Chapter 7
Emerging Technologies for the Recovery of Microbial Bioactive Compounds

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Abstract For agriculture, industry, and society as a whole, microbially derived bioactive compounds are extremely important. They are frequently used as active components in food additives, medicine, and agriculture. Archaea, bacteria, fungus, protozoa, algae, and viruses are among the few examples of microorganisms. A vast array of distinctive compounds produced by the diversity of microorganisms has emerged as a viable source for cutting-edge biotechnology. Microorganisms are simple to grow and enable a more effective generation of natural bioactive compounds than do plants. In contrast to synthetic bioactive chemicals, most microbial ones are noncytotoxic and nonmutagenic. There is a huge variety of microorganisms, but very few of them have been cultivated and looked at for the generation of secondary metabolites. The pharmaceutical and neutraceutical industries are very interested in the phenols, flavonoids, steroids, and alkaloids that have been discovered in microalgae, bacteria, yeast, and actinomycetes. More extensive research is required in order to better understand and make the most use of these microbial bioctives because their mode of action has not yet been fully clarified. Taking advantage of nature's rich biodiversity, this could also result in the development of new medicines and applications.

7.1 Introduction

The diversity of microscopic microorganisms, including bacteria, protozoa, fungi, archaea, algae, and viruses, provides a range of special bioactive substances of pharmacological value that must be made commercially available for the benefit of mankind. A numeric value of around 23,000 secondary metabolites extracted from

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microbes are been discovered so far. Among them 42% is contributed by both actinomycetes and fungi and rest of the 16% is bagged by eubacteria [1]. Bioactive compounds comprise of growth hormones, antitumor agents, pigments, antibiotics, etc., which comprehensively participates in the pharmaceuticals industries [2]. Scientists have recently focused their attention on cutting-edge techniques that allow the extraction of biologically active compounds without their degradation. Examples of these techniques include microwave-assisted extraction, enzyme-assisted extraction, supercritical fluid extraction, pressurized liquid extraction, and ultrasound-assisted extraction [3]. These efficient and emerging extraction methods are better than traditional methods in terms of performance, maximum yield, short processing time, and its ecologically sound approach. Earlier traditional methods of extraction include liquid–liquid extraction (LLE), solid–liquid extraction (SLE), and extraction in a Soxhlet system. The major stumbling block in the use of traditional method of extraction is the requirement of large amount of hazardous solvents and lengthy extraction durations [4].

Plant-based bioactive compounds, such as medicinal plants herbs shrubs, fruits, vegetables, cereals, and so forth, have historically provided the industrial market for these substances. However, due to climatic and regional variation, this method has certain drawbacks, due to which it affects the chemical content of plants and the challenge of ensuring the quality of agricultural products [5]. The industrial production of bioactive substances hence requires robust and sustainable manufacturing employing contemporary biotechnological technologies. Since the introduction of cell culture technologies, metabolic engineering, and synthetic biology, the biotechnological generation of bioactive substances has been intensively researched [6]. Large-scale fermentors are used for the optimum production of bioactive compounds. Instead of using wild-type organisms, this approach typically uses target transgenic organism with desired properties under precisely regulated process conditions. Microbes have been drawing more interest among these production hosts due to their unique characteristics in comparison to other organisms or tissues, such as rapid growth, ease of cultivation, and simplicity of genetic manipulation.

The best strategy for enhanced recovery of bioactive compounds will be selection of appropriate extraction technique, solvent type, and microbial class when using microorganisms. Although traditional organic solvent extraction methods are widely used, accessible, and enable quick extraction of bioactive compounds, they should be phased out gradually because they use a lot of solvents and run the risk of thermal denaturation or transformation of compounds of interest [7].

With regard to enhance production of desired bioactive compounds with less or no harmful impact on the target bioactives, novel extraction techniques, such as ultrasonic, enzyme, and microwave aided extraction alone or in combination, have illustrated significant advantages over conventional approaches:

7.2 Microwave-Assisted Extraction (MAE)

In particular, MAE has been used expansively for the extraction of bioactive compounds from plant materials [8, 9]. Two optimum frequencies are being utilized 915 MHz and 2450 MHz for ignition in both industrial and residential settings. Microwave has an electromagnetic property. According to Kaderides et al. [10], the heating effect produced by the microwave is the key mechanism of MAE, which enables higher temperature for extraction and a faster mass transfer rate. Microwave have the tendency to penetrate into the material into certain depths and cause interaction with the polar constituent in it that result in direct heating or bulk heating inside the solvent body and the sample matrix [11]. Figure 7.1 depicts the closed type microwave system.

Microwave-assisted extraction is functional only within a closed or open loop, so as the closed or open system's pressure rising above or remaining below atmospheric pressure, respectively. The schematic designs of a closed system with homogenous radiation are shown in Fig. 7.1 along with those of an open system with focused radiation. If temperature and pressure control systems are present in the equipment, the closed MAE system may also perform extraction under controlled temperature and pressure in a sealed vessel with uniform microwave heating. Due to the extraction solvent's boiling point being raised by the increased pressure in the closed vessel, it can reach higher working temperatures than the open system [12]. Despite the extreme pressure and temperature in the reactor that lead to efficient and fast extraction with less solvent consumption, they also increase the safety risks and equipment control requirement. An advance green process through microwave-assisted extraction of bioactive metabolites from *Arthrospira Platensis* (cyanobacteria) and evaluation of its bioactivity was conducted. Numerous microbiota-derived compounds have been synthesized by microorganisms and one of the important one is bioactive peptides. These peptides are known for their regulating cell cycle and cell signaling. It also plays an important role in maintaining hypertension, hyperglycemia, and damaged proteins. There are other bioactive compounds that are secreted by microorganisms which have same potential in maintaining other important biochemical aspects [13]. Extraction of these bioactive compounds is necessary, and some of the important methods include.

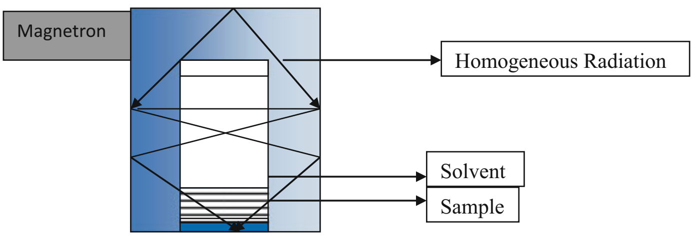

Fig. 7.1 Closed-type microwave system

7.3 Ultrasound-Assisted Extraction Method

Ultrasound extraction method of bioactive compounds is being performed at both industrial and laboratory scale. Ultrasonic wave causes cavitations in particle that arises due to collision, disturbance, and disruption of atoms present in the particle. Cavitations cause pores in the material results in mass transfer rate and solvent into biomass [14]. The big advantage of using ultrasound mediated extraction is that it can be set up with different configuration depending upon the nature of extraction. Ultrasound extraction method can be used with a variety of solvents like ethanol, water, acetone, methanol, ethyl acetate, and ethanol, but critical things are to be carried out by ultrasound-mediated extraction at lower temperature, which is necessary for maintaining the integrity of thermosensitive compounds [15]. Keeping this in mind, the extraction of gallic and ergatsterol form *Agaricus bisporus* was done with the cavitations method in which ethanol was being used as solvents [16]. Figure 7.2 demonstrates the principle of UAE.

7.4 Enzyme-Mediated Extraction

Enzyme-mediated extraction is useful for extracting phyto-chemicals from their respective associated cell wall. Presence of hemicelluloses, cellulose, and lignin in higher concentration makes difficult for conventional-based extraction method for extracting bioactive compounds [17]. Enzyme-mediated extraction provides an

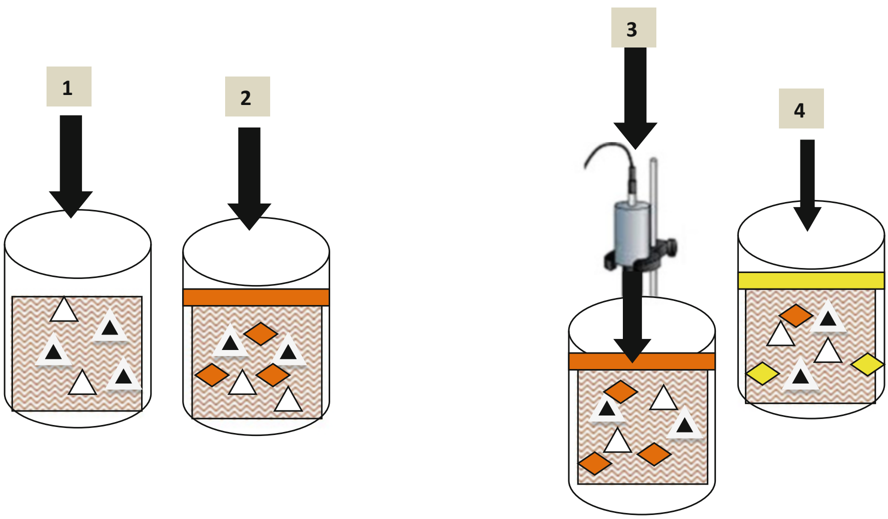

Fig. 7.2 Principle of the UAE. (1) The sample is added into the cylindrical vessel. (2) Addition of solvent for extraction. (3) The sample is brought into touch with the sonotrode. Sample sonication takes place. (4) The sample is prepared for cleanup

Table 7.1 List of bioactive compounds extracted from microorganisms, plants, and algae via enzyme-mediated extraction method

S. No.	Source	Bioactive compound	Enzyme	Reference
1.	*C. annuum baydgi*	Carotenoids and capsaicinoids	Cellulase, hemicellulase, pectinase	Salgado-Roman et al. [18]
2.	*Codium fragile*	Uronic acid, sugars, sulfates, and proteins	Cellulase, beta-glucanase, Ultraflo, Neutrase	Kulshreshtha et al. [19]
3.	*Fucus distichus*	Fucoidans	Alginate lyase	Nguyen et al. [20]
4.	*Helianthus annuus L.*	Carotenoids	Viscozyme	Ricarte et al. [21]
5.	*Haematococcus pluvialis*	Astaxanthin	Flavourzyme	Poojary et al. [22]

alternative method to it by employing enzymes like alpha-amylase, cellulose, hemicellulose, and pectinase, which are known to be involved in the digestion of cell wall [11]. Enzyme-mediated extraction offers several advantages like nature friendly and lower consumption of energy as compared to other techniques. Reduction of toxic solvents and efficient extraction of volatile and thermal compounds is also one of its bid advantage over conventional methods (Table 7.1). Extracted volatile compounds can be used in providing flavors, fragrnance, and pigments [17]. However, one disadvantage of using enzyme-mediated extraction is using larger volume of substances as it can be highly expensive [23].

7.5 Pressurized Liquid Extraction

According to Nieto et al. [24], PLE is an extraction method that inculcates expelling of analytes from solid matrixes by giving high temperatures (T_{extr}) and pressures (P_{extr}), typically up to 200 °C and over 200 bar, respectively, without reaching the critical point using liquid solvents [25]. These optimal conditions provide improved matrix kinetics by increasing solubility and mass transfer rates, which in turn increase solvent diffusivity [26]. The solvent and the sample are simultaneously administered into the extraction cell. The extraction cell comprises an oven chamber and a pressure valve, which altogether contributes in attaining suitable temperature and pressure in order to extract the compound present in the sample. Then, the extracted compound is cooled and collected in a moving steel chamber. Li et al. [27] depicted the automated pressurized liquid extraction (APLE) method for lipid extraction from dried cells of the oleaginous yeast species *Rhodosporidium toruloides* and *Cryptococcus curvatus*.

7.6 Application

Plant-derived polyphenols such as stilbenes and curcuminoid comprising one or more hydroxyl group acquired from phenylalanine or tyrosine aromatic amino acids are well popularized in food and cosmetic industries. Properties such as antioxidant and anti-inflammatory make them suitable for the prevention of heart disruption and cancer. Genetically engineered bacterium such as *E.coli and* yeast *Saccharomyces cerevisiae* are used for the production of polyphenols commercially because of their ease of cultivation in laboratory under modulated fermentation condition. At present, the use of novel extraction strategies for the extraction of polyphenol bioactives from microbes is in trend, and many new complex structures and biosynthetic metabolic pathways have been elucidated [28, 29].

Table 7.2 lists the biologically active compounds retrained from microbes.

Proteins are considered as essential component in maintaining good health by its consumption, and amino acids are the basic constituents' building blocks for its activity. Leucine, isoleucine, valine, threonine, lysine, methionine, phenyl- alanine, and tryptophan are among the eight amino acids essential required by the human body. All amino acids are commercially produced except for glycine, methionine, and aspartate. *C. glutamicum is* the most prominent strain for the production of amino acids and generally regarded as safe due to its resistant capacity against phage infection. Also *E.coli* is used for the industrial production of amino acids because it is stable at high fermentation temperatures [39, 40]. Also, vitamins which are

Table 7.2 The biologically active compounds retrained from microbes

Bioactive Compounds	Examples	References
1. Polysaccharides	Alginate, cellulose, fucoidan, laminarin, agar, carragenan, furcellaran, mannan, porphyrin, xylan, amylase, amylo-pectin, pectin, xylan, cellulose	Ghosh et al. [30]
2. Lipids/fatty acids	PUFAs, omega3 fatty acids: eicosapentaenoic acid, decosahexanoic acid; omega 6 fatty acids: γ linolenic acid and arachidonic acid	Priyadarshani and Rath [31]
3. Poly-phenols	Polyphenols, with flavonoids, stilbenes, and curcuminoids	Dudnik et al. [32]
4. Pigments	Phycocyanin, phycoerythrin, carotenoids, catotenes: γ carotene and β carotene, lycopene, xanthophylls, chlorophyll, etc.	García-López et al. [33]
5. Antioxidants	Tocopherol, mycosporine-like amino acids	Young and Lowe [34]
6. Proteins and amino acids	Spirulina	Wan et al. [35]
7. Minerals	Ca, Mg, Zn, CO, Na, I, B	Hou [36]
8. Hormones	Auxins, gibberellins, ethylene, cytokinins	Mazzoli et al. [37]
9. Vitamins	$B_{12,}$ K, C, E, D, A	Watanabe and Bito [38]

majorly required in the human body that too in very minute quantities are not synthesized inside the human body so required uptake externally. Vitamin B_{12} cobalamine, vitamin B_2 riboflavin, vitamin C ascorbic acid, and β carotene are produced industrially via chemical transformation reaction [41, 42] .

7.7 Conclusion

The capacity to produce bioactive compounds by microbes via different novel extraction techniques is discussed in this review, along with their significance as cutting-edge sources of naturally occurring bioactive compounds. Microorganisms have simple growth requirements and are easily cultivated under lab condition to produce desired bioactive compound with enhanced productivity via modulating the temperature, pressure, volume, and other necessary constituents. Compared to synthetic bioactive compounds such as antioxidants, the majority of microbial antioxidants are nonmutagenic and noncytotoxic. There is an immense heterogeneity in the microbial population, but its potential of producing bioactive compounds for many microbes are yet to be discovered that may have high biotechnological and pharmaceutical significance in the present era.

The pharmaceutical and nutraceutical sectors are becoming increasingly interested in the production of flavonoids, phenols, alkaloids, vitamins, and steroids using microorganisms including microalgae, bacteria, yeast, actinomycetes, and mushrooms. A few fungi-derived substances with promising antioxidant activity include isopestacin, astaxanthin, pestacin, and polysaccharides. These substances are employed as functional ingredients in food, nutraceutical products, cosmetics, and pharmaceuticals. In-depth investigation and research are required to increase the productivity of popularizing bioactive compounds from fungus.

In particular, *Saccharomyces cerevisiae* is the primary species used in the generation of bioactive substances. Long back yeast is considered as a good food supplement in food industries and is well employed for the production of beverages. Torularhodin, peroxiredoxins, and thioredoxins carotenoids that are gaining attention for its multifunctional role as antioxidant, anticancer, and antimicrobial property opens new possibilities for the discovery of new drugs having high economic importance. More thorough research is required to advance knowledge and maximizes the usage of these microbial bio-active because their mode of action has not been fully investigated. The creation of novel medications and applications that make use of nature's rich biodiversity may result from this as well.

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