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Review

Electrochemical Methods for Protein and Lipid Recovery and Valorization from Waste Activated Sludge

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Abstract

Waste activated sludge (WAS), traditionally considered a by-product of biological wastewater treatment, is increasingly recognized as a valuable source of proteins, lipids, nutrients, and other biomolecules with potential for resource recovery and environmental sustainability. Electrochemical technologies have emerged as promising approaches for WAS valorization because they can promote extracellular polymeric substance disruption, microbial cell lysis, and biomolecule release under controllable conditions. This review examines recent advances in the electrochemical recovery of proteins and lipids from WAS, focusing on the mechanisms governing their release, including reactive oxygen species generation, electron transfer, electroporation, and electro-osmotic effects. Electrooxidation, electro-Fenton, electrocoagulation, bioelectrochemical systems, and hybrid electrochemical processes are compared in terms of treatment conditions, recovery performance, and preservation of biomolecule quality. Particular emphasis is placed on the ability of these technologies to recover proteins and lipids while limiting excessive oxidation that may compromise their structural integrity and subsequent applications. The potential integration of recovered biomolecules into sludge biorefineries for producing biofuels, biomaterials and other value-added products is discussed within a circular bioeconomy framework. Overall, electrochemical technologies represent a versatile pathway for converting WAS from an environmental liability into a source of high-quality biomolecules and renewable products, supporting the transition of wastewater treatment plants toward integrated resource recovery and more sustainable environmental management.

Keywords: waste activated sludge; electrochemical technologies; protein recovery; lipid recovery; sludge biorefinery

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1. Introduction

Wastewater treatment is essential for protecting water resources and reducing the environmental impacts associated with municipal and industrial discharges. However, biological wastewater treatment also generates substantial quantities of excess sludge, including waste activated sludge (WAS), whose treatment and management represent important environmental and economic challenges. Conventional sludge-management

routes include land application, anaerobic digestion, incineration, and landfilling, but increasing attention is being directed toward recovering resources from wastewater and sludge rather than considering these streams exclusively as wastes requiring treatment or disposal [1,2]. This transition provides opportunities to recover energy, nutrients, organic compounds, and other value-added products while improving resource utilization within wastewater-treatment systems [1,3].

WAS is a heterogeneous matrix containing microbial biomass, extracellular polymeric substances (EPS), and associated organic and inorganic components. Among the recoverable organic fractions, proteins and lipids are particularly attractive because of their potential subsequent valorization in agricultural products, biofuels, bio-based materials, and biochemical feedstocks [3–5]. However, proteins and lipids can be associated with the EPS matrix, microbial cell envelopes, intracellular compartments, and sludge flocs, which restricts their accessibility. Conventional approaches, including thermal, alkaline or acidic treatment, enzymatic treatment, mechanical disruption, and solvent-based extraction, can promote sludge disruption and biomolecule solubilization or extraction, but their performance and effects on the target compounds depend strongly on the treatment conditions [4,5].

The structural organization of WAS therefore represents an important physicochemical barrier to biomolecule recovery. Disruption of the EPS matrix can increase the accessibility of extracellularly associated compounds, whereas intracellular proteins and membrane-associated lipids may additionally require permeabilization or disruption of microbial cells. Importantly, biomolecule mobilization or solubilization does not necessarily constitute final recovery because subsequent separation, concentration, extraction, and purification may be required to obtain a useful protein- or lipid-rich fraction [4,5]. This distinction is particularly important when comparing treatment technologies because increases in soluble protein, soluble chemical oxygen demand (SCOD), or sludge disintegration primarily indicate enhanced accessibility or solubilization rather than recovery of a separated product.

Electrochemical technologies have emerged as promising and controllable approaches for sludge treatment and resource recovery [6,7]. Depending on the electrochemical technology and operating conditions, sludge transformation may involve direct electron-transfer reactions, electrokinetic effects, reactive-species generation, local changes in pH and redox conditions, and, when sufficiently strong local electric fields are achieved, electroporation [6]. These effects can promote sludge disintegration and increase the accessibility of sludge-associated compounds. Electrochemical systems also provide operational control through parameters such as current density or applied voltage, treatment time, electrode material and configuration, and reactor conditions. Nevertheless, energy consumption, electrode stability, and scale-up remain important considerations for practical implementation [6,7].

Available studies support the potential of electrochemical processes for sludge treatment and resource recovery, including sludge disintegration, dewaterability improvement, phosphorus release and recovery, and enhanced accessibility or conversion of sludge-associated organic matter [6–9]. However, much of the available literature focuses on sludge disintegration, dewatering, nutrient recovery, or bulk indicators of organic-matter solubilization rather than the subsequent separation and final recovery of specific protein and lipid products. Consequently, the relationship between electrochemical treatment conditions, biomolecule mobilization, downstream recovery, and final product characteristics requires further clarification.

Previous reviews have addressed electrochemical sludge treatment and resource recovery [6], electrochemical sludge dewatering and alternative uses [7], separation and purification of value-added biocompounds from WAS [4], and protein recovery from sludge [5]. In contrast, the present review specifically examines the application of electro-

chemical methods to the mobilization and recovery of both proteins and lipids from WAS, integrating electrochemical mechanisms, operating conditions, downstream separation and recovery, product quality, and subsequent valorization pathways.

WAS is the primary feedstock considered in this review. Studies involving primary sludge, scum, other biomass sources, or synthetic wastewater are identified separately and included only when they provide relevant comparative or mechanistic information; their results are not considered directly equivalent to those obtained from WAS. This distinction is important because sludge composition and biomolecule accessibility depend strongly on the origin and characteristics of the feedstock.

Accordingly, an important knowledge gap remains between electrochemically enhanced sludge disintegration and the recovery of well-characterized protein- and lipid-rich products. This review addresses this gap by critically examining the mechanisms and operating conditions governing electrochemical biomolecule mobilization, the distinction between release, solubilization, extraction, and final recovery, and the downstream processes required for product separation and valorization. Particular attention is given to protein and lipid recovery performance, product quality, energy and scale-up considerations, and integration with complementary resource-recovery processes. Through this approach, the review evaluates the potential of electrochemical methods to transform WAS from a residual treatment burden into a source of value-added products and contribute to more resource-efficient and environmentally sustainable wastewater management.

2. Wastewater Activated Sludge as a Biomolecular Reservoir

2.1. Generation and Characteristics of WAS

Waste activated sludge (WAS) is an unavoidable by-product of biological wastewater treatment processes and constitutes one of the major residual streams generated by wastewater treatment plants (WWTPs). During secondary treatment, microorganisms convert dissolved and particulate organic matter into biomass, a fraction of which must be periodically removed to maintain process stability and treatment efficiency [3]. Sludge management and disposal can account for a substantial fraction of the operating costs of conventional WWTPs [9,10], highlighting the need to develop sustainable valorization strategies. Consequently, wastewater treatment is increasingly shifting from conventional sludge disposal toward resource recovery and circular-economy approaches, in which WAS can be considered a potential feedstock for recovering energy, nutrients, and value-added compounds [3,9–13].

From a physicochemical perspective, WAS is a heterogeneous matrix composed of water, microbial biomass, extracellular polymeric substances (EPS), organic particulates, inorganic components, and adsorbed contaminants [4,12,14]. Volatile solids commonly constitute a major fraction of sludge total solids, while the organic matter contains proteins, carbohydrates, lipids, nucleic acids, and humic-like substances [4,14]. Their relative abundance varies substantially with wastewater composition, treatment configuration, sludge retention time, operating conditions, and the analytical methods used for characterization [4,11]. Therefore, compositional values reported for WAS should be interpreted according to their corresponding reporting basis (e.g., total solids, volatile solids, or EPS).

A defining characteristic of WAS is its complex hierarchical structure. Organic compounds are associated with microbial cells and the surrounding EPS matrix, which contributes to microbial aggregation and floc stability [14]. EPS comprises a heterogeneous mixture of proteins, polysaccharides, humic substances, lipids, nucleic acids, and other organic and inorganic constituents [14,15]. These structural associations influence sludge dewaterability, biodegradability, mass transfer, and biomolecule accessibility [11,15,16]. Consequently, understanding both WAS composition and floc architecture is essential for

developing technologies capable of enhancing biomolecule mobilization and facilitating subsequent extraction and recovery. Figure 1 summarizes the structural organization, biochemical composition, and main factors influencing the composition of WAS.

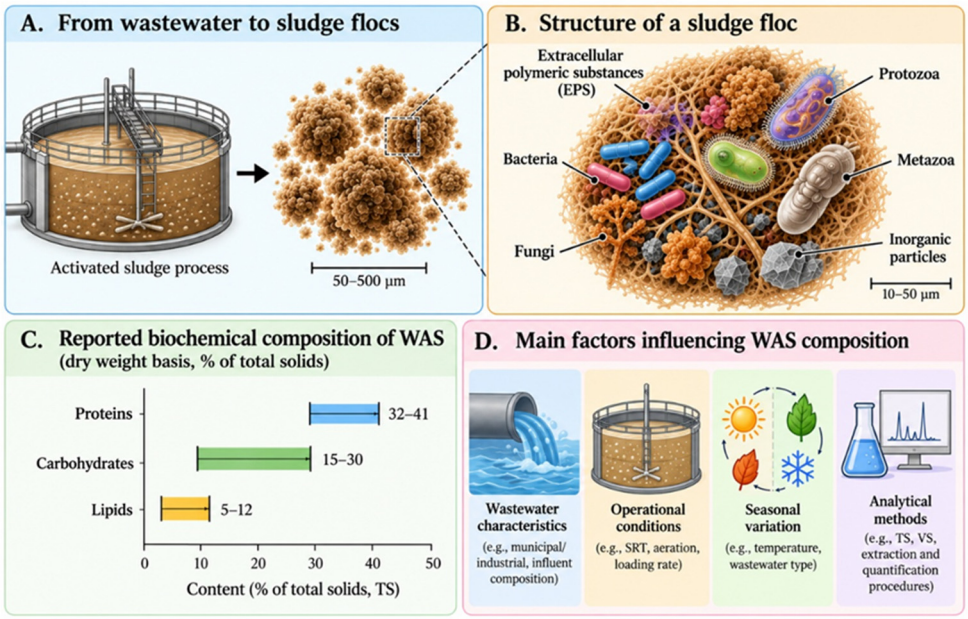


Figure 1. Composition and structural organization of waste activated sludge (WAS). (**A**) Formation of WAS during the activated sludge process. (**B**) Main structural components of a sludge floc, including extracellular polymeric substances (EPS), microorganisms, and inorganic particles. (**C**) Representative biochemical composition of WAS expressed as a percentage of total solids (% TS), including proteins (32–41%), carbohydrates (15–30%), and lipids (5–12%) [17]. These values represent literature-reported ranges and may vary according to sludge origin, wastewater characteristics, operating conditions, and analytical methods. (**D**) Main factors influencing WAS composition, including wastewater characteristics, operational conditions, seasonal variation, and analytical methods. Structural and compositional information was compiled from the literature [14–17].

2.2. Protein Occurrence and Distribution in WAS

Proteins are among the most abundant organic constituents of WAS, although their reported abundance varies considerably depending on wastewater characteristics, treatment configuration, operational conditions, and the analytical and reporting basis used [5,12,13,18]. Their abundance is largely associated with the microbial biomass present in activated sludge systems, where proteins perform essential structural, metabolic, enzymatic, and transport functions. Proteins are also important components of extracellular polymeric substances (EPS), where they contribute to microbial aggregation and floc structure [19,20]. Consequently, the substantial protein content of WAS makes this fraction an attractive target for resource recovery and subsequent valorization [5,21,22].

Proteins in WAS can occur intracellularly within microbial cells, in association with EPS, or in the soluble phase [5,18,23]. However, their association with cellular structures and the complex EPS matrix can restrict their accessibility [19,20,24,25]. Effective protein recovery therefore generally requires disruption or modification of these structural associations to promote protein mobilization and release, followed by appropriate separation and purification steps [21–23,26].

From a resource-recovery perspective, sludge proteins can be processed into protein-rich fractions, peptides, amino acids, and other nitrogen-containing products with potential for subsequent valorization [21–23,26,27]. However, the suitability of the recovered products depends not only on extraction or recovery yield but also on their composition, purity, molec-

ular characteristics, and intended application [22,23,26,27]. Treatment severity is particularly important because conditions that promote sludge disruption and protein mobilization can also induce protein hydrolysis and other molecular modifications [21–23,26,27]. Therefore, operating conditions should be optimized to achieve sufficient protein mobilization while limiting undesirable transformations according to the intended downstream application. In this context, electrochemical technologies are particularly attractive because treatment intensity can be controlled through operating parameters such as current density or applied voltage, treatment time, electrode material, and reactor conditions. Nevertheless, enhanced protein release or solubilization should not be considered equivalent to selective protein recovery, and subsequent separation, purification, and product characterization remain necessary.

2.3. Lipid Occurrence and Distribution in WAS

Lipids constitute an important fraction of the organic matter present in WAS, although their abundance is generally lower than that of proteins. Reported lipid contents vary considerably depending on sludge origin, wastewater characteristics, treatment configuration, sludge retention time, and analytical methodology [22,28–30]. Lipid contents ranging from approximately 46 to 157 mg g^{-1} dry solids have been reported for different municipal sewage sludges [28]. Other studies have reported lipid concentrations on different analytical bases, further illustrating the variability associated with sludge characteristics and lipid-extraction procedures [29,30]. Therefore, quantitative lipid contents should be interpreted according to the sludge type, analytical method, and reporting basis used rather than directly compared across studies.

Sludge lipids comprise a heterogeneous mixture of compounds associated with microbial biomass and other organic fractions of the sludge. These compounds include phospholipids, glycerides, free fatty acids, and other extractable lipid species [28,30,31]. Fatty acid profiles of sewage sludge commonly contain substantial proportions of C16 and C18 fatty acids, supporting the potential use of sludge-derived lipid fractions as feedstocks for biodiesel and other oleochemical applications [28,30,31]. However, lipid accessibility depends strongly on the sludge matrix and the location and chemical form of the lipid fraction. Lipids associated with microbial cells and sludge flocs may therefore require disruption and appropriate extraction procedures before they can be effectively recovered [28–31].

Effective lipid recovery consequently involves not only increasing lipid accessibility but also extracting and separating the target fraction from the complex sludge matrix. Treatment and extraction conditions are important because they can influence lipid yield, composition, and the characteristics of the recovered fraction [28–31]. Accordingly, evaluation of lipid recovery should consider the analytical basis used to quantify lipids, the composition of the extracted fraction, and its suitability for the intended downstream application. Other sludge-derived streams, such as primary scum and fat-rich materials, may contain substantially different lipid fractions and should be distinguished from WAS when comparing recovery performance [32]. In addition, lipid valorization can follow routes other than direct extraction of native sludge lipids; for example, hydrolyzed sludge can serve as a substrate for the production of new intracellular lipids by oleaginous microorganisms [33]. These pathways should be distinguished from direct lipid recovery because the recovered lipids originate from microbial conversion rather than from the native lipid fraction initially present in WAS.

3. Challenges in Protein and Lipid Extraction

3.1. Sludge Architecture

The recovery of proteins and lipids from WAS is inherently constrained by its complex and heterogeneous architecture. Rather than existing exclusively as freely extractable

compounds, these biomolecules are associated with microbial cells, extracellular polymeric substances (EPS), and other organic and inorganic components within sludge flocs [34,35]. This structural organization creates physical barriers that restrict mass transfer and reduce the accessibility of cell-associated and intracellular biomolecules. Furthermore, the compact structure of sludge flocs can create diffusion limitations that hinder the penetration of treatment or extraction agents, thereby limiting biomolecule mobilization and subsequent extraction [34,35].

3.2. Extracellular Polymeric Substances (EPS)

EPS constitute a major structural component of activated sludge flocs and can represent an important barrier to biomolecule extraction. EPS comprise a heterogeneous mixture of proteins, polysaccharides, nucleic acids, lipids, humic substances, and other organic and inorganic constituents and are commonly operationally differentiated into loosely bound (LB-EPS) and tightly bound (TB-EPS) fractions [34–37]. These matrices contribute to floc stability through hydrogen bonding, electrostatic and hydrophobic interactions, and multivalent-cation bridging, while also retaining water and organic matter [34,37]. Because proteins, lipids, and other organic compounds can be incorporated into or associated with the EPS matrix, their mobilization may require disruption or modification of this structural network [34,36].

3.3. Cellular Barriers

In addition to the EPS matrix, microbial cell envelopes constitute an important barrier to the accessibility of intracellular and membrane-associated biomolecules. Cell walls and cytoplasmic membranes restrict the release of intracellular organic matter, and effective mobilization therefore requires sufficient permeabilization or disruption of these structures [35–38]. Thermal and other pretreatment methods can promote cell disruption and increase sludge solubilization; however, the extent of these effects depends strongly on treatment severity and sludge characteristics [38,39]. Excessively severe treatment may also alter the chemical characteristics of released organic compounds, which should be considered when subsequent recovery and valorization are intended.

3.4. Protein–Lipid–EPS Interactions

Proteins, lipids, polysaccharides, humic substances, and inorganic ions participate in multiple physicochemical interactions within the sludge matrix. Hydrogen bonding, electrostatic interactions, hydrophobic interactions, and interactions mediated by multivalent cations contribute to the organization and stability of EPS and microbial aggregates [34,37,40]. In particular, cations such as Ca^{2+}, Mg^{2+}, and Fe^{3+} can interact with negatively charged functional groups and contribute to bridging within the extracellular matrix [37,40]. Collectively, these interactions can restrict biomolecule mobility and accessibility, explaining why a high protein or lipid content does not necessarily translate into a high extraction or recovery yield.

3.5. Limitations of Conventional Extraction Technologies

Conventional approaches investigated for sludge disintegration and biomolecule extraction include thermal treatment, alkaline and acid treatment, ultrasonication, mechanical disruption, enzymatic treatment, and solvent-based extraction [18,38,39,41]. These approaches can promote sludge disintegration, organic-matter solubilization, and biomolecule accessibility; however, their performance and resource requirements depend strongly on the treatment method and operating conditions [38,39,41]. Depending on the technology, limitations may include energy and chemical requirements, solvent consumption, prolonged

processing, or the need for subsequent separation and purification [41]. Moreover, severe treatment conditions can alter the composition and molecular characteristics of released organic compounds [38,39]. Therefore, conventional extraction processes should be evaluated not only in terms of biomolecule release or extraction yield but also with respect to treatment severity, resource consumption, downstream processing requirements, and the characteristics of the recovered products.

3.6. Biomolecule Accessibility as the Main Bottleneck

The recovery of value-added compounds from WAS depends not only on their abundance but also on their accessibility within the sludge matrix. In this context, biomolecule accessibility refers to the extent to which sludge-associated proteins, lipids, and other target compounds can be mobilized or made available for subsequent extraction and recovery. In WAS, these biomolecules occur at different structural locations. Proteins can be associated with the EPS matrix, cell envelopes, and intracellular compartments, whereas lipids can occur within cell membranes, intracellular storage structures, and other sludge-associated organic fractions. Polysaccharides and humic substances are predominantly associated with the EPS matrix, while nucleic acids are mainly intracellular, although extracellular nucleic acids can also occur within EPS [13,15]. As illustrated in Figure 2, the hierarchical organization of sludge flocs, EPS, microbial cell envelopes, and intracellular compartments creates structural barriers that influence biomolecule accessibility.

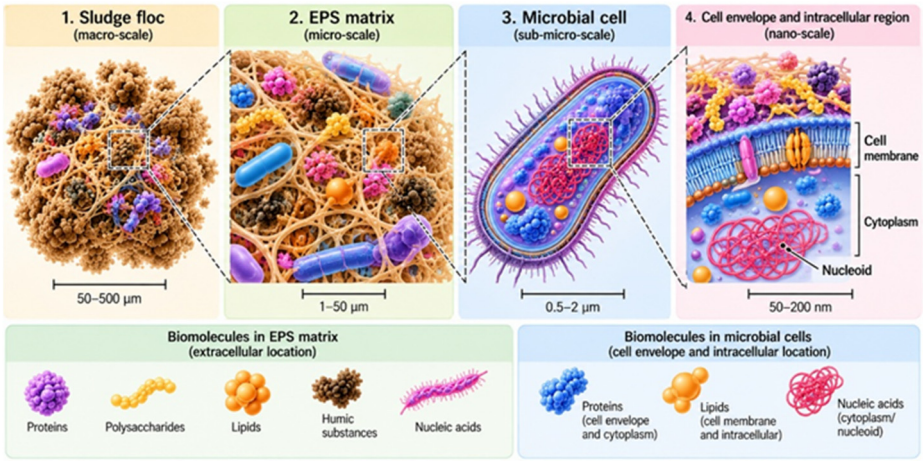


Figure 2. Hierarchical organization of waste activated sludge (WAS) and distribution of target biomolecules within the extracellular polymeric substance (EPS) matrix and microbial cells. The schematic illustrates structural organization from the sludge floc to the cell envelope and intracellular region [14,15].

Effective recovery therefore requires treatment strategies that account for the structural location of the target compound. Disruption or modification of the EPS matrix can increase the accessibility of extracellularly associated compounds, whereas mobilization of intracellular proteins and lipids may additionally require permeabilization or disruption of microbial cell envelopes. Increasing biomolecule accessibility is particularly relevant to sludge valorization because the availability of sludge-associated organic matter influences its subsequent conversion or recovery into value-added products [42]. However, increasing treatment severity can also induce undesirable transformations of released compounds; therefore, operating conditions should be controlled according to the intended recovery pathway and product characteristics. Electrochemical technologies are of particular interest in this context because their treatment intensity can be adjusted through operating parameters such as current density or applied potential, electrode material, and treatment

time. Nevertheless, enhanced accessibility or biomolecule release does not constitute final recovery, which requires subsequent extraction, separation, and, depending on the intended product, purification. Figure 2 summarizes the hierarchical structural organization of WAS and the principal extracellular, membrane-associated, and intracellular locations relevant to biomolecule accessibility and subsequent recovery.

4. Electrochemical Mechanisms for Protein and Lipid Mobilization

Electrochemical treatment can recover proteins and lipids from WAS since uses processes that include electron-transfer reactions at electrode interfaces, in situ generation of reactive oxygen species (ROS), membrane electroporation, microbial cell lysis, extracellular polymeric substance (EPS) degradation, and floc fragmentation. Rather than acting independently, these mechanisms form a coupled cascade that progressively disrupts sludge structure and improves the accessibility of intracellular and extracellular biomolecules [43–45].

4.1. Direct Electron-Transfer Reactions

The first step in electrochemical sludge treatment involves electron-transfer reactions at the electrode–liquid interface. At the anode, oxidation reactions remove electrons from electroactive species, whereas cathodic reactions drive reduction processes. Water electrolysis and interfacial electron-transfer reactions can generate localized pH gradients and reactive intermediates, which may contribute to structural modifications of sludge-associated organic matter [43,44].

At the anode, water oxidation can proceed toward oxygen evolution, releasing protons and electrons, as represented by Equation (1):

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad (1)$$

In electrochemical oxidation systems, water oxidation at the anode surface can also generate adsorbed hydroxyl radicals, M(•OH), where M represents an active site on the electrode surface. This interfacial reaction can be represented by Equation (2):

$$M + H_2O \rightarrow M(\bullet OH) + H^+ + e^- \quad (2)$$

In parallel with water oxidation, electroactive organic species can undergo direct anodic electron transfer. For a generic neutral organic species R, one-electron oxidation can be represented as:

$$R \rightarrow R^{\bullet +} + e^- \quad (3)$$

At the cathode, water reduction can produce molecular hydrogen and hydroxide ions, generating a locally alkaline environment:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (4)$$

The simultaneous occurrence of anodic and cathodic reactions can therefore establish localized pH gradients within the electrochemical system. In locally acidic regions, protonation of ionizable functional groups such as carboxylates can occur according to Equation (5):

$$R\text{–}COO^- + H^+ \rightarrow R\text{–}COOH \quad (5)$$

These changes in local pH and surface charge can modify electrostatic interactions within the EPS matrix and affect floc stability. In addition, direct electron transfer and electrode-generated reactive species can oxidize susceptible functional groups in proteins, lipids, and other EPS-associated organic compounds, contributing to sludge disintegration and solubilization [43,44].

4.2. Reactive Oxygen Species Generation

Reactive oxygen species (ROS) can play an important role in electrochemical sludge treatment. Depending on the electrochemical system and operating conditions, relevant species may include hydroxyl radicals ($^{\bullet}OH$), superoxide ($O_2^{\bullet-}$), hydroperoxyl radicals ($HO_2^{\bullet}$), hydrogen peroxide (H_2O_2), and, under specific conditions, singlet oxygen ($^1O_2$) [44–46]. These species can react with organic components of the sludge matrix, promoting EPS modification, membrane damage, and organic-matter solubilization. ROS-mediated oxidation can also affect membrane lipids and proteins, increasing membrane permeability and facilitating biomolecule release. However, the formation and relative contribution of individual ROS depend strongly on electrode material, applied current or potential, pH, dissolved oxygen, electrolyte composition, and reactor configuration [44–46].

One-electron reduction of dissolved oxygen can produce superoxide:

$$O_2 + e^- \rightarrow O_2^{\bullet-} \quad (6)$$

Protonation of superoxide produces the hydroperoxyl radical:

$$O_2^{\bullet-} + H^+ \leftrightarrow HO_2^{\bullet} \quad (7)$$

Hydroperoxyl radicals can contribute to hydrogen peroxide formation:

$$2\ HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \quad (8)$$

Hydrogen peroxide can undergo one-electron reduction to form hydroxyl radicals:

$$H_2O_2 + e^- \rightarrow {}^{\bullet}OH + OH^- \quad (9)$$

ROS can subsequently react with susceptible functional groups in sludge-associated organic compounds. For example, hydroxyl radicals can oxidize thiol groups:

$$\text{R-SH} + {}^{\bullet}OH \rightarrow \text{R-S}^{\bullet} + H_2O \quad (10)$$

Oxidation of alcohol groups in polysaccharide-associated structures can be represented schematically as:

$$\text{R-CH}_2\text{OH} + 2\ {}^{\bullet}OH \rightarrow \text{R-CHO} + 2H_2O \quad (11)$$

Lipid peroxidation can be initiated by hydrogen abstraction from a susceptible lipid substrate:

$$RH + {}^{\bullet}OH \rightarrow R^{\bullet} + H_2O \quad (12)$$

The resulting carbon-centered radical can subsequently react with molecular oxygen to form a peroxyl radical:

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \quad (13)$$

Equations (10)–(13) are simplified representations of possible ROS-mediated reaction pathways rather than elementary mechanisms. Collectively, these reactions illustrate how ROS can contribute to EPS modification, membrane damage, and increased biomolecule accessibility [44–46].

However, ROS generation has a dual role in biomolecule recovery. Controlled ROS exposure can facilitate EPS disruption and membrane permeabilization, thereby enhancing the release of proteins and lipids. In contrast, excessive ROS generation or prolonged electrochemical treatment can promote secondary oxidation of the released biomolecules, including oxidation of susceptible amino acid residues and lipid peroxidation, potentially reducing product integrity and subsequent valorization potential. Therefore, electrochemical conditions should be optimized to achieve sufficient sludge disruption while limiting

over-oxidation. In particular, current density or applied potential and treatment time should be controlled to regulate the extent of ROS generation and exposure, while electrode material and electrolyte composition should be selected according to the desired oxidation pathway. Consequently, the optimal operating window is not necessarily the condition producing the highest degree of sludge oxidation but rather the one providing sufficient biomolecule release with minimal subsequent degradation [44–46].

4.3. Electroporation

In addition to electrochemical oxidation, sufficiently strong electric fields can affect microbial cell membranes through electroporation. The electric field induces a transmembrane potential that can alter membrane permeability and promote the formation of nanoscale pores in the phospholipid bilayer [47,48]. Depending on the electric-field strength and exposure conditions, electroporation may be reversible, resulting in temporary membrane permeabilization, or irreversible, leading to persistent loss of membrane integrity. These effects can increase the accessibility and release of intracellular proteins, lipids, enzymes, and other cellular components [47,48].

For an approximately spherical cell exposed to a uniform external electric field, the induced transmembrane potential can be approximated using the Schwan equation:

$$\Delta\Psi m = 1.5\ Er\ \cos\theta \quad (14)$$

where $\Delta\Psi m$ is the induced transmembrane potential, E is the local electric-field strength, r is the cell radius, and θ is the angle between the electric-field direction and the position vector normal to the membrane surface.

Electroporation can occur when the induced transmembrane potential reaches a sufficiently high value, with threshold values commonly reported on the order of approximately 0.2–1.0 V depending on cell type, membrane properties, pulse characteristics, and exposure conditions [47,48]. Above the relevant threshold, membrane permeability can increase substantially and may progress toward irreversible membrane damage under sufficiently severe conditions. In WAS, such membrane permeabilization can facilitate the release of intracellular biomolecules and may also increase their exposure to electrochemically generated reactive species. However, because the local electric field experienced by microbial cells depends on reactor geometry, electrode configuration, conductivity, and operating conditions, electroporation should not be inferred solely from the applied reactor voltage or current.

4.4. Cell Lysis

Under sufficiently severe electrochemical conditions, oxidative damage and membrane destabilization can lead to loss of membrane integrity and cell lysis. Cell lysis can release intracellular proteins, lipids, nucleic acids, and soluble metabolites into the liquid phase, thereby increasing the soluble organic fraction of sludge. This process can enhance the accessibility of intracellular biomolecules for subsequent extraction and recovery. However, excessive oxidative treatment may also promote secondary degradation of released biomolecules, potentially affecting their integrity and subsequent valorization. Therefore, electrochemical operating conditions should be optimized to achieve sufficient cell disruption while limiting degradation of the target compounds [43,49,50].

ROS-mediated oxidation of cellular and membrane-associated organic compounds can involve hydrogen abstraction, as illustrated in Equation (12). The resulting carbon-centered radicals can react with molecular oxygen to form peroxyl radicals, as shown in Equation (13). Subsequent radical reactions may promote further oxidation and fragmentation of susceptible cellular components, contributing to membrane damage and cell lysis [49,50].

4.5. EPS Disruption

Extracellular polymeric substances (EPS) form an important structural matrix of activated sludge flocs and can restrict the accessibility of sludge-associated biomolecules. EPS comprise a heterogeneous mixture of proteins, polysaccharides, humic substances, lipids, nucleic acids, and inorganic components that contribute to floc stability through hydrogen bonding, electrostatic interactions, hydrophobic interactions, and multivalent-cation bridging [50,51]. Depending on the electrochemical technology and operating conditions, electrochemical treatment can modify the EPS matrix through oxidation of susceptible organic components, changes in local pH and charge interactions, and disruption of intermolecular associations. ROS-mediated reactions can modify proteins, polysaccharides, and other EPS constituents, weakening the structural organization of the matrix and promoting the transfer of bound organic matter into more soluble or accessible fractions [50,51]. These changes can facilitate the mobilization of EPS-associated biomolecules and increase the accessibility of microbial cells. However, EPS disruption and increases in soluble organic matter or soluble protein should not be interpreted as direct evidence of final biomolecule recovery, which requires subsequent separation and collection of the target fraction.

4.6. Floc Structural Changes

Electroosmotic transport is another electrokinetic phenomenon relevant to sludge treatment. Under an applied electric field, electroosmosis can promote water movement and contribute to sludge dewatering, although this effect does not directly demonstrate protein or lipid recovery [52].

Electrochemical treatment can alter the structural organization of sludge flocs through changes in the EPS matrix, electrostatic interactions, and cation-mediated associations. Depending on the electrochemical technology and operating conditions, these effects can weaken pre-existing floc structures and promote fragmentation into smaller aggregates, potentially increasing the surface area available for mass transfer and improving the accessibility of sludge-associated organic matter [53]. Changes in floc structure may also reduce diffusion limitations and facilitate interactions between electrochemically generated reactive species and sludge components.

Multivalent cations such as Ca^{2+} can contribute to EPS stabilization by bridging negatively charged functional groups. Disruption of these cation-mediated associations can be represented schematically as:

$$(\mathrm{EPS} - \mathrm{Ca} - \mathrm{EPS}) \rightarrow 2\mathrm{EPS}^{-} + \mathrm{Ca}^{2+} \quad (15)$$

This equation schematically represents the disruption of a calcium-mediated bridge between negatively charged EPS functional groups rather than an elementary chemical reaction.

However, the extent and direction of floc structural changes are technology-dependent. In particular, electrocoagulation can promote charge neutralization and the formation of new aggregates through electrochemically generated Fe- or Al-based coagulant species; therefore, aggregation during electrocoagulation should not be interpreted as disruption of the original WAS floc structure. Accordingly, changes in particle size, sludge solubilization, or dewaterability should be considered indicators of structural modification rather than direct evidence of protein or lipid recovery [53].

4.7. Biomolecule Release Pathways

Protein and lipid mobilization from waste activated sludge depends on their location within the heterogeneous sludge matrix and on the electrochemical technology and operating conditions. Proteins and lipids may occur in the soluble phase, within loosely or tightly associated EPS fractions, at microbial cell envelopes, or in intracellular compart-

ments. Consequently, their accessibility and release can differ according to their structural association with the sludge matrix and the extent of EPS modification and cell-envelope disruption [43,45,53,54].

As illustrated in Figure 3, several technology- and condition-dependent mechanisms can contribute to biomolecule mobilization, including direct electron-transfer reactions, ROS-mediated oxidation, membrane permeabilization or electroporation, EPS disruption, cell lysis, and changes in floc structure. These mechanisms may occur simultaneously or contribute to different extents rather than constituting a universal sequential pathway. EPS modification can increase the accessibility of extracellularly associated compounds, whereas mobilization of intracellular proteins and membrane-associated or intracellular lipids may additionally require sufficient permeabilization or disruption of microbial cells.

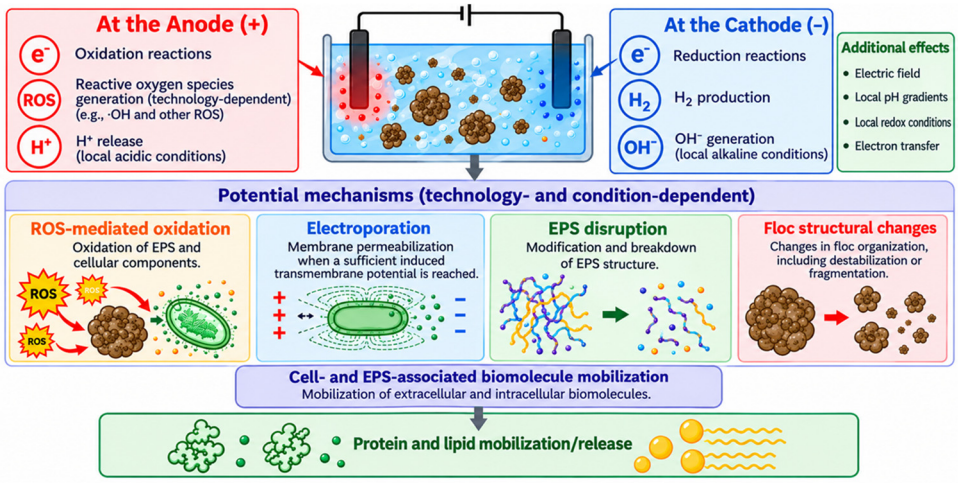


Figure 3. Schematic representation of the main electrochemical reactions and technology- and condition-dependent mechanisms contributing to protein and lipid mobilization from waste activated sludge (WAS), including ROS-mediated oxidation, electroporation, EPS disruption, and floc structural changes.

The extent of protein and lipid mobilization therefore depends on the interaction between sludge characteristics, biomolecule location, electrochemical technology, and treatment severity. Importantly, increased biomolecule release or solubilization does not constitute final recovery. Subsequent separation, extraction, concentration, and, where required, purification, are necessary to obtain useful protein- or lipid-rich fractions. Electrochemical operating conditions should therefore be optimized to provide sufficient biomolecule accessibility and release while limiting excessive oxidation and degradation of the target compounds [43,45,53,54].

5. Protein and Lipid Release

Proteins and lipids are valuable constituents of WAS with potential for subsequent recovery and valorization [55–57]. Electrochemical treatment can promote their mobilization through EPS disruption, membrane permeabilization, cell lysis, and ROS-mediated oxidation, depending on the technology and operating conditions [55–63]. However, excessive oxidative treatment may compromise protein and lipid integrity, highlighting the need to balance biomolecule release with preservation of product quality [55–63].

Importantly, biomolecule release or solubilization does not constitute final recovery. Subsequent separation, extraction, concentration, and, where necessary, purification is required to obtain useful protein- or lipid-rich fractions. Therefore, increases in soluble protein, soluble organic matter, or sludge disintegration should not be interpreted as direct measures of final recovery.

5.1. Electrochemical Approaches

Several electrochemical technologies have been investigated for sludge disintegration, biomolecule mobilization, and resource recovery from WAS. These technologies differ considerably in their electrochemical mechanisms, electrode configurations, reactive species generated, operational requirements, and effects on sludge-associated organic matter. Among them, electrooxidation, electro-Fenton, electrocoagulation, and bioelectrochemical systems have received considerable attention. Depending on the technology and operating conditions, these approaches can promote EPS modification, cell disruption, organic-matter solubilization, or subsequent separation and conversion processes, thereby increasing the accessibility of proteins and other sludge-associated compounds. However, enhanced protein release or solubilization should not be interpreted as selective protein recovery unless a separated protein-rich product is subsequently obtained and characterized.

5.1.1. Electrooxidation

Electrooxidation (EO) is an electrochemical treatment in which organic matter can be transformed through direct electron transfer at the anode surface and indirect oxidation mediated by electrochemically generated reactive species. Depending on the electrode material, electrolyte composition, and operating conditions, reactive oxygen species such as hydroxyl radicals ($^{\bullet}OH$) can contribute to EPS modification, membrane damage, and sludge disintegration, thereby increasing the accessibility and mobilization of extracellular and intracellular biomolecules [64–66].

The performance of EO is strongly influenced by electrode material, current density or applied potential, treatment time, electrolyte composition, and sludge characteristics. Electrode materials investigated for electrochemical oxidation include boron-doped diamond (BDD), titanium-based mixed metal oxide (MMO) electrodes, graphite, and dimensionally stable anodes (DSA) [64–68]. Electrode material influences oxygen-evolution behavior and the formation and interaction of reactive species at the electrode surface, thereby affecting the extent of organic-matter oxidation and sludge disintegration. Under appropriate conditions, EO can increase soluble organic matter and protein concentrations through EPS modification and cell disruption. However, increases in soluble protein or other soluble organic fractions indicate enhanced mobilization or solubilization rather than final biomolecule recovery.

Treatment severity is particularly important because excessive oxidative conditions can promote secondary oxidation of released proteins and lipids, potentially affecting their molecular integrity and subsequent valorization. Therefore, current density or applied potential and treatment time should be optimized to achieve sufficient sludge disruption and biomolecule mobilization while limiting over-oxidation [65,67,68].

EO can be implemented using relatively simple reactor configurations and provides direct control through electrical operating parameters. However, electrical energy requirements, electrode fouling or degradation, and the potential for over-oxidation remain important considerations for practical application [64–68].

5.1.2. Electro-Fenton (EF)

Electro-Fenton (EF) combines electrochemical reactions with Fenton chemistry to generate hydroxyl radicals ($^{\bullet}OH$). In typical EF systems, hydrogen peroxide (H_2O_2) is generated electrochemically through the cathodic reduction of dissolved oxygen and subsequently reacts with ferrous ions (Fe^{2+}) to produce $^{\bullet}OH$. Depending on the reactor configuration, Fe^{3+} can also be electrochemically reduced to Fe^{2+}, contributing to catalyst regeneration and sustained Fenton reactions [67–70].

Cathodic generation of hydrogen peroxide can be represented as:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (16)$$

Hydroxyl radicals are subsequently generated through the Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (17)$$

Electrochemical reduction of Fe^{3+} can contribute to the regeneration of Fe^{2+}

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (18)$$

The resulting reactive species can oxidize susceptible components of EPS and microbial cell envelopes, promoting EPS modification, membrane damage, sludge disintegration, and increased accessibility of sludge-associated organic matter [67–70]. However, these effects depend on the operating conditions and should not be interpreted as direct evidence of selective protein or lipid recovery.

EF performance is influenced by several interrelated parameters, including pH, iron concentration, current density or applied potential, dissolved oxygen availability, electrode characteristics, and treatment time [67–72]. Treatment severity is particularly important because excessive ROS generation or prolonged exposure can promote secondary oxidation of released proteins and lipids, potentially reducing their molecular integrity and subsequent valorization potential. Therefore, operating conditions should be optimized to provide sufficient sludge disruption and biomolecule mobilization while limiting over-oxidation.

Compared with conventional EO, EF involves additional homogeneous Fenton reactions in the liquid phase and can therefore provide a different pathway for ROS generation and sludge oxidation. However, its practical implementation may require pH adjustment, iron addition or management, subsequent neutralization, and control of iron-containing residuals, depending on the reactor configuration [68–72]. These additional chemical and operational requirements should be considered together with electrical energy consumption and downstream processing when evaluating EF for biomolecule mobilization and subsequent recovery from WAS.

5.1.3. Electrocoagulation

Electrocoagulation (EC) typically employs sacrificial aluminum or iron electrodes that dissolve under an applied electric current, generating metal ions that subsequently form hydrolyzed species and metal hydroxide precipitates. These species can promote charge neutralization, adsorption, and sweep flocculation, leading to the aggregation and separation of suspended and colloidal material [73,74]. At the cathode, hydrogen evolution can additionally promote flotation of the resulting aggregates.

Unlike oxidation-based electrochemical processes, the formation of new aggregates during EC should not be interpreted as disruption of the pre-existing WAS floc or EPS structure. Depending on the operating conditions, EC may instead capture soluble or colloidal organic compounds through adsorption or incorporation into Fe- or Al-based flocs. Consequently, proteins and other sludge-derived compounds mobilized into the liquid phase may be redistributed into the coagulated fraction rather than remaining in solution [73–75].

EC may therefore be particularly relevant as a conditioning and separation process within an integrated biomolecule-recovery scheme. However, its contribution should be evaluated by distinguishing sludge conditioning and phase separation from biomolecule mobilization and final recovery. The distribution and characteristics of protein- or lipid-containing fractions after EC depend on electrode material, current density, pH, treatment time, and the resulting metal hydroxide species [73–75].

5.1.4. Bioelectrochemical Systems

Bioelectrochemical systems (BES), including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs), couple microbial metabolism with extracellular electron transfer to electrodes. In sludge-treatment applications, these systems can promote the conversion of biodegradable organic matter while enabling energy or resource recovery, depending on the reactor configuration and operating conditions [73–76]. Their contribution to protein and lipid recovery differs from that of oxidation-based electrochemical technologies because BES primarily rely on biologically mediated substrate conversion and electron transfer rather than intensive ROS-mediated oxidation.

At the anode, electroactive microorganisms oxidize biodegradable organic substrates and transfer the resulting electrons to the electrode. For example, the anodic oxidation of acetate can be represented as follows:

$$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^- \quad (19)$$

This reaction illustrates electron release during microbial substrate oxidation; the actual stoichiometry depends on the organic compounds being metabolized. Electron transfer to the anode can occur through direct extracellular electron-transfer mechanisms involving redox-active cell components or conductive structures, or through mediated electron transfer involving soluble redox-active compounds.

In MFCs using oxygen as the terminal electron acceptor at the cathode, the cathodic reaction can be represented as:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (20)$$

In MECs configured for hydrogen production, application of an external voltage can drive cathodic proton reduction:

$$2H^+ + 2e^- \rightarrow H_2 \quad (21)$$

When integrated with sludge treatment, microbial hydrolysis, biodegradation, cell turnover, and changes in EPS organization can increase the accessibility of sludge-associated organic matter [74–78]. However, increases in organic-matter solubilization or conversion should not be interpreted as direct evidence of selective protein or lipid recovery. The extent to which proteins and lipids are mobilized, transformed, or retained depends on the microbial community, reactor configuration, substrate characteristics, and operating conditions.

BES can therefore provide opportunities for integrating WAS treatment with biological conversion and energy or resource recovery. Nevertheless, evidence for the direct recovery of separated protein- or lipid-rich products from BES remains limited compared with measurements of sludge degradation, organic-matter conversion, and energy-related outputs. Consequently, protein and lipid recovery should be evaluated using product-specific mass balances and characterization rather than inferred from overall sludge degradation or solubilization [74–78].

5.2. Hybrid Systems

Hybrid systems combine electrochemical treatment with physical, chemical, or biological processes to enhance sludge disintegration, biomolecule mobilization, or subsequent resource recovery. Such combinations may exploit complementary mechanisms and, under appropriate conditions, reduce the treatment severity required from an individual process [79–81]. However, their performance depends strongly on the sequence and operating conditions of the combined treatments.

In electrochemical–alkaline systems, alkaline conditions can promote sludge disintegration, modification of the EPS matrix, and hydrolysis or solubilization of sludge-associated

organic matter, thereby increasing biomolecule accessibility. The electrochemical step can subsequently or simultaneously contribute through electron-transfer reactions, local pH and redox changes, and, depending on the system, generation of reactive species [79,82]. The relative contribution of each treatment should nevertheless be determined using appropriate single-treatment controls before synergistic effects are attributed to the combined process.

Electrochemical treatment can also be combined with ultrasonication. Acoustic cavitation and the associated shear forces can disrupt sludge flocs and modify the EPS matrix, potentially improving mass transfer and increasing the accessibility of sludge-associated compounds to subsequent electrochemical reactions [80,83]. Depending on the operating conditions, such combinations may enhance sludge solubilization or biomolecule mobilization; however, reductions in treatment time or energy consumption should be demonstrated using comparable single-treatment controls and a consistent energy-normalization basis.

Other hybrid configurations may integrate electrochemical treatment with enzymatic, biological, Fenton-based, photocatalytic, or other advanced oxidation processes [81,84,85]. These combinations provide opportunities to couple complementary mechanisms of sludge disruption, biomolecule mobilization, and downstream conversion. Nevertheless, enhanced solubilization or protein release does not necessarily represent higher final protein recovery or improved product quality. Final recovery requires subsequent separation and characterization of the target fraction, and potential improvements in energy efficiency or biomolecule integrity should be evaluated quantitatively.

Importantly, the contribution of the electrochemical step should be distinguished from that of accompanying chemical, thermal, ultrasonic, biological, or other treatments. When appropriate single-treatment controls are unavailable, improvements in sludge disintegration, biomolecule mobilization, or recovery should be attributed to the combined treatment rather than exclusively to its electrochemical component [79–85].

5.3. Recovery Performance

The reported performance of electrochemical treatments for protein and lipid mobilization from WAS depends on sludge characteristics, reactor configuration, electrode material, and operating conditions [64,66,69]. Comparisons among studies remain challenging because reported endpoints range from sludge disintegration and soluble chemical oxygen demand (SCOD) to protein solubilization, extraction yield, and final recovery.

Electrooxidation (EO) can enhance sludge disintegration and biomolecule mobilization through direct and mediated oxidation mechanisms [64–68]. Electro-Fenton (EF) treatment can similarly promote organic-matter solubilization through electrochemically supported Fenton reactions and reactive oxygen species generation [69–72]. However, EO may involve substantial electrical energy consumption, whereas EF can require additional pH adjustment and iron management. Both processes require careful control of treatment severity to limit secondary oxidation of released biomolecules.

In contrast, electrocoagulation (EC) primarily promotes charge neutralization, aggregation, and phase separation through electrochemically generated Fe- or Al-based coagulants [73–75]. Consequently, its performance should be evaluated by considering the distribution of target compounds between liquid and coagulated fractions rather than soluble concentrations alone. Bioelectrochemical systems (BES), meanwhile, mainly support biologically mediated organic-matter conversion and energy or resource recovery [76–78], with limited evidence of isolated protein- or lipid-rich products.

Overall, these technologies fulfill complementary rather than directly interchangeable functions. EO and EF are more closely associated with oxidative biomolecule mobilization, EC with conditioning and separation, and BES with biological conversion. Therefore, technology selection should be guided by the intended product, demonstrated recovery

performance, product quality, energy and reagent requirements, and downstream processing needs. Importantly, differences in analytical endpoints prevent direct comparisons of protein or lipid recovery efficiency unless equivalent product-specific measurements are available. Table 1 summarizes the operating conditions and reported performance endpoints of representative electrochemical studies.

Table 1. Study-by-study comparison of electrochemical approaches for biomolecule release and resource recovery from sewage sludge.

Electrochemical Method	Sludge/Target	Electrode(s)/ Operating Conditions	Performance, Analytical Basis & Energy *	Main Limitations	Ref.
Electrooxidation	Municipal secondary-settler return sludge (7.0 g L^{-1}); EPS proteins and cell lysis	Ti/SnO_2-Sb anode; Ti mesh cathode; 18 V; 90 min; initial pH 6.8–7.0	Soluble-EPS protein increased from 5.98 to 244.99 mg L^{-1} (Lowry assay; ~41-fold). Sludge disintegration: 25.35%; cell lysis: 20.15%. Final protein/lipid recovery: NR; energy: NR	Possible biomolecule oxidation; energy demand; final recovery not demonstrated	[66]
Electro-Fenton	WAS; protein and polysaccharide solubilization	Fe electrodes; pH 3; 2.5 A dm^{-2}; H_2O_2: 1.8 g L^{-1}; 1 h	Soluble protein: 166.62 → 536.62 mg L^{-1}; protein release: 68.95%; polysaccharide release: 65.22%. Final protein/lipid recovery: NR; energy: NR	Acidic pH and H_2O_2 requirements; potential protein oxidation; final protein/lipid recovery not demonstrated	[70]
Electrocoagulation	Municipal sewage sludge; Soluble organics	Fe electrodes; pH 7; 150 A m^{-2}; 30 min	DD, SCOD, TSS/TOC. DD: 84.23%; SCOD: 675.2 mg L^{-1}. Protein/lipid recovery: NR; operating cost: EUR 0.078 m^{-3}	Electrode consumption; metal-containing solids; limited biomolecule selectivity	[71]
Bioelectro-chemical systems (BES)	WAS; organic-matter/resource recovery	Carbon-based MEC electrodes; 0.6 V; anaerobic operation	Methane/VS and humic-substance analyses. CH_4: 155.5–158.2 mL g^{-1} VS. Protein/lipid recovery: NR; comparable energy basis: NR	Long treatment; microbial dependence; scale-up complexity	[74]
Hybrid electrochemical systems	Primary sludge; solubilization/re-source recovery	Alkaline–ultrasound + MEC; pH 10, 2 h; US: 20 kHz, 0.5 W mL^{-1}, 80 min;	Pretreatment DS: 67.2%; enhanced subsequent resource conversion in the MEC. Protein/lipid recovery: NR; comparable energy basis: NR	Multistep process; chemical/energy inputs; difficult attribution of individual effects	[79]

* Performance values are reported according to the analytical endpoints and calculation bases of the original studies. Protein/lipid release or solubilization is not considered equivalent to final recovery unless a subsequent recovery step is demonstrated. Energy consumption is reported only when its value and normalization basis can be verified. NR: not reported; WAS: waste activated sludge; VS: volatile solids; SCOD: soluble chemical oxygen demand; EPS: extracellular polymeric substances; DD: disintegration degree; TSS: total suspended solids; TOC: total organic carbon; BES: bioelectrochemical systems; MEC: microbial electrolysis cell; DS: degree of solubilization; US: ultrasound.

6. Valorization Pathways for Recovered Biomolecules

Electrochemical treatment can contribute to the valorization of waste activated sludge (WAS) by increasing the accessibility of recoverable constituents and facilitating subsequent separation or conversion processes [86–90]. Depending on the treatment configuration, potential recovery pathways may target protein- and lipid-rich fractions alongside other resources, such as volatile fatty acids, nutrients, methane, hydrogen, or biopolymers. How-

ever, electrochemical treatment represents one component of an integrated recovery scheme rather than a complete biorefinery process. The following sections focus on the downstream recovery and valorization of proteins and lipids mobilized from WAS.

6.1. Protein Valorization Pathways

The valorization of proteins recovered from WAS depends not only on recovery yield but also on product purity, molecular integrity, composition, and functionality. Consequently, treatment and downstream processing conditions should be selected to achieve sufficient protein mobilization and recovery while limiting undesirable modifications that could restrict subsequent applications [87,91].

One potential valorization route involves the production of protein hydrolysates through controlled enzymatic or chemical hydrolysis. These hydrolysates contain peptides and free amino acids that may serve as nitrogen-rich substrates for microbial processes or as feedstocks for agricultural and biochemical applications [91–93]. Depending on their composition and molecular characteristics, peptide-rich fractions may also exhibit functional properties such as antioxidant or metal-chelating activity; however, these properties should be experimentally verified for the recovered product before specific applications are proposed.

Recovered protein fractions may also provide feedstocks for bio-based materials or further biochemical conversion [90,92]. Following hydrolysis, amino acids and peptides can potentially be used as substrates in microbial processes for the production of value-added compounds, including organic acids, enzymes, biosurfactants, pigments, and other biochemicals [87,91,94]. The feasibility of these pathways depends on substrate composition, purity, microbial requirements, and the presence of potentially inhibitory sludge-derived compounds.

Agricultural valorization represents another potential pathway for protein hydrolysates and amino-acid-rich fractions. Protein hydrolysates have been investigated for applications such as plant biostimulants and nitrogen-containing agricultural products [92,94]. However, when these fractions originate from WAS, their suitability should be established through product-specific characterization, including nutrient composition, contaminants, pathogens, and compliance with applicable safety requirements.

6.2. Lipid Valorization Pathways

Lipids recovered from WAS represent a potentially valuable feedstock for biofuel and biochemical applications. Sludge-derived lipid fractions may contain phospholipids, glycerides, free fatty acids, and other extractable organic compounds, with their composition depending on sludge characteristics and the extraction procedure [95–97]. Consequently, evaluation of lipid recovery should consider not only extraction yield but also lipid-class distribution, free fatty acid content, fatty acid composition, purity, and oxidative deterioration, particularly when subsequent valorization is intended.

One of the most extensively investigated valorization routes for sludge-derived lipids is biodiesel production. Triglycerides can be converted into fatty acid alkyl esters through transesterification, whereas free fatty acids require esterification or other appropriate conversion routes. The substantial presence of C16 and C18 fatty acids reported in sewage-sludge lipid fractions supports their potential use as biodiesel feedstocks [96–99]. However, the extracted fraction may also contain free fatty acids, phospholipids, pigments, and other co-extracted compounds that can affect subsequent conversion and may require additional separation or purification.

Beyond biodiesel production, recovered lipid fractions and their constituent fatty acids may provide feedstocks for oleochemical and other bio-based applications, including surfactants, lubricants, specialty esters, and related products [95,98,100]. Their suitability for these applications depends on the composition, purity, and physicochemical characteristics

of the recovered fraction. Accordingly, product-specific characterization and downstream processing are necessary before the potential value of sludge-derived lipids can be established.

Lipid valorization may also involve biological conversion pathways in which sludge-derived organic substrates are used to support microbial production of new intracellular lipids or other value-added compounds. Such pathways should be distinguished from the direct extraction and recovery of native sludge lipids because the resulting products are generated through microbial conversion rather than recovered directly from the lipid fraction initially present in WAS. Overall, the available evidence indicates that electrochemical treatment may facilitate lipid accessibility as part of an integrated recovery process, but subsequent extraction, phase separation, and product purification remain necessary to obtain a usable lipid-rich fraction.

6.3. Integrated Protein–Lipid Recovery

Integrated recovery of proteins and lipids from WAS provides an opportunity to utilize multiple organic fractions from a common feedstock. Because these biomolecules can occur at different locations within the sludge matrix, including the EPS matrix, microbial cell envelopes, and intracellular compartments, treatment strategies that modify these structural barriers may increase the accessibility of both fractions [101–103]. Electrochemical treatment can contribute to this process by promoting EPS modification, membrane permeabilization, cell disruption, and biomolecule mobilization, depending on the technology and operating conditions. However, concurrent mobilization of proteins and lipids should not be considered equivalent to their simultaneous recovery as separated products.

The operating conditions required for effective mobilization may differ according to the location and characteristics of the target biomolecules. Current density or applied potential, treatment time, electrode material, electrolyte composition, and other reactor conditions should therefore be optimized to achieve sufficient sludge disruption while limiting undesirable protein oxidation and lipid peroxidation [102,104]. Following electrochemical treatment, selective downstream separation is required to obtain distinct protein- and lipid-rich fractions, and their recovery yield, purity, and molecular characteristics should be evaluated independently.

Recovered protein- and lipid-rich fractions can subsequently be directed toward complementary valorization pathways according to their composition and quality [101–105]. Such an integrated approach provides opportunities for more complete utilization of WAS-derived organic matter and for the production of multiple value-added fractions from a single feedstock. However, its overall economic and environmental benefits depend on the additional energy, reagents, separation and purification requirements, product yields and quality, and management or further utilization of the remaining sludge fraction.

Overall, the protein, lipid, and integrated protein–lipid recovery and valorization pathways discussed in Sections 6.1–6.3 are summarized in Figure 4. Following electrochemical mobilization of sludge-associated biomolecules, downstream separation and fractionation can generate protein-rich, lipid-rich, or combined fractions for subsequent valorization into agricultural products, bio-based materials, biofuels, oleochemicals, and fermentation-derived products. These pathways highlight the potential for integrating electrochemical treatment with downstream recovery and conversion processes to support the multiproduct valorization of WAS. Importantly, the configurations shown in Figure 4 represent potential pathways based on the available literature rather than a single experimentally demonstrated integrated process.

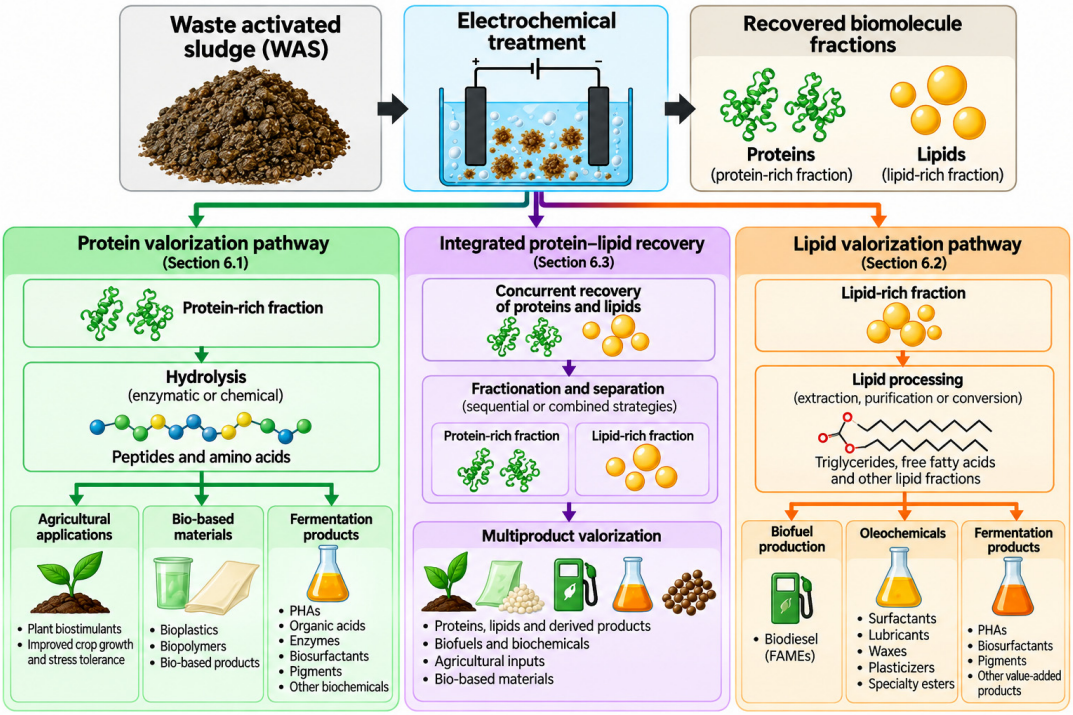


Figure 4. Proposed protein and lipid recovery and valorization pathways from waste activated sludge (WAS) following electrochemical treatment.

6.4. Circular Bioeconomy Implications

Electrochemical technologies can contribute to integrated WAS resource-recovery strategies by promoting sludge disintegration, biomolecule mobilization, conditioning, separation, or biological conversion, depending on the technology and operating conditions [106–108]. Compared with conventional approaches such as chemical hydrolysis, thermal treatment, ultrasonication, and solvent extraction, electrochemical processes may reduce dependence on externally added chemicals in some configurations. However, their performance, energy requirements, and ability to preserve biomolecule integrity depend strongly on operating conditions, while differences in sludge characteristics and reported performance metrics limit direct comparisons [102–108].

The contribution of electrochemical methods to a circular bioeconomy depends on the complete recovery chain rather than biomolecule mobilization alone. Downstream separation, concentration, and purification must be considered alongside product composition, quality, and intended applications. In addition, sludge-associated contaminants, including heavy metals, pathogens, organic micropollutants, and microplastics, may restrict the suitability of recovered products [109–111]. Pilot-scale validation, techno-economic assessment, and life-cycle assessment are therefore needed to establish the feasibility of integrated recovery schemes [106–112].

Table 2 compares conventional and electrochemical approaches for protein and lipid recovery in terms of operating conditions, reported extraction or recovery performance, and principal advantages and limitations. It also highlights the limited availability of directly comparable data on the purity and quality of recovered products.

Table 2. Comparison of conventional and electrochemical technologies relevant to protein and lipid recovery and valorization from sewage sludge.

Method	Representative Operating Conditions	Reported Performance	Main Advantages/Limitations	Ref.
Thermal–alkaline treatment	pH 13; 70 °C; 9 h; optimized at pH 13.23 and 77.18 °C	60.72% protein extraction; ~62.07% protein recovery under optimized conditions	Effective protein extraction/high pH and heating requirements; downstream purification required	[22]
Ultrasonic–alkaline treatment	pH 12; 1.7 W mL^{-1}; 24 min; sludge moisture content: 94%	81.4% protein extraction efficiency	High and rapid protein extraction/alkali consumption and ultrasonic energy demand	[102]
Hydrothermal treatment	125–250 °C; maximum lipid recovery at ~200 °C	WAS lipid recovery increased from 5.35 to 11.55 wt%	Enhances lipid extraction through hydrothermal disruption/high temperature and energy requirement	[103]
Direct liquid–liquid extraction (LLE)	Sludge acidification; hexane; room temperature; three extraction stages	91% lipid recovery *	High lipid recovery without sludge drying/organic-solvent consumption and recovery required	[104]
Electrooxidation (EO)	EPT: 8–15 V, 0.5–1.5 h	EPT: 15–30% enhanced EPS-matrix destruction at 8–15 V for 0.5 h; 59% higher hydrolysis rate and 28% higher digestibility at 15 V for 1.5 h.	Rapid and controllable WAS disruption and organic-matter mobilization; limited chemical addition/excessive oxidation and energy input require optimization	[43]
Electro-Fenton (EF)	Electrochemically supported Fenton treatment; acidic conditions	68.95% protein release and 65.22% polysaccharide release, vs. 45.25% and 35.22%, respectively, for thermo-alkaline treatment in the same study	Strong protein/EPS mobilization; favorable direct comparison with thermo-alkaline treatment/pH and Fe management and over-oxidation require control	[70]
Electrocoagulation (EC)	Fe electrodes; pH 7; 150 A m^{-2}; 30 min	84.23% sludge disintegration degree; SCOD = 675.2 mg L^{-1}	Effective sludge disintegration/conditioning and enhanced soluble-organic accessibility/electrode consumption; final protein/lipid recovery not quantified	[71]
Bioelectrochemical systems (BES)	MEC at 0.5 V combined with ultrasonic pretreatment	3.68-fold higher biogas production; 2.56-fold higher cathodic H_2 recovery; 1.48-fold higher VSS removal	Couples sludge-organic conversion with energy/resource recovery/longer biological operation; performance reflects integrated treatment	[106]
Hybrid electrochemical systems	Integrated mechano-electro-biological process	~91.4% TOC recovery; subsequent conversion to SCP representing >63% of TOC	High multiproduct valorization potential/contributions of individual treatment steps must be distinguished	[107]

Reported values correspond to study-specific analytical endpoints and should not be interpreted as directly equivalent protein or lipid recovery efficiencies. Protein release, EPS destruction, sludge disintegration, solubilization, and hydrolysis indicate enhanced biomolecule accessibility or mobilization rather than final recovery unless subsequent separation and collection of the target product are demonstrated. BES and hybrid-system results represent integrated resource-conversion processes and therefore should not be interpreted as isolated protein or lipid recovery efficiencies. Product purity and downstream purification requirements are discussed separately in the text. EO, electrooxidation; EPT, electrochemical pretreatment; EF, electro-Fenton; EC, electrocoagulation; BES, bioelectrochemical system; MEC, microbial electrolysis cell; LLE, liquid–liquid extraction; EPS, extracellular polymeric substances; SCOD, soluble chemical oxygen demand; VSS, volatile suspended solids; TOC, total organic carbon; SCP, single-cell protein. * Result obtained from primary sludge rather than WAS and included as comparative evidence for conventional lipid extraction.

The purity of proteins and lipids recovered from sewage sludge depends not only on extraction efficiency but also on process selectivity and subsequent purification steps. For protein recovery, thermal–alkaline extraction can achieve protein extraction rates of approximately 60.7–62.1%; however, the resulting solution is a protein-rich fraction rather than a purified protein product, and further purification is required because other soluble organic components may be co-extracted [22]. Similarly, conventional lipid extraction can provide high recovery; for example, direct liquid–liquid extraction recovered up to 91% of the available lipid fraction from primary sludge [104]. Nevertheless, solvent-extractable fractions may contain free fatty acids and other extractable organic compounds and should not necessarily be considered pure lipids.

Electrochemical treatments can enhance sludge disintegration and the mobilization of proteins and other sludge-associated organic compounds [105–110], but quantitative information on the purity of subsequently recovered protein- and lipid-rich fractions remains scarce. Therefore, although electrochemical processes show promising potential for biomolecule mobilization and subsequent recovery, the available literature does not yet demonstrate that they consistently produce protein or lipid fractions of higher purity than conventional methods. Standardized characterization of recovered fractions and appropriate downstream purification should therefore be considered in future studies to enable reliable comparison of product purity among technologies [111,112].

7. Challenges and Future Research Needs

Electrochemical methods provide a promising platform for coupling waste activated sludge (WAS) treatment with the recovery of value-added products. Their controllable operating conditions and ability to promote sludge disintegration, extracellular polymeric substance (EPS) modification, and biomolecule mobilization create opportunities for integration with downstream resource-recovery processes. However, translation of these advantages into practical applications requires further progress in methodological standardization, product recovery and characterization, energy efficiency, electrode durability, reactor scale-up, and integrated process design. Future research should therefore evaluate the complete recovery chain, from electrochemical mobilization to product separation, purification, and final valorization.

7.1. Standardization, Product Recovery, and Quality

One of the principal barriers to comparing electrochemical sludge treatment studies is the absence of standardized experimental methodologies. Reported performance varies considerably because studies employ different sludge sources, solids concentrations, electrode materials and configurations, reactor designs, operating conditions, extraction procedures, and analytical methods [113–116]. Moreover, sludge disintegration, soluble chemical oxygen demand (SCOD), soluble protein, EPS modification, extraction yield, and final product recovery are sometimes used as performance indicators without clearly distinguishing the corresponding process endpoints. Future studies should therefore differentiate biomolecule mobilization or release, solubilization, extraction, and final recovery and report the initial target content, recovered mass, recovery yield, purity, and calculation basis whenever final recovery is demonstrated. Energy consumption should likewise be reported using a clearly defined normalization basis to facilitate comparison among technologies.

Product quality should be evaluated together with recovery yield. Electrochemical treatment can increase biomolecule accessibility, but released proteins and lipids may remain mixed with soluble organic matter, cell debris, inorganic constituents, and other sludge-derived compounds. Consequently, downstream operations such as solid–liquid separation, membrane separation, precipitation, concentration, solvent extraction, or other

product-specific purification processes may be required. Protein characterization should consider parameters such as purity, molecular integrity, amino acid composition, oxidation state, and application-specific functionality, whereas lipid characterization may include lipid-class distribution, free fatty acid content, fatty acid composition, purity, and oxidative deterioration [117–120]. Because excessive treatment can promote protein oxidation and lipid peroxidation, future research should identify operating conditions that provide sufficient biomolecule mobilization while limiting secondary degradation. Quantitative assessment of product purity and functionality will be particularly important for determining whether enhanced biomolecule release can be translated into useful recovered products.

7.2. Energy, Electrode Durability, and Reactor Scale-Up

Energy consumption is a major consideration for the practical implementation of electrochemical WAS treatment. Electrical requirements depend on current density or applied voltage, treatment time, electrode material and spacing, conductivity, sludge solids concentration, reactor configuration, and mass-transfer conditions [121,122]. Electrolyte composition can also influence electrochemical reaction pathways, as demonstrated by studies examining the effects of halide and carbonate ions on the transformation of organic contaminants [123]. Comparison among studies remains difficult because energy consumption is reported using different normalization bases or, in some cases, only electrical operating parameters are provided. Future studies should therefore report operating conditions together with energy consumption using clearly defined bases, such as treated sludge volume, initial or removed solids, and, where final recovery is demonstrated, the amount of recovered product. In addition, the energy and resource requirements associated with downstream separation, concentration, and purification should be included when evaluating the overall process, because electrochemical mobilization alone does not constitute final product recovery. These downstream requirements can substantially influence the economic viability of obtaining protein- or lipid-rich products from WAS.

The overall resource demand extends beyond electricity. Depending on the electrochemical technology, additional inputs may include pH-control reagents, supporting electrolytes, iron or other catalysts, and sacrificial Fe or Al electrodes. Electrode fouling, passivation, catalyst deactivation, and corrosion can further reduce process efficiency and increase maintenance and replacement requirements [121,124,125]. Development of durable and cost-effective electrode materials, together with strategies for electrode regeneration and fouling control, is therefore important for long-term operation. Consequently, the economic viability of electrochemical protein and lipid recovery should be assessed using comprehensive techno-economic analyses that account for electrochemical treatment, consumables, electrode replacement, and downstream separation and purification.

These considerations become increasingly important during scale-up. Most electrochemical sludge studies have been conducted using laboratory-scale batch systems, whereas practical implementation requires reactors capable of treating heterogeneous, high-solids sludge while maintaining adequate mass transfer, current distribution, and operational stability. Electrode surface area, spacing and arrangement, mixing, hydraulic configuration, conductivity, and reactor geometry can substantially influence reactor performance [126,127]. Scale-up requirements also differ among electrooxidation, electro-Fenton, electrocoagulation, and bioelectrochemical systems because these technologies involve different electrode configurations, reaction environments, and material requirements. Long-term continuous or semi-continuous operation and pilot-scale studies using representative WAS are therefore needed to determine whether laboratory-scale improvements can be maintained under practically relevant operating conditions [121,126,127].

7.3. Integrated Electrochemical Resource-Recovery Configurations

Integrated electrochemical configurations can combine biomolecule mobilization with downstream separation and conversion processes, including membrane separation, precipitation, solvent extraction, fermentation, anaerobic digestion, and nutrient recovery. Such integration may enable the utilization of multiple WAS-derived fractions, including protein- and lipid-rich products [127–130].

The performance of these configurations should be evaluated across the complete recovery chain, with the contribution of individual treatment steps distinguished through appropriate controls. When such controls are unavailable, enhanced performance should be attributed to the integrated process rather than to the electrochemical component alone.

Bioelectrochemical integration with anaerobic processes may also couple organic-matter conversion with energy recovery [130,131]. However, diverting biodegradable compounds toward higher-value products may reduce their availability for methane production. Therefore, the allocation of carbon and other resources among competing recovery pathways should be considered when designing multi-product WAS valorization strategies.

7.4. Advanced Electrodes and Data-Assisted Process Optimization

Advances in electrode materials and reactor configurations provide opportunities to improve electron transfer, mass transport, electrochemical activity, and process efficiency [121,126]. Three-dimensional electrode configurations are particularly relevant because increased electrode surface area and enhanced mass transfer can improve electrochemical reactor performance [126]. The use of graphite rod anode arrays for electrochemical dye oxidation further illustrates how electrode arrangement can be adapted to promote contaminant transformation, although such results do not establish selective protein or lipid recovery from WAS [132]. The effectiveness of advanced oxidation processes depends on the generation and reactivity of oxidizing species, which are influenced by the selected treatment conditions [133]. Nevertheless, increased electrochemical activity or oxidant generation does not necessarily translate into selective biomolecule recovery. Future electrode development should therefore consider not only electrochemical activity but also durability, cost, fouling resistance, material consumption, and performance under the complex physicochemical conditions of WAS. Particular attention should be given to achieving sufficient sludge disruption and biomolecule mobilization without promoting excessive oxidation of the released compounds. Recent advances in electrode materials for electrochemical detection of organic pollutants also illustrate developments in electrode surface design and analytical functionality, although their applicability to WAS treatment and biomolecule recovery remains to be demonstrated [134].

Data-assisted optimization may complement advances in electrode and reactor design. Electrochemical WAS treatment involves numerous interacting variables, including current density, applied voltage, electrode configuration, pH, conductivity, sludge composition, solids concentration, and treatment time. Artificial intelligence, machine learning, and advanced process-control approaches could assist in identifying relationships among these variables and optimizing operating conditions for specific treatment and recovery objectives [135,136]. However, reliable implementation will require sufficiently large, standardized, and experimentally validated datasets. These approaches should therefore complement mechanistic understanding and experimental validation rather than replace them.

7.5. Techno-Economic and Environmental Outlook

The practical implementation of electrochemical WAS valorization ultimately depends on whether the value generated through resource recovery can justify the associated energy, material, and downstream-processing requirements. Comprehensive techno-economic

assessments should consider capital investment, electricity and reagent consumption, sacrificial electrode use, electrode replacement and maintenance, reactor operation, downstream separation and purification, and the quantity and value of recovered products [129,137]. The scale and configuration of wastewater infrastructure may also influence the feasibility of implementing resource-recovery technologies [138].

Environmental assessment should similarly consider the complete treatment and recovery chain. Life-cycle assessment provides an appropriate framework for determining whether potential reductions in residual waste management and substitution of virgin resources compensate for electricity, chemicals, electrodes, solvents, and other process inputs [139]. Potential trade-offs with established sludge-management routes should also be included. In particular, diversion of biodegradable organic matter toward protein, lipid, or other product recovery may influence anaerobic digestion and methane production [130]. Therefore, environmental benefits should be evaluated at the system level rather than inferred solely from increased biomolecule release or extraction.

The fate of sludge-associated contaminants is another important consideration. Heavy metals, pathogens, organic micropollutants, and microplastics may remain in the residual sludge or partition into recovered fractions depending on the treatment and separation strategy. Consequently, recovery yield alone is insufficient to establish product suitability. Product-specific contaminant characterization and compliance with applicable safety requirements are particularly important when agricultural, feed-related, or other sensitive applications are proposed.

Integration with low-carbon or renewable electricity may reduce the electricity-related environmental burden of electrochemical treatment and facilitate its incorporation into more sustainable wastewater-treatment configurations [127,137,140]. Nevertheless, renewable power alone does not establish the overall sustainability of the complete recovery process. Economic and environmental advantages should therefore be demonstrated through comparative techno-economic and life-cycle assessments at relevant scales.

Future research should prioritize standardized recovery metrics, selective downstream separation, product-quality characterization, and pilot-scale validation supported by techno-economic and life-cycle assessments. These efforts are essential to establish the practical feasibility of electrochemical protein and lipid recovery from WAS.

8. Conclusions

Based on the evidence evaluated in this review, the following conclusions can be drawn:

- Electrochemical technologies can enhance WAS disintegration, EPS modification, and the mobilization of sludge-associated proteins and lipids. However, the available evidence more consistently demonstrates biomolecule release and organic-matter solubilization than the final recovery of separated protein- or lipid-rich products. This distinction remains essential when interpreting reported treatment efficiencies.
- Electrochemical operating conditions must be selected according to the target biomolecule and intended recovery pathway. Current density or applied voltage, treatment time, pH, electrode material, and sludge characteristics influence both biomolecule mobilization and the risk of oxidative degradation. Consequently, no universally optimal operating conditions can be established from the available studies. Optimization should balance release efficiency, molecular integrity, and energy and reagent consumption rather than maximize sludge disintegration alone.
- Electrochemical technologies perform different and potentially complementary functions. Electrooxidation and electro-Fenton primarily support oxidative sludge disruption and biomolecule mobilization, whereas electrocoagulation contributes mainly to conditioning and phase separation. Bioelectrochemical systems are more closely associated with

biological conversion and energy or resource recovery. Direct comparisons of protein and lipid recovery efficiency remain limited because studies frequently report different analytical endpoints rather than equivalent measurements of isolated products.

- Downstream processing is a determining factor in the practical recovery of proteins and lipids. Enhanced solubilization does not guarantee selective extraction, high product purity, or preservation of functionality. Appropriate separation and purification strategies, together with measurements of recovered mass, purity, molecular integrity, and contaminant content, are therefore required to establish whether electrochemical treatment produces fractions suitable for subsequent valorization.
- The potential applications of recovered proteins and lipids depend on their composition and quality. Protein-rich fractions may be directed toward hydrolysates, biochemical feedstocks, or bio-based materials, whereas lipid-rich fractions may support biofuel and oleochemical production. Nevertheless, these applications should be regarded as product-dependent opportunities rather than demonstrated outcomes of electrochemical treatment alone. The presence of sludge-associated contaminants and possible treatment-induced degradation must be assessed before specific uses can be established.
- The practical feasibility of electrochemical WAS valorization remains dependent on the performance of the complete recovery chain. Hybrid configurations may integrate complementary treatment, separation, and conversion steps, but their individual contributions should be distinguished experimentally. Future studies should prioritize standardized product-specific recovery metrics, energy and material balances, product-quality characterization, continuous or pilot-scale validation, and comparative techno-economic and life-cycle assessments. These evaluations are necessary to determine whether electrochemical recovery offers measurable advantages over conventional sludge-management and resource-recovery alternatives.

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