

Chapter 7

Agro-Industrial Waste as Substrates for the Production of Bacterial Pigment



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Abstract There is worldwide interest in process development for the production of pigments from natural sources due to a serious safety problem with many artificial synthetic colorants, which have widely been used in foodstuff, cosmetic, and pharmaceutical manufacturing processes. Low-cost by-products and residues of agro-industrial origin have shown their potential in production of different pigments by diverse group microorganisms and to explore the possibility of pigment production by different microbial isolates from numerous sources on various substrates. The main applications of recycled wastes are enzyme production, organic acid isolation, pigment extraction, bioactive compound production, etc. Therefore, more regulatory approval and capital investments are required to bring these value-added products in the commercial market. The conversion of agro-industrial residues to important substances may not only provide future dimension to researchers but also reduce the current environmental hazards.

Keywords Agro-industrial waste · Microbes · Fermentation · Pigments · Low-cost substrate

7.1 Introduction

Agro-industrial wastes are those which are generated from the food as well as agricultural industries or from the agricultural practices. Most of the agro-industrial wastes are untreated and underutilized and these untreated wastes produce completely different issues with climate change by increasing the number of

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greenhouse gasses (Bos and Hamelinck 2014). These wastes also cause serious disposal problems (Rodríguez-Couto 2008). However, only a small portion of all waste generated during agro-industrial processes is recovered as by-products, and the vast majority of them are not considered as viable for further use and are discarded directly into the environment or are responsible for major expenses with proper disposal. The environmental damage caused by agricultural waste leads to focus on more research on the utilization of agricultural waste. Even though agricultural residues are produced in large quantities in developing countries, they are mainly utilized as animal feed and landfills (Salihu et al. 2012).

At present, extensive agricultural waste disposal methods not only fail to effectively convert and utilize agricultural resources but also cause serious environmental pollution (Dai et al. 2018). It is a worldwide concern to dictate the improvement of alternative cleaner and renewable bioenergy resources (Okonko et al. 2009). Most of the wastes generated by agro-based food industries are high in nutrients and can form breeding grounds for disease-causing microbes if left unprocessed and inadequately treated. Interestingly, these wastes can serve as raw materials for the production of value-added products or as a source of renewable energy (Ravindran et al. 2018). The utilization of these agro-industrial wastes for the production of useful products emphasizes their biotechnological potentials for efficient value addition (Pandey and Soccol 2000; Pandey et al. 2000a, b, c; Soccol and Vandenberghe 2003).

Agricultural residues contain variability in composition like high amount of proteins, sugars, and minerals. Due to high nutritional composition, these residues not described as “wastes” but considered as raw materials for other product formation and developments (Sadh et al. 2018). The availability of these nutrients in raw materials offers appropriate environments for the growth of microorganisms. Agro-industrial waste and their complex organic contents constitute a significant source of residual nutrients which serve as rich media for microbial growth and production of the enzymes (Martins et al. 2011). In recent times, agricultural wastes have been made to use in biotechnological processes such as production of value-added compounds and substrates for microbial isolation.

7.1.1 Agro-Industrial Waste: A Scenario

The use of agricultural and agro-based industry wastes as raw materials can help to reduce the production cost and contributed in recycling of waste as well to make the environment eco-friendly (Sadh et al. 2018). These wastes may be used as low-cost raw materials for the production of other value-added compounds, with the expectancy of reducing the production costs (Bhatia et al. 2012). The environmental concern is because most of the agro-industrial wastes contain phenolic compounds and/or other compounds of toxic potential; which may cause deterioration of the environment when the waste is discharged to the nature. The agro-industrial wastes may be used in these processes as solid support, carbon, nitrogen, and/or mineral sources, which would allow obtaining more economical fermentation processes

avoiding the use of expensive chemical components in the media formulation (Table 7.1). As a consequence, more economical processes could be established for implementation on an industrial scale (Mussatto et al. 2012).

Agro-industrial wastes are generated during the industrial processing of agricultural products. Those derived from agricultural activities include materials such as

Table 7.1 Agro-industrial waste management

S. No.	Source of waste	Possible ways of utilization	Remarks	References
1.	Fruit wastes	Landfilling or incineration	Emission of methane, carbon-di-oxide, and other toxic substances	Dhillon and Kaur (2016), Deng et al. (2012)
2.	Apple pomace	Animal feed	High sugar content, low digestibility, low vitamin, and mineral content	Vendruscolo et al. (2008)
3.	Rice straw, sweet potato waste, sawdust, potato waste, corn stalks, sugarcane bagasse, and sugar beet waste	Biofuels	Decrease the deforestation by reducing our dependence on forest woody biomass	Duhan et al. (2013), Kumar et al. (2014, 2016)
4.	Raw residual coconut milk, raw residual pineapple juice	Bioethanol production by <i>Saccharomyces cerevisiae</i>	Alternative to replace fossil fuels is the production of bioethanol from agro-industrial wastes	Domínguez-Bocanegra et al. (2015)
5.	Vegetable's waste like potato peel, carrot peel, and onion peel. Banana stem	Bioethanol—Fermentation technique— <i>Saccharomyces cerevisiae</i>	Increases the yield by microbial fermentation	Mushimiyimana and Tallapragada (2016)
6.	Rice straw and corn stalks	Biofuel— <i>Aspergillus niger</i> and <i>Trichoderma viride</i>	Bioethanol	El-Tayeb et al. (2012)
7.	Sugarcane molasses	Biofuel— <i>Zymomonas mobilis</i>	Bioethanol	Cazetta et al. (2007)
8.	Wheat straw, sugarcane bagasse, maize straw, paddy straw	SSF— <i>Bacillus licheniformis</i>	α -Amylase	Kaur et al. (2015)
9.	Oil cake—coconut, groundnut, cotton seed, gingelly, or soybean	SSF— <i>Pseudomonas</i> sp.	Lipase	Faisal et al. (2014)
10.	Fruit peel waste	<i>Aspergillus niger</i>	Invertase	Mehta and Duhan (2014)

straw, stem, stalk, leaves, husk, shell, peel, lint, seeds, pulp, or stubble from fruits, cereals, bagasses or sweet sorghum milling, spent coffee grounds, brewer's spent grains, and many others. These wastes are generated in large amounts throughout the year and are the most abundant renewable resources on earth. They are mainly composed by sugars, fibers, proteins, and minerals, which are compounds of industrial interest (Mussatto et al. 2012).

A huge amount of organic residues and related effluents are produced every year through the food processing industries like juice, chips, meat, confectionary, and fruit industries. These organic residues can be utilized for different energy sources. The residue of different fruits and vegetables such as fruit and vegetable peels is commonly known as a waste or no use. But many researches focused on these peels and got good results. So these wastes are considered as a valuable raw material for the production of various pharmaceutical products (Parashar et al. 2014). The maximum percentage of antioxidant activity was observed in pomegranate peel than lemon and orange peel (Singh and Immanuel 2014).

7.2 Microbial Fermentation

Agro-industrial by-products that frequently cause serious environmental problems can be possibly used as inexpensive carbohydrate sources for microbial fermentations, thus decreasing their initial high biological oxygen demand (BOD) while obtaining biochemical compounds like pigments suitable for pharmaceutical, chemical, and food industries (Sarvamangala and Aparna 2016). Low-cost by-products and residues of agro-industrial origin have shown their potential in production of different pigments by diverse group of microorganisms. Researchers have shown a great interest in the processing of agro-industrial wastes for fermentation processes in the development of value-added products (Fig. 7.1).

The utilization of such materials as substrates for microbial cultivation intended to produce cellular proteins, organic acids, mushrooms, biologically important secondary metabolites, enzymes, prebiotic oligosaccharides, and as sources of fermentable sugars in the second generation ethanol production has been reported (Sánchez 2009). Notably, the microbial enzymes can be the products themselves as well as tools in these bioprocesses. Agro-industrial wastes are valuable sources of lignocellulosic materials. The lignocellulose is the main structural constituent of plants and represents the primary source of renewable organic matter on earth. It can be found at the cellular wall and is composed of cellulose, hemicellulose, and lignin, plus organic acids, salts, and minerals (Pandey et al. 2000a; Hamelinck et al. 2005). Being rich in cellulose, hemicellulose as well as in other nutrients, these wastes are one of the main reasons for pollution and therefore should be used as potential substrates for microbial fermentation rather than considering them as wastes (Rodríguez-Couto 2008).

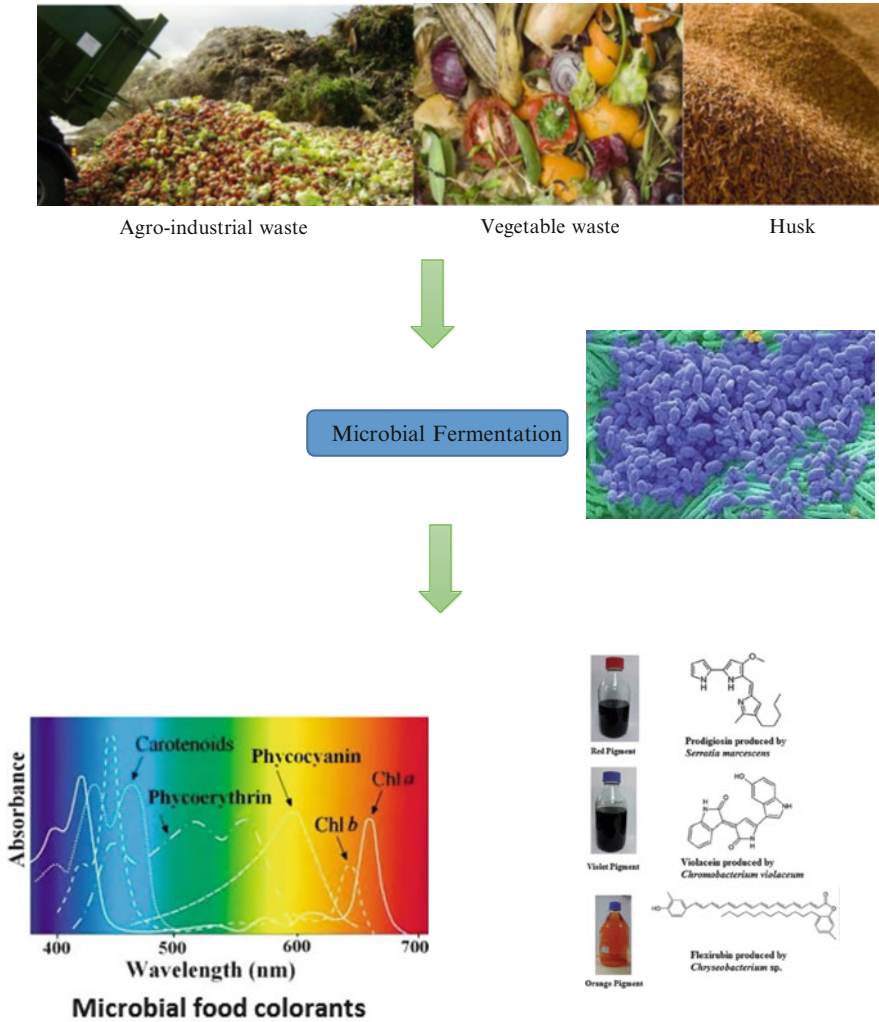


Fig. 7.1 Applications of agro-industrial wastes (Venil et al. 2014)

7.3 Pigment Production

Ever since, natural colors from spices and herbs, fruits, and vegetables have been part of the everyday diet of humans. Fruit by-products have become an important source of those pigments and colors, mainly because they present high color stability and purity. Bio-based pigments have several advantages such as biodegradability, zero or less toxicity, and eco-friendliness with their synthetic counterparts (Yusof 2017). Therefore, a lot of attention is now being undertaken for the synthesis of biocolorants from wastes using the microorganisms (Venil et al. 2013). In the

present scenario, researchers have shown a great interest in the processing of waste for fermentation processes in the development of value-added products like microbial pigments. Utilization of waste not only eliminates the disposal problems but also solves the problem of environment pollution.

Microorganisms such as bacteria, mold, and fungi produce different types of pigments depending on their sources. Some well-studied microbial strains that have potential of bio-pigment production from wastes are belonging to genera *Monascus*, *Rhodotorula*, *Aspergillus*, and *Penicillium*. For example, the following species are chiefly reported for bio-pigment production: *Alteromonas rubra*, *Rugamonas rubra*, *Streptovorticillium rubrirecticuli*, *Streptomyces longisporus*, *Serratia marcescens*, *Pseudomonas magnesorubra*, *Vibrio psychroerythrous*, *S. rubidaea*, *Vibrio gazogenes*, etc. (Dufossé 2006; Méndez et al. 2011; Panesar et al. 2015). Along this line, variety of substrates and microorganisms has been tested. Beta-carotene synthesis by citrus products, carotenoids production using whey ultrafiltrate, sauerkraut brine, and peat extract, riboflavin in concentrated rectified grape must, astaxanthin on grape juice are some promising studies (Korumilli 2014). A wide range of bacterial strains such as *Serratia marcescens*, *Pseudomonas magnesorubra*, *Vibrio psychroerythrous*, *S. rubidaea*, *Vibrio gazogenes*, *Alteromonas rubra*, *Rugamonas rubra*, *Streptovorticillium rubrirecticuli*, and *Streptomyces longisporus* have shown their potential in pigment production (Krishna 2008).

Among the natural pigments produced by bacteria reported so far (Table 7.2), most researches have focused on yellow and red pigment production, carotenoid from *Phaffia rhodozyma* (Vazquez et al. 1997), *Micrococcus roseus* (Chattopadhyay et al. 1997), *Brevibacterium linens* (Guyomarc'h et al. 2000) and *Bradyrhizobium* sp. (Lorquin et al. 1997) and xanthomonadin from *Xanthomonas campestris* pv. (Poplawsky et al. 2000). The research concerning violacein has mainly focused on its medical application. In addition to its application in dyeing fabrics (Shirata et al. 2000), violacein has also exhibited cytotoxic activity in human colon cancer cells (de Carvalho et al. 2006), antileishmanial (Leon et al. 2001), antiulcerogenic (Duran et al. 2003), antiviral, antibiotic, antitumoral, and anti-Trypanosomacruzi activities (Andrighetti-Frohner et al. 2003). Recently, prodigiosin has been considered effective as a biological control agent against harmful algae in natural marine environments besides its role in textile dyeing and medicinal uses.

Microbial pigments offer the following benefits and advantages (Hendry and Houghton 1997; Babitha 2009): Easy propagation and wide strain selection; high versatile and productive over other sources; fermentation is inherently faster and more productive production compared with any other chemical process; easy to manipulate genes; simple and fast culturing techniques allowing continuous bioreactor operation; structural complexity suits for industrial needs; microbial pigments extracted using simple liquid–liquid extraction technique minimizing operation cost; cheap substrates used for bulk production.

Many investigations have been performed to reduce the costs and optimize the pigment production (Table 7.3); and factors such as carbon and nitrogen source are very important to consider on the selection of agro-industrial waste as substrates (Panesar and Kennedy 2012). A wide spread natural substrate is milk whey; it

Table 7.2 Natural pigments produced by bacteria (Malik et al. 2012)

Bacteria	Pigments	Color	Applications
<i>Agrobacterium aurantiacum</i> <i>Paracoccus carotinifaciens</i> <i>Xanthophyllomyces dendrorhous</i>	Astaxanthin	Pink-red	Feed supplement
<i>Rhodococcus maris</i>	Beta-carotene	Bluish-Red	Used to treat various disorders such as erythropoietic protoporphyria Reduces the risk of breast cancer
<i>Bradyrhizobium</i> sp. <i>Haloferax alexandrinus</i>	Canthaxanthin	Dark-red	Colorant in food, beverage, and pharmaceutical preparations
<i>Corynebacterium insidiosum</i>	Indigoidine	Blue	Protection from oxidative stress
<i>Rugamonas rubra</i> <i>Streptoverticillium rubrirecticuli</i> <i>Vibrio gazogenes</i> <i>Alteromonas rubra</i> <i>Serratia marcescens</i> <i>Serratia rubidaea</i>	Prodigiosin	Red	Anticancer, immunosuppressant, antifungal, algicidal Dyeing (textile, candles, paper, ink)
<i>Pseudomonas aeruginosa</i>	Pyocyanin	Blue-green	Oxidative metabolism, reducing local inflammation
<i>Chromobacterium violaceum</i> <i>Janthinobacterium lividum</i>	Violacein	Purple	Pharmaceutical (antioxidant, Immunomodulatory, antitumoral, antiparasitic activities) Dyeing (textiles) Cosmetics (lotion)
<i>Flavobacterium</i> sp. <i>Paracoccus zeaxanthinifaciens</i> <i>Staphylococcus aureus</i>	Zeaxanthin	Yellow	Used to treat different disorders, mainly with affecting the eyes
<i>Xanthomonas oryzae</i>	Xanthomonadin	Yellow	Chemotaxonomic and diagnostic markers

contains lactose, proteins, and minerals, principally. Biological wastewater treatment technologies can assist in safe disposal of whey within environmental specifications, but these are expensive (Marova et al. 2012) becoming an attractive low-cost substrate for microbial production of carotenoids.

Table 7.3 Production of microbial pigments utilizing agro-industrial waste (Panesar et al. 2015)

Microbes	Media	Pigment	References
Submerged fermentation			
<i>Rhodotorula rubra</i>	Whey medium containing coconut water	Yellow pigment	Kaur et al. (2008)
<i>Sporidiobolus salmonicolor</i>	Yeast-salt medium	Carotenoids	Valduga et al. (2009)
<i>R. glutinis</i>	Tomato waste-based medium	Carotenoids	Silveira et al. (2008)
<i>Rhodospirium paludigenum</i>	Urea KH ₂ PO ₄ , MgSO ₄	Carotenoids	Yimyoo et al. (2011)
Solid-state fermentation			
<i>Monascus ruber</i>	Broken rice-based medium, packed bed of long grain rice-based medium, jackfruit seed powder-based medium	Red and yellow pigment	Vidyalaksmi et al. (2009)
<i>Monascus purpureus</i>	Corn meal, coconut residue, peanut meal, soybean meal-based medium	Red pigment	Nimnoi and Lumyong (2011)

7.4 Application of Bacterial Pigments

Bacterial pigments have wide range of applications in the pharmaceutical, food, and textile industry and have been discussed below.

7.4.1 Pharmaceutical Industry

Investigation of most of the pigmented bacteria has shown the efficiency in clinical applications of pigments for treating various diseases such as antibiotic, anticancer, and immune-suppressive properties (Venil et al. 2013). The property of bacteria to produce bio-pigments is used to produce medically important products. Pharmaceutical industry uses many microbial pigments in their products. Many pigmented secondary metabolites have significant potential clinical applications and many research works are going on for treating many diseases such as cancer, leukemia, diabetes mellitus etc. (Kumar et al. 2015). Bacterial pigments with fluorescence are employed in laboratories to label antibodies and indicate the progress of specific reaction. The pigments also play an important role in maintaining the health of human skin; melanin is used in sun block creams to protect the skin from UV radiation (Rao et al. 2017).

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 (Kim et al. 2012). The nutraceuticals functions of these xanthophylls and carotenes also help to prevent problems such as heart attacks and strokes (Long 2004). A red pigment, astaxanthin is important carotenoids

which has great commercial value, and is also used as pharmaceuticals feed. A strong therapeutics molecules prodigiosins are known for their immune-suppressive anti-cancer properties (Han et al. 1998). It has high commercial applications as prodigiosin possesses antibacterial, antifungal, anti-protozoal, cytotoxic, and anti-inflammatory properties (Panesar et al. 2015). *Hahella chejuensis* produces a pigment which is also known to have immune suppressant and antitumor properties (Kim et al. 2008).

7.4.2 Food Industry

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 show many negative impacts on health when consumed. Some fermentation-derived pigments, such as β -carotene, are now in use in the food industry. Various pigments provide a good appearance with additional nutritive and medicinal values such as antibiotic, antioxidants (Yangilar and Yildiz 2016). Demand for natural food colorants is more than its availability in food industry. Many natural colors are available, in which microbial colorants play important role as food coloring agent as their production and down streaming process are easy.

Natural colors are environment friendly and moreover serve as the dual need for visually attractive colors and health benefits in food colorants of probiotic (Nagpal et al. 2011). Therefore, pigments from microbial sources are good alternative. In addition, natural colorants will not only be beneficial to human health but will also be helpful for the maintenance of biodiversity. Some natural food colorants have commercial potential for use as antioxidants (Tuli et al. 2015). Thus bacterial colorants in addition to being environment friendly, can also serve the dual need for visually appealing colors and probiotic health benefits in food products (Venkatasubramanian et al. 2011). They are considered safe and approved by FDA. The successful marketing of pigments derived from bacteria, both as a food color and a nutritional supplement depend on consumer safety and freshness of the products.

7.4.3 Textile Industry

Textile industries use large amount of pigments mostly the synthetic ones. Synthetic dyes are widely available at an economical price and produce a wide variety of colors and have many drawbacks such as toxicity, mutagenicity, and carcinogenicity properties leading to various health problems such as skin cancer and allergies (Srikanlayanukul et al. 2006; Gurav et al. 2011). Hence, consumers demand for dyes of natural origin as colorants. Use of natural pigments in the textile industry is eco-friendly and noncarcinogenic. For successful commercial use of natural dyes for

any particular fiber, the appropriate and standardized techniques for dyeing for that particular fiber natural dye system need to be adopted (Venil et al. 2013). Therefore to obtain newer shade with acceptable color fastness behavior and reproducible color yield, appropriate scientific dyeing techniques/procedures are to be derived.

Microbial pigments are eco-friendly colorants applicable to dyeing textile fabrics (Chadni et al. 2017). Many microbial pigments were used to dye different types of fabric. Prodigiosin from *Vibrio* spp. can dye wool, nylon, acrylics, and silk. By using tamarind as a mordant, pigment from *Serratia marcescens* can color up to five types of fabric, including acrylic, polyester microfiber, polyester, silk, and cotton (Yusof 2008). Ahmad et al. (2012) observed the potentiality of prodigiosin and violacein in batik making. Kumar et al. (2015) reported that red pigment prodigiosin from *Vibrio* sp., *Serratia* sp., and violet pigment from *Chromobacterium violaceum* are suitable for textile industry for dyeing of all fibers including cotton, wool, silk, nylon, and acrylic fibers.

7.5 Conclusions

Microbes utilize the waste for their growth through fermentation process and produce novel secondary metabolites. Therefore in recent years, various agro-industrial residues have been used as a substrate or additive for pigment production which may represent an added value to the industry thereby reducing the production cost. Extracted bacterial pigments can be used in food, pharmaceuticals, textiles, cosmetics, and also in food research and for the development of functional foods. The use of agricultural and agro-based industry wastes as raw materials not only eliminates the disposal problems but also helps to reduce the production cost contributed in recycling of waste as well to make the environment eco-friendly.

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