Food Science and Engineering
https://ojs.wiserpub.com/index.php/FSE/

UNIVERSAL WISER PUBLISHER

Review

Cost-Effective Purification of Waste-Derived Enzymes Beyond Chromatographic Approaches: A Sustainable Perspective

Marcelli Powzum Amorim, Vitória Dassoler Longo, Larissa Capeletti Romani, Helen Treichel*

Laboratory of Microbiology and Bioprocess, Federal University of Fronteira Sul, Erechim, RS, CEP 99700-000, Brazil
Email: helen.treichel@uffs.edu.br

Received: 9 October 2025; **Revised:** 19 November 2025; **Accepted:** 3 December 2025

Abstract: The increasing generation of agro-industrial and food waste poses a significant environmental challenge, but also represents an opportunity to obtain high-value biocompounds, such as enzymes. In this context, this article provides a review of the primary purification methods for enzymes derived from waste, with a focus on low-cost and environmentally friendly techniques. Three key methodologies are addressed: precipitation, Three-Phase Partitioning (TPP), and Aqueous Two-Phase Systems (ATPS). Precipitation stands out for its simplicity and cost-effectiveness, and is often employed as an initial purification step. TPP combines extraction and purification into a single process and can be optimized using green solvents. Meanwhile, ATPS enables efficient separation of biomolecules in liquid-liquid systems composed of polymers or salts. The analyzed studies demonstrate that these techniques are promising for recovering enzymes with high activity and yield from low-cost sources while minimizing environmental impact. The review concludes that technological gaps still hinder large-scale application, highlighting the need for more efficient, sustainable, and economically viable methods to valorize waste and advance the circular bioeconomy. Furthermore, the techniques presented in this study highlight the potential for greater economic viability compared to traditional chromatography methods. In addition, they can be scaled up to industrial levels while adhering to sustainability guidelines.

***Keywords*:** Agro-industrial, food waste, precipitation, Three-Phase Partitioning (TPP), Aqueous Two-Phase Systems (ATPS)

1. Introduction

For many years, the use of natural resources, food, and energy has been guided by a model called the linear economy. This model is based on the concept of "extract-produce-discard". However, this economic model is no longer viable, since the world population is expected to reach 9 billion people by 2050 [1]. Inadequate disposal practices pose a significant challenge to achieving the United Nations Sustainable Development Goals (SDGs) and to ensuring a cleaner environment for future generations [2]. With the increase in population, there is consequently an increase in food production and in the amounts of agricultural waste. Therefore, a shift from a linear model to a circular model is necessary. This is to preserve resources, manage waste, and ensure sustainable development for future generations [1].

The growing environmental and public health problems associated with food waste disposal are an increasingly significant global challenge. In developed countries, food waste has reached unprecedented levels, with an estimated

DOI: https://doi.org/10.37256/fse.7120268837

61% of all food produced not being consumed, contributing to significant environmental degradation [3]. Specifically, approximately 2.5 billion tons of food are wasted every year [4]. When improperly managed, this waste decomposes in landfills, leading to the emission of greenhouse gases, contamination of water supplies, and the proliferation of disease vectors, posing serious health risks [5].

A key objective is to establish a system that minimizes food losses during production, where 30% of food waste occurs. These residues can serve as raw materials for high-value-added products through eco-friendly processes. Such waste materials are low-cost, homogeneous in composition, rich in carbohydrates, and abundantly available, making them highly suitable for microbial conversion into bioactive compounds with significant technological and economic value [6]. Recovering biocompounds from waste, such as proteins, enzymes, humic acids, lipids, and short-chain fatty acids, presents a promising economic opportunity [7].

Enzymes catalyze essential biological processes and are safer than synthetic Chemicals [8]. They represent a high-value product, with the industrial enzyme market projected to reach 11.2 billion by 2029, up from 7.9 billion in 2024, at a Compound Annual Growth Rate (CAGR) of 7.2% [9].

Enzymes can be produced through the fermentation of various waste streams, including agricultural and food waste. Numerous studies have demonstrated the successful application of these enzymes in hydrolyzing multiple organic waste materials, including sugarcane bagasse, beet pulp, bread waste, and municipal solid organic waste [10, 11].

While commercial enzyme applications often use crude preparations, pharmaceutical and biomedical applications require purified forms [12]. To fully understand the structure-function relationship of a protein, its characteristics must be studied post-purification. Protein purification involves isolating specific proteins for detailed research on their enzymatic, physiological, biochemical, and structural properties [13].

Conventional purification techniques include fractional precipitation, ion exchange, affinity chromatography, and gel filtration [14]. However, most of these methods are costly, time-consuming, and involve multi-step protocols, resulting in low yields and high operational expenses, rendering them impractical for large-scale applications [15, 16]. Moreover, these methods conflict with the goal of waste-derived enzyme production, as they fail to align with a "greener," environmentally friendly, and circular economy.

In this context, this study aims to review methods for purifying enzymes derived from waste materials. The objective was to compile current knowledge and explore simpler, more cost-effective techniques for valorizing these biocompounds, rather than high-cost chromatographic methods.

2. Discussion

2.1 *Precipitation*

Precipitation has been widely used in enzyme purification. This method utilizes the different solubilities of protein molecules (enzymes) in various solvents to isolate and purify specific enzymes within a mixture [17]. Depending on the solvent and/or salt used, it can be categorized into salt precipitation and organic solvent precipitation, as shown in Figure 1. The latter process potentially denatures enzymes; therefore, the extraction should be performed at lower temperatures.

Ammonium sulfate is the most widely used salt in this technique, as most proteins are precipitated at high molarity, and it has a density that does not interfere with the sedimentation of a wide range of these biocompounds [18]. Ammonium sulfate is a low-cost salt with high water solubility, acting as a chaotropic agent that increases water entropy. It enhances hydrophobic interactions and reduces protein flexibility, thus making the protein's tertiary structure more stable and preventing separation [19].

This technique promotes a protein concentration effect while simultaneously purifying the protein. However, precipitation has low separation capability due to its low specificity and is typically used as a first purification step [18, 20].

The advantages of precipitation compared to other methods lie in its relative simplicity, effectiveness, selectivity, and ease of implementation. Precipitation studies have been conducted for the recovery of biorefinery products (mainly bulk proteins, but also enzymes and humic acids) from a wide range of sources, particularly various types of waste, whether urban or industrial. Isoelectric precipitation is one of the most commonly used methods, with ammonium

sulfate being the agent that achieves the highest recovery yields [7].

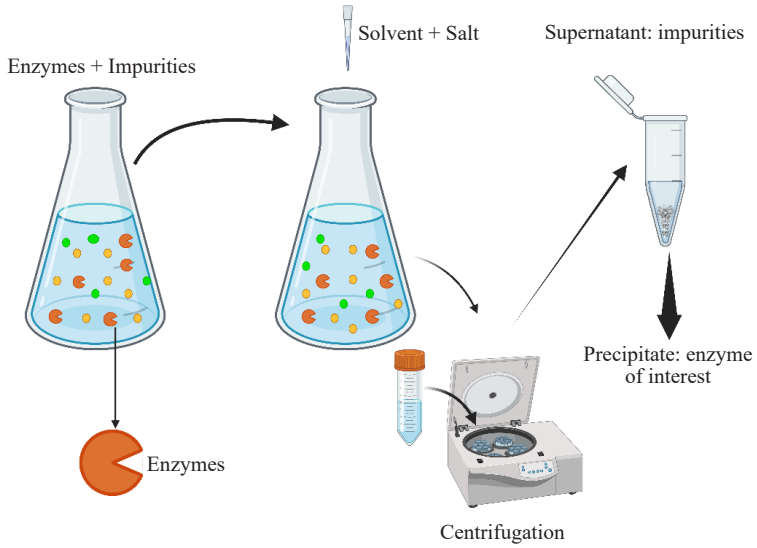

Figure 1. Enzyme purification by the precipitation method

In this context, Table 1 presents recent studies on enzyme purification by precipitation from various waste sources using ammonium sulfate and solvents. Enzymes such as cellulases, amylases, and proteases were obtained from the composted residues of a type of mushroom through precipitation by the gradual addition of ice-cold acetone, resulting in extracts with good activity, especially for cellulase [21]. Another study utilized tangerine peel and an organic solvent to obtain xylanase, achieving a 31.34% recovery [22]. It is evident that precipitation yields low purification rates and is generally regarded as a preliminary method for enzyme separation in most studies. Subsequently, enzymes undergo more refined, yet more costly, purification methods.

Luo et al. [23] explain that, depending on the solvent, precipitation can be categorized mainly into two methods: precipitation with saline solution and precipitation with organic solvent, as observed in the data presented in Table 1. Most studies employ the first method, which presents higher purification efficiencies, as is the case with ammonium sulfate. The data in Table 1 emphasize that ammonium sulfate is a prominent salt in this technique. Furthermore, other types of salts can be used, such as magnesium sulfate, sodium sulfate, sodium chloride, and sodium phosphate [24]. Studies on the specific activity of trypsin in crude visceral extracts reported an initial activity of 0.06 U/mg. After purification by precipitation with ammonium sulfate, the activity increased 3.7 times, reaching 0.22 U/mg [25].

The data presented in Table 1 demonstrate that precipitation is a method that does not yield high results, although the data for protease (73.32%) and amylase (78.38%) are satisfactory. However, for specific enzymes such as amylase, it afforded satisfactory yields, particularly when considering its extreme simplicity and low cost. The combination of waste-derived enzymes and this type of purification method significantly contributes to waste minimization, aligning with the principles of sustainability and the circular economy. The data in Table 1 suggest that the hydrolase class exhibits high purification rates with ammonium sulfate salt. Furthermore, high activity is noted for the L-asparaginase (106.66%) enzyme, a non-peptide bond hydrolase.

Table 1. Examples of enzymes derived from waste purified by the precipitation method

Enzyme	Biomass	Salt/solvent	Recovery (%)	Purification fold	Reference
Protease	Fish processing waste	Ammonium sulphate	73.20	2.1	[26]
Amylase	Rice bran	Ammonium sulphate	78.38	1.84	[27]
Cellulase	Hypogaea shells	Ammonium sulphate	29.64	2.06	[28]
Xylanase	Tangerine peel	Cold acetone	31.34	1.97	[22]
Amylase, cellulase, and protease	Oyster mushroom	Cold acetone	-	-	[21]
Pectinase	Cherimoya and cherimoya pulp	Cold acetone	66.60/24.20	-	[29]
Lipase	Grape and cottonseed oil	Ammonium sulphate/ Acetone/Ethanol	-	3.74/16.76/10.72	[30]
L-asparaginase	Vegetable peels and agro waste	Ammonium sulphate (80%)	106.66	0.99	[31]

Proteases represent one of the most widely used enzyme groups globally in industry, with diverse applications in the food, pharmaceutical, and biotechnological fields. This class of enzymes is typically extracted from living organisms, such as microorganisms [32]. However, fish processing waste generates large quantities of residues that could be raw materials for the extraction of this type of enzyme. Numerous byproducts result from the processing of fish waste. Despite this, fishery byproducts are frequently used in low-value-added applications [33]. The studies listed in this article demonstrate that enzymes, such as proteases, can be extracted from waste and purified through a precipitation process. It becomes possible to achieve satisfactory purification rates using only salts without the need for chromatography, a method that is commonly used and has a high associated cost.

The use of fish waste as a nutrient source is an economical approach. In addition, the enzymes obtained exhibit high thermostability, a characteristic that warrants in-depth analysis for the application and sustainable production of proteases [32, 34]. Future studies can focus on mass production, substrate specificity, and catalytic mechanisms to optimize production and purification for industrial and environmental applications.

2.2 *Three-Phase Partitioning (TPP)*

TPP is a partial purification method that combines extraction, separation, and purification processes into a versatile technology. Figure 2, as demonstrated in TPP, typically shows three phases formed by adding a certain amount of inorganic salt (usually ammonium sulfate) and an organic solvent (generally tert-butanol) to the crude extract. Proteins, including enzymes, accumulate in the intermediate phase. At the same time, low-molecular-weight pigments and lipids separate into the upper phase. And polar compounds (e.g., sugars) remain in the lower phase [35-37].

Several principles are involved in TPP. Among these are saline precipitation, isoionic precipitation, cosolvent precipitation, electrostatic forces, osmotic forces, cosmotropic precipitation, conformational compaction, and changes in protein hydration, which are responsible for the formation of the protein precipitate at the interface [38, 39].

Although the TPP system presents many advantages over traditional extraction and separation technologies, several aspects require further study and improvement. More “green” and efficient methods for extracting bioactive molecules could be developed based on the traditional TPP system. Tert-butanol, for example, is widely used in TPP systems and is a volatile, flammable, and environmentally harmful solvent. In this context, the exploration of “green” solvents as alternatives to tert-butanol becomes essential. Furthermore, greater attention should be paid to the effect of the TPP system on the bioactivity of the target molecules [40].

Deep Eutectic Solvents (DES) have been reported as a promising alternative to toxic solvents. They consist of two or more components (a hydrogen bond acceptor and one or more hydrogen bond donors) that are intentionally prepared through techniques such as heated stirring, grinding, or freeze-drying, among others [41]. Martins et al. [42] recently

defined a deep eutectic solvent as “a mixture of two or more pure compounds for which the eutectic point temperature is below that of an ideal liquid mixture, exhibiting significant negative deviations from ideality ($\Delta T2 > 0$)”, where $\Delta T2$ represents the temperature depression that is the difference between the ideal and real eutectic point.

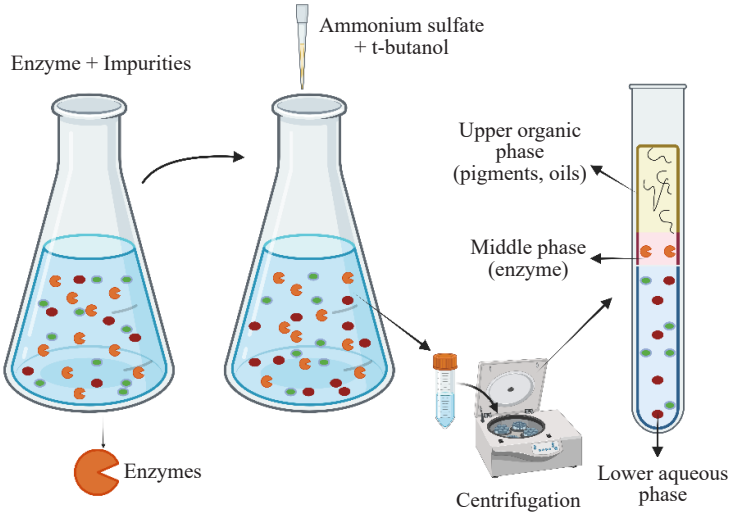

Figure 2. Schematic drawing of a three-phase partitioning system for purifying enzymes

These solvents offer several advantages over conventional solvents, including simple synthesis, environmental friendliness, low or non-toxicity, and promising recyclability. Due to these properties, they represent potential substitutes for traditional organic solvents in extraction and isolation procedures [43, 44]. The physicochemical properties of deep eutectic solvents are one of the main reasons behind the growing interest of researchers in these solvents. In addition to exhibiting low volatility, non-flammability, low vapor pressure, and low chemical and thermal stability, deep eutectic solvents are chemically tunable, allowing them to be designed for specific applications, given the wide variety of compounds that form them [43]. Deep eutectic solvents are currently among the most considered and investigated solvents. Their highly interesting properties increase the possibility of replacing other conventional solvents in numerous sectors, especially industrial ones.

In the TPP process, organic reagents can be used as replacements for tert-butanol. Among them, n-butanol stands out, as it can provide higher yields since its chain structure and molecular size prevent penetration into the protein structure, contributing to the stabilization of the triphasic system [45, 46].

Therefore, integrating TPP with deep eutectic solvents presents an interesting approach that is considered an innovative and current method for isolating and purifying bioactive ingredients such as enzymes derived from waste materials. Another alternative to tert-butanol, frequently reported in recent studies, is dimethyl carbonate. A green solvent with low toxicity and a reduced risk of skin irritation [47]. Ethanol, ethyl acetate, isobutanol, and isopropyl can also serve as substitutes, though the choice depends on the purification efficiency each solvent can provide. Studies have demonstrated better results with dimethyl carbonate. For polysaccharide extraction, Zhang [46] showed that the combination of lauric acid and terpineol represents an efficient and sustainable alternative to the use of t-butanol.

Additionally, many studies have explored the combined use of TPP with ultrasound or microwave irradiation methods to enhance enzymatic purification yields. In hydrolase purification, the authors achieved 142.6 ± 1.9% recovery and a purification factor of 2.38 ± 0.02, with the percentage recovery increasing up to 1.17 times when combining microwave-assisted extraction with TPP [48].

Table 2 shows that the enzymes with the highest purification yields were bromelain (244.00%), protease (253.50%), and collagenolytic protease (232.00%). The authors report that some properties of the TPP system may explain this better yield. The higher the interfacial tension (resulting from the addition of more ammonium sulfate and tert-butanol), the greater the relative amount of protein. However, a lower interfacial tension (10-0.1 $mN \cdot m^{-1}$) provides a mild

condition for protein precipitation, which may also contribute to the preservation of enzymatic activity [49]. Sarkar et al. [50] demonstrated an inverse relationship between the amount of salt needed to precipitate a protein and its molecular weight.

Comparing the data in Table 1 with those in Table 2 demonstrates that TPP achieves significantly higher purification yields, often exceeding 100%. Furthermore, it facilitates handling at room temperature, thus preserving the accessibility of active biomolecules and stabilizing the protein structure. Critical parameters, such as salt concentration, the ratio of enzyme extract to solvent, and pH, are crucial for obtaining different yields. The specific type of enzyme to be purified is also, naturally, a determining factor. Another point to note is that the TPP system still has limited studies regarding the purification of enzymes obtained from waste. This is an area that deserves further investigation, given its proven efficiency in purifying various commercial enzymes or those extracted from microorganisms.

Table 2. Examples of enzymes derived from wastes purified by the TPP method.

Enzyme	Biomass	Yield (%)	Purification fold	Reference
Protease	Banana stem postharvest	127.3	4.2	[51]
Bromelain	Pineapple crown waste	244.00	3.4	[52]
Polyphenol oxidase	Waste potato peels	98.30	19.7	[53]
Peroxidase	Zucchini heads	159.00	10.0	[54]
Laccase	Hardwood of beech	73.00	24.0	[55]
Peroxidase	Orange peels	93.96	18.2	[56]
Protease	Papaya peels	253.50	15.8	[57]
Collagenolytic protease	Intestinal viscera of peacock bass	232.00	5.0	[58]
Protease	Fish wastes	-	3.1	[35]
Lipase	Residue from the hepatopancreas of white shrimp	87.41	3.5	[59]
Protease	Olive residue	56.25	5.3	[60]
Protease	*Solanum dubium* seeds	93.30	2.0	[61]

Although precipitation is a cost-effective and straightforward method, its application in industrial production yields low product purity. In this context, it is pertinent to consider the significant contribution of the solvents used in this method to environmental pollution. In the search for more environmentally sustainable techniques, TPP systems that use deep eutectic solvents pave the way for a new generation of scalable green solvents.

The data in Table 2 demonstrate excellent purification results using the TPP method. Particularly for certain enzymes of technological interest in the environmental field (peroxidase and protease), especially in the bioremediation of emerging pollutants. Enzymatic bioremediation has been attracting growing interest in innovative processes known as “eco-processes”. Offering a promising alternative to replace chemical catalysts with biological catalysts capable of converting highly complex and environmentally hazardous molecules into smaller, non-toxic ones [62, 63].

Peroxidase enzymes are a pertinent example, as they serve as biocatalysts exploited for environmental purposes by biotechnological industries. These protein structures participate in the oxidation/precipitation of hazardous contaminants, rendering them biodegradable and enabling their conversion into value-added products for specific applications [64].

Hydrolases, such as protease (Table 2), can catalyze the initial step of ester bond hydrolysis, and this initial regulatory metabolic reaction accelerates the degradation of pollutants. They can catalyze the first stage of ester bond hydrolysis, a regulatory metabolic process that enhances the degradation of pesticides containing ester groups, such as

organophosphates, pyrethroids, and carbamates [65].

Although some purification techniques have been developed in recent years, their large-scale applicability is still limited. Most methods are expensive, time-consuming, and involve multiple steps, such as chromatography. Considering that purification methods account for 60 to 80% of production costs [66], the demand for accessible and efficient bioseparation methods, such as TPP, is becoming increasingly urgent. It is reported that 70% of the potential savings compared to conventional chromatographic methods can be achieved using TPP [67].

A direct comparison between a chromatography process and TPP reveals a significant cost reduction when the chromatography process is replaced with TPP. The method has a good reputation for increasing the activity of purified enzymes. In addition to stabilizing this activity, it is also possible to immobilize such an enzyme simultaneously. Immobilization results in an industrial biocatalyst with active and robust catalytic potential.

2.3 *Aqueous Two-Phase System (ATPS)*

ATPS are a type of liquid-liquid extraction that offers distinct advantages. It is a widely used technique for separation and extraction, employed not only in the pharmaceutical and chemical industries but also in various other fields, particularly in the handling of unstable substances [68, 69].

Beijerinck first described ATPS in the late 19th century. However, they were rediscovered and first applied in the 1950s by Albertsson to isolate and separate plant organelles and cellular fragments [70]. This method is an efficient and economically viable approach for enzyme purification. Phase separation occurs when two mutually incompatible solutes are mixed in water, resulting in two distinct aqueous phases with different compositions, depending on their relative affinity for each phase [71-73].

The system consists of two compounds dissolved in an aqueous medium, which, at concentrations above a specific threshold, undergo liquid-liquid demixing [74]. It is a highly versatile extraction system, as it can be formed using a variety of different constituents [71, 75]. Implementing this method does not require expensive equipment or reagents and is considered a promising approach due to its high yield, high selectivity, high degree of purification, and excellent reproducibility [76]. ATPs are a suitable environment for biomolecules due to the high water content in both phases (80-95%), and are widely used in the separation and purification processes of biological materials [77].

ATPS has been successfully applied to the purification of various enzymes, including lipase [78], peroxidase [79], and amine dehydrogenase [80], achieving high yields and selectivities.

The most widely studied types of ATPS can be formed by mixing two hydrophilic polymers (polymer/polymer), such as Polyethylene Glycol (PEG) and dextran; a polymer and a high-melting-point salt (polymer/salt), typically dibasic salts or alcohol/salt systems. In polymer/polymer systems, molecular weight and relative hydrophobicity are frequently manipulated to control phase separation and molecular partitioning [81, 82]. A PEG/dextran ATPS using low molecular weight PEG exhibits a narrower biphasic region than systems with PEG at high molecular weight. A lower hydration capacity at high molecular weight PEG creates more unfavorable interactions with hydrophilic dextran, thereby expanding the biphasic region [83]. Souza et al. [79] demonstrated the superiority of ATPS, yielding clearer extracts with higher specific activity and purification factors compared to conventional methods.

In polymer/salt ATPS, the Gibbs free energy of hydration of salts plays a crucial role in defining the biphasic region within the ternary phase diagram. For instance, when using the same polymer, the biphasic region of an ATPS expands with salts exhibiting more negative Gibbs free energy of hydration values (i.e., greater solvation capacity) and contracts with salts showing more positive Gibbs free energy of hydration values [84]. This phenomenon, known as the salting-out effect, is frequently discussed in studies of salt-based ATPS and their partitioning aptitudes. The partition coefficient may also vary depending on the nature of the salts and the system's net charge, which influences the distribution of salt ions between the upper and lower phases [85]. The basis of ATPS separation is the selective distribution of components between two phases. Factors such as the charge of the biomolecule or surface properties, such as hydrophobicity, are crucial [77].

The typical visual representation of an ATPS is a phase diagram. Although the system is ternary, consisting of two polymers (or salt and a polymer) and water, it is often simplified as a binary representation. Two phases will form if the system contains polymers (or a polymer and a salt) at concentrations represented by a point above the binodal curve [86]. The resulting phases differ in composition: the upper phase is predominantly polymer, while the lower phase concentrates polymer (or salt) (Figure 3). Even with the construction of a phase diagram for potential ATPS systems,

their thermodynamic stability is not easily predictable. Increasing polymer and salt concentrations leads to phase separation and the formation of ATPS, resulting in partial dehydration of the solutions. This dehydration is only partial because both phases remain water-rich [87].

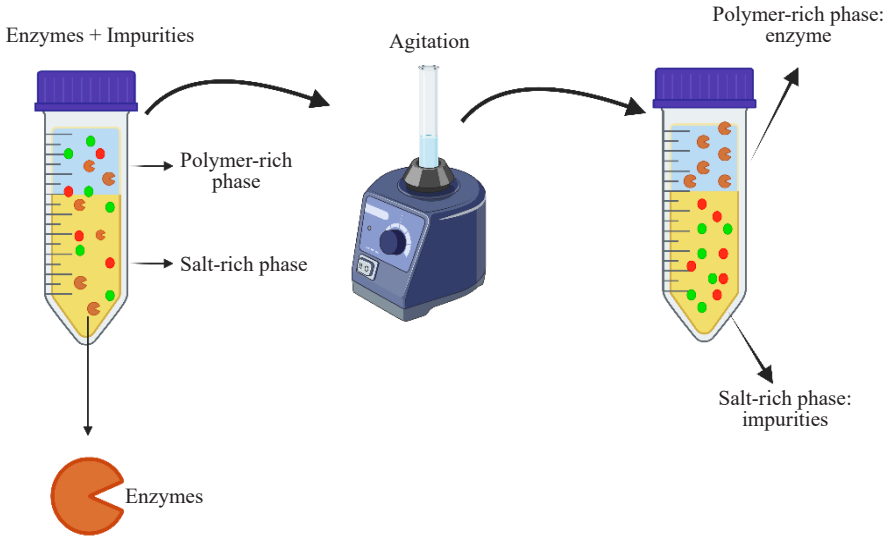

Figure 3. Enzyme purification by ATPS

The partitioning of biomolecules and impurities within the system can be influenced by the choice of phase-forming agent or through the addition of a displacing agent (e.g., neutral salts) [88, 89]. In numerous studies, sodium chloride has been widely employed to drive target biomolecules into the preferred phase (separating impurities), achieving biomolecule yields exceeding 95% [90]. ATPS represent a significant advancement in liquid-liquid extraction techniques, demonstrating considerable promise for environmental protection [91]. Organic salts, such as citrates, tartrates, oxalates, and acetates, are considered more environmentally friendly alternatives to inorganic salts due to their lower environmental impact and reduced corrosion potential [92].

Alencar et al. [93] explain that when the enzyme predominates in the upper phase, the partition coefficient (K) is greater than zero. One of the main variables affecting the partition coefficient is pH. It induces protein partitioning by altering the charge of biomolecules or the proportion of charged molecules [94]. The higher the pH value, the greater the influence on enzyme partitioning into the PEG phase. Furthermore, ATPS is influenced by several factors, including the size and conformation of biomolecules, molecular charge, ionic properties, phase composition, electrical potential between phases, polymer concentration, and polymer molecular size [95].

According to the data presented in Table 3, enzymes derived from microorganisms, such as fungi or yeasts, stand out for providing high purification yields. According to Brígida et al. [96], organic materials such as peels, leaves, and food waste are suitable sources for enzyme purification using ATPS, as they yield consistent values across the studied variables. This is likely due to their homogeneous and well-defined biomass, unlike enzyme extraction from microorganisms, which is influenced by numerous variables.

An analysis of the data in Table 3 reveals that ATPS is an efficient system for enzyme purification. The most significant purification factors were for the enzymes protease (96.87%), lipase (94.65%), and xylose (97.30%). The values of these incomes can be explained by depending on pH, as protein partitioning behavior is strongly influenced by their positive or negative charges, which are determined by their isoelectric points. Typically, positively charged proteins partition into the lower phase, while negatively charged proteins migrate to the upper phase [97, 98].

Table 3. Examples of enzymes purified by the ATPS method

Enzyme	Source	Yield (%)	Purification fold	References
Lipase	Tilapia viscera	64.45	6.30	[99]
Peroxidase	Yacon potato peel	33.87	4.66	[79]
Lipase	Pequi seed	86.39	4.48	[100]
Protease	Estuary of Guanabara Bay/Rio de Janeiro	96.87	1.69	[101]
Xylose reductase	Cashew apple waste hydrolysis	97.30	7.05	[102]
Cysteine protease	Papaya peel	26.38	4.08	[103]
Lipase	Porcine pancreas	94.65	4.36	[104]
Lipase	Soil contaminated with residual oil	87.71	24.14	[105]

The data presented in Table 3 underscore that enzymes such as lipase yielded higher purification efficiencies. Agricultural and industrial residues represent a highly sustainable and low-cost source for lipase extraction. Lipases are versatile biocatalysts capable of mediating a wide range of reactions, including hydrolysis, aminolysis, esterification, and alcoholysis [106]. Furthermore, studies have demonstrated the applicability of these enzymes in environmental bioremediation. For instance, previous work has reported the degradation of the synthetic polyester polycaprolactone by lipases [107]. Other investigations have demonstrated the bioremediation of slaughterhouse wastewater using lipases [108].

Moreover, proteases, which also warrant emphasis based on the data in Table 3, have been successfully employed for the bioremediation of proteinaceous waste and feathers [109, 110]. It has been previously demonstrated that alkaline protease achieved 75% deproteinization of shrimp processing residues [111].

Peroxidases, which also exhibited satisfactory purification factors (Table 3), hold significant potential for application in the bioremediation of various pollutants. As documented in previous studies, these enzymes are capable of degrading volatile organic compounds (e.g., benzene, toluene, ethylbenzene, and xylene), phenolic and non-phenolic substances, as well as polycyclic aromatic hydrocarbons [112-114].

Due to their environmental protection, continuous operation, and ease of amplification, ATPS have been frequently used [115]. ATPS, composed of short-chain alcohols and salts, have attracted increasing attention due to their low viscosity and high mass transfer efficiency, particularly for compositions that can form stable biphasic systems and possess a wide phase formation window [116]. ATPS is proving to be an emerging purification technology. With attractive properties such as time savings, simplicity, and easy scaling up, which make its application economically viable [117].

Innovative techniques that integrate ATPS methods can achieve satisfactory results [93, 118, 119]. From this perspective, they could be alternatives to conventional methods. Furthermore, they offer equal or greater productivity compared to traditional methods. Techniques like ATPS are environmentally friendly, with lower consumption of organic solvents and energy, thus aligning with global sustainability principles.

Recent studies have emphasized that techniques for potential recovery and reuse of phase-forming components in ATPs are crucial for economic viability and sustainability [120]. Efficient recovery methods reduce waste and operational costs, allowing the reuse of polymers, salts, and other agents [121]. Techniques such as ultrafiltration, precipitation, and evaporation can be used with polymers like PEG. These are precipitated and redissolved, while salts are recovered through crystallization [122-124]. Closed-loop systems and advanced methods, such as membrane technologies and selective extraction, increase recovery efficiency and component integrity. This practice reduces environmental impact and supports the large-scale industrial application of ATPS [87]. These principles align with the principles of green chemistry and the Sustainable Development Goals (SDGs). This could be a promising method to replace chromatography.

According to Bekavac et al. [87], despite the advantages of ATPS, several challenges need to be addressed for its widespread adoption in biomanufacturing industries, including understanding the maximum capacity of these systems, addressing the limitations of predictive design, and comparing them with existing platforms in terms of economic and environmental sustainability. Experimental design methodologies have been employed to optimize the purification process conditions in ATPS; however, detailed models that predict the partitioning behavior of biomolecules remain scarce. In this context, it becomes essential that studies be targeted.

3. Conclusions

This review study highlights the current lack of sophisticated and modern, yet low-cost, methods for enzymatic purification. The lower-cost techniques examined in this review (precipitation, TPP, and ATPS) demonstrate that affordable techniques remain limited despite significant technological advancements in enzymatic purification.

It is inconsistent to employ expensive methods for purifying enzymes derived from waste materials, where the primary objectives are cost reduction and process sustainability, without achieving proper waste valorization. Although separation systems have evolved, most still rely on basic separation techniques discovered many years ago. While these techniques can be combined with other methodologies or with each other to enhance purity, yield, and selectivity, they can also preserve enzyme integrity and even increase enzymatic activity during processing, thereby transforming waste into valuable bioproducts.

The purified form of a biomolecule, such as an enzyme, allows for greater volumetric activity and prevents undesirable side reactions caused by contaminating byproducts. Despite these benefits, TPP is primarily confined to laboratories and still faces some challenges in being implemented in industrial applications.

Future research in this field should focus on optimizing performance through computational modeling, artificial intelligence, and the development of novel materials. Emphasizing studies that enhance the efficiency of techniques while adhering to sustainable principles is crucial for effective waste management and the development of high-value enzymes for diverse industrial applications. Through this comprehensive review, which outlines the limitations of current techniques and the need for novel methodologies, we aim to pave the way for scientific exploration and innovation in low-cost enzymatic purification.

Acknowledgments

The authors thank the Brazilian Funding Agencies: Brazilian National Council for Scientific and Technological Development (CNPq-302484/2022-1), Coordination of the Superior Level Staff Improvement (CAPES-001), the support of the Bioprocess and Biotechnology for Food Research Center (Biofood), which is funded through the Research Support Foundation of Rio Grande do Sul (FAPERGS-22/2551-0000397-4 and 24/2551-0001209-5), Federal University of Fronteira Sul (UFFS).

Funding

CAPES, CNPq, and FAPERGS

Conflict of interest

On behalf of my co-authors, I declare that we have no conflict of interest related to this article.

References

[1] Hoang AT, Pandey A, Huang Z, Nižetić S, Le AT, Nguyen XP. Biofuels an option for agro-waste management. In: *Environmental Sustainability of Biofuels: Prospects and Challenges.* Elsevier; 2023. p.27-47.

[2] Ezeorba TPC, Okeke ES, Mayel MH, Nwuche CO, Ezike TC. Recent advances in biotechnological valorization of agro-food wastes (AFW): Optimizing integrated approaches for sustainable biorefinery and circular bioeconomy. *Bioresource Technology Reports*. 2024; 26: 101823.

[3] Igini M. *10 food waste statistics in America.* 2022. Available from: https://earth.org/food-waste-in-america/ [Accessed 10th December 2025].

[4] World Wildlife Fund. *Driven to waste: The global impact of food loss and waste on farms.* 2021. Available from: https://www.worldwildlife.org/publications/driven-to-waste-the-global-impact-of-food-loss-and-waste-on-farms [Accessed 10th December 2025].

[5] Guimarães NS, Reis MG, Fontes LDA, Zandonadi RP, Botelho RBA, Alturki HA, et al. Plate food waste in food services: A systematic review and meta-analysis. *Nutrients*. 2024; 16(10): 1429.

[6] Pal P, Singh AK, Srivastava RK, Rathore SS, Sahoo UK, Subudhi S, et al. Circular bioeconomy in action: transforming food wastes into renewable food resources. *Foods*. 2024; 13(18): 3007.

[7] Núñez D, Oulego P, Collado S, Riera FA, Díaz M. Separation and purification techniques for the recovery of added-value biocompounds from waste activated sludge: a review. *Resources, Conservation and Recycling*. 2022; 182: 106327.

[8] Gupta R, Beg QK, Lorenz P. Bacterial alkaline proteases: molecular approaches and industrial applications. *Applied Microbiology and Biotechnology*. 2002; 59(1): 15-32.

[9] Markets and Markets. *Industrial enzymes market by type (carbohydrases, proteases, lipases, polymerases & nucleases), application (food & beverages, bioethanol, feed, detergents, wastewater, soil, and oil treatment), source, formulation, and region-global forecast to 2029*. Available from: https://www.marketsandmarkets.com/Market-Reports/industrial-enzymes-market-237327836.html [Accessed 10th December 2025].

[10] Marzo C, Díaz AB, Caro I, Blandino A. Conversion of exhausted sugar beet pulp into fermentable sugars from a biorefinery approach. *Foods*. 2020; 9(10): 1351.

[11] Ladakis D, Stylianou E, Ioannidou SM, Koutinas A, Pateraki C. Biorefinery development, techno-economic evaluation and environmental impact analysis for the conversion of the organic fraction of municipal solid waste into succinic acid and value-added fractions. *Bioresource Technology*. 2022; 354: 127172.

[12] Sriariyanun M, Kitiborwornkul N, Tantayotai P, Rattanaporn K, Show PL. One-pot ionic liquid-mediated bioprocess for pretreatment and enzymatic hydrolysis of rice straw with ionic liquid-tolerance bacterial cellulase. *Bioengineering*. 2022; 9(1): 17.

[13] Paulshock BZ. Coping on amphetamines. *The New England Journal of Medicine*. 1970; 282(6): 346.

[14] Morisseau C, Beetham JK, Pinot F, Debernard S, Newman JW, Hammock BD. Cress and potato soluble epoxide hydrolases: purification, biochemical characterization, and comparison to mammalian enzymes. *Archives of Biochemistry and Biophysics*. 2000; 378(2): 321-332.

[15] Gagaoua M, Boucherba N, Bouanane-Darenfed A, Ziane F, Nait-Rabah S, Hafid K, et al. Three-phase partitioning as an efficient method for the purification and recovery of ficin from Mediterranean fig *(Ficus carica L.)* latex. *Separation and Purification Technology*. 2014; 132: 461-467.

[16] Gaur R, Sharma A, Khare SK, Gupta MN. A novel process for extraction of edible oils. *Bioresource Technology*. 2007; 98(3): 696-699.

[17] Baloch KA, Patil U, Yesilsu AF, Benjakul S. Characteristics and application of lipase from Asian seabass liver fractionated using aqueous two-phase partition technique for defatting fish skin before collagen extraction. *Turkish Journal of Fisheries and Aquatic Sciences*. 2023; 23(12): TRJFAS24000.

[18] Yazici EY, Ahlatci F, Yilmaz E, Celep O, Deveci H. Precipitation of zinc from cyanide leach solutions using Trimercapto-s-triazine (TMT). *Hydrometallurgy*. 2020; 191: 105206.

[19] Fathi F, Kasra-Kermanshahi R, Moosavi-Nejad Z, Qamsari EM. Partial purification, characterization and immobilization of a novel lipase from a native isolate of *Lactobacillus fermentum*. *Iranian Journal of Microbiology*. 2021; 13(6): 871-877.

[20] Mariam SHS, Ooi CW, Tan WS, Janna OA, Arbakariya A, Tey BT. Purification of rabbit polyclonal immunoglobulin G with ammonium sulphate precipitation and mixed-mode chromatography. *Separation and Purification Technology*. 2015; 144: 133-138.

[21] Devi SK, Prabhu N, Monika S, Swapna NM, Vaishali S, Gajendran T. Sequestration and purification of essential enzyme from *Pleurotus florida* compost waste and its application studies. *Journal of Applied Biology &*

Biotechnology. 2020; 8(1): 64-68.
[22] Al Mousa AA, Abo-Dahab NF, Hassane AMA, Gomaa AERF, Aljuriss JA, Dahmash ND. Harnessing *Mucor* spp. for xylanase production: statistical optimization in submerged fermentation using agroindustrial wastes. *BioMed Research International*. 2022; 2022: 3816010.
[23] Luo W, Zhang J, Ahmmed MK, Sakai K, Shahidi F, Zhi Z, et al. Valorization of animal by-product enzymes: Advancing sustainable food processing through innovative extraction, purification, and application strategies. *Trends in Food Science & Technology*. 2025; 156: 104870.
[24] Zamani A, Rezaei M, Madani R, Habibi Rezaie M. Trypsin enzyme from viscera of common kilka (*Clupeonella cultriventris caspia*): purification, characterization, and its compatibility with oxidants and surfactants. *Journal of Aquatic Food Product Technology*. 2014; 23: 237-252.
[25] Andric A, Wagner E, Heberle A, Holzknecht M, Weiss AKH. Extraction and purification of FAHD1 protein from swine kidney and mouse liver. *Journal of Visualized Experiments*. 2022; 180: e63333.
[26] Saranya R, Jayapriya JT, Tamil Selvi A. Purification, characterization, molecular modeling and docking study of fish waste protease. *International Journal of Biological Macromolecules*. 2018; 118: 569-583.
[27] Paul JS, Beliya E, Tiwari S, Patel K, Gupta N, Jadhav SK. Production of biocatalyst α-amylase from agro-waste 'rice bran' by using *Bacillus tequilensis* TB5 and standardizing its production process. *Biocatalysis and Agricultural Biotechnology*. 2020; 26: 101648.
[28] Sulyman AO, Igunnu A, Malomo SO. Isolation, purification and characterization of cellulase produced by *Aspergillus niger* cultured on *Arachis hypogaea* shells. *Heliyon.* 2020; 6(12): e05668.
[29] García NM, Cely NM, Méndez PA. Study of the pectinase production from soursop and cherimoya pulp for agroindustrial waste reduction in Colombia. *Waste and Biomass Valorization*. 2024; 15(11): 6357-6365.
[30] Tacin MV, Massi FP, Fungaro MHP, Teixeira MFS, de Paula AV, de Carvalho Santos-Ebinuma V. Biotechnological valorization of oils from agro-industrial wastes to produce lipase using *Aspergillus sp.* from Amazon. *Biocatalysis and Agricultural Biotechnology*. 2019; 17: 369-378.
[31] Acharya R, Kanungo T, Gupta N. Screening and purification of lasparaginase production by *Aspergillus quadrilineatus* using agro wastes and vegetable peels. *Current Trends in Biotechnology and Pharmacy*. 2025; 19(2): 2322-2329.
[32] Anandharaj M, Sivasankari B, Siddharthan N, Rani RP, Sivakumar S. Production, purification, and biochemical characterization of thermostable metalloprotease from novel Bacillus alkalitelluris TWI3 isolated from tannery waste. *Applied Biochemistry and Biotechnology*. 2016; 178(8): 1666-1686.
[33] Dash S, Shyamasuta S, Dash P. Evaluation of various possibilities of underutilized fish processing biomass as a value-added product: A waste-to-wealth approach. In: *Innovations in Fermentation and Phytopharmaceutical Technologies*. Elsevier; 2022. p.437-455.
[34] Fahmy NM, El-Deeb B. Optimization, partial purification, and characterization of a novel high molecular weight alkaline protease produced by *Halobacillus sp.* HAL1 using fish wastes as a substrate. *Journal of Genetic Engineering and Biotechnology*. 2023; 21(1): 48.
[35] Akkaya SN, Almansour A, Altintas R, Sisecioglu M, Adiguzel A. Purification, characterization, optimization, and docking simulation of alkaline protease produced by *Brevibacillus agri* SAR25 using fish wastes as a substrate. *Food Chemistry*. 2025; 471: 142816.
[36] Chew KW, Ling TC, Show PL. Recent developments and applications of three-phase partitioning for the recovery of proteins. *Separation & Purification Reviews*. 2019; 48(1): 52-64.
[37] Yan JK, Wang YY, Qiu WY, Ma H, Wang ZB, Wu JY. Three-phase partitioning as an elegant and versatile platform applied to nonchromatographic bioseparation processes. *Critical Reviews in Food Science and Nutrition*. 2018; 58(14): 2416-2431.
[38] Dogan N, Tari C. Characterization of three-phase partitioned exo-polygalacturonase from *Aspergillus sojae* with unique properties. *Biochemical Engineering Journal*. 2008; 39(1): 43-50.
[39] Garg R, Thorat BN. Nattokinase purification by three phase partitioning and impact of t-butanol on freeze drying. *Separation and Purification Technology*. 2014; 131: 19-26.
[40] Chen X, Cai C, Tan Z. Deep eutectic solvents-based three-phase partitioning for tomato peroxidase purification: A promising method for substituting t-butanol. *Food Chemistry*. 2022; 393: 133379.
[41] Shoshtari-Yeganeh B, Giesy JP, Yeganeh MS, Badibostan H. Deep eutectic solvents (DESs) in microextraction of Parabens: A review. *Microchemical Journal*. 2024; 201: 110699.
[42] Martins MAR, Pinho SP. Insights into the nature of eutectic and deep eutectic mixtures. *Journal of Solution Chemistry*. 2019; 48(7): 962-982.
[43] El Achkar T, Greige-Gerges H, Fourmentin S. Basics and properties of deep eutectic solvents: a review.

Environmental Chemistry Letters. 2021; 19(4): 3397-3408.
[44] Hansen BB, Spittle S, Chen B, Poe D, Zhang Y, Klein JM, et al. Deep eutectic solvents: A review of fundamentals and applications. *Chemical Reviews*. 2021; 121(3): 1232-1285.
[45] Liu Z, Yu D, Li L, Liu X, Zhang H, Sun W, et al. Three-phase partitioning for the extraction and purification of polysaccharides from the immunomodulatory medicinal mushroom inonotus obliquus. *Molecules*. 2019; 24(3): 403.
[46] Zhang Y, Zhong C, Liu S, Du L, Ma H, Zhang H, et al. Ultrasonic assisted three-phase partitioning extraction and bioactivities validation of polysaccharides from *Flammulina velutipes* residues. *Food Chemistry: X*. 2025; 31: 103177.
[47] Wang YY, Ma H, Yan JK, Wang KD, Yang Y, Wang WH, et al. Three-phase partitioning system with dimethyl carbonate as organic phase for partitioning of exopolysaccharides from *Phellinus baumii*. *International Journal of Biological Macromolecules*. 2019; 131: 941-948.
[48] Salvi HM, Yadav GD. Extraction of epoxide hydrolase from *Glycine max* using microwave-assisted three-phase partitioning with dimethyl carbonate as green solvent. *Food and Bioproducts Processing*. 2020; 124: 159-167.
[49] Sonar MP, Rathod VK. Extraction of type II antidiabetic compound corosolic acid from *Lagerstroemia speciosa* by batch extraction and three phase partitioning. *Biocatalysis and Agricultural Biotechnology*. 2020; 27: 101694.
[50] Sarkar S, Manna MS, Bhowmick TK, Gayen K. Priority-based multiple products from microalgae: review on techniques and strategies. *Critical Reviews in Biotechnology*. 2020; 40(5): 590-607.
[51] Nugrahani I, Muharrifani Y, Al Iman A. Protease from banana stem postharvest (*Musa × paradisiaca*): Extraction, purification, and its potential milk-clotting activity. *International Journal of Biological Macromolecules*. 2025; 315: 144622.
[52] Gul A, Khan S, Arain H, Khan H, Ishrat U, Siddiqui M. Three-phase partitioning as an efficient one-step method for the extraction and purification of bromelain from pineapple crown waste. *Journal of Food Processing and Preservation.* 2022; 46(11): e16973.
[53] Niphadkar SS, Rathod VK. Ultrasound-assisted three-phase partitioning of polyphenol oxidase from potato peel (*Solanum tuberosum*). *Biotechnology Progress*. 2015; 31(5): 1340-1347.
[54] Al-Madhagi H, Yazbik V, Abdelwahed W. One-step isolation and purification of peroxidase from zucchini heads. *Electronic Journal of Biotechnology*. 2023; 66: 30-37.
[55] Wasak A, Drozd R, Grygorcewicz B, Jankowiak D, Rakoczy R. Purification and recovery of laccase produced by submerged cultures of *Trametes versicolor* by three-phase partitioning as a simple and highly efficient technique. *Polish Journal of Chemical Technology*. 2018; 20(4): 88-95.
[56] Vetal MD, Rathod VK. Three phase partitioning a novel technique for purification of peroxidase from orange peels (*Citrus sinenses*). *Food and Bioproducts Processing*. 2015; 94: 284-289.
[57] Chaiwut P, Pintathong P, Rawdkuen S. Extraction and three-phase partitioning behavior of proteases from papaya peels. *Process Biochemistry*. 2010; 45(7): 1172-1175.
[58] Oliveira VM, Assis CRD, Costa HMS, Silva RR, França RCP, Santos JF, et al. Separation and partial purification of collagenolytic protease from peacock bass (*Cichla ocellaris*) using different protocol: precipitation and partitioning approaches. *Biocatalysis and Agricultural Biotechnology*. 2020; 24: 101509.
[59] Kuepethkaew S, Sangkharak K, Benjakul S, Klomklao S. Use of TPP and ATPS for partitioning and recovery of lipase from Pacific white shrimp (*Litopenaeus vannamei*) hepatopancreas. *Journal of Food Science and Technology*. 2017; 54(12): 3880-3891.
[60] Zerizer H, Boughachiche F, Mebarki A, Sinacer O, Rachedi K, Ait Kaki A. Partitioning purification, biochemical characterization, and milk coagulation efficiency of protease from a newly *Streptomyces sp.* isolate. *Brazilian Journal of Microbiology*. 2024; 55(3): 2179-2187.
[61] Nasr AIAM, Mohamed Ahmed IA, Hamid OIA. Characterization of partially purified milk-clotting enzyme from sunflower (*Helianthus annuus*) seeds. *Food Science & Nutrition*. 2016; 4(5): 733-741.
[62] Shakerian F, Zhao J, Li SP. Recent development in the application of immobilized oxidative enzymes for bioremediation of hazardous micropollutants-A review. *Chemosphere*. 2020; 239: 124716.
[63] Stadlmair LF, Letzel T, Drewes JE, Grassmann J. Enzymes in removal of pharmaceuticals from wastewater: a critical review of challenges, applications and screening methods for their selection. *Chemosphere*. 2018; 205: 649-661.
[64] Chen Z, Wan C. Biological valorization strategies for converting lignin into fuels and chemicals. *Renewable and Sustainable Energy Reviews*. 2017; 73: 610-621.
[65] Bhatt P, Zhou X, Huang Y, Zhang W, Chen S. Characterization of the role of esterases in the biodegradation of organophosphate, carbamate, and pyrethroid pesticides. *Journal of Hazardous Materials*. 2021; 411: 125026.

[66] Maskey B, Shrestha S, Shrestha A, Sangroula P, Karki DB, Shrestha NK. Single step three phase partitioning for the purification of bromelain enzyme from pineapple (*Ananas comosus*) fruit. *Journal of Food Measurement and Characterization*. 2025; 19(8): 6108-6118.
[67] Rajendran DS, Chidambaram A, Kumar PS, Venkataraman S, Muthusamy S, Vo DVN, et al. Three-phase partitioning for the separation of proteins, enzymes, biopolymers, oils and pigments: a review. *Environmental Chemistry Letters*. 2023; 21(2): 911-934.
[68] Polyakova M, Diekmann A, Grünewald M. Overview of innovative technologies in liquid-liquid extraction regarding flexibility. *Chemical Engineering & Technology*. 2020; 92(12): 1941-1952.
[69] Ribeiro SC, Monteiro GA, Cabral JMS, Prazeres DMF. Isolation of plasmid DNA from cell lysates by aqueous two-phase systems. *Biotechnology and Bioengineering.* 2002; 78(4): 376-384.
[70] Albertsson PÅ. Chromatography and partition of cells and cell fragments. *Nature*. 1956; 177(4513): 771-774.
[71] Buarque F, Gautério G, Coelho M, Lemes A, Ribeiro B. Aqueous two-phase systems based on ionic liquids and deep eutectic solvents as a tool for the recovery of non-protein bioactive compounds—a review. *Processes*. 2022; 11(1): 31.
[72] Capela EV, Valente AI, Nunes JCF, Magalhães FF, Rodríguez O, Soto A, et al. Insights on the laccase extraction and activity in ionic-liquid-based aqueous biphasic systems. *Separation and Purification Technology*. 2020; 248: 117052.
[73] Santos AG, Buarque FS, Ribeiro BD, Coelho MAZ. Extractive fermentation for the production and partitioning of lipase and citric acid by *Yarrowia lipolytica*. *Process Biochemistry*. 2022; 122: 374-385.
[74] Xu S, Zhu Q, Xu S, Yuan M, Lin X, Lin W, et al. The phase behavior of n-ethylpyridinium tetrafluoroborate and sodium-based salts ATPS and its application in 2-chlorophenol extraction. *Chinese Journal of Chemical Engineering*. 2021; 33: 76-82.
[75] Lee SY, Khoiroh I, Ling TC, Show PL. Aqueous two-phase flotation for the recovery of biomolecules. *Separation & Purification Reviews*. 2016; 45(1): 81-92.
[76] Molino JVD, Lopes AM, Viana Marques DDA, Mazzola PG, da Silva JL, Hirata MH, et al. Application of aqueous two-phase micellar system to improve extraction of adenoviral particles from cell lysate. *Biotechnology and Applied Biochemistry*. 2018; 65(3): 381-389.
[77] Raji F, Rahbar-Kelishami A. Evaluation of biocompatible aqueous two-phase systems with the double interface for the recovery of biomolecules. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 2021; 624: 126823.
[78] Dobreva V, Zhekova B, Dobrev G. Use of aqueous two-phase and three-phase partitioning systems for purification of lipase obtained in solid-state fermentation by *Rhizopus arrhizus*. *The Open Biotechnology Journal*. 2019; 13(1): 27-36.
[79] Souza DH, Fernandes M, Kempka AP, Magalhães MD, Trevisan V, Skoronski E. Yacon (*Smallanthus sonchifolius*) peel as a promising peroxidase source for the treatment of phenolic wastewater. *Biocatalysis and Agricultural Biotechnology*. 2022; 39: 102254.
[80] Wessner M, Bommarius B, Brandenbusch C, Bommarius AS. Purification of chimeric amine dehydrogenase using a tailor-made aqueous two-phase system-A case study. *Journal of Molecular Liquids*. 2021; 323: 114991.
[81] Iqbal M, Tao Y, Xie S, Zhu Y, Chen D, Wang X, et al. Aqueous two-phase system (ATPS): an overview and advances in its applications. *Biological Procedures Online*. 2016; 18(1): 18.
[82] Ooi CW, Tey BT, Hii SL, Kamal SMM, Lan JCW, Ariff A, et al. Purification of lipase derived from *Burkholderia pseudomallei* with alcohol/salt-based aqueous two-phase systems. *Process Biochemistry*. 2009; 44(10): 1083-1087.
[83] Pereira JFB, Kurnia KA, Cojocaru OA, Gurau G, Rebelo LPN, Rogers RD, et al. Molecular interactions in aqueous biphasic systems composed of polyethylene glycol and crystalline vs. liquid cholinium-based salts. *Physical Chemistry Chemical Physics*. 2014; 16(12): 5723.
[84] Jorge AMS, Pereira JFB. Aqueous two-phase systems-versatile and advanced (bio)process engineering tools. *Chemical Communications*. 2024; 60(84): 12144-12168.
[85] Ramesh V, Murty VR. Partitioning of thermostable glucoamylase in polyethyleneglycol/salt aqueous two-phase system. *Bioresources and Bioprocessing*. 2015; 2(1): 25.
[86] Murari GF, Penido JA, Machado PAL, Lemes LR, Lemes NHT, Virtuoso LS, et al. Phase diagrams of aqueous two-phase systems formed by polyethylene glycol + ammonium sulfate + water: equilibrium data and thermodynamic modeling. *Fluid Phase Equilibria*. 2015; 406: 61-69.
[87] Bekavac N, Benković M, Jurina T, Valinger D, Gajdoš Kljusurić J, Jurinjak Tušek A, et al. Advancements in aqueous two-phase systems for enzyme extraction, purification, and biotransformation. *Molecules*. 2024; 29(16): 3776.

[88] Azevedo AM, Gomes AG, Rosa PAJ, Ferreira IF, Pisco AMMO, Aires-Barros MR. Partitioning of human antibodies in polyethylene glycol-sodium citrate aqueous two-phase systems. *Separation and Purification Technology*. 2009; 65(1): 14-21.
[89] Kaplanow I, Stecker J, Schembecker G, Merz J. Multistage processing of tunable aqueous polymer phase impregnated resins (TAPPIR®). *Chemical Engineering & Technology*. 2018; 41(7): 1324-1330.
[90] Kress C, Sadowski G, Brandenbusch C. Novel displacement agents for aqueous 2-phase extraction can be estimated based on hybrid shortcut calculations. *Journal of Pharmaceutical Sciences*. 2016; 105(10): 3030-3038.
[91] Yang Z, Wang J, Wang H, Xie H, Zhang Y, Wang M. Simultaneous extraction and purification of polysaccharides and proteins from *Pleurotus ostreatus* using an aqueous two-phase system. *Journal of Food Science*. 2025; 90(1): e17674.
[92] Ulloa G, Coutens C, Sánchez M, Sineiro J, Fábregas J, Deive FJ, et al. On the double role of surfactants as microalga cell lysis agents and antioxidants extractants. *Green Chemistry*. 2012; 14(4): 1044.
[93] Alencar VNS, Nascimento MC, Ferreira JVDS, Batista JMDS, Cunha MNC, Nascimento JM, et al. Purification and characterization of fibrinolytic protease from Streptomyces parvulus by polyethylene glycol-phosphate aqueous two-phase system. *Annals of the Brazilian Academy of Sciences*. 2021; 93(suppl 4): e20210335.
[94] Silva OS da, Gomes MHG, de Oliveira RL, Porto ALF, Converti A, Porto TS. Partitioning and extraction protease from Aspergillus tamarii URM4634 using PEG-citrate aqueous two-phase systems. *Biocatalysis and Agricultural Biotechnology*. 2017; 9: 168-173.
[95] da Silva OS, Alves RO, Porto TS. PEG-sodium citrate aqueous two-phase systems to in situ recovery of protease from *Aspergillus tamarii* URM4634 by extractive fermentation. *Biocatalysis and Agricultural Biotechnology*. 2018; 16: 209-216.
[96] Brígida AIS, Buarque FS, Nogueira VLR, Melo VMM, Guisán JM, Ribeiro BD, et al. Partial purification of crude lipase extract from *Yarrowia lipolytica*: Precipitation, aqueous two-phase systems (ATPS), and immobilization methods. *Cleaner Chemical Engineering*. 2023; 6: 100105.
[97] Carvalho T, Finotelli PV, Bonomo RCF, Franco M, Amaral PFF. Evaluating aqueous two-phase systems for *Yarrowia lipolytica* extracellular lipase purification. *Process Biochemistry*. 2017; 53: 259-266.
[98] Padilha GS, Osório WR. Economic method for extraction/purification of a *Burkholderia cepacia* lipase with potential biotechnology application. *Applied Biochemistry and Biotechnology*. 2019; 189(4): 1108-1126.
[99] Patchimpet J, Sangkharak K, Eiad-ua A, Klomklao S. Thermoseparating aqueous two-phase system for lipase recovery and partitioning from *Nile tilapia* viscera: Biochemical properties and effect of ultrasound. *Journal of Molecular Liquids.* 2021; 331: 115721.
[100]Nascimento PA, Alves AN, dos Santos KA, Veloso CM, Santos LS, da Costa Ilhéu Fontan R, et al. Partitioning of pequi seed (*Caryocar brasiliense Camb*.) lipase in aqueous two-phase systems composed of PEG/2-propanol + ammonium sulfate + water. *Brazilian Journal of Chemical Engineering*. 2021; 38(4): 957-965.
[101]Buarque FS, Carniel A, Ribeiro BD, Coelho MAZ. Selective enzymes separation from the fermentation broth of *Yarrowia lipolytica* using aqueous two-phase system based on quaternary ammonium compounds. *Separation and Purification Technology*. 2023; 324: 124539.
[102]Lemos LTM, de Medeiros LL, Ribeiro-Filho N, de Sousa CAB, de Melo Santos SF, da Silva FLH. Xylose reductase generated by yeast *Candida tropicalis* purified using an aqueous two-phase system optimised by RSM. *Food Chemistry*. 2025; 490: 145069.
[103]Singla M, Sit N. Isolation of papain from ripe papaya peel using aqueous two-phase extraction. *Journal of Food Measurement and Characterization*. 2023; 17(2): 1685-1692.
[104]De Carvalho Batista Muniz I, de Oliveira RM, Alves AN, da Silva EC, Santos BS, de Souza Junior EC, et al. Partition of porcine pancreatic lipase in ATPS composed of PEG/salt and alcohol/salt: a thermodynamic study. *Brazilian Journal of Chemical Engineering*. 2023; 40(3): 873-883.
[105]Akhter K, Sadaf E, Bibi A, Ayyub J, Ghous T, Akhtar T. Effective lipase purification from *Bacillus cereus* NC7401: a novel approach using an alcohol-/salt-based aqueous two-phase system. *Chemical Papers*. 2024; 78(2): 963-971.
[106]Chandra P. Peningkatan hasil belajar sejarah kebudayaan Islam melalui model cooperative learning tipe team games tournament (TGT) di MTs Al-Quraniyah Bengkulu [Improving learning outcomes of Islamic cultural history through the cooperative learning model type team games tournament (TGT) at MTs Al-Quraniyah Bengkulu]. *At-Tajdid: Journal of Education and Islamic Thought*. 2020; 4(1): 1-20.
[107]Wang L, Liu T, Sun H, Zhou Q. Transesterification of para-hydroxybenzoic acid esters (parabens) in the activated sludge. *Journal of Hazardous Materials.* 2018; 354: 145-152.
[108]Sarmah N, Revathi D, Sheelu G, Yamuna Rani K, Sridhar S, Mehtab V, et al. Recent advances on sources and industrial applications of lipases. *Biotechnology Progress*. 2018; 34(1): 5-28.

[109]Kumar L. Role of proteases in bioremediation of temple protein-containing waste with special reference to Mangalnath, Ujjain (MP)-India. *Indian Journal of Pure & Applied Biosciences*. 2020; 8(3): 602-607.
[110]Isaac GS, Abu-Tahon MA. Dehairing capability of alkaline keratinase produced by new isolated *Cochliobolus hawaiiensis* AUMC 8606 grown on chicken feather. *Romanian Biotechnological Letters*. 2016; 22(6): 12147-12154.
[111]Bhandari S, Poudel DK, Marahatha R, Dawadi S, Khadayat K, Phuyal S, et al. Microbial enzymes used in bioremediation. *Journal of Chemistry*. 2021; 2021: 1-17.
[112]Deshmukh R, Khardenavis AA, Purohit HJ. Diverse metabolic capacities of fungi for bioremediation. *Indian Journal of Microbiology*. 2016; 56(3): 247-264.
[113]Kadri T, Rouissi T, Kaur Brar S, Cledon M, Sarma S, Verma M. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. *Journal of Environmental Sciences*. 2017; 51: 52-74.
[114]Godambe T, Fulekar M. Bioremediation of Petrochemical Hydrocarbons (BTEX)-Review. *Journal of Environmental Science and Pollution Research*. 2017; 3(3): 189-199.
[115]Zhang Y, Han L, Zou L, Zhang M, Chi R. Development of an SVR model for microwave-assisted aqueous two-phase extraction of isoflavonoids from *Radix Puerariae*. *Chemical Engineering Communications*. 2021; 208(7): 1005-1016.
[116]Lin Y, Zeng H, Wang K, Lin H, Li P, Huang Y, et al. Microwave-assisted aqueous two-phase extraction of diverse polysaccharides from *Lentinus edodes*: Process optimization, structure characterization and antioxidant activity. *International Journal of Biological Macromolecules*. 2019; 136: 305-315.
[117]Ferreira AM, Passos H, Okafuji A, Tavares APM, Ohno H, Freire MG, et al. An integrated process for enzymatic catalysis allowing product recovery and enzyme reuse by applying thermoreversible aqueous biphasic systems. *Green Chemistry*. 2018; 20(6): 1218-1223.
[118]Hu J, Liu J, Huang X, Di D, Pei D. Efficient extraction of polysaccharides from *Lycium barbarum* L. by aqueous two-phase system combined with tissue-smashing extraction. *Industrial Crops and Products*. 2022; 184: 115036.
[119]Wang L, Li W, Liu Y, Zhi W, Han J, Wang Y, et al. Green separation of bromelain in food sample with high retention of enzyme activity using recyclable aqueous two-phase system containing a new synthesized thermo-responsive copolymer and salt. *Food Chemistry*. 2019; 282: 48-57.
[120]Dimitrijević D, Bösenhofer M, Harasek M. Liquid-liquid phase separation of two non-dissolving liquids—a mini review. *Processes*. 2023; 11(4): 1145.
[121]Sankaran R, Show PL, Yap YJ, Lam HL, Ling TC, Pan GT, et al. Sustainable approach in recycling of phase components of large scale aqueous two-phase flotation for lipase recovery. *Journal of Cleaner Production*. 2018; 184: 938-948.
[122]Han J, Fang S, He X, Wang L, Li C, Wu J, et al. Combination of aqueous two-phase flotation and inverse transition cycling: Strategies for separation and purification of recombinant *β*-glucosidase from cell lysis solution. *Food Chemistry*. 2022; 373: 131543.
[123]Cutshaw A, Frost H, Uludag-Demirer S, Liu Y, Liao W. Protein extraction, precipitation, and recovery from *Chlorella sorokiniana* using mechanochemical methods. *Energies*. 2023; 16(12): 4809.
[124]Lohfink KC, Baumgärtner K, Kirsch M, Rhein F, Diehl M, Nirschl H. Online monitoring of continuous aqueous two-phase flotation (ATPF) for the development of an autonomous control strategy. *Chemical Engineering Science*. 2024; 297(4): 120287.