Chapter 6
Application of Alternative Technologies for the Recovery of Bioactive Compounds from Microbial Sources

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Abstract Indiscriminate chemical compound usage, such as agrochemicals, pesticides, fungicides, antibiotics, drugs, and other synthetic products, such as dyes, polymers, and heavy metals, have devastated soils, waters, and even living beings themselves. In this way, the development process for providing bioactive compounds for human, animal, and environmental health is one of the most urgent needs to enhance the balance between human exploitation and nature. These needs focus on obtaining healthy and safe products obtained in sustainable processes. Therefore, the search for natural compounds that can be used as nutrients, natural pesticides, and antibacterial or anticancer agents is increased.

Keywords Bioactive · Metabolites · Antimicrobial · Ultrasound-assisted extraction

6.1 Introduction

Plants, animals, and microorganisms are valuable sources of natural products with bioactivity [1, 2]. The natural products market was valued at USD 189 billion in 2021. This market is projected to reach USD 300 Billion by 2030 [3]. Natural compound research from plants is a well-known field. However, further research regarding natural compounds obtained from microorganisms is necessary.

Microorganisms produce primary metabolites such as amino acids, carbohydrates, proteins, and enzymes [3, 4]. Moreover, microorganisms can produce secondary metabolites with potential use for conservation or protection. These compounds are recognized by their bioactive properties, such as antimicrobial, antioxidant, anticarcinogenic, antiparasitic, and anti-inflammatory [2]. Therefore, the production and recovery of these compounds allow for obtaining high-value products. As can be observed in Table 6.1, microorganisms such as Archaea, bacteria, fungi, yeast, algae, and even some parasites produce bioactive compounds.

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Table 6.1 Bioactive compounds derived from different microbial sources

Source	Organism	Bioactive compound (s)	Bioactive properties	References
Archaea	*Haloferax larsenii HA1*	Halocin	Antibacterial protein and cell protector	Kumar and Tiwari [5]
	Halorubrum sp. *SH1*	Bacterioruberin	Antioxidant	de la Vega et al. [6]
Bacteria	***Streptomyces***			
	Streptomyces anulatus NEAE-94	Unsaturated and saturated fatty acids, alkenes, fatty acid esters, alkanes, and triterpenes	Antimicrobial activity against *Staphylococcus aureus*	El-Naggar et al. [7]
	Streptomyces globisporus BU2018	Exopolysaccharides	Antioxidant	Abdel-Aziz et al. [8]
	Streptomyces tunisialbus sp	Fatty acids and menaquinones	Antimicrobial activity against gram-positive, and gram-negative bacteria, yeast, and filamentous fungi	Ayed et al. [9]
	Streptomyces sp. BO7	Biphenyls	Antibacterial, antioxidant, and anticancer	Taechowisan et al. [10]
	Bacillus			
	Bacillus licheniformis	Bacitracin	Antimicrobial	Ali et al. [11]
	Bacillus subtilis	Fengycin	Antifungal	Wu et al. [12]
	Pseudomonas			
	Pseudomonas cedrina	Biomass extract rich in diketopiperazines	Anticancer	Sánchez-Tafolla et al. [13]
	Lactobacillus			
	Lactobacillus coryniformis NA-3	Exopolysaccharides (α-rhamnose, α-mannose, α-galactose, and α-glucose)	Antioxidant and antibiofilm	Xu et al. [14]
Fungus	*Aspergillus* fumigatus MF029	Chaetominine, sphingofungin, emodin, chaetominine, sphingofungin, and trypacidin	Antitubercular activity	Song et al. [15]
	Aspergillus fumigatus	Biomass extract rich in phenolic compounds (rutin, quercetin, caffeic acid, kaempferol, and ellagic acid)	Antibiofilm, antiproliferative, antioxidant, and antimutagenic	Kaur et al. [16]

(continued)

Table 6.1 (continued)

Source	Organism	Bioactive compound (s)	Bioactive properties	References
	Fusarium redolens	Biomass extract rich in chrysophanol and fumaric acid	Antimicrobial	Nazir et al. [17]
Yeast	*Metschnikowia yeast genus*	Alkaloids, antibiotics, and long-chain fatty acids	Antifungal	Fernandez-San Millan et al. [18]
Microalgae	*Nannochloropsis gaditana*	Omega-3 eicosapentaenoic acid	Prevention of cardiovascular diseases	Martínez et al. [19]
	Spirulina (Arthrospira platensis)	Phycocyanin	Anti-inflammatory antioxidant, antiviral, immunity-boosting, and anticancer	Lauceri et al. [20]
	Nannochloropsis oculata and *Porphyridium purpureum*	Biomass extract rich in anticancer and antioxidant activities	Anticancer	Garcia-Parra et al. [21]

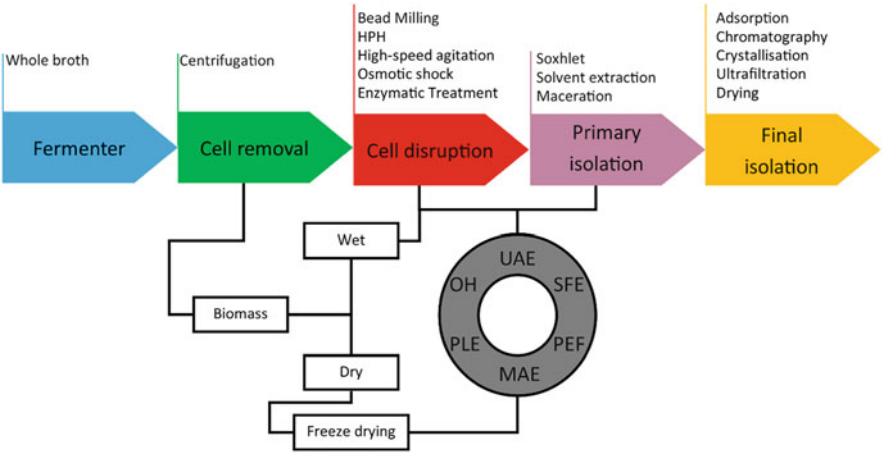

Fig. 6.1 Steps involved in the recovery of bioactive compounds from microorganisms

The production of the compounds depends on the environmental or culture media conditions. Moreover, most of these bioactive compounds could have high demand in the medical, pharmacological, and food industries [22].

The recovery of bioactive compounds from microbial sources comprises several steps in a downstream process. A further selection of recovery and purification steps will depend on whether the product is inside or outside the cell. Figure 6.1 represents the main steps involved in the recovery of bioactive compounds from microorganisms. Cell disruption is an initial step that is fundamental in the recovery of

compounds, and thus, the disruption method choice is crucial. In this context, the cell wall composition also influences the disruption method performance. For example, some microalgae with high cellulose, glucose, and mannose contents have more rigid cell walls. Moreover, cells in the stationary phase or growth in rich nutrient media can have strong cell walls, which influences the selection of the disruption method and its parameters [23].

Among conventional disruption methods, mechanical and nonmechanical methods such as bead milling, high-pressure homogenization (HPH), osmotic shock, and enzymatic treatments are used for bioactive compound recovery. In the past decades, many alternative technologies have been explored for bioactive extraction from microbial sources, such as ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), pulsed electric field extraction (PEF), microwave-assisted extraction (MAE), and others (Fig. 6.1). These alternative approaches are considered environmentally friendly and enhance extraction yields. In this context, the extraction with no previous cell disruption represents an excellent alternative to less energy-consuming process development. Alternative extraction techniques such as UAE, PEF, and MAE are mechanical methods that apply mechanical forces by waves or electric currents to break the cellular membrane.

6.2 Bioactive Compounds from Microbial Sources

Microorganisms produce novel antimicrobial, antitumoral, and anti-inflammatory molecules. Moreover, these compounds have potential applications in the biotechnological, nutraceutical, pharmaceutical, and environmental industries [24]. As shown in Table 6.1, bioactive compounds with antimicrobial, antifungal, and antioxidant activities are produced by Archea, Prokaryotic, and Eukaryotic domains. Moreover, the production of macromolecules such as amino acids, proteins, lipids, and carbohydrates is influenced by temperature, pH, humidity, aeration, and substrate. In this context, the interaction between these parameters and niches like oceans, mangroves, and caverns, even specific parts of plants or animal represent new opportunities to find novel bioactive compounds [25]. However, further research is necessary to identify the easy-to-cultivate and most productive microorganisms to scale up the production on a large scale for their subsequent application. Next, mainly microbial sources of bioactive compounds are described.

6.2.1 Main Microbial Sources of Bioactive Compounds

6.2.1.1 Archea Bioactive Compounds

Archaea compounds are produced under extreme conditions such as salt saturation, high temperature, and elevated UV radiation [26]. *Archea* produces bioactive

compounds such as exopolysaccharides, carotenoids, and proteins. These compounds have potential applications in biomedical, pharmaceutical, cosmetic, environmental, and industrial fields. Gómez-Villegas et al. [27] reported strains of *Haloarchaea* as a potential source of compounds. *Haloferax larsenii* HA1 [28] produces halocins and sulfolobicins [29]. Halocins and sulfolobicins are proteinic compounds that could be used as food preservative because it causes cellular deformation and release of cell contents leading to cell death. Carotenoids are produced by *Haloarcula japónica*, *Halobacterium salinarum*, and *Halococcus morrhuae*. Moreover, extremozymes are produced by *Pyrococcus furiosus*, *Thermococcus littoralis*, and *Thermus aquaticus* [30]. On the other hand, some pigments, such as carotenoids, bacteriorhodopsin, and bacterioruberin, are produced by *Halobacterium Salinarum*. These compounds help cells to adapt to hypersaline conditions by acting as a water barrier, allowing ions and oxygen molecules to pass through the cell membrane. Therefore, these compounds can be used as antioxidants and photoprotective in food and cosmetics.

6.2.1.2 Bacteria Bioactive Compounds

Actinomycetes are one of the most reported genera that produce bioactive compounds. For example, *Streptomyces* spp. can synthesize microbial compounds such as vinaceuline, bafilomycin, and antimycin [24]. Moreover, antioxidant compounds such as violacein and prodigiosin are produced by *Streptomyces rubrireticuli* and *S. longisporus ruber* [31]. These molecules or their derivatives are known for their antimalarial, antibacterial, and anticancer activities. Other strains of the genera *Bacillus, Pseudomonas, Myxobacteria, Cyanobacteria* [1], and *Lactobacillus* [32] can produce other bioactive molecules. For example, the antimicrobial compounds bacitracin and bacilysin are produced by *B. liquenoformes* and *B. subtilis*, respectively [33]. On the other hand, *Pseudomonas* spp. (*P. aeruginosa, P. fluorescent,* and *Pseudomonas chlororaphisin*) produce antimicrobial compounds such as pyocyanin [3].

Lactic acid bacteria (LAB), such as *Lactococcus* and *Pediococcus*, have been reported to produce bacteriocins. Bacteriocins are known as immunomodulators with antimicrobial activity [32]. Phenolic compounds with antioxidant properties such as chlorogenic acid and gallic acid are produced by several cyanobacterial species [31]. On the other hand, fabclavines, xenocoumacins, xenorhabdins, and PAX peptides are antiparasitic compounds identified in *Xenorhabdus* and *Photorhabdus* [34]. Strains of *Photorhabdus luminescens* and *Xenorhabdus nematophila* showed anti-trypanosomal activity and potential use to develop novel drugs against Chagas disease [35]. Prokaryotes are an excellent choice for bioactive compound production due to their metabolic versatility and easy handling in the laboratory. Moreover, synthetic biology tools or heterologous systems could enhance bioactive compound production [31, 36].

6.2.1.3 Fungi and Yeast Bioactive Compounds

Fungi are eukaryotic organisms known to habit almost all ecological niches of the Earth, especially where there are organic sources and are in a state of decomposition. Many bioactive compounds are generated after mycelial growth and can affect direct sporulation. For example, *Aspergillus nidulans* and *Fusarium gramineaum* produce linoleic acid and zearalenone. Moreover, *Alternaria alternata* produces melanins as a proactive compound from UV rays. In addition, *Aspergillus terreus* has been recognized to produce lovastatin, which has metabolic activity. The most reported groups of secondary metabolites in this domain are polyketides, no ribosomal peptides, and terpenes [3]. Some endophytic fungi isolated from plants possess antimicrobial, antioxidant, and cytotoxic activities [37]. For example, compounds with antiprotozoal, antibacterial, and antiviral activity have been found in endophytic fungi such as *Colletotrichum, Diaporthe, Fusarium, Trichoderma, Penicillium*, and *Xylariagenera* [25]. Moreover, yeasts and other nonfilamentous eukaryotic microorganisms produce metabolites with antifungal such as piperidine and protoemetine (alkaloids), p-coumaroyl quinic acid (phenylpropanoid), which are produced by *Metschnikowia pulcherrim*a [18]. Moreover, the yeast used in fermented beverage production (wine, beer) produces alcohols such as tyrosol, which is responsible for the flavor in fermented beverages and is recognized for its antioxidant and cardioprotective properties [38]. Yeast can also be used as a model cell for genetic engineering assays to produce compounds derivate from plants when the expression in a complex system is required [39].

6.2.1.4 Microalgae Bioactive Compounds

Microalgae are found in oceans, fresh and wastewater, and extreme environments. Microalgae are an excellent source of metabolites such as fatty acids, carbohydrates, proteins, vitamins, and bioactive compounds [19]. Antimicrobial and anticancer compounds have been found in microalgae [21]. For example, phenolic compounds and hydroxycinnamic acids such as gallic acid, chlorogenic acid, ferulic acid, and caffeic acid have been found in *Chlorella vulgaris, Haematococcus pluvialis, Diacronema lutheri, Phaeodactylum tricornutum, Tetraselmissuecica, Ankistrodesmussp., Spirogyrasp., Euglena cantabrica, Caespitella pascheri, and Porphyridiumpurpureum* [31]. Metabolites such as exopolysaccharides with immunomodulatory, anti-inflammatory, antiviral, antifungal, and antibacterial capacities are produced by *Porphyridium* sp., *Arthospira* sp., and *Chlorella* sp. [21]. On the other hand, some specific compounds, such as polyunsaturated aldehydes with anticancer activity, are found in marine diatoms [40]. Therefore, microalgae could be an excellent source of novel bioactive compounds with multiple applications.

6.3 Production of Bioactive Compounds

During the past few decades, the bioactive compounds market from microbial sources has been growing due to its impact on the agriculture, food, and pharmaceutical industries. For example, the agroindustry uses bioactive compounds for pest control and plant growth promotion. Therefore, to take advantage of all properties offered by microorganisms, it is necessary to develop a sustainable process that produces bioactive compounds at low cost and high quality and effectiveness. Next, an overview of the main compounds produced by microbial sources is presented. Almost all bioactive compounds from microorganisms are related to their antimicrobial activity. Currently, there is a concern regarding the increase in antimicrobial resistance. As mentioned before, some antimicrobial molecules can disrupt the cell membrane. For example, membrane synthesis is inhibited by lipopeptides and polymyxin produced by *Bacillus* sp. and *Paenibacillus polymyxa* [33], respectively. In that context, antibiotics such as streptomycin, gentamicin, and tetracycline are produced by *Streptomyces griseus*, *Micromonospora purpurea*, and *Streptomyces aureofasciencs* [24]. These antibiotics inhibit protein synthesis in cells. Many of these compounds are recovered from marine microorganisms, which grow in extreme temperatures, under osmotic stress [41]. On the other hand, compounds such as bacteriocins isolated from the gastrointestinal tract are recognized for their immunomodulatory properties and antimicrobial capacity. Moreover, bacteriocins are used as a food preservative [32].

In the case of microalgae, the antimicrobial activity is related to the overproduction of fatty acids that can reduce the ability to breathe and cause cell death [24]. On the other hand, antifungal compounds such as glycolipids produced by *Bacillus licheniformis* can inhibit *Aspergillus niger* [2]. Antioxidant compounds such as polyphenols, carotenoids, or exopolysaccharides are produced by *Aspergillus* spp. *and Artrospira* sp., among others. These compounds can scavenge free radicals and are recognized for their photo-protective properties. Exopolysaccharides are high-molecular-weight carbohydrate polymers with radical scavenging activities, metal chelation activity, and lipid peroxidation inhibition [31]. These compounds are one of the most exploited bioactive substances due to their antiaging capacity. Other bioactive properties, such as anticholinesterase, antituberculosis, and antimalarial activity, have been shown in microorganisms. Further research is necessary to explore the microbial capacity to obtain bioactive compounds. Combined with the develop alternative extraction processes can allow the obtaining of pure and safe molecules with application in the medical field, pharmaceuticals, food, and environment industries.

6.3.1 Conventional Extraction Processes

Conventional extraction methods such as maceration, Soxhlet, solvent extraction, and hot reflux extraction have been used for compound recovery from microbial sources. Although they consume large quantities of solvents and employ longer extraction times, these processes are recognized for their simplicity and low-cost implementation [42]. Generally, they are used as a reference to compare with alternative technologies. Maceration is a straightforward extraction process carried out at ambient temperature under agitation. Bioactive compounds from *Pleurotus ostreatus* were recovered by maceration after 90 min at 25 °C, 150 rpm, using water and ethanol as extraction solvents [43]. Different fractions rich in proteins and phenolic compounds were recovered depending on the solvent proportion. For example, a mixture with 95% ethanol enhances protein extraction, while 50% ethanol increases the content of phenolic compounds. In a similar approach, Daud et al. [44] recovered red pigments from the fungus *Monascus purpureus* at 30 °C but during 16 h under agitation (180 rpm). The red pigment solubility depends on the solvent polarity, where the best solvent was 60% ethanol, which allows a maximum yield of 207 AU/g dry fermented solids. On the other hand, hot water extraction is used for obtaining polysaccharides from mushrooms. For example, polysaccharides were obtained from *Ganoderma resinaceum* by hot reflux extraction at 100 °C for 8 h [45].

Recently, Soxhlet extraction of oil from the microalgae *Spirogyra* [46] and *Chlorella pyrenoidosa* [47] was studied using extraction times ranging between 1 and 4 h at boiling temperatures depending on the solvents (n-Hexane and 2-Methyltetrahydrofuran). Oil extraction from *Spirogyra* required previous drying and milling pretreatments to enhance the extraction process due to a finer algae size causing better contact with the solvent. Moreover, usually, the more dried, the higher yield. In the drying/dehydration processes, freeze-drying is one of the most used methods. Low temperatures employed by freeze-drying keep the integrity of the compounds. However, freeze-drying is energy consuming, which could limit its application at the industrial scale. In this context, the direct extraction from wet biomass is getting more attention in the scientific community. For example, wet biomass was used as raw material in the oil recovery from *Chlorella pyrenoidosa* by Soxhlet [47]. Although lipid extraction is enhanced, total fatty acids content presented a reduction. Therefore, cell pretreatment influences extraction performance in conventional extraction processes as well as in alternative techniques. Thus, further research is necessary to establish the optimal process conditions. As mentioned, high temperatures and long extraction times are characteristics of conventional extraction methods. Therefore, energy consumption and thermally compound degradation are drawbacks that must be overcome. However, these techniques will continue to be used to compare alternative or novel technologies.

6.3.2 *Alternative Extraction Technologies*

Over the past decades, the recovery of bioactive compounds using alternative technologies has been focused on by researchers in many fields, such as food, chemical, or biotechnology. Generally, alternative extraction methods are more feasible than conventional extraction methods. The use of solvents generally recognized as safe (GRAS), the shorter extraction times, and the higher extraction yields are the main advantages of these methods. Among the most popular alternative extraction methods, ultrasound, microwaves, supercritical fluids, pressurized fluids, and electric fields are the most used technologies for bioactive extraction from microbial sources. Next, the concepts and applications of alternative extraction methods will be presented.

6.3.2.1 Ultrasound-Assisted Extraction (UAE)

Ultrasound is one of the most used extraction technologies employed in the bioactive compound recovery from fruits, vegetables, herbs, spices, seeds, and microorganisms. In UAE, cell disruption is caused by an acoustic phenomenon known as cavitation. In cavitation, the ultrasound waves generate rarefaction and compression cycles, creating gas bubbles in the cytoplasm. Once the bubbles have reached a maximum size, they collapse and release large amounts of energy (5000 K and 2000 atm) [48]. The cell wall is disrupted due to mechanical effects, the solvent has more intimate contact with the target compounds, and the extraction rates are enhanced [49]. Extraction parameters such as ultrasound power, frequency, temperature, solvent, type of device, and extraction time influence the extraction process. As shown in Table 6.2, temperatures ranging from 25 °C and 70 °C are used for compound recovery. Ultrasonic power from 100 W to 1000 W and short extraction times are used (e.g., minutes). Regarding extraction solvents, GRAS solvents such as water, ethanol, buffers, and deep eutectic solvents (DESs) are used. DESs are recognized as environmentally friendly, inexpensive, and chemically stable [50].

Depending on the microorganism, the effect on extraction parameters can be different. Generally, an increase in the ultrasound power, temperature, and extraction time increases the recovery of the compounds. The increase in the ultrasonic power enhances the cavitation, the cell structure is disrupted faster, and the solvent penetrates more efficiently [51]. The increase in temperature enhances the solubility of the compounds. Moreover, the denaturation of the membrane can be promoted by temperature [52]. However, excessive temperature or ultrasonic power may trigger the degradation of the compounds through the failures of the chemical structure or the generation of ROS [53]. Regarding frequency, cell disruption is enhanced by the acceleration caused by higher frequencies. The higher the frequency, the smaller the cavity sizes and the faster the bubbles collapse [48].

Table 6.2 UAE applications of bioactive compounds from microbial sources

Species	Compound (s) recovered	Extraction conditions	References
Morchella importuna	Polysaccharides	62 °C, 600 W, 31 min, choline chloride/oxalic acid (DESs)	Pan et al. [50]
Dictyosphaerium sp.	Polysaccharides	50 °C, 500 W, 50 min, water	Chen et al. [51]
Saccharomyces cerevisiae	Polysaccharides	70 °C, 1000 W, 8 h, 0.2 M sodium hydroxide	Eom et al. [58]
Arthrospira Platensis	Lutein/zeaxanthin	60–70 °C, 10 min, methanol	Sam et al. [59]
Chlorella vulgaris and *Porphyridium purpureum*	Carotenoids	70% power, ethanol (60%)	Vintila et al. [60]
Nannochloropsis gaditana	Omega-3 long chain-polyunsaturated fatty acids	50 °C,100 W, 30 min, ethanol	Castejón and Marko [61]
Diaporthe schini	Antioxidant compounds	25 °C, 400 W, pulsed mode (0.93), 15 min, ethanol	da Rosa et al. [62]
Saccharomyces cerevisiae, saccharomyces boulardii, Metschnikowia fructicola andTorulaspora delbrueckii	Mannoproteins	80% amplitude, 4 min, 0.1 M phosphate buffer, pH 6.5	Snyman et al. [63]
Grifola frondosa	Polysaccharides	65 °C, 4.5 h, water	Ji et al. [64]
Agrocybe cylindracea	Dietary fiber	Ultrasonic-assisted enzymatic method, the α-amylase concentration of 1.50%, protamex concentration of 1.20%, 150 W	Jia et al. [65]
Haematococcus pluvialis	Astaxanthin	25 °C, 80% amplitude, pulsed mode (3 min off and 12 min on), $(NH_4)_2SO_4$ salt solution/2-propanol	Khoo et al. [66]
Porphyridium cruentum and *Porphyridium purpureum*	Proteins, carbohydrates, lipids, fatty acids and phycoerythrin	30 °C, 100 W, 13–15 min, 50 mM Na-phosphate buffer (*P. cruentum*), and water (*P. purpureum*)	Ardiles et al. [67]

6.3.2.2 Supercritical Fluid Extraction (SFE)

SFE uses substances at temperatures and pressures above their critical point. These substances are known as supercritical fluids (SCF). Above the critical point, the fluids can diffuse as gas and has liquid solvation power [54]. For SFE, generally, before extraction, the microbial biomass is freeze-dried and disrupted. For instance, a ball mill is used to enhance the extraction of intracellular compounds from *Scenedesmus almeriensis* [55] and *Nannochloropsis* sp. [56]. CO_2 is the most used

Table 6.3 SFE applications of bioactive compounds from microbial sources

Species	Compound (s) recovered	Extraction conditions	References
Scenedesmus almeriensis	Lutein	65 °C, 550 bar, 14.48 g/min CO_2	Mehariya et al. [55]
Nannochloropsis sp.	Omega-3 fatty acids	75 °C, 550 bar, 14.48 g/min CO_2	Leone et al. [56]
Aurantiochytrium sp.	Omega-3 fatty acids and phenolic compounds	80 °C, 300 bar, 12 g/min CO_2	De Melo et al. [68]
Diaporthe schini	Antioxidant compounds	40 °C, 250 bar, 4 g/min CO_2, biomass:Ethanol, 1:1.5 (w/v)	da Rosa et al. [69]
Usnea subfloridana	Usnic acid	85 °C, 150 bar, 2 mL/min CO_2	Boitsova et al. [70]
Schizochytrium sp.	Docosahexaenoic acid (DHA)	77 °C, 465 bar, 5 mL/min CO_2, 1.25 mL/min ethanol	Rodríguez-España et al. [73]
Inonotus obliquus	Triterpenoids	50 °C, 350 bar, 3 mL/min CO_2	Huynh et al. [74]
Coccomyxa onubensis	Lutein and phenolic compounds	70 °C, 400 bar, 2 mL/min CO_2, 2.30 mL/min ethanol	Ruiz-Domínguez et al. [75]
Haematococcus pluvialis	Astaxanthin	50 °C, 500 bar, 2 L/min CO_2	Espinosa Álvarez et al. [76]
Chlorella vulgaris	Phenolic compounds	60 °C, 250 bar, 40 g/min CO_2 (ethanol 10% w/w)	Georgiopoulou et al. [77]

solvent in SFE, and it is recognized as safe (GRAS), inexpensive, has low toxicity, readily available, and has an easily accessible critical point (31 °C and 73.8 bar) [57]. Temperatures between 40 °C and 85 °C and pressures between 250 and 550 bar are suitable for bioactive compound extraction (Table 6.3). The selectivity of the CO_2 is modified by changing the temperature and pressure. For example, the solvent density increases as the temperature increases, and the solvent density increases as the pressure increases. Thus, the solubility of the intracellular compounds is enhanced by increasing the pressure at a constant temperature. This behavior has been observed in the recovery of omega-3 fatty acids and phenolic compounds from *Nannochloropsis* sp. [56] and *Aurantiochytrium* sp. [68].

Although CO_2 is the most common SFC used for bioactive compound extraction from microbial sources by SFE, it only allows the extraction of nonpolar compounds as lipids. Thus, CO_2 is used mainly for lipid or fatty acid extraction from microbial sources such as microalgae, as shown in Table 6.3. On the other hand, for polar compound extraction (e.g., phenolic compounds), a co-solvent such as ethanol is necessary. For instance, ethanol is used as a co-solvent for bioactive compound extraction from fungi [69], microalgae [55], and lichen [70]. SFE allows obtaining higher purity extracts while solvent recycling is possible.

Table 6.4 MAE applications of bioactive compounds from microbial sources

Species	Compound (s) recovered	Extraction conditions	References
Nannochloropsis oceanica	Proteins	40 °C, 700 W, 30 min, choline acetate	Motlagh et al. [72]
Rhizopus oryzae	Chitosan	300 W, 22 min, 1 N NaOH	Sebastian et al. [81]
Haematococcus pluvialis	Astaxanthin	75 °C, 700 W, 7 min dimethyl sulfoxide	Aslanbay Guler et al. [82]
Auxenochlorella Protothecoides	Lipids	2.8 kW, 200 μs pulse, cell suspension	Zhang et al. [83]
Chlorella vulgaris and Botryococcus braunii	Lipids	400 W, 40 s, cell suspension	Rokicka et al. [84]
Kappaphycus alvarezii	β-Carotene, chlorophyll, antioxidants	45 °C, 170 W, 12.5–14.5 min 80% methanol	Baskararaj et al. [85]
Psylocibe cubensis	Psilocin and psilocybin	50 °C, 600 W, 5 min, 60% methanol	Polo-Castellano et al. [86]
Lactococcus lactis	Menaquinones	50 °C, 600 W, 5 min, ethanol	Lee et al. [87]
Porphyridium cruentum and *Porphyridium purpureum*	Proteins, carbohydrates, lipids, fatty acids, and phycoerythrin	200 W, 60 s, 50 mM Na-phosphate buffer/ water (54:46 v/v)	Ardiles et al. [67]

6.3.2.3 Microwave-Assisted Extraction (MAE)

MAE is an alternative technology recognized by the shorter extraction time and the use of GRAS solvents, which increase extraction yields and preserve the integrity of the extracts. MAE has several applications, mainly in the food industry, regarding the extraction of bioactive compounds. In the compound recovery from microorganisms, microwaves are applied to a cell suspension prepared with an organic solvent. The cell suspension can be prepared using wet or dried biomass. Among organic solvents, dielectric or polar solvents such as water or ethanol are preferred. Microwaves with frequencies ranging between 300 MHz and 300 GHz cause fast boiling of the intracellular liquid, which increases the internal pressure and the size expansion of the cells, producing cell disruption [71]. However, although microwaves can cause cell disruption, previous cell disruption (e.g., high-pressure or bead milling) of microorganisms such as microalgae is recommended before MAE [72]. This pretreatment increases the cell wall disruption and enhances the extraction yields. Microwave power, temperature, solvent, extraction time, and matrix are the main parameters that influence MAE. Interaction between solvent and compounds is fundamental because the target compound should be highly soluble, and the solvent must have a high dielectric constant. Solvents such as water, methanol, and ethanol can absorb high amounts of microwave energy, and as shown in Table 6.4, these

solvents are used for compound recovery from microorganisms. Temperature and extraction time are related. Higher temperatures and longer extraction times allow an increase in extraction yields [78]. For example, as can be observed in Table 6.4, the recovery of compounds from microorganisms is performed using temperatures and extraction times up to 75 °C and 30 min. However, exposure to high temperatures during prolonged times triggers compound degradation.

6.3.2.4 Pulsed Electric Field (PEF) Extraction

PEF is a nonthermal technology with growing interest in biotechnology industries for cell disruption due to its many advantages. PEF is an environmentally friendly process with shorter extraction times that increases extraction yields, avoiding triggering the degradation of the bioactive compounds [79]. In PEF, the sample is placed in the treatment chamber where a uniform and strong electric field is applied. The pass of short high-voltage electric pulses causes an electropermeabilization of the cell membranes without altering the bioactive compounds [80]. This permeabilization allows the recovery of the compounds from the microorganisms, minimizing the formation of cell debris with further simplification of the downstream operations.

As can be observed in Table 6.5, electrical impulses ranging between 15 kV/cm and 40 kV/cm are enough to allow the recovery of the compounds from the microorganisms. Although the effects of the electric field strength depend on the matrix characteristics, this range generates the irreversible permeabilization of microbial cells [88]. During membrane permeabilization, many transmembrane pores are formed, which enhances solvent penetration and further extraction of the bioactive compounds. For example, in the extraction of carotenoids from *Xanthophyllomyces dendrorhous* after PEF at 20 kV/cm for 135 μs, 80% of permeabilization was obtained, which increases the extraction yield up to 70% of total carotenoids contained in the yeast suspension. Moreover, extraction parameters such as extraction time, pulse width, conductivity, and pulse frequency can also influence permeabilization and PEF efficiency. For example, the increase from 25 kV/cm to 40 kV/cm in the electric field in the extraction of lipids from *Chlorella* cells increases lipid extraction [89]. However, when the electric field strength reaches a threshold value, the lipid extraction yield decreases due to the release of other compounds and the generation of large amounts of cell debris. This technology could represent many advantages at the industrial scale due to its low-energy consumption and easy incorporation into the processing line [90].

6.3.2.5 Other Alternative Technologies

Other alternative technologies are used for bioactive compound recovery from vegetal sources but with less intensive application in microbial sources. For example, pressurized liquid extraction (PLE) uses solvents above their boiling point but

Table 6.5 PEF applications of bioactive compounds from microbial sources

Species	Compound (s) recovered	Extraction conditions	References
Chlorella vulgaris	Water-soluble proteins, carbohydrates, and lipids	25 °C, 20 kV/cm, 100 kJ/kg_{SUSP}, 5 μs of pulse width, water (1 h), ethyl acetate (3 h)	Carullo et al. [80]
Chlorella	Lipids	35 kV/cm, the conductivity of 400 μS/cm, water,30 min	Zhang et al. [89]
Chlorella pyrenoidosa	Lipids	25 °C, 20 kV/cm, 6 μs of pulse width, chloroform/methanol	Han et al. [101]
Nannochloropsis oculata	Carbohydrates, proteins, and pigments	30 °C, 40 kV/cm, 10 μs of pulse width, water,30 min	Zhang et al. [102]
Saitozyma podzolica	Lipids	20 °C, 15 kV/cm, 1 μs of pulse width, ethanol and hexane,	Gorte et al. [103]
Xanthophyllomyces dendrorhous	Carotenoids, astaxanthin	25 °C, 20 kV/cm, 3 μs of pulse width, ethanol	Aguilar-Machado et al. [104]

below their critical point, applying high pressures. The high pressure allows deeper penetration of the solvent, and the temperature reduces the solvent viscosity, enhancing the extraction of the compounds [78]. Currently, PLE is mainly applied to contaminant detection in several areas. However, PLE can also be used for compound recovery from microorganisms after drying and cell disruption. Unsaturated fatty acids and carotenoids have been recovered from oleaginous yeasts [91] and microalgae [92–94] using temperatures ranging from 80 °C to 150 °C and pressures of 100 bar. An alternative to PLE is continuous pressurized solvent extraction (CPSE), which uses lower temperatures and pressures than PLE, keeping similar or even higher yields. For example, carotenoids and phycobiliproteins from *Cyanobium* sp. LEGE 06113 by CPSE at 70 °C and 1.5 mL/min (ethanol) have been recovered using CPSE [95].

Ultrahigh pressure extraction (UHPE) is similar to PLE but uses higher pressures (up to 8000 bar). This variation allows performing the extraction process without previous cell disruption due to higher pressure can break down the cell membrane. For example, UHPE (one cycle at 6000 bar at 50 °C) enhanced the extraction of carotenoids from *Haematococcus pluvialis* and *Porphyridium cruentum* microalgae compared with PLE [96]. However, the performance also depends on the microorganisms and type of compound. For example, although UHPE (one cycle at 1000 bar and 50 °C) was not superior to PLE in the carotenoid extraction from *Nannochloropsis oceanica*, UHPE increased the extraction of polyunsaturated fatty acids [93].

Ohmic heating (OH) is based on the Joule effect, where an electric current flows through resistive materials such as the cell wall [97]. Heating in OH is faster and more homogeneous than traditional thermal treatments. Moreover, OH causes cell wall breakdown, enhancing the mass transfer of intracellular compounds. For example, the ethanolic extracts obtained from *Cyanobium* sp. by OH (70 °C,

5 min, and 20 kHz) showed high antioxidant capacity [95]. Moreover, yields and antioxidant activity obtained by OH were better than the extraction by homogenization. Recently, OH has been applied in bioactive compound recovery from microalgae. In this context, nutrients from *Coelastrella* sp. LFR1 [98] were recovered by OH at 217 V/cm and 100 °C, showing higher performance for cell disruption. This combination allows the yield increase of chlorophyll and proteins in microalgae biomass. OH is also used for the recovery of bioactive compounds from *Spirulina platensis*. This photosynthetic cyanobacterium is recognized for producing antioxidant, antiviral, anti-cancer, and anti-inflammatory compounds. Ferreira-Santos et al. [99, 100] reported the feasibility of OH in the recovery of intracellular compounds using temperatures between 30 °C and 50 °C, 4 V/cm, and 20 kHz of frequency. OH is a technology with higher extraction yields, lower energy consumption, and shorter extraction times than conventional extraction processes with potential use at an industrial scale (Table 6.5).

6.4 Conclusions and Future Perspectives

Microorganisms can be an excellent source of valuable compounds with applications in several industries. Antibiofilm, antiproliferative, antioxidant, antimicrobial, anti-inflammatory, and antimutagenic activities are found in bioactive compounds recovered from microbial sources. Depending on the localization of the compounds, different pretreatments and extraction techniques can be explored. Pretreatment and extraction techniques are applied depending on the localization of the compounds. Pretreatment as drying or cell disruption is necessary for intracellular compounds. Some alternative extraction techniques like UAE, MAE, and OH allow simultaneous cell disruption and extraction. Alternative extraction technologies have higher yields and shorter extraction times than conventional processes. However, the initial cost and the lack of scaling-up criteria still are the main shortcomings. Therefore, large-scale systems development and further research regarding process optimization are necessary. To the extent that industrial-scale equipment and economically viable processes are developed, these alternative technologies could be more extensively used.

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