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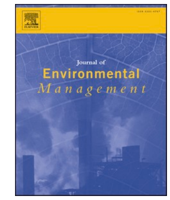

Review

Sludge dewatering: A review of conventional methods and microbial assisted approaches

Yunpeng Chen [a], Chonlong Chio [a], Rishnika Boteju [a], Guimeng Lu [a], Qing-Lai Dang [b], Wensheng Qin [a,*]

[a] Department of Biology, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario, P7B 5E1, Canada
[b] Faculty of Natural Resources Management, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario, P7B 5E1, Canada

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ABSTRACT

Sludge dewatering remains a major challenge in wastewater treatment due to strong water binding within extracellular polymeric substances and the complex structure of microbial flocs. Conventional mechanical, chemical, and physicochemical conditioning methods primarily remove free and interstitial water but are less effective in disrupting extracellular polymeric substance associated bound water, and are often linked to high chemical consumption, elevated energy demand, and increased downstream environmental pressures. This review synthesizes recent advances in sludge dewatering, focusing on extracellular polymeric substance architecture, water state distribution, and microbial-assisted mechanisms. By integrating key performance indicators, including capillary suction time, specific resistance to filtration, dry solids content, nuclear magnetic resonance relaxation characteristics, and extracellular polymeric substance fractionation, a unified framework is established linking sludge microstructure, water binding modes, and dewatering performance. Microbial-assisted strategies, including bioaugmentation, *in situ* activation of indigenous consortia, and enzyme-assisted conditioning, are evaluated in terms of direct extracellular polymeric substance degradation and indirect physicochemical modulation, such as changes in pH, ionic strength, redox conditions, and electrical conductivity. Reported improvements vary with sludge type and operating conditions, and many strategies showing strong laboratory performance demonstrate inconsistent results during scale up. Emerging hybrid microbial chemical and microbial physical processes are therefore examined to clarify how microbial pretreatment enhances bound water release while reducing chemical dosage and energy demand. Sustainability is assessed in terms of resource use, carbon emissions, and impacts on downstream sludge utilization. This review provides a mechanism-oriented framework to support process selection and optimization of sustainable sludge dewatering technologies.

1. Introduction

Sludge management remains a major challenge in wastewater treatment. Municipal wastewater treatment plants generate approximately 45 million tonnes of sewage sludge dry solids annually worldwide, and this amount is expected to increase as treatment standards become more stringent (Di Giacomo and Romano, 2022; Gao et al., 2020). Activated sludge is highly hydrated, with moisture contents commonly exceeding 85% (Cao et al., 2021). In such systems, microbial cells and extracellular polymeric substances (EPS) form a hydrophilic, viscoelastic floc matrix that retains water through hydrogen bonding, electrostatic interactions, and capillary forces, thereby limiting dewatering efficiency (Y. Liu and Fang, 2003). As a result, sludge thickening, dewatering, and disposal are highly resource-intensive and may account for 40–60% of total wastewater treatment plant operating costs (Domini et al., 2022).

Sludge dewatering is governed not only by total moisture content, but also by sludge microstructure and water distribution. Sludge water is generally classified as free water, interstitial water, and bound water. Free and interstitial water can be removed relatively readily by mechanical force, whereas bound water is more strongly retained within microbial flocs and the EPS matrix (Y. Li et al., 2025; Tsang and Vesilind, 1990). Conventional dewatering therefore often combines centrifugation, belt pressing, or filter pressing with chemical or physicochemical conditioning to promote floc aggregation or partially disrupt the EPS matrix (Tuncal and Mujumdar, 2023). However, these treatments

* Corresponding author.
E-mail address: wqin@lakeheadu.ca (W. Qin).

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usually require substantial chemical and energy inputs, may introduce secondary environmental burdens, and can affect downstream sludge utilization, including anaerobic digestion and energy recovery (Cao et al., 2021; Capodaglio and Callegari, 2023).

Although sludge dewaterability is commonly evaluated using capillary suction time (CST), specific resistance to filtration (SRF), EPS composition, and water distribution, these indicators are often reported separately. As a result, efforts to relate sludge microstructure to water binding states and dewatering performance remain limited, and a unified interpretive framework is still lacking in sludge dewatering research (B. Wu et al., 2018).

Microorganism-assisted sludge dewatering has recently attracted increasing attention as a potential complement to conventional conditioning. This approach relies on microbial activity and extracellular enzymes to modify sludge physicochemical properties, mainly through EPS degradation and restructuring (Zavala and Patlan, 2016; J. Zhou et al., 2014). Previous studies have shown that microbial action can disrupt key EPS components, especially proteins and polysaccharides, thereby reducing bound water retention and improving dewaterability, as reflected by lower CST and SRF values (Kang et al., 2023). Relevant enzymes include proteases, glycosidases, amylases, and lipases, which can break protein crosslinks, polysaccharide backbones, and hydrophobic domains within tightly bound EPS, thereby weakening the viscoelastic matrix and promoting bound water release (Nesterov et al., 2024; Wang et al., 2024a).

Microbial effects vary across EPS fractions, and a clear and consistent EPS nomenclature is therefore essential. In this review, EPS is classified into three operational fractions: soluble EPS (S-EPS), loosely bound EPS (LB-EPS), and tightly bound EPS (TB-EPS). S-EPS refers to dissolved or colloidal biopolymers in the bulk liquid or weakly associated with sludge flocs. LB-EPS denotes the outer, weakly attached EPS layer that can be removed by relatively mild extraction, whereas TB-EPS denotes the inner, cell associated EPS fraction that is more strongly retained within the floc matrix and generally requires stronger extraction. When the collective term bound EPS (B-EPS) is used, it refers specifically to LB-EPS plus TB-EPS. These terms are applied consistently throughout this review.

Microbial conditioning can also act through indirect pathways. These include disruption of cation bridging, suppression of EPS synthesis, organic acid driven solubilization, and biosurfactant-mediated modification of interfacial properties (Higgins et al., 2004; Sobeck and Higgins, 2002; Win and Song, 2023). Despite this progress, important knowledge gaps remain. The quantitative relationship between EPS transformation and sludge water distribution is still insufficiently

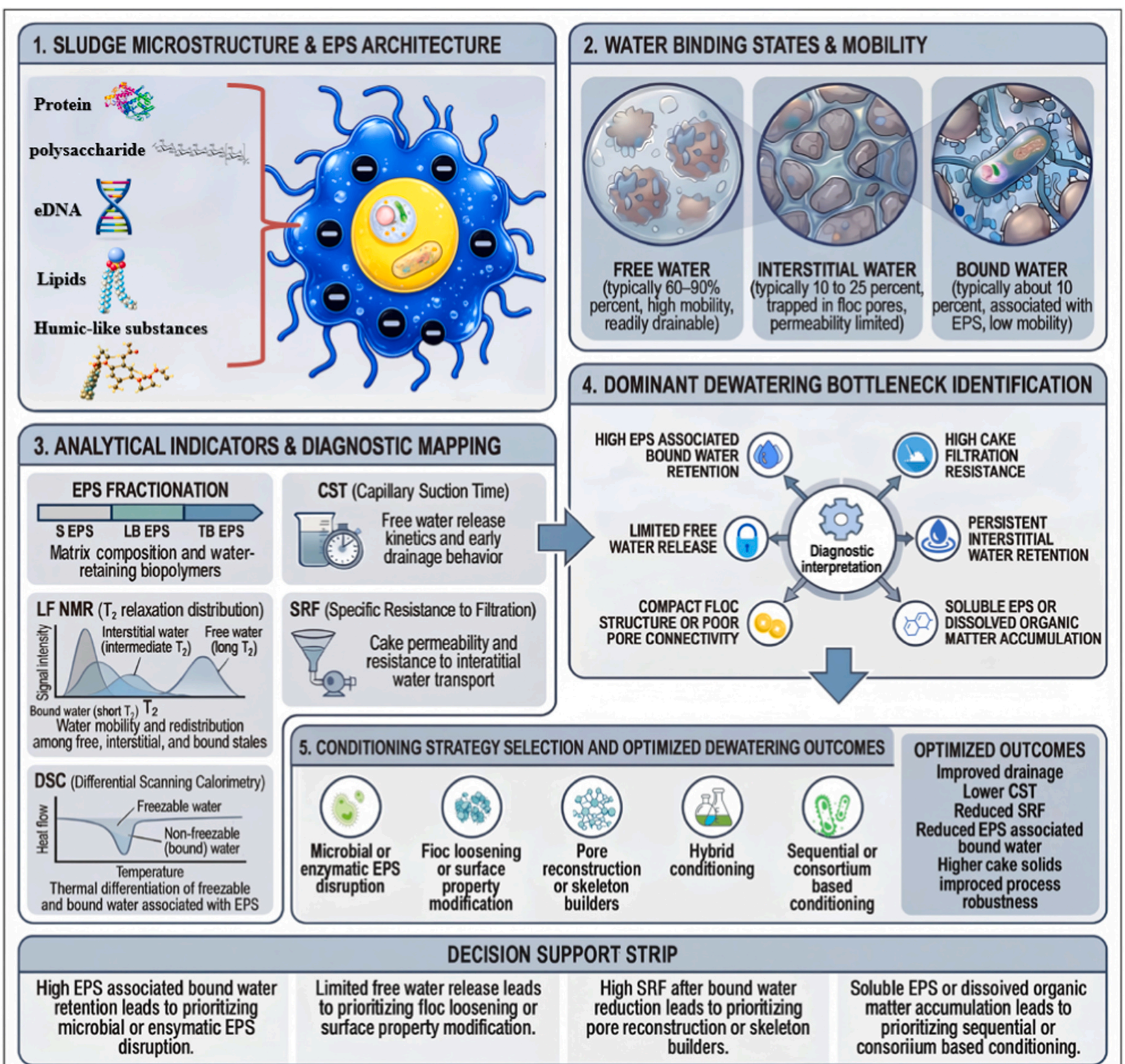

Fig. 1. Integrated framework of sludge microstructure, water binding, diagnostic indicators, and dewatering optimization.

defined. It also remains unclear how microbial activity interacts with physicochemical pretreatments and how biological conditioning can be translated to full scale systems under variable operating conditions.

To address these gaps, this review proposes a unified mechanistic framework (Fig. 1) linking sludge microstructure, EPS architecture, water binding states, analytical indicators, and conditioning strategy selection. Within this framework, sludge dewaterability is governed by interactions among floc structure, EPS associated water retention, and the distribution and mobility of free, interstitial, and bound water. Key indicators, including EPS fractionation, CST, SRF, differential scanning calorimetry (DSC), and low-field nuclear magnetic resonance (LF NMR), are therefore interpreted collectively to identify the dominant dewatering bottleneck, supporting strategy selection, and guide process optimization.

2. Fundamentals of sludge dewatering and water retention mechanisms

In sewage sludge, a substantial fraction of water is retained within the biopolymeric matrix formed by microbial cells and extracellular polymeric substances (EPS), and therefore behaves differently from free water (Sponza, 2003). Sludge dewatering performance is determined by how this water is distributed, how strongly it is associated with solids, and how the floc matrix responds to external force. These features are controlled by the combined effects of colloidal particles, EPS architecture, and mechanical stress, which together define the achievable solid-liquid separation efficiency (Higgins and Novak, 1997).

2.1. Physicochemical characteristics of sewage sludge

Sewage sludge is a heterogeneous colloidal system composed of organic particles, inorganic minerals, microbial cells, and EPS. These components form irregular bio-flocs that entrap water both within and around the microbial aggregates. Sludge water occurs mainly as free water, interstitial water, and bound water, each differing in its degree of association with the solid matrix (Vaxelaire and Cezac, 2004). Because sludge commonly contains more than 85% water and these water fractions are unevenly distributed, dewatering remains intrinsically difficult (Lee, 1996).

Sludge particles usually carry a net negative surface charge due to the ionization of carboxyl, hydroxyl, and phosphate groups in EPS and microbial cell walls (H. Liu and Fang, 2002). This charge promotes electrostatic repulsion and contributes to colloidal stability. At the same time, sludge contains particles spanning a broad size range, including bacteria, cell fragments, organic debris, and fine inorganic matter. This polydispersity creates heterogeneous floc structures and pore networks, which directly influence cake compressibility, permeability, and filtration behavior (Wilen et al., 2003).

Sludge also exhibits viscoelastic and thixotropic behavior, reflecting deformation of the polymeric matrix and floc restructuring under shear (Eshtiaghi et al., 2013). Its viscosity and yield stress vary with shear rate and solids concentration, indicating a non-Newtonian response during flow and dewatering (Tixier and Guibaud, 2003). Together with strong water-solid interactions mediated by EPS, these rheological properties increase resistance to compression and filtration. As a result, sludge behaves more like a semi-solid gel than a simple suspension, which restricts water drainage under mechanical pressure.

2.2. Water states and measurement methods in sludge

Water in sludge is distributed across different binding states within the floc structure and EPS matrix. According to interaction strength and removal difficulty, it is generally classified as free water, interstitial water, and bound water (Vaxelaire and Cezac, 2004). Free water occupies relatively large voids between flocs and can be removed readily by settling or mild mechanical force. Interstitial water is retained within pores and channels inside flocs, where its mobility is constrained by capillary forces, pore connectivity, and sludge compressibility. Bound water is more strongly associated with microbial cells and hydrophilic EPS functional groups through hydrogen bonding, ionic interactions, and related physicochemical forces, making it the most difficult fraction to remove. Although bound water usually accounts for a smaller proportion of total water, it is widely regarded as the principal barrier to deep dewatering (Tsang and Vesilind, 1990). As shown in Fig. 1, these water states arise from sludge microstructure, EPS architecture, floc compactness, pore connectivity, and interfacial interactions rather than existing as independent compartments.

These factors operate across multiple structural levels, meaning that sludge dewaterability cannot be reliably evaluated using a single indicator. Instead, multiple analytical methods are required to capture the relationships among sludge structure, water binding state, and dewatering performance. Capillary suction time (CST) reflects the rate of water release and is commonly used as an indicator of sludge drain ability (Meeten and Smeulders, 1995). Specific resistance filtration (SRF) reflects resistance during pressure-driven filtration and is more closely related to cake permeability and limitations in interstitial water transport (Coackley and Jones, 1956). Differential scanning calorimetry (DSC) distinguishes freezable and non-freezable water based on thermal behaviour and thereby estimates the fraction of tightly retained water associated with the sludge matrix (Freire, 1995). Low field nuclear magnetic resonance (LF NMR) differentiates water populations with different binding strengths through T2 relaxation time distributions, allowing nondestructive characterization of water mobility and distribution within sludge structure (Liang et al., 2023). In parallel, EPS fractionation identifies S-EPS, LB-EPS, and TB-EPS, thereby indicating which EPS domains are most closely associated with structural stability and water retention.

These indicators should be interpreted collectively rather than independently. EPS fractionation identifies matrix components associated with water retention, LF NMR and DSC characterize water mobility and binding strength, and CST and SRF reflect the resulting engineering performance. Together, they provide a more rigorous basis for evaluating sludge dewaterability than any single method alone.

This integrated framework is summarized in Fig. 1. Sludge microstructure and EPS architecture determine the distribution of free, interstitial, and bound water, and the combined responses of EPS fractionation, LF NMR, DSC, CST, and SRF help identify the dominant dewatering bottleneck and guide conditioning strategy selection. For example, increased TB EPS is often associated with stronger water retention, reflected by a larger bound water fraction in DSC and shorter T2 components in LF NMR. If CST decreases but SRF remains high, water release may have improved while cake permeability remains limited, indicating that interstitial water transport remains the main constraint. By contrast, concurrent reductions in CST, SRF, bound water signals, and EPS content suggest broader matrix restructuring, improved water mobility, and enhanced overall dewaterability.

2.3. Role of extracellular polymeric substances (EPS)

EPS forms the primary biopolymeric matrix of sewage sludge and is mainly composed of proteins, polysaccharides, humic-like substances, and extracellular DNA (eDNA). These components form a hydrated three-dimensional network that surrounds microbial cells and fine particles. Through this matrix, EPS supports floc formation, regulates surface properties, and provides abundant hydrophilic functional groups such as COOH, OH, and NH_2 that bind water through hydrogen bonding and ionic interactions (Flemming, 2001).

The amount and composition of EPS strongly influence sludge dewaterability (Table 1). High EPS content generally increases viscosity, gel strength, and water retention. In particular, a higher protein to polysaccharide ratio (PN/PS) is often associated with poorer dewatering because protein rich matrices usually exhibit stronger cohesion and

Table 1
Effects of EPS composition on water binding behavior and sludge dewaterability.

EPS characteristic	Structural role	Water binding effect	Dewatering impact	Indicators	Key references
Total EPS	Hydrated viscoelastic matrix around cells and particles	Retains bound and interstitial water	Increases compressibility and limits water release	Higher CST and SRF; lower DS	(Flemming, 2001; J. Zhou et al., 2014)
Protein content (PN)	Hydrophobic and cohesive EPS component	Strong water retention	Often worsens dewaterability at high levels	Higher CST and SRF	(B. Wu et al., 2018; J. Zhou et al., 2014)
Polysaccharide content (PS)	Supports gel elasticity and floc stability	Moderate water retention	Effect depends on structure	Variable CST and SRF	(Z. Guo et al., 2020; Xiao et al., 2020)
PN/PS ratio	Indicates EPS balance and cohesion	Higher ratio strengthens water retaining network	Often associated with poorer dewatering	Higher CST and SRF	(B. Wu et al., 2018; J. Zhou et al., 2014)
Extracellular DNA (eDNA)	EPS backbone component	Increases rigidity and immobilized water	Raises cake resistance	Higher SRF; more short T_2 signals	(X.-Y. Yu et al., 2019; Zhang et al., 2019)
LB EPS	Loosely bound outer EPS layer	Affects interstitial water mobility	Influences filtration and permeability	Changes in CST and SRF	Z. Guo et al. (2020)
TB EPS	Tightly bound EPS near cells	Retains bound water	Main barrier to deep dewatering	Stronger bound water signals by DSC and LF NMR	Tsang and Vesilind (1990)

stronger water binding capacity (J. Zhou et al., 2014). These characteristics reduce water mobility within flocs and hinder the release of interstitial water and bound water during mechanical compression.

Within the EPS matrix, different fractions contribute differently to water retention. S-EPS mainly exists in the liquid phase or weakly associated outer regions and is more closely related to colloidal stability and soluble organic release. LB-EPS is associated with the outer floc layer and more strongly influences floc permeability and interstitial water mobility. TB-EPS is the innermost and most strongly retained fraction and is typically the dominant EPS barrier to bound water release and deep dewatering. Accordingly, B-EPS in this review refers collectively to LB-EPS and TB-EPS.

Because EPS is central to both floc stability and water retention, its degradation or restructuring has become a key route for improving sludge dewaterability. Reducing total EPS, altering PN/PS, or disrupting the three-dimensional EPS network can weaken colloidal stability and facilitate water release. These effects underpin many microbial assisted conditioning processes, in which microbial enzymes and metabolites selectively hydrolyze or transform EPS to improve subsequent dewatering performance (Huang et al., 2022; J. Zhou et al., 2014).

3. Conventional sludge dewatering and conditioning techniques

Conventional sludge dewatering relies on mechanical separation, often assisted by chemical or physicochemical conditioning to overcome colloidal stability and EPS mediated water retention. For process selection, the key issue is which water fraction a method mainly targets, which structural barrier it modifies, and what penalty it introduces elsewhere in the treatment train. As summarized in Table 2, mechanical dewatering mainly removes free water and part of interstitial water, whereas chemical conditioning primarily improves interstitial water release and cake formation by altering surface charge, floc structure, and the outer EPS region. Thermal, microwave, ultrasonic, and electro dewatering methods become more relevant when persistent cake resistance or EPS associated bound water limits further drying, although these approaches often increase energy demand, soluble COD release, or downstream complexity. (Cao et al., 2021; Vaxelaire and Cezac, 2004; Xiao and Abbt-Braun, 2020).

3.1. Mechanical dewatering

Mechanical dewatering remains the core full-scale sludge reduction step in wastewater treatment plants and is commonly implemented by centrifuges, belt filter presses, and plate frame filter presses. Although these systems differ in force application and hydraulic configuration, they all remove mobile water by gravity, pressure, or centrifugal acceleration rather than by directly disrupting EPS associated water binding (Cao et al., 2021; Vaxelaire and Cezac, 2004).

Mechanical dewatering is therefore most effective for free water and part of interstitial water, but much less effective for water strongly retained within the EPS matrix. Its performance declines when dewaterability is controlled mainly by high EPS content, poor cake permeability, and floc compressibility rather than by bulk water volume alone. This is why biological sludges usually require prior conditioning and why presses and centrifuges often reach a practical dryness ceiling even under optimized operation (Cao et al., 2021; Vaxelaire and Cezac, 2004).

Mechanical dewatering is therefore the essential bulk water removal step rather than a complete solution to poor dewaterability. Its advantages are operational maturity, robustness, and broad engineering applicability, but increasing pressure or rotational intensity does not proportionally improve removal of strongly retained water. As a result, DS may increase while the underlying physicochemical limitation remains unresolved, especially when downstream transport, drying, incineration, or valorization requires a drier cake. Because dewatering and deep dewatering also contribute substantially to plant wide energy demand, DS should not be evaluated independently of energy input. (Chang et al., 2023a; Chu et al., 2005).

3.2. Chemical conditioning

Chemical conditioning is widely used because it directly targets the colloidal and interfacial factors that limit solid liquid separation. Its function is not simply to enlarge flocs, but to reduce electrostatic repulsion, reorganize EPS, and facilitate water migration during subsequent mechanical dewatering. Inorganic coagulants such as ferric chloride, ferric sulfate, alum, and poly aluminum chloride mainly act through hydrolysis, charge neutralization, and sweep aggregation, whereas cationic polymer flocculants mainly act through adsorption and bridging. Accordingly, chemical conditioning is generally more effective for improving interstitial water release and cake formation than for removing water strongly retained within the inner EPS matrix (Bolto and Gregory, 2007; Cao et al., 2021; Sobeck and Higgins, 2002).

Inorganic coagulants destabilize sludge by neutralizing negative surface charge and aggregating fine particles into denser flocs. Multivalent metal ions also interact with EPS proteins and polysaccharides, compress the diffuse double layer, and reduce surface hydration. These effects mainly improve drainage and interstitial water mobility. However, the benefit is strongly dependent. Excess hydrolyzed metal species and hydroxide precipitates may accumulate on particle surfaces or within inter floc spaces, producing smaller and more compact aggregates. Under pressure, these flocs collapse more easily, block drainage pathways, and reduce cake permeability even when initial aggregation appears improved (Z. Guo et al., 2020; Sobeck and Higgins, 2002).

Polymeric flocculants also show an optimum dosage window. At

Table 2
Comparison of sludge dewatering and conditioning technologies by target water fraction, floc and EPS effects, and process implications.

Category	Representative techniques	Main water fraction targeted	Main structural action	Typical DS range (%)	Main operational penalty	Likely downstream implication	Process selection implication	Representative references
Mechanical dewatering	Centrifugation; belt filter press; plate frame filter press	Free water; part of interstitial water	Limited direct EPS disruption; governed mainly by floc permeability and cake compressibility	15–25	Energy demand; limited deep dewatering; sensitive to sludge properties	Usually preserves sludge chemistry, but low final dryness may increase transport and disposal burden	Best as the baseline step for bulk water removal	(Cao et al., 2021; Chang et al., 2023b; Vaxelaire and Cezac, 2004)
Chemical conditioning	Ferric salts; aluminum salts; PAC; cationic polymer flocculants	Mainly interstitial water; part of weakly retained water	Charge neutralization; adsorption bridging; partial EPS contraction; floc strengthening	20–30	Continuous chemical demand; overdosing may cause restabilization, dense cakes, and lower permeability	May increase ash and inorganic loading; may affect digestion and nutrient recovery	Best when poor dewatering is driven by colloidal instability and weak cake structure	(Bolto and Gregory, 2007; He et al., 2024; Pasciucco et al., 2024; Sobeck and Higgins, 2002)
Thermal treatment	Thermal hydrolysis; moderate or high temperature heating	Interstitial water and EPS associated bound water	Strong disruption of EPS hydration, macromolecular structure, and cell integrity	25–40	High heat demand; greater equipment complexity	Shifts organics to the liquid phase may benefit or complicate downstream digestion	Best when EPS controlled resistance and bound water dominate, especially with energy recovery	(Chang et al., 2023b; Neyens et al., 2004; Zhang et al., 2019)
Microwave and ultrasonic pretreatment	Microwave irradiation; ultrasound	Interstitial water and part of EPS associated bound water	Rapid heating or cavitation; floc fragmentation; EPS solubilization; cell disruption	Variable	High specific energy demand; possible rise in soluble COD if over intensified	May support digestion when integrated, but can increase filtrate treatment burden	Best as an integrated pretreatment rather than a standalone route to higher cake dryness	(Jakoi et al., 2021; Martinez et al., 2015; Zhao et al., 2024)
Electro dewatering	Electro dewatering after cake formation	Residual interstitial water and poor mobile water after mechanical dewatering	Electro osmotic transport through formed cake; limited direct EPS disruption	30–45	Higher energy demand at high dryness; rising resistance; Joule heating; electrode corrosion; pH gradients	Reduces transport burden, but adds electrical and materials management requirements	Best as a deep dewatering step after bulk water removal	(Conrardy et al., 2016; Mahmoud et al., 2010, 2011)

suitable doses, long cationic chains bridge particles and EPS clusters into larger, stronger aggregates. Once surfaces become saturated, however, additional polymer can no longer form effective bridges. Instead, polymer segments may overcompensate surface charge or create steric hindrance, thereby restabilizing previously destabilized particles. Overdosed polymers may also form flocs that appear large but are dense and highly compressible. During filtration or pressing, these flocs deform, close internal pores, and produce a less permeable cake. This is why many sludge systems show an optimum polymer dose, after which SRF and cake moisture rise again rather than continue to decrease (Bolto and Gregory, 2007; Z. Guo et al., 2020).

Chemical conditioning can also alter EPS mediated water retention more subtly through metal ion cross linking or polymer induced contraction of gel like domains. Even so, its main value remains colloidal destabilization, floc strengthening, and interstitial water transport rather than removal of strongly retained water.

Its main limitation is reduced downstream compatibility. Metal salts increase the inorganic load and ash content of sludge cake, and residual coagulants may impair subsequent anaerobic digestion. Wu et al. (2022) showed that both PAC and PFS altered methane production during anaerobic digestion of waste-activated sludge, with stronger inhibition under PFS. Pasciucco et al. (2024) likewise reported that aluminum-based coagulants reduced methane yield and that inhibition increased with dosage. Chemical conditioning is therefore highly effective when rapid solid-liquid separation is the main objective, but less attractive when the treatment train prioritizes anaerobic digestion, phosphorus recovery, or low chemical dependency (Pasciucco et al., 2024; Y. Wu et al., 2022).

3.3. Physicochemical dewatering methods

Physicochemical methods become more relevant when dewatering is limited by sludge structure rather than by the presence of mobile water alone. Compared with routine mechanical dewatering or standard chemical conditioning, these approaches act more directly on EPS water interactions, residual cake resistance, and water that remains inaccessible after conventional separation (H. Liu and Fang, 2002; Vaxelaire and Cezac, 2004).

Thermal treatment is a representative example. Heating weakens hydrogen bonding, alters EPS conformation, promotes solubilization of proteins and polysaccharides, and can lyse microbial cells, thereby converting part of the retained water into more removable forms. It is therefore more effective than routine mechanical or chemical conditioning when the dominant barrier is EPS associated bound water. However, this benefit must be balanced against high heat demand and greater soluble organic release, which may complicate liquor handling unless the process is integrated with downstream digestion and energy recovery (Chang et al., 2023a; Neyens et al., 2004; X. Zhang et al., 2019).

Microwave and ultrasonic pretreatments also attack the sludge matrix through rapid heating, cavitation, shear, and floc disruption. They can improve subsequent dewatering by releasing EPS associated water, but they also increase soluble COD and energy demand. Their engineering value therefore depends heavily on process integration, particularly whether the released soluble organics are recovered through anaerobic digestion rather than becoming an additional treatment burden (Jakoi et al., 2021; Martinez et al., 2015; Zhao et al., 2024).

Electro dewatering occupies a different position because it is generally applied after cake formation. It removes residual water through electro-osmotic flow and is mainly used as a deep dewatering step after conventional presses or centrifuges approach their dryness limit. This makes it particularly useful when residual cake resistance, rather than bulk water removal, is the dominant constraint. However, performance is highly sensitive to current, cake thickness, cake dryness, and electrical resistance. Energy demand rises sharply at higher solids contents, while Joule heating, pH gradients, and electrode corrosion complicate long-term operation and scale up (Conrardy et al., 2016; Mahmoud et al., 2010, 2011).

Overall, conventional methods should be compared by the water fraction they target, the structural level at which they act, and their operational or downstream penalties. Mechanical dewatering remains the base step for bulk water removal, chemical conditioning is most effective when poor dewaterability is driven by colloidal instability and weak cake structure, and thermal, microwave, ultrasonic, and electro dewatering methods are more relevant when EPS associated bound water or residual cake resistance becomes the main bottleneck. This comparative logic, rather than DS improvement alone, underpins Table 2. (Chang et al., 2023a; Vaxelaire and Cezac, 2004).

4. Microbial assisted dewatering: mechanisms and applications

Based on the integrated framework shown in Fig. 1, microbial assisted dewatering can be evaluated through a stepwise diagnostic logic. Sludge microstructure and EPS architecture are first examined to identify the main structural barriers to water release. EPS fractionation, low field nuclear magnetic resonance (LF NMR), and differential scanning calorimetry (DSC) are then interpreted together to determine whether poor dewaterability is mainly associated with bound water retention, interstitial water retention, or restricted water mobility. Capillary suction time (CST) and specific resistance to filtration (SRF) are subsequently used to verify whether the dominant engineering limitation is insufficient water release or high filtration resistance. Conditioning strategies can then be selected according to the identified bottleneck, providing a practical basis for targeted diagnosis and optimization.

4.1. Concept and classification

Microbial-assisted dewatering refers to sludge conditioning strategies that intentionally use microorganisms or their metabolic activities to improve dewaterability by modifying EPS, floc structure, and interfacial physicochemical properties. Unlike conventional chemical or physicochemical pretreatments, these approaches rely on biological processes such as enzymatic hydrolysis, metabolic regulation, and redox-driven transformation. Their main advantage is that they target the structural and interfacial causes of water retention rather than merely promoting bulk solid-liquid separation (Ben Hamed et al., 2025; Goncalves et al., 2023).

Mechanistically, microbial-assisted dewatering can be divided into two broad routes: direct microbial action and indirect microbial conditioning. As illustrated in Fig. 2, direct microbial action mainly involves enzymatic degradation of EPS macromolecules, which promotes depolymerization of the EPS network, weakens floc cohesion, and facilitates the release of interstitial water and bound water. By contrast, indirect microbial conditioning acts through interfacial modification and environmental regulation, including biosurfactant production and metabolism-driven changes in pH, redox potential, ionic strength, osmotic pressure, and EPS ionization, thereby enhancing water migration and floc restructuring (Kang et al., 2023; Kurade et al., 2016; Murugesan et al., 2016).

Direct microbial action is typically associated with extracellular enzymes such as proteases, glycosidases, and nucleases, which hydrolyze proteins, polysaccharides, and extracellular DNA within the EPS matrix. This reduces gel strength, increases pore connectivity, and promotes the release of water retained in the sludge matrix. Indirect microbial conditioning, in contrast, may markedly improve CST or SRF even without a large immediate decrease in total EPS concentration, because the dominant effect is environmental regulation rather than bulk EPS depolymerization. For example, bioacidification using *Acidithiobacillus ferrooxidans* reduced CST to below 10 s and lowered SRF by more than 94 percent in anaerobically digested saline sludge, while a biogenic flocculant produced by the same microorganism reduced CST and SRF by about 74 percent and 89 percent, respectively (Kurade et al., 2016; Murugesan et al., 2016). These two routes may operate independently or synergistically, depending on sludge properties and operating conditions.

4.2. Enzymatic and biochemical mechanisms

The effectiveness of microbial assisted dewatering is largely governed by enzymatic and biochemical modification of the EPS matrix rather than by simple reduction of bulk water content. Because EPS is chemically heterogeneous and spatially stratified, enzymatic conditioning should be viewed as a set of component specific reactions acting

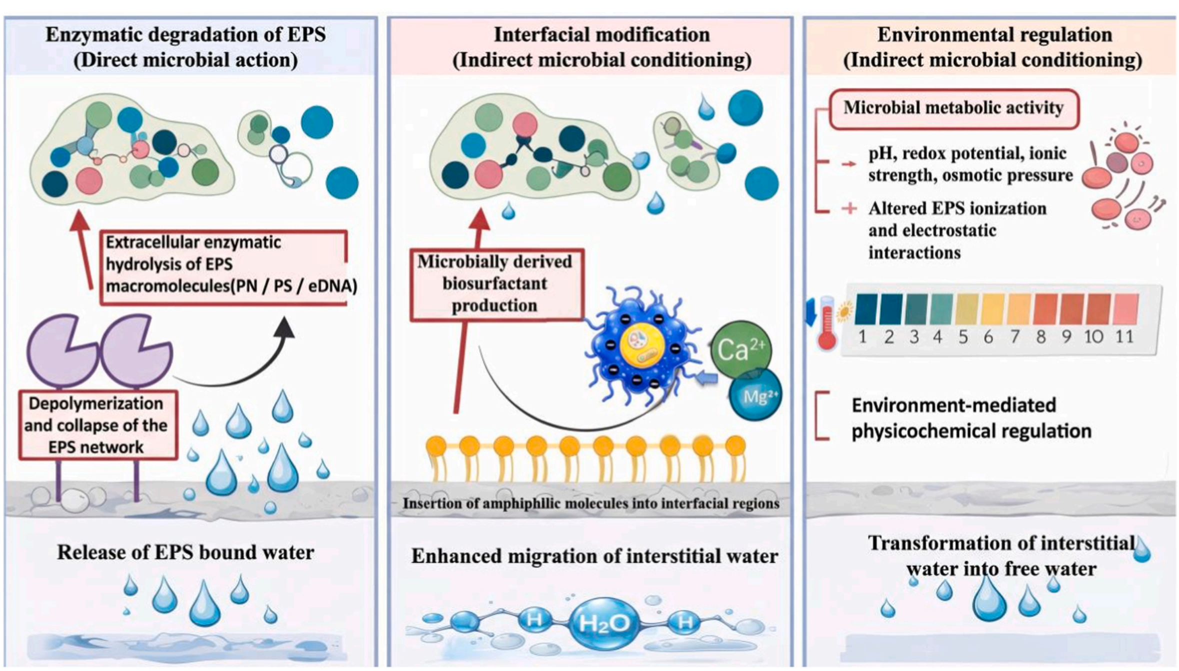

Fig. 2. Conceptual framework of microbial assisted sludge dewatering mechanisms.

on proteins, polysaccharides, and cell associated structures across different EPS layers. This is important because changes in water content do not always correspond to improved CST or filtration behavior (Lin et al., 2020; G.-H. Yu et al., 2008).

Single enzyme systems provide clearer mechanistic specificity. Proteases mainly attack protein related EPS structures, whereas cellulase or alpha amylase target polysaccharide rich domains. Lysozyme differs because it primarily disrupts microbial cell walls and can redistribute proteins and polysaccharides among S-EPS, LB-EPS, and TB-EPS fractions. This specificity makes single enzyme systems useful for identifying which EPS component is most related to poor dewaterability. However, selective degradation of one component does not necessarily improve engineering performance. Pei et al. (2010) showed that protease and cellulase each increased CST and produced no obvious improvement in sludge solids content after centrifugation. Kang et al. (2023) likewise reported that although alpha amylase and neutral protease reduced sludge water content to 68.67% and 69.82%, CST still increased after enzyme conditioning. These findings indicate that partial degradation may release soluble fragments or fine particles that worsen filtration behavior (Kang et al., 2023; Pei et al., 2010).

Mixed enzyme systems generally provide broader substrate coverage and are more likely to destabilize heterogeneous sludge matrices. Their advantage lies in functional complementarity: protease can expose carbohydrate domains embedded in protein-rich aggregates, while amylase or other polysaccharide-degrading enzymes can open gel-like networks and improve access to inner matrix regions. Accordingly, mixed enzyme treatment often produces greater sludge solubilization than single enzyme treatment. Yang et al. (2010) found that mixed enzyme treatment had a stronger effect on sludge solubilization than single enzyme treatment, and that a protease to amylase ratio of 1:3 increased solids hydrolysis from 10% in the control to 68.43% at 50 °C. Chen et al. (2015) further showed that enzyme feeding mode significantly affected sludge lysis efficiency and that alpha amylase plus protease gave better solubilization than alternative addition sequences (Chen et al., 2015; Q. Yang et al., 2010).

From an engineering perspective, single enzyme systems are easier to standardize and interpret, whereas mixed enzyme systems are generally more effective for complex sludge but require optimization of enzyme ratio, sequence, pH, temperature, and reaction time. Importantly, the best dewatering strategy is not always the one that causes the greatest decrease in total EPS. Lin et al. (2019) showed that combined lysozyme and cationic polyacrylamide reduced water content to 57.79%, mainly by disrupting the microbial cell wall and EPS structure and converting intracellular water and part of the bound water into free water. Later studies further showed that lysozyme altered EPS spatial distribution, reduced viscosity and shear modulus, weakened water adsorption, and promoted transformation of bound water into more mobile fractions (Lin et al., 2019, 2020; Lin and Li, 2022). Overall, single enzyme systems provide clearer mechanistic resolution, whereas mixed enzyme systems are more suitable for broad EPS disruption in heterogeneous sludge.

4.3. *Microbial taxa and metabolic features*

The performance of microbial-assisted dewatering depends strongly on microbial community composition and associated metabolic capabilities. Reported systems commonly involve hydrolytic microorganisms, biosurfactant-producing microorganisms, or mixed consortia capable of coordinated EPS transformation and environmental regulation. However, their effectiveness is not constant across sludge types, because sludge characteristics determine EPS composition, substrate accessibility, and the metabolic pathways that can be activated. For example, structural EPS was reduced more extensively in waste activated sludge than in aerobic granular sludge during anaerobic conversion, and dewaterability during anaerobic digestion of waste activated sludge first deteriorated and only later recovered, reflecting dynamic changes in LB-EPS and water distribution. These findings show that microbial conditioning must be interpreted in relation to sludge type rather than generalized across all systems.

Among hydrolysis-oriented microorganisms, enzyme producing taxa are central because extracellular enzymes can directly attack proteinaceous and polysaccharide components of EPS. Representative enzyme producing strains of *Bacillus subtilis* have been reported to secrete cellulase, xylanase, protease, and amylase, whereas *Paenibacillus polymyxa* strains can exhibit β 1,3 glucanase, cellulase, amylase, and protease activities (F. Yang et al., 2024; Yao et al., 2025). However, hydrolytic potential does not guarantee the same dewatering outcome under different conditions. Mixed yeast conditioning reduced proteins, polysaccharides, and nucleic acids in EPS by 60.43 ± 2.73%, 18.94 ± 2.39%, and 48.30 ± 3.37%, respectively, while reducing CST by 17.19 ± 1.16% (X. Y. Yu et al., 2019). *Talaromyces flavus* S1 improved sludge dewaterability by 48.1%, and its mycelium decreased CST by 74.0% in sterilized sludge but by 43.7% in non-sterilized sludge (H. Liu et al., 2017). This contrast suggests that background microbiota, substrate competition, and matrix complexity can strongly affect pathway expression and final efficiency (H. Liu et al., 2017).

By contrast, biosurfactant or bioemulsifier associated taxa may improve dewatering mainly through interfacial modification. *Pseudomonas* species are well known for lipopeptide biosurfactants, whereas *Acinetobacter* species are associated with bio emulsifier production and, in some cases, rhamnolipid biosurfactants (Dong et al., 2016; Mujumdar et al., 2019; L. Zhou et al., 2024). These metabolites can reduce surface tension and weaken hydrophobic or colloidal interactions within sludge-like matrices. Their importance is especially evident in oily or hydrophobic sludge, where interfacial destabilization may be more important than direct EPS hydrolysis. In floated oily sludge, rhamnolipid treatment at 300 to 1000 mg/L, pH 5 to 7, and 10 to 60 °C directly separated 50 to 80% of water, with lower pH exerting a stronger effect than temperature (Long et al., 2013). This suggests that biosurfactant mediated pathways may outperform hydrolysis dominant routes in sludge systems where emulsion stability is a major barrier (Long et al., 2013).

At the community level, mixed consortia often outperform single strains because different metabolic steps can proceed simultaneously. One group may depolymerize structural EPS, another assimilates the released oligomers, and others further modify the surrounding physicochemical environment. This likely contributes to the stronger robustness of mixed or sequential systems. For instance, a polygalacturonate degrading consortium increased structural EPS degradation from 47.6% to 85.2% and raised waste activated sludge destruction from 11.5% to 28.4% (Z.-Y. Hu et al., 2023). In a sequential bioleaching system, *Mucor* sp. ZG 3 reduced dissolved organic carbon by 65.2% and low molecular weight DOM by 76.2%, thereby alleviating inhibition on subsequent iron-oxidizing bacteria and ultimately lowering SRF by 40.0% compared with the conventional process (Wang et al., 2024b). Overall, microbial taxa should be interpreted not only by taxonomy, but by their ability to activate different pathways under distinct sludge and operating conditions. Hydrolysis dominant microorganisms are generally more effective when accessible protein and polysaccharide rich EPS fractions are abundant, whereas biosurfactant related pathways are more advantageous in hydrophobic sludge. Mixed and sequential consortia often provide the greatest robustness because they integrate complementary functions across multiple transformation steps (Z.-Y. Hu et al., 2023; Wang et al., 2024b).

4.4. *Application modes and operational parameters*

Microbial assisted dewatering can be implemented through bioaugmentation, *in situ* activation of indigenous microorganisms, enzyme assisted conditioning, and combined hybrid processes. These modes differ in response speed, controllability, dependence on microbial growth, and strength of available quantitative evidence.

Bioaugmentation introduces selected strains or microbial products to enhance EPS degradation or floc reconstruction. Its advantage is that the function of the added agent is relatively explicit, which makes the response easier to interpret. Quantitative evidence shows meaningful improvement, but performance varies with product type and sludge properties. For example, the cationic chitosan like bio flocculant BF01314 produced from *Citrobacter youngae* reduced CST by about 57 percent and SRF by about 87 percent while increasing cake solids to 23.2 percent, whereas a *Bacillus megaterium* derived biopolymer reduced CST and SRF by about 40 percent and 38 percent, respectively, and increased dry solids to 28.6 percent when used with PAC (Z. Guo et al., 2020; Hatta et al., 2023).

In situ activation stimulates indigenous microorganisms through adjustment of pH, aeration, redox conditions, or nutrient supply. This avoids inoculum preparation and may reduce material input, but compared with bioaugmentation or enzyme systems, its performance is less standardized and more dependent on plant specific sludge characteristics. It is most promising when the native community already has the required functional potential and operating conditions can be controlled selectively.

Enzyme assisted conditioning bypasses microbial growth by directly applying crude or formulated enzymes, allowing more predictable control over EPS hydrolysis kinetics. It offers the clearest mechanistic interpretation and faster catalytic response, but its effectiveness depends strongly on how well the enzyme system matches sludge specific EPS composition.

Combined hybrid processes currently provide the strongest quantitative evidence. In these systems, microbial conditioning acts as a biological pretreatment that lowers structural resistance before a second driving force is applied. A short time bioleaching plus polyferric sulfate process achieved a 94.0 percent reduction in SRF and an 11.6 percent reduction in sludge cake moisture after 12.5 h, while reaching performance comparable to 24 h of standalone bioleaching. Likewise, microbial fuel cell conditioning before electrodewatering reduced final sludge moisture from 76.5 percent to 54.8 percent, showing that microbial pretreatment improved sludge response to the electric field rather than simply adding another unit operation (Li et al., 2023a; Shen et al., 2023). These side-by-side comparisons make hybrid systems the most convincing option when the goal is a measurable engineering advantage.

Across all modes, key parameters include inoculum or enzyme dosage, pH, treatment time, and the evolution of EPS relative to dry solids. However, EPS reduction alone should not be used as the sole performance indicator. It should be interpreted together with CST, SRF, final DS, and, where possible, bound water change.

4.5. Advantages and challenges

Microbial assisted dewatering offers several potential advantages over conventional conditioning, but these should be interpreted carefully. Its clearest advantage is reduced dependence on external chemical dosing. By weakening EPS structure, altering floc properties, or promoting biological aggregation, microbial systems may avoid the residual metal or polymer load associated with chemical conditioning, which is important because PAC, PFS, and related coagulants can impair anaerobic digestion and reduce methane yield, especially at high dosage (Pasciucco et al., 2024; Y. Wu et al., 2022).

A second advantage is mechanistic selectivity. Rather than acting only on bulk water removal, microbial assisted strategies target the biological and physicochemical causes of poor dewaterability, particularly EPS related water retention. Quantitative studies show that microbial or enzyme mediated systems can substantially reduce CST, SRF, or sludge water content under suitable conditions, although the extent of improvement varies across sludge types and operating modes. Microbial assisted dewatering should therefore be viewed not as universally superior, but as a targeted route for alleviating EPS dominated dewatering bottlenecks while reducing reliance on high chemical input (Hatta et al., 2023; Kang et al., 2023; Kurade et al., 2016).

Claims of lower energy demand and better sustainability also require caution. Although microbial systems often operate under milder conditions than thermal hydrolysis or electrochemical deep dewatering, longer reaction time, inoculum preparation, pH control, aeration, mixing, and larger reactor volume may offset this apparent advantage. Existing life cycle and optimization studies likewise show that the condition yielding the best dewatering performance is not always the one with the lowest environmental burden or cost (S. Wang et al., 2023; Wang et al., 2024b). At present, microbial assisted dewatering should therefore be regarded as a promising rather than fully established sustainable option.

Several challenges still limit large-scale implementation. Performance depends strongly on microbial adaptability and functional stability, which vary with sludge origin, composition, and operational history. Compared with chemical conditioning, biological processes usually require longer reaction times and more complex control of pH, temperature, redox state, and nutrient balance, which may reduce throughput in full scale plants. In addition, laboratory and pilot scale results do not always translate directly to continuous, high-load systems. Further progress will therefore require improved microbial selection, better process control, and more robust hybrid designs (Y. Liu and Fang, 2003; Shen et al., 2023; Wang et al., 2024b). Representative microbial conditioning systems and their reported dewatering outcomes are summarized in Table 3.

5. Integrative and sustainable dewatering strategies

5.1. Hybrid microbial chemical and microbial physical processes

To address the limitations of standalone conditioning, hybrid processes combining microbial treatment with chemical or physical dewatering steps have attracted increasing interest. Their value lies not simply in adding a microbial stage but in improving sludge response to the subsequent process. By partially degrading EPS, weakening floc structure, and releasing retained water, microbial pretreatment can reduce structural resistance before oxidation, flocculation, or electro dewatering. Hybrid performance should therefore be judged against the corresponding standalone process using CST, SRF, cake moisture, DS, treatment time, and, where available, energy or reagent demand. A hybrid system can be considered advantageous only when it achieves better dewatering or a similar endpoint with lower operational intensity (Li et al., 2023a; Shen et al., 2023).

The clearest evidence comes from microbial chemical systems. In short time bioleaching plus polyferric sulfate conditioning, Li et al. (2023b) reported a 94.0% reduction in SRF and an 11.6% reduction in cake moisture after 12.5 h, while achieving performance comparable to 24 h of standalone bioleaching. In pilot scale operation, cake moisture reached about 59.2%. The BS + H_2O_2 process also achieved SRF and CST reduction ratios of 90.3% and 80.9%, respectively, after only 30 min, outperforming comparison processes over the same period (Li et al., 2023a). In bioleaching Fenton conditioning, bound water, SRF, and CST further decreased from 3.95 g g^{-1}, 6.16×10^{12} m kg^{-1}, and 130.6 s to 3.15 g g^{-1}, 2.81×10^{11} m kg^{-1}, and 33 s, respectively (Y. Li et al., 2024). Together, these results show that microbial chemical coupling can produce faster and deeper improvement than standalone bioleaching or oxidation.

A similar pattern is seen when microbial products are combined with conventional coagulants or flocculants. Guo and Chen (2017) reported that a microbial bio flocculant alone produced a sludge cake DS of 19.3% and an SRF of 4.8×10^{12} m kg^{-1}, whereas combining the same bio flocculant with PAC increased DS to 24.1% and reduced SRF to 3.0×10^{12} m kg^{-1}. This finding indicates that the microbial component provided flocculation capacity, while PAC strengthened charge neutralization and aggregate consolidation. Among microbial physical hybrids, coupling microbial conditioning with electro dewatering also provides a clear benchmark. Shen et al. (2023) showed that standalone electro

Table 3
Overview of microbial based conditioning systems for sludge dewatering.

Microbial system/treatment	Sludge type	Targeted EPS component	EPS reduction (%)	CST reduction (%)	SRF reduction (%)	Final DS (%)	Conditioning time	Reference
Chitosan-like bioflocculant BF01314 (*Citrobacter youngae*)	Waste activated sludge (WAS)	Total EPS matrix	Not reported	57.3	87.1	23.2	≥3 min	Hatta et al. (2023)
Biogenic flocculant produced by *Acidithiobacillus ferrooxidans*	Anaerobically digested sludge (ADS)	Total EPS and bound water	Not reported	~74.0	~89.0	~30.0	Not reported	Kurade et al. (2016)
Bio-acidification using *Acidithiobacillus ferrooxidans* culture	Anaerobically digested saline sewage sludge	Total extractable EPS	Not reported	Not reported	>94.0	Not reported	24 h	Murugesan et al. (2016)
Biopolymer from piggery wastewater (*Bacillus megaterium*)	Biological sludge (WAS)	Total EPS	Not reported	40.0	38.2	28.6	Not reported	Z. Guo et al. (2020)
Iron-oxidizing bacteria (*Acidithiobacillus ferrooxidans*) as biogenic flocculant	Activated sludge and anaerobically digested saline sludge	Total EPS and floc matrix	Not reported	Not reported	Not reported	Not reported	Hours; exact time not reported	(Wong et al., 2016)

dewatering produced a final sludge moisture content of 64.0%, whereas microbial fuel cell conditioned sludge reached 54.8% under the same 25 V, 12 min treatment. This 9.2 percentage point decrease indicates that microbial pretreatment improved sludge response to the electric field rather than merely adding another step. Lv et al. (2024) further reported that a microbial fuel cell powered electro Fenton system coupled with chitosan quaternary ammonium salt reduced sludge cake water content, CST, and SRF to 61.21%, 15.6 s, and 1.02×10^{12} m kg^{-1}, respectively. Even so, the strongest evidence in this category still comes from studies that directly benchmark hybrid electro assisted systems against standalone comparators. Overall, hybrid microbial conditioning should be considered superior only when it demonstrably outperforms a standalone comparator or reaches a similar endpoint with less time, lower energy input, or lower chemical demand. At present, the strongest comparative evidence supports microbial coupling with flocculation and electro dewatering, whereas combinations involving skeleton builders or thermal assistance remain mechanistically plausible but still lack sufficient side by side benchmarking (Table 4). Hybrid strategies are therefore promising, but their value depends on demonstrated comparative advantage rather than conceptual synergy alone (J. Guo and Chen, 2017; Lv et al., 2024; Shen et al., 2023).

5.2. Engineering scale up barriers and pilot scale evidence for full scale implementation

Despite promising laboratory results, the main barriers to full scale implementation of microbial assisted sludge dewatering are engineering rather than purely biological. Most scale-up evidence still comes from acidophilic bioleaching and fungal conditioning, but existing reports show that implementation is feasible under selected conditions. In China, sludge bioleaching progressed from pilot testing to commercial operation, with reported treatment capacities increasing from 50 t/d of conventionally dewatered sludge in one plant to more than 20 plants with a combined capacity approaching 6000 t/d of sludge at 80% moisture content (Hu et al., 2015; Zhang et al., 2025). These cases demonstrate feasibility but also show that successful scale up depends on sludge characteristics, reactor design, and integration with downstream dewatering (W. Hu et al., 2015; H. Zhang et al., 2025).

The first barrier is feed variability, especially solids concentration, alkalinity, and digestion status. In bioleaching systems, high buffering capacity can suppress acidification and weaken dewatering improvement as solids concentration increases (W. Yang et al., 2020). Bioleaching that is effective for undigested sludge may also perform poorly

Table 4
Classification and functional roles of skeleton builders used in sludge dewatering and their synergistic interactions with bio and physico-chemical conditioning.

Category	Representative materials	Standalone structural role in dewatering	Hybrid configuration with reported performance advantage	Comparative evidence relative to single-process conditioning	Key references
Inert inorganic granular skeleton builders	Diatomite, kaolin, bentonite, coal ash	Provide rigid, non-compressible particles that preserve pore channels and reduce cake compressibility	Fenton oxidation + skeleton builders	Significant synergistic effects were reported; under optimal conditions, sludge cake water content decreased to 49.5 ± 0.5%	(Bao et al., 2024; H. Liu et al., 2017)
Fibrous skeleton builders	Synthetic fibers, cellulose fibers, rice husk fibers	Form entangled three-dimensional networks that improve pore connectivity and mechanical support during filtration	CPAM + polypropylene fibers	Sludge water content decreased from 96.2% to 65.9%, and cake compressibility coefficient decreased from 1.13 to 0.89	(Bao et al., 2024; Y. Yang et al., 2023)
Carbon-based porous skeleton builders	Biochar, activated carbon, sludge-derived char	Supply rigid and porous carbonaceous scaffolds that improve permeability and drainage	FeCl3 conditioned sludge cake derived biochar used as skeleton builder	Compared with FeCl3 alone, SRF decreased by 63.9%, net sludge solids yield increased by 39.2%, and net sludge water removal reached 98.36%	(Bao et al., 2024; Y. Wu et al., 2016)
Carbon-based porous skeleton builders	Sludge-based particle electrodes/biochar	Act simultaneously as conductive media, adsorbents, and skeleton particles in electro-assisted systems	Biochar + electrolysis	CST and SRF decreased by 58.12% and 81.01%, respectively, and net sludge solids yield increased by 87.05% relative to raw sludge	H. Yu et al. (2022)
Industrial by-product and reactive skeleton builders	Granulated blast furnace slag, coal gangue, incineration ash, fly ash residues	Serve as low-cost rigid fillers or reactive mineral supports that improve cake strength and permeability	Quicklime + granulated blast furnace slag	Optimized performance was reported at pH 10.2, 0.34 g g^{-1} DS, and 14 min contact time, but no clear standalone comparator was given in the abstract	(Bao et al., 2024; Ramachandra and Devatha, 2020)

for anaerobically digested sludge because high alkalinity and limited ferrous iron inhibit acidification and acid thiobacillus growth (H. Zhang et al., 2025). Full scale implementation therefore requires feed classification, alkalinity management, and solids specific process control rather than a universal inoculation strategy (W. Yang et al., 2020; H. Zhang et al., 2025).

A second barrier is reaction time and hydraulic throughput. Biological conditioning is usually slower than chemical conditioning, which increases reactor volume requirements and limits daily treatment capacity. Li et al. (2023b) addressed this by coupling short duration bioleaching with polyferric sulfate flocculation. Under optimized conditions, SRF was reduced by 94.0% and sludge cake moisture by 11.6% after 12.5 h, while pilot scale dewatering produced a cake moisture content of about 59.2%. This suggests that scale up is more realistic when microbial conditioning is integrated into a hybrid front end process rather than operated as a fully standalone unit (Li et al., 2023a).

A third barrier is inoculum management and process stability. Tao et al. (2021) showed this in bench and pilot scale fungal conditioning with *Penicillium simplicissimum* NJ12. At a 5% inoculum volume fraction, SRF decreased from 1.97×10^{13} to 3.52×10^{11} m/kg and CST from 32 to 12 s within 3 d. However, stable operation required sludge recycling at a ratio of 1:2, and fresh inoculum still had to be replenished because performance was maintained for only three successive cycles. This indicates that inoculum retention, recycling design, and refresh frequency are key engineering variables (Tao et al., 2021).

A fourth barrier is reactor operation and process integration. Pilot ferrous sulfate bioleaching required two 4 m^3 bioreactors, 20% v/v inoculum, initial acidification to pH 4.0, and 4.0 g/L ferrous sulfate heptahydrate (Mercier et al., 2006). Although that study focused mainly on stabilization and metal removal, it illustrates the operational demands of scale up, including inoculum loading, electron donor supply, acidity control, and compatible reactor hydraulics. Engineering reports further suggest that successful sludge bioleaching is usually coupled with diaphragm filter pressing or similar high pressure dewatering systems rather than implemented in isolation (W. Hu et al., 2015; H. Zhang et al., 2025).

Overall, available pilot and engineering evidence suggests that successful scale up is most likely when four conditions are met: sludge is characterized in advance for buffering capacity, solids concentration, and digestion status; pH and substrate supply are tightly controlled; inoculum recycle and replenishment are built into process design; and the biological step is integrated with existing dewatering infrastructure, often in hybrid form. Future studies should therefore move beyond bench scale CST and SRF improvement and evaluate residence time, reactor footprint, inoculum logistics, feed variability, downstream compatibility, and long-term operational stability under realistic plant conditions (Li et al., 2023a; Tao et al., 2021; W. Yang et al., 2020; H. Zhang et al., 2025).

5.3. Sustainability and life cycle considerations

The sustainability of sludge dewatering technologies should be evaluated on a whole system rather than inferred from dewatering efficiency alone. Recent life cycle assessment and life cycle cost studies show that conditioning routes with similar dewatering outcomes can differ substantially in environmental and economic burdens because reagent production, energy use, and downstream requirements vary across configurations. In pilot scale sludge dewatering, Wang et al. (2023) showed that advanced oxidation-based conditioners differed markedly in both environmental impact and total cost, and that better dewaterability did not necessarily correspond to better sustainability. A subsequent life cycle linked response surface analysis further showed that the environmentally and economically preferred operating window could be 6–68% lower in environmental impact and 15–70% lower in cost than the dewaterability only optimum (Wang et al., 2024b). These findings indicate that future studies should report CST, SRF, and final DS together with specific energy consumption, reagent demand, and carbon related indicators rather than using dewaterability as the sole decision criterion.

A second sustainability issue is downstream compatibility. Improved dewaterability does not necessarily improve overall resource recovery if conditioning chemistry impairs anaerobic digestion or sludge reuse. Wu et al. (2022) showed that both PAC and PFS inhibited methane production during anaerobic digestion of waste activated sludge, with stronger inhibition under PFS. This is especially relevant to hybrid and chemically assisted systems, because a process that lowers cake water content but suppresses methane recovery may shift burdens rather than reduce them. Future evaluation of microbial and hybrid conditioning should therefore integrate dewatering and post treatment performance, including methane yield, residual metal accumulation, and compatibility with reuse pathways, rather than treating dewatering as an isolated unit operation (Y. Wu et al., 2022).

5.4. Research outlook

Several priorities emerge directly from the gaps identified in this review. First, the field needs a standardized, fraction resolved EPS analysis. Recent work has emphasized that inconsistent EPS extraction and analytical methods remain a major barrier to cross study comparison and mechanistic interpretation (Ben Hamed et al., 2025). At the same time, strain level evidence shows that EPS degradation does not necessarily improve dewaterability because microbial treatment may affect S EPS, LB EPS, and TB EPS differently and may also generate hydrophilic products that offset the benefit of EPS loss (Kang et al., 2023; Lin et al., 2020; Pei et al., 2010). A practical next step is therefore to establish benchmark protocols based on consistent operational definitions and extraction sequences for S EPS, LB EPS, and TB EPS, and to pair these measurements with LF NMR, DSC, FTIR, and fluorescence analyses on the same sludge samples. These datasets should then be linked quantitatively to CST, SRF, and final DS across municipal, digested, and industrial sludges.

Second, future research should move from descriptive microbiome profiling to omics guided dewatering design. Metagenomic analysis has already shown that improved bioleaching based dewaterability can be associated with genes involved in iron transport and electron transfer, while recent meta transcriptomic datasets provide functional information linked to wastewater process conditions (Y. Li et al., 2024; Mahajna et al., 2025). These advances support enzyme or consortium design tailored to sludge specific EPS composition. A practical strategy would be to compare high performing and low performing conditioning consortia using metagenomics and transcriptomics, identify pathways most strongly associated with bound water release, and then formulate targeted enzyme mixtures for protein rich and polysaccharide rich EPS matrices.

Third, data driven control should progress from offline prediction to real time optimization. ANN based models have already been developed to predict sludge dewaterability from physicochemical properties and conditioning parameters, while deep learning-based imaging has enabled sludge moisture prediction within seconds (Kowalczyk and Kamizela, 2021; Li et al., 2023a; Xu et al., 2025). In parallel, reinforcement learning for wastewater process control and machine learning assisted microbiome engineering have begun to be demonstrated in activated sludge related systems (Croll et al., 2023; S. Zhang et al., 2025). A logical next step is therefore to develop machine learning models for real time optimization of microbial conditioning, using online moisture, conductivity, rheology, and image derived features together with offline EPS and microbial markers to optimize inoculum dosage, conditioning time, redox conditions, and co-conditioner addition.

Fourth, cross scale transferability must become an explicit research objective. Large-scale comparison of activated sludge communities has shown that laboratory and full-scale bioreactors differ substantially in community structure, which helps explain why promising bench scale strategies are often difficult to reproduce in practice (Ma et al., 2023). Future studies should therefore validate microbial and hybrid dewatering strategies at pilot scale across multiple sludge sources and seasons, using harmonized metadata on sludge origin, solids concentration, ionic composition, EPS fractions, operational history, and dewatering endpoints. Pilot scale work should also adopt multi objective evaluation that combines dewatering performance with digestion compatibility, energy use, reagent demand, and life cycle burden, because the environmentally or economically preferred operating window may not coincide with the dewaterability only optimum.

6. Conclusions

Sludge dewatering remains fundamentally constrained by the structure, composition, and spatial organization of EPS. However, the main conclusion of this review is not simply that microbial conditioning is promising, but that its value is selective rather than universal. Microbial methods are most suitable for replacing or reducing high dosage chemical conditioning, especially metal salt and polymer based pretreatments used mainly to improve flocculation and interstitial water release, because these conventional approaches often increase ash content, leave residual chemicals in sludge cake, and impair downstream anaerobic digestion or resource recovery (Pasciucco et al., 2024; Y. Wu et al., 2022).

By contrast, microbial conditioning is unlikely to replace mechanical dewatering itself, since mechanical processes remain necessary for bulk free water removal. A second high value judgment is that not all hybrid approaches should be prioritized equally. Based on the available comparative evidence, priority should be given to microbial coupling with chemical oxidation or flocculation and to microbial coupling with electrodewatering, because these configurations have already demonstrated measurable advantages over standalone comparators in CST, SRF, cake moisture, or treatment time. In contrast, combinations involving skeleton builders or thermal assistance remain mechanistically plausible, but their engineering value is still supported more by conceptual rationale than by rigorous side by side benchmarking. Future development should therefore focus on hybrid routes that already show reproducible comparative gains, rather than on all plausible combinations. A third high value judgment concerns engineering translation. The primary constraint is not any single factor in isolation, but the lack of predictive and controllable process performance across heterogeneous sludge matrices. In practice, this appears as unstable microbial response, variable residence time requirements, weak transferability from laboratory sludge to full scale sludge, and insufficient linkage between microbial activity and online dewatering indicators. The field therefore no longer needs further proof that microorganisms can influence sludge dewaterability. It needs frameworks that make this influence predictable, scalable, and operationally robust. The most urgent priorities are standardized EPS fractionation and water state characterization, omics guided design of microbial consortia and enzyme systems, and data driven monitoring and control that connect microbial activity with real time dewatering performance under realistic plant conditions (Ma et al., 2023; Wang et al., 2024b; S. Zhang et al., 2025).

Taken together, the most defensible future role of microbial assisted dewatering is not as a standalone green substitute for all existing technologies, but as a selective replacement for chemically intensive conditioning and as a biological pretreatment within quantitatively validated hybrid systems. Progress along this route, supported by pilot scale benchmarking across representative sludge types, will be essential for moving microbial assisted dewatering from proof of concept toward full scale engineering implementation.

CRediT authorship contribution statement

Yunpeng Chen: Writing – original draft, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Chonlong Chio:** Writing – review & editing. **Rishnika Boteju:** Resources. **Guimeng Lu:** Software. **Qing-Lai Dang:** Supervision. **Wensheng Qin:** Supervision.

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Data availability

No data was used for the research described in the article.

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