Supercritical carbon dioxide is a suitable solvent for the extraction of carotenoids because of the low polarity of these compounds. The carbon dioxide extraction process is selective in the presence of more polar pigments such as chlorophyll a.	[123]
High pressure homogenization	High efficiency of disruption cell High recovery of thermolabile compound	-	Elderberry (Sambucus nigra L.) pomace	Extraction of anthocyanins	By the application of high pressure, it is possible to obtain anthocyanin-rich extracts from elderberry pomace possessing high antioxidant activity	[124]

Green extraction method	Advantages	Limitation	Materials studied	Aim of study	Outcomes	References
	Economically of industrial scale-up					
Enzyme and high pressure	Increased yield of value added cell components Short extraction process Low amount of solvent consumption	Require to understand the enzymes' catalytic property and mode of action before use. The cost of enzymes are expensive. Difficult to scale-up as its depend on environmental conditions.	Tomato waste	Carotenoids extraction	To obtain increased yield recovery of carotenoids (lycopene)	[125-126]
Ultrasonication	Efficient of mixing effect	Scale-up not feasible Non-selective release Elevated operational cost Degradation of thermo-labile compounds	Orange processing waste	Optimisation of beta carotene	To observe the amount, antioxidant activity and colour parameters of carotenoids extracted	[127]
CO_2 depressurisation	Have ability in cell membrane modification Reduce intracellular pH High removal of vital constituent from cell and cell membranes Enhance the availability	slight time-consuming	Botryococcusbrauni i (microalga)	Extraction of carotenoid and chlorophyll	Microscopic images showed partial cell disruption by rapid depressurisation improved the extraction of microalga compounds	[128]

Green extraction method	Advantages	Limitation	Materials studied	Aim of study	Outcomes	References
	of extracted compounds					
Fermentation	Increase yield of production Economically feasible to produce low bulk, and high value product	Heat build up Occasional of fungal and bacteria contamination Limitation of microbial types that can be used	Shrimp waste	Carotenoids	Fermentation was found to be superior to acid ensilaging with respect to stability and extractability of carotenoids from the shrimp waste	[129]
Bead milling	Considerably effective against cell walls with considerable rigidity. The apparatus can be actively cooled to prevent heat-dependent damage to the pigment meant to be extracted	Results can be unpredictable and fine-tuning is required. Can consume considerable amounts of energy if parameters are not optimized	Sporobolomyces ruberriumus H110	Carotenoids	Extraction methods by using planetary mill with 135 mg of glass beads or irregular quartz stone with agitation speed of 200 rpm	[130]

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6. CONCLUSION AND FUTURE PERSPECTIVE

Biotechnological production of microbial pigments is a promising industrial strategy because it enables to aid in increasing natural products of additives for consumers, as well as contribute to environmental preservation. This review summarizes information regarding strategies, methods and biotechnological parameters that modulate the production of microbial pigments. The application of microbial pigments by using suitable methods, right parameters of fermentation conditions and extractions lead to facilitate microbial pigments to produce high yields with affordable cost. However, there is lack of literature describing definitive mechanisms by which these microbial pigments may be used.

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