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Review

Hypersaline organic wastewater treatment: Biotechnological advances and engineering challenges

Yan-Qing Zhang [a], Jing-Long Han [a], Hao-Yi Cheng [a], Hong-Cheng Wang [a], Tie-Jun Liu [b], Bin Liang [a,*], Ai-Jie Wang [a,c]

[a] State Key Laboratory of Urban Water Resource and Environment, School of Eco-Environment, Harbin Institute of Technology, Shenzhen, 518055, China
[b] Guangdong Provincial Key Laboratory of Intelligent and Resilient Structures for Civil Engineering, Harbin Institute of Technology, Shenzhen, 518055, China
[c] State Key Laboratory of Urban Water Resource and Environment, School of Environment, Harbin Institute of Technology, Harbin, 150090, China

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ABSTRACT

The sustainable treatment of hypersaline organic wastewater (HSOW) remains a significant challenge in industrial wastewater management, as conventional approaches often fail to meet stringent discharge standards and low-carbon sustainability targets. Halotolerant and halophilic microbial strains offer promising solutions, yet their application is hindered by limited stress resistance, thus hindering effective treatment and achieving near-zero liquid discharge. In this review, we systematically examine endogenous strategies, such as microbial mutualism and genetic engineering, alongside exogenous approaches, including functional materials, electrical and magnetic stimulation, and 3D bioprinting, to improve microbial resilience in hypersaline environments. Furthermore, we propose an integrated treatment framework that combines physicochemical and biochemical processes, leveraging biological detoxification and biological desalination to enhance the treatment of HSOW while minimizing environmental impact and carbon emissions. By advancing the understanding of microbial stress adaptation and optimization strategies, this review provides critical insights into the development of sustainable, low-carbon wastewater treatment solutions.

1. Introduction

Hypersaline organic wastewater (HSOW) (>1 wt% salinity) is produced from industrial processes such as fine chemical manufacturing, as well as pharmaceuticals, pesticides, and leather production, and typically contains high concentrations of inorganic salt ions (e.g., Na^+, Cl^-, Ca^{2+}, SO_4^{2-}) along with substantial amounts of organic matter and even refractory organics [1,2]. Effectively treating HSOW remains a considerable challenge for the water treatment industry. Numerous reviews have summarized HSOW treatment, with most types adopting physicochemical (e.g., coagulation–flocculation, advanced oxidation, membrane technologies, electrochemical techniques) and biological methods (e.g., membrane bioreactors, constructed wetlands, halophilic functional bacteria enhanced treatment), among others [3–10]. Halophilic bacteria (e.g., *Psychrobacter*) and archaea (e.g., *Haloferax volcanii*) have shown the potential to effectively treat certain types of HSOW [11,12]. Despite extensive research, these treatments face various technological constraints, such as high energy consumption and biological activity inhibition. Therefore, identifying and optimizing the key factors that minimize these adverse effects would facilitate the advancement of physicochemical and biochemical treatment processes.

With enterprises' current emphasis on their operations' cost-effectiveness and low-carbon emissions, biological treatment technologies are increasingly favored for their low-carbon footprint and minimal amounts of secondary pollution compared to those of alternatives [13,14]. However, the high salinity and toxicity of pharmaceutical and chemical wastewater can severely inhibit microbial activity [15]. Therefore, optimizing functional communities, improving biological stress resistance, and synergistically transforming organic pollutants under high-inhibition conditions are crucial for reducing hazardous mixed-salt waste and maximizing the low-carbon benefits of biologically treating HSOW [16]. Moreover, improving microbial halotolerance is essential for maintaining

* Corresponding author.
E-mail addresses: liangbin1214@hit.edu.cn, liangbin1214@163.com (B. Liang).

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stability and efficiency in industrial applications, particularly when the performance of halophilic bacteria and archaea is compromised by salinity fluctuations. Both endogenous and exogenous enhancement strategies have become indispensable for addressing these challenges. Endogenous enhancement is primarily focused on improving the halotolerance mechanisms of microbial strains or communities, including promoting microbial halotolerance mutualism and employing genetic engineering for targeted strain modification to enhance biological survival in hypersaline environments [17–20]. In contrast, exogenous enhancement strategies leverage external energy interventions (e.g., electrical and magnetic stimulation) to significantly bolster microbial halotolerance by boosting extracellular electron transfer and activating cellular stress response mechanisms [21,22]. Exogenous enhancement also involves the application of functional materials (e.g., three-dimensional [3D] bioprinting carriers) to promote microbial adhesion and biofilm formation, further strengthening functional microorganisms' resistance to environmental fluctuations [23,24]. Developing effective exogenous enhancement methods offers broad application prospects for the engineered treatment of HSOW [25].

Notably, increasing microbial survival under hypersaline stresses through these enhancement strategies can sustain the synergistic degradation of recalcitrant organics by functional microbial communities, facilitating the deconstruction and detoxification of pollutants and creating favorable conditions for the mineralization of low-concentration recalcitrant organics [26]. Furthermore, microbial-mediated nitrate and sulfate reduction can lower salinity in HSOW, reducing the interference of inorganic salts in subsequent physicochemical processes [27]. In summary, combining endogenous and exogenous enhancement strategies to stabilize biological treatment under high-salinity and high-toxicity conditions while achieving detoxification and salt reduction will provide robust technical support for the low-carbon and efficient treatment of HSOW.

In this regard, our review offers a comprehensive summary of enhanced strategies for improving microbial halotolerance, focusing on their potential to optimize low-carbon treatment processes (Fig. 1). We mainly elucidated two key points: (1) methods and mechanisms for enhancing microbial halotolerance in hypersaline environments through both endogenous and exogenous reinforcements and (2) the core role of biological detoxification and biological desalination in physicochemical–biochemical coupling processes for HSOW treatment. We aim to provide a scientific basis for the biological treatment of HSOW and offer recommendations for optimally integrating it with physical–chemical processes.

2. Limitations of physicochemical and biochemical technologies

We searched the Web of Science database using the keywords “high-salt wastewater,” “hypersaline wastewater,” and “high-salinity wastewater”; 614 relevant publications from January 2000 to September 2024 were identified. Publications and citations related to hypersaline wastewater have increased sharply over the past five years (Supplementary Material Fig. S1a), indicating the topic's emergence as a significant research hotspot. A co-occurrence analysis of high-frequency keywords shows that research primarily focuses on physicochemical and biochemical treatment technologies and their removal efficiency (Supplementary Material Fig. S1b). While previous reviews have detailed these treatment technologies [5,8], these methods still face various engineering limitations and challenges. Physicochemical technologies for treating HSOW, such as advanced oxidation processes (AOPs), membrane technology, and evaporative crystallization, are significantly limited. High salinity reduces wastewater treatment efficiency by interfering with electron transfer and oxidant activity, accelerates membrane fouling, generates toxic byproducts such as chlorate and bromate [28,29], and increases energy consumption and equipment corrosion [7,30–32]. Although studies have demonstrated that certain halophilic bacteria can remove chlorates and bromates [33], these challenges still threaten wastewater treatment systems' stability and long-term operation. Biochemical methods, although energy-efficient and less toxic than their alternatives, are hampered by high salt concentrations that inhibit microbial growth and enzymatic activity and by varying organic types and concentrations that affect biodegradation efficiency [34,35] (Fig. 2). Therefore, an in-depth exploration of the mechanisms and application prospects of enhancing microbial halotolerance is crucial for optimizing the efficiency and reliability of HSOW biological treatment and for providing key information leading to the achievement of near zero liquid discharge (ZLD).

3. Endogenous enhancement of microbial resistance to hypersaline stress

Enhancing the intrinsic halotolerance of functional microorganisms in hypersaline environments is crucial for their survival and adaptability. Effectively manipulating endogenous halotolerance mechanisms is a strategy for strengthening microbial resistance to salt stress. Directed evolution under high salt concentrations can help researchers select mutant strains with improved halotolerance. Importantly, metabolic interactions among bacterial strains significantly augment the halotolerance of functional communities. Genetic engineering techniques can improve the synthesis and secretion of compatible solutes (e.g., betaine, ectoine, proline) by a genetic circuit design that clones and expresses key genes. These strategies are essential for advancing microbial halotolerance.

3.1. Halotolerant mutualism mechanism of microbial communities

3.1.1. Syntrophy in extreme environments

In natural environments, certain microorganisms collaborate rather than compete for nutrients, performing specific transformations that they cannot achieve independently or utilizing metabolites to sustain their survival. This type of microbial interaction, known as syntrophy, is vital for microbial communities to persist in extreme environments [36,37]. Symbiotic relationships typically involve microorganisms sharing the same microhabitat, in which the metabolic products of one species are utilized by another. For instance, *Marinobacter* sp. N4, isolated from a halophilic environment, uses phenanthrene as its sole carbon source and converts phenanthrene to 1-hydroxy-2-naphthoic acid (1H2N). *Halomonas* sp. G29 then converts 1H2N to 1,2-dihydroxynaphthalene (1,2-DHN), which can be further transformed into salicylic acid (SALA) via strain N4. Subsequently, strain G29 can convert SALA to catechol, while strains N4 and G29 utilize SALA for complete phenanthrene mineralization through the catechol 2,3-dioxygenase and 1,2-dioxygenase pathways, respectively [38]. This symbiosis supports bacterial survival in hypersaline conditions. Similarly, methanogens and sulfate-reducing bacteria often coexist in hypersaline marine environments. For example, methane consumption in hypoxic marine sediments is mediated by two archaeal communities (ANME-1 and ANME-2) coexisting with sulfate-reducing bacteria. Their growth kinetics suggest that the proliferation of sulfate-reducing bacteria may be promoted by anaerobic methanogenic communities [39]. A metabolic cross-feeding study shows that the *Halorubrum* and *Marinococcus* strains can co-exist at salinities of up to 25% [40], indicating that specific substance exchanges between microorganisms can increase the survival of functional communities in hypersaline environments.

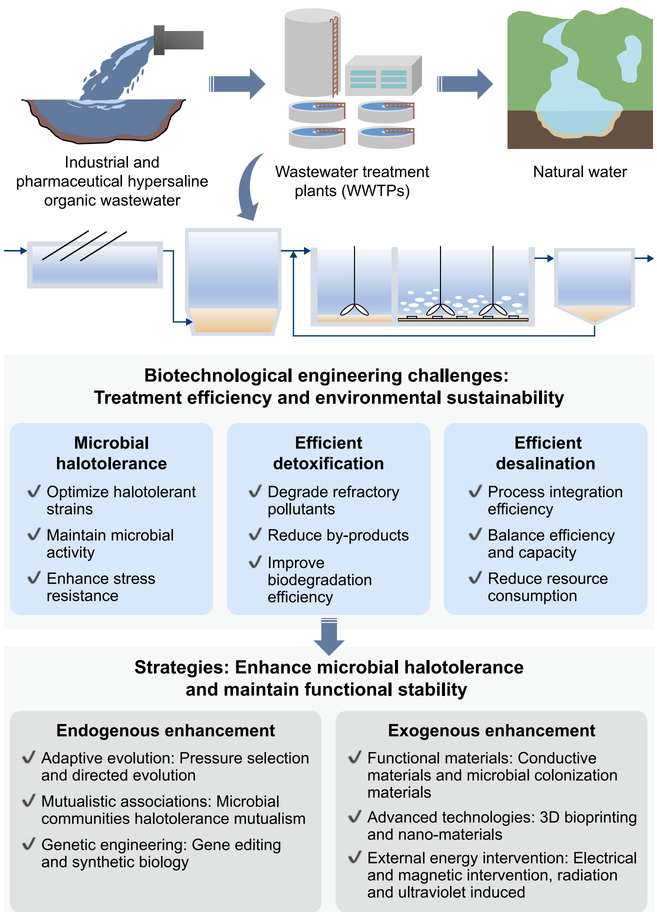

Fig. 1. Biotechnological challenges and enhancement strategies for hypersaline organic wastewater (HSOW) treatment. An overview of the key biotechnological challenges in HSOW treatment, including the maintenance of microbial activity and the optimization of process efficiency in hypersaline environments, that highlights proposed endogenous and exogenous enhancement strategies for improving microbial halotolerance. These strategies aim to optimize the effectiveness of biological treatment processes under hypersaline stress, focusing on improving biological detoxification and biological desalination efficiency.

3.1.2. Compatible solute strategy for osmotic pressure regulation

Most microbial cells use a compatible solute strategy for managing osmotic pressure by accumulating neutral, small organic molecules that can be synthesized internally or absorbed from the environment [41]. In some cases, compatible solutes such as betaine and trehalose can serve as effective carbon and nitrogen sources [42,43]. When nitrogen is abundant, *Ectothiorhodospira halochloris* accumulates extracellular proline. However, when nitrogen starvation occurs, trehalose accumulates and fully replaces ectoine, thereby releasing nitrogen for cell growth [43]. Absorbing these solutes from the medium is generally more energy-efficient than synthesizing them [44], making absorption the preferred method for regulating cellular osmotic pressure. Most bacteria can transport and absorb at least one compatible solute [45]. For instance, *Bacillus subtilis* possesses five types of transport systems: OpuA, OpuB, OpuC (ABC-type transport proteins), OpuD (secondary transport proteins that are members of the betaine/carnitine/choline transport [BCCT] family), and OpuE (which belongs to the sodium/solute transport protein family). OpuE also functions as a proline transport protein whose transcription is regulated by high osmotic pressure, highlighting its crucial role in cellular osmotic adaptation [46,47]. Therefore, the exogenous addition of compatible solutes can enhance the efficiency of biological treatment processes in HSOW and solid waste management. For instance, adding glycine betaine to food waste containing 1.16% NaCl increases methane production approximately sixfold compared to the control group, significantly improving the anaerobic digestion of saline food waste [48]. However, the impact of exogenous betaine addition varies among different microorganisms. In anaerobic mutualistic bacterial communities, betaine increases the growth of acetic acid-utilizing bacteria, acetic acid-utilizing methanogens, and butyric acid-utilizing bacteria, while its effect on propionic acid-producing bacteria is relatively weak [49], possibly due to the lack of corresponding transporters. The addition of glycine betaine also regulates the secretion of extracellular polymeric substances (EPSs). For example, supplementing with glycine betaine at a salinity of 3.5% increases total EPS content from 12.50 ± 0.05 mg g^{-1} of cell dry weight to 24.58 ± 0.96 mg g^{-1}. Compared to conditions with 0% salinity, this supplementation reduces electrostatic repulsion and cell compaction, resulting in a more compact protein structure. Moreover, glycine betaine enhances the release of exogenous electron shuttles (e.g., flavins, c-type cytochromes) and increases the relative abundance of salt-tolerant coding genes (e.g., *kdpB*, *betA*, *opuD*, *epsP*), effectively mitigating osmotic stress in microorganisms [50,51].

3.1.3. Excretion and absorption of compatible solutes

Bacteria can release compatible solutes into the environment; other bacterial strains can then absorb and utilize these solutes. For instance, *Halomonas salina* DSM 5928 secretes ectoine at a release rate that often surpasses the absorption rate, resulting in a non-equilibrium state. Ectoine synthesis is not limited by intracellular threshold concentrations [52], and its total secreted concentration remains unaffected by NaCl levels in the culture medium. Interestingly, the production efficiency of ectoine is increased at lower NaCl concentrations [53]. Similarly, *Escherichia coli* secretes glycine betaine when grown in a medium with high osmotic pressure and supplemented with choline (Fig. 3a), entering other cells through ProU and ProP transporters [54]. Additionally, introducing exogenous compatible solutes under high-salinity conditions does not enhance the growth of *Bacillus subtilis* MGA3. These bacteria cannot resist high osmotic stress by absorbing compatible solutes or hydrolyzing proline-containing peptides; they can only synthesize L-glutamic acid. Interestingly, about 70% of newly synthesized L-glutamic acid is excreted in hypersaline environments [55] (Fig. 3b). This efflux provides readily available raw materials for other strains in the bacterial community that can utilize L-glutamic acid. When extracellular osmotic pressure decreases, bacteria maintain internal osmotic pressure by releasing low-molecular-weight solutes. For example, *Corynebacterium glutamicum* prioritizes the excretion of compatible solutes such as glycine betaine and proline via osmotic regulation channels rather than carriers, with the excretion mediated by the reversal of the glycine betaine uptake channel [56]. A similar betaine efflux is observed in *Salmonella enterica*, which helps regulate intracellular betaine levels [17] (Fig. 3c).

3.1.4. Compatible solute sharing in microbial communities

In microbial communities such as biofilms, bacteria with high-affinity betaine uptake systems may benefit from betaine efflux (Table 1), and glycine betaine transporters are widely present in microorganisms. A previous study analyzed 60 BCCT family transporters containing glycine betaine across 83 bacterial genomes [57], showing that strains unable to synthesize glycine betaine can transport and absorb it via related transporter proteins. Notably, even under sustained steady-state conditions with high osmotic pressure, bacteria appear to excrete and reabsorb compatible solutes [54,58], suggesting a synthesis-release-recapture cycle that

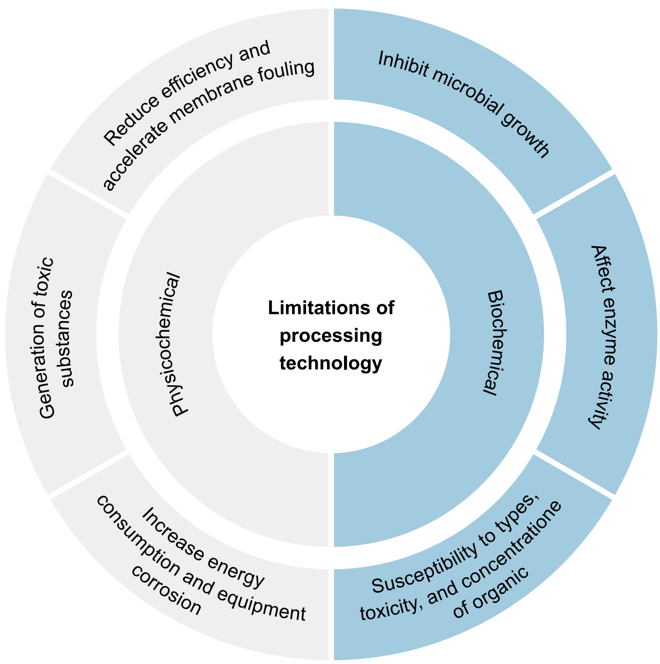

Fig. 2. Limitations of physicochemical and biochemical hypersaline organic wastewater treatment technologies. Physicochemical treatment technologies are primarily constrained by high rates of energy consumption and potentially excessive secondary pollution, while the inhibitory effects of high salinity on microbial activity significantly limit biochemical treatment.

may help cells fine-tune their intracellular pressure during cell division [59]. Extracellular compatible solutes or precursor substances such as L-glutamic acid can also be absorbed and utilized by other bacteria, indicating that sharing compatible solutes in microbial communities has the potential to enhance halotolerance.

Microbial communities may adapt to salt stress through interdependent mechanisms promoting survival and reproduction. Two compatible solute transport vectors, OpuD and PutP, in *Vibrio cholerae* are identified as facilitating the transportation and absorption of glycine betaine and proline. However, *V. cholerae* lacks the *bet* gene for synthesizing glycine betaine and can only synthesize ectoine (Fig. 3d). Transporters for ectoine have been found in other halophilic bacteria, suggesting that bacteria may expand their osmotic adaptation mechanisms by coexisting with others that synthesize multiple compatible solutes [60,61]. Further research has confirmed that supplementing discarded culture media with glycine betaine or other strains (such as *Vibrio fluvialis*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus*, which can synthesize glycine betaine de novo in the presence of choline) can promote *V. cholerae* growth and biofilm development (Table 1) [18]. These findings demonstrate that compatible solute sharing may be a cooperative mechanism in microbial communities since solutes provided by other bacteria enhance the survival of *V. cholerae* in marine environments by regulating osmotic adaptability and biofilm formation. Additionally, betaine promotes the growth of *V. cholerae* at high salt concentrations and reinforces its surface adhesion by inducing *vps* gene transcription. Thus, bacteria can bolster their response to osmotic stress by forming biofilm communities, increasing the halotolerance of individual bacteria in hypersaline environments. These interdependencies enable more efficient adaptation to osmotic stress, fostering the survival of functional microbial communities in extreme environments. In HSOW treatment, these cooperative mechanisms can be harnessed to improve microbial performance, such as higher bioremediation efficiency and better halotolerance. By utilizing the interspecies exchange of compatible solutes, such as glycine betaine and ectoine, microbial communities can be optimized to maintain key metabolic functions, thus providing a low-energy, sustainable approach to HSOW treatment.

3.2. Genetic engineering enhances microbial halotolerance

Genetic engineering has demonstrated a remarkable potential for boosting microbial halotolerance. Through gene editing and transgenic techniques, microbial genomes can be precisely modified to increase their resistance to high salinity [62].

3.2.1. Key genes and pathways for halotolerance

The gene *proU* is closely associated with the transport of glycine betaine, and its non-specific expression has been confirmed to support the growth of engineered bacteria under hypersaline stress. The *proU* operon was subcloned from *E. coli* onto a broad host vector, pMMB206, and transferred to a microbial community composed of four *Pseudomonas* species (Fig. 4a). Under the control of the tac-lac promoter, the constructed *Pseudomonas* sp. exhibits enhanced halotolerance and effective hydrocarbons degradation, whereas the wild-type *Pseudomonas* cannot grow at a concentration of 5.84% NaCl (Table 1) [63]. This engineered bacterial consortium provides an effective solution for treating hypersaline industrial wastewater. Moreover, while *Pseudomonas putida* KT2440 displays strong environmental stress tolerance, the introduction of *betB*-encoded betaine-aldehyde dehydrogenase and Na^+/H^+ antiporter *EcnhaA* from *E. coli* significantly enhances the growth of strain KT2440 at 4% salinity. The co-expression of *EcnhaA* and *betB* increases the maximum halotolerance of strain KT2440-*EcnhaA-betB* to 5% salinity [64]. Additionally, the engineered strain KT2440-*EcnhaA-betB* can degrade 56.70% of benzoic acid and 95.64% of protocatechuic acid at 4% salinity within 48 h, whereas the wild-type strain KT2440 exhibits no biodegradation under the same conditions (Table 1). These examples highlight the potential of genetic engineering to maintain strain functionality under

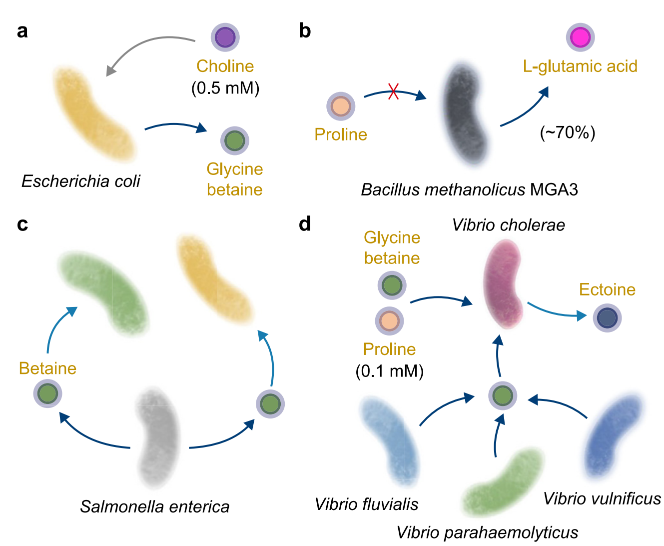

Fig. 3. Mechanisms of microbial compatible solute sharing. **a**, *Escherichia coli* synthesizes and excretes glycine betaine using choline as a precursor. **b**, *Bacillus subtilis* MGA3 excretes approximately 70 % of newly synthesized L-glutamic acid under hypersaline conditions. **c**, *Salmonella enterica* excretes betaine for utilization by other strains. **d**, Adding glycine betaine or discarded culture media of strains (*Vibrio fluvialis*, *Vibrio vulnificus*, *Vibrio parahaemolyticus*) that secrete glycine betaine promotes the growth of *Vibrio cholerae* in hypersaline environments.

Table 1
Summary of endogenous and exogenous methods for enhancing microbial halotolerance.

Enhancement pattern	Applied technology	Salinity (%)	Specific strain, material, or method	Results	References
Endogenous enhancement	Halotolerant mutualism	4.09	*Salmonella enterica*	Bacteria can enhance their halotolerance to betaine efflux in *Salmonella enterica*	[17]
		2.92	*V. cholerae*, *V. fluvialis, V. vulnificus*, and *V. parahaemolyticus*	Supplementing discarded culture media containing betaine from *V. fluvialis*, *V. vulnificus*, and *V. parahaemolyticus* promoted the growth of *V. cholerae*	[18]
	Genetic engineering	5.80	*E. coli*, *Pseudomonas*	The transfer of the *proU* operon to *Pseudomonas* resulted in 25-fold increase in salt protection	[63]
		4	*Pseudomonas putida* KT2440	The engineered strain KT2440-*EcnhaA-betB* degraded 56.70 % of benzoic acid and 95.64 % of protocatechuic acid within 48 h	[64]
		7	*Halomonas elongata*	The engineered salt-inducible *HopGadBmut* gene enhanced γ-aminobutyric acid accumulation in *Halomonas elongata*	[66]
		10	*Halomonas elongata* ATCC 33174	The transfer of plasmid pSH15 from *E. coli* to *Halomonas* strains enhanced halotolerance	[73]
Exogenous enhancement	Functional materials	2.34	Magnetite (100 g L^{-1})	Higher COD removal efficiencies (90.2 ± 0.5 % vs. 73.1 ± 1.9 %) and methane production rates (4082 ± 334 mL STP) d^{-1} vs. (2640 ± 120 mL STP) d^{-1} than the non-amended control group	[78]
		3.51	Nitrogen-doped carbon nanotube catalysts (CoCe@NCNTs)	A stable norfloxacin removal efficiency of 64.1 %; the PN/PS ratio in the biofilm was 1.94 times higher than that of the control group	[81]
		2–35	Polyvinyl alcohol sodium alginate (PVA + SA)	Crude oil was effectively removed by PVA + SA-immobilized communities, with an average degrading ratio of 19.42–31.45 mg L^{-1} d^{-1})	[82]
	Electrical intervention	0–5	Low-voltage stimulation (1.2 V)	Higher COD removal rate (93 % vs. 53 %) under 50 g L^{-1} NaCl than the non-stimulated group	[94]
		7	Electric fields	Improved performance of phenolic wastewater treatment (86 % vs. 68 %)	[98]
	Magnetic intervention	0–2	50 mT SMF	Enhanced halotolerance of aerobic granular sludge with COD and TN removal rates of 100 % and 72.9 %, respectively.	[102]
		15	Iron oxide magnetic nanoparticles (IOMNPs)	Halophilic bacterial growth increased more than twofold by removing bile acids with IOMNPs (4 g L^{-1})	[106]
	Radiation	15–20	Gamma irradiation	A mutant of *Halomonas* sp. YJPS3-3 with increased halotolerance	[108]
	Ultraviolet rays	15	15 W UV lamp irradiated at a distance of 25 cm	Intracellular betaine content of the mutant strain *Halomonas* sp. UV-1 reached a maximum of 190 μg mg^{-1}	[111]

hypersaline stress.

3.2.2. Transposon mutagenesis for enhanced halotolerance

In recent years, the transposon insertion mutant system has emerged as a powerful genetic tool across various biological systems. Using a Tn5-based transposon insertion mutagenesis system, the researchers developed a salt-tolerant engineering strain: *Zymomonas mobilis* ZMT2 (Fig. 4b). This mutant strain, which has the halotolerance gene *himA* inserted into its transposon, exhibits a significantly higher rate of sugar-to-ethanol conversion compared to that of the wild-type strain *Z. mobilis* ZM4 under a maximum of 2% NaCl stress [65]. These results show that transposon-based genetic tools can effectively increase microbial halotolerance and improve industrial applications.

3.2.3. Compatible solutes, biofilm formation, and membrane composition

Enhancing the accumulation of compatible solutes, boosting biofilm formation, and modifying the composition of bacterial cell membranes are key strategies for improving the halotolerance of bacterial strains in hypersaline environments [66–68]. For instance, introducing the engineered salt-inducible *HopGadBmut* gene into the genome of *Halomonas elongate* (Fig. 4c), which produces L-glutamic acid, increases the intracellular accumulation of high concentrations of γ-aminobutyric acid as an osmotic protectant (Table 1), thereby improving their halotolerance [66]. By heterologously expressing *E. coli*'s *pfs* and *luxS* genes in *Z. mobilis*, a universal quorum-sensing signal molecule, autoinducer 2 (AI-2), is generated to control cell morphology and strengthen stress resistance [69]. The exogenous expression of *pfs* significantly enhances biofilm formation, increasing the tolerance of *Z. mobilis* to organics and hypersaline stress. Additionally, in the salt-tolerant strain *Saccharomyces cerevisiae* BY4741 (Y00) obtained through nitrosoguanidine mutagenesis, the modular assembly of the genes *cds1* and *cho1*, related to salt-stress tolerance, reduces the ratio of anionic to zwitterionic phospholipids in strain Y03, thereby improving the strain's salt tolerance (7% NaCl) [68]. Ultimately, the targeted utilization of genes associated with cell membranes enhances the survival of microorganisms in hypersaline environments.

3.2.4. Enhancing halotolerance in non-halophilic bacteria through synthetic biology

Synthetic biology offers promising avenues for enhancing microbial halotolerance. Recent research employed the halophilic bacterium *Halomonas cupida* J9 as a platform to identify robust promoters of the construction of engineered strains capable of degrading 25 mg L^{-1} of *p*-nitrophenol in seawater within 6 h, demonstrating its potential for *in situ* bioremediation [70]. Future applications may leverage this approach to develop diverse salt-tolerant bioremediation platforms for HSOW treatment.

Enhancing the halotolerance of non-halotolerant bacteria offers another promising avenue for research. Gene transfer between non-halophilic and moderately halophilic bacteria has been demonstrated through conjugation (Fig. 4d) [71,72]. For instance, a shuttle vector (pHS15) derived from a small plasmid isolated from *Halomonas elongata* ATCC 33174 has been shown to transfer from *E. coli* to various *Halomonas* strains [73]. Consequently, gene exchange between non-halophilic and halophilic bacteria in hypersaline environments may increase the halotolerance of non-halophilic bacteria. Currently, many bacteria capable of degrading various recalcitrant organic compounds have been identified; however, most are ineffective in treating hypersaline industrial organic wastewater due to their lack of halotolerance. Constructing mobile plasmids with higher stress resistance and halotolerance may significantly improve the resilience of functional communities

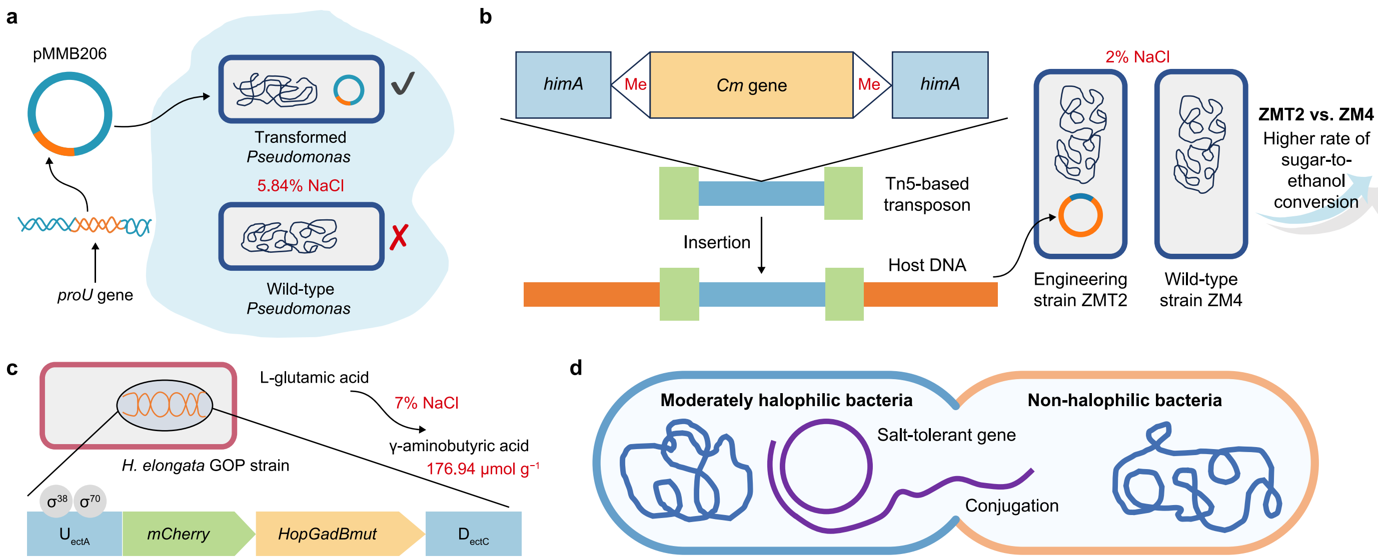

Fig. 4. Genetic engineering to enhance microbial halotolerance. **a**, The *proU* operon was subcloned into the host-vector pMMB206 and introduced into *Pseudomonas.* This genetically engineered *Pseudomonas* demonstrated enhanced halotolerance and improved hydrocarbon biodegradation efficiency compared to the wild-type strain. **b**, A halotolerant strain, *Zymomonas mobilis* ZMT2, was engineered using a Tn5-based transposon insertion system carrying the *Cm* gene and *Me* mosaic end into the genome. ZMT2 exhibited significantly higher sugar-to-ethanol conversion rates under 2 % NaCl stress compared to the wild-type strain ZM4. **c**, Introducing the *HopGadBmut* gene into the genome of *Halomonas elongata* GOP strain enhanced the conversion of L-glutamate into high concentration γ-aminobutyric acid as an osmoprotectant. The U_{ectA} is located upstream of *ectA* and may bind to the osmotically induced factor σ38 and vegetative factor σ70. *ectA* encodes L-2,4-diaminobutyric acid acetyltransferase, *ectC* encodes ectoine synthase, and *mCherry* encodes a red fluorescent reporter protein. **d**, Gene transfer between non-halophilic and moderately halophilic bacteria can be achieved through conjugation, thereby potentially enhancing the halotolerance of non-halophilic bacteria.

in wastewater treatment systems. This approach can also alleviate the bottleneck in the biological treatment of HSOW (e.g., antibiotic wastewater, petrochemical wastewater) and has significant practical engineering applications.

3.2.5. Challenges in genetic engineering for halotolerance

While genetic engineering holds great promise, several challenges remain. One of them lies in the complexity of salt-stress mechanisms, which involve multiple metabolic pathways and cellular responses. Current approaches may not fully account for the intricate interactions between halotolerance and other stress factors, such as oxidative stress and nutrient deprivation. Furthermore, the potential trade-offs between halotolerance and other essential microbial functions, such as growth rate or substrate utilization, should be addressed. These limitations could guide further research toward optimizing genetic modifications for more robust and efficient microbial strains.

4. Exogenous enhancement of microbial resistance to hypersaline stress

Integrating functional materials with external energy intervention has emerged as an effective exogenous strategy to enhance microbial halotolerance and improve the efficiency of biological treatment of HSOW. Compared to endogenous enhancement methods, exogenous enhancement allows microorganisms to conserve more energy in hypersaline environments, thereby facilitating their adaptation to hypersaline stress.

4.1. Functional materials enhance microbial halotolerance and effective colonization

High salinity typically diminishes bioreactor efficiency in wastewater treatment; however, incorporating functional materials can notably boost HSOW treatment.

4.1.1. Conductive materials

Various studies have demonstrated conductive materials' effectiveness in improving anaerobic biodegradation under hypersaline stress [74–76]. Conductive materials, such as powdered activated carbon (PAC) and magnetite, enrich microorganisms that are highly capable of cation transport. They facilitate direct interspecies electron transfer (DIET), improve K^+ uptake, and enhance the synthesis or transport of compatible solutes (e.g., glycine betaine, ectoine, trehalose, proline), thereby providing functional microorganisms with more energy to withstand hypersaline stress (Fig. 5a) [77]. Similarly, the addition of magnetite significantly improves the efficiency of anaerobic treatment of HSOW, achieving a higher chemical oxygen demand (COD) removal rate (90.2 ± 0.5%) at concentrations of 100 g L^{-1} magnetite compared to the non-amended control group (73.1 ± 1.9%) (Table 1). This method also enriches salt-tolerant bacteria, with the relative abundance of *Pseudomonas* increasing from 7.4% to 15.5% [78]. Additionally, microorganisms in hypersaline environments use sodium and potassium pumps to regulate osmotic pressure. Carbon nanotubes (CNTs), which contain narrow hydrophobic inner pores resembling microbial membrane channels [79], have shown the potential to mimic potassium pore proteins, thereby facilitating transmembrane K^+ transport [80]. CNTs could also theoretically serve as channels for transporting compatible solutes, constituting a potential solution for enhancing bacterial halotolerance (Fig. 5a).

The bioelectrochemical system (BES) constitutes a promising approach to increasing microbial salt tolerance, particularly when using nitrogen-doped carbon nanotube catalysts (CoCe@NCNTs) (Fig. 5a). The ratio (0.55) of nicotinamide adenine dinucleotide and its reduced counterpart (NAD^+/NADH) in the anodic biofilm is significantly higher with $CoCe_{0.5}$@NCNTs compared to the control group with other Co and Ce doping ratios. This ratio is crucial for electron generation via NAD^+ reduction, accelerating microbial metabolic activity. Moreover, with CoCe@NCNTs, the protein-to-polysaccharide (PN/PS) ratio in the biofilm under hypersaline conditions (3.5% NaCl) is 1.94 times greater than in the control group (Table 1) [81], highlighting how the stable electrochemical performance of the cathode improves the salt resistance of anodic microorganisms.

4.1.2. Cell immobilization techniques

Cell immobilization techniques, such as entrapment in a matrix or polymer, encapsulation, cross-linking, and combined methods, are widely used to strengthen the stress resistance of microorganisms (Fig. 5b). In a previous study, a crude-oil- degrading microbial community was embedded in sodium alginate (SA) and polyvinyl alcohol sodium alginate (PVA + SA). As salinity increased from 2% to 35%, the crude-oil-removal efficiencies for the free microbial community, the SA-immobilized community, and the PVA + SA-immobilized community decreased from 90% to 31.85%, 54.84%, and 58.13%, respectively [82]. Immobilization with SA and PVA + SA notably enhanced bacterial halotolerance (Table 1). Furthermore, immobilizing electrogenic microorganisms (*Shewanella oneidensis* MR-1) in BES using graphite/alginate granules increased coulombic efficiency by 1.7 times and enhanced halotolerance [83].

4.1.3. Biocompatible carriers for enhanced microbial colonization

Effective microbial colonization is essential for optimizing bioreactor performance in HSOW treatment. Microbial colonization is governed by mechanisms including surface adhesion, biofilm development, metabolic adaptability, and interspecies interactions [84]. Microbial colonization in complex aquatic environments is influenced by environmental factors, such as temperature and salinity, and the physicochemical properties of the materials available for colonization. Prior research on microbial colonization in marine environments explored using various artificial matrices, such as stainless steel, glass, ceramics, and plastics (e.g., polyvinyl chloride, polyurethane). Polyurethane demonstrated high species diversity and colonization rates in microbial communities [85]. A previous study investigated the effects of substrate properties (e.g., surface charge, hydrophobicity, roughness, hardness) on bacterial adhesion to plastics and identified substrate hardness as a critical factor in bacterial colonization [86]. Developing biocompatible carriers to improve the adhesion and biofilm formation of functional communities in hypersaline environments and conducting in-depth analyses of interactions between microorganisms and functional materials would significantly advance the biologically enhanced treatment of HSOW.

4.2. 3D bioprinting technology improves microbial halotolerance

Bacteria demonstrate remarkable adaptability, allowing them to thrive in diverse ecological niches and enhancing their survival in harsh environments through the formation of biofilms [87]. Recent advances in 3D printing technology, a form of rapid prototyping, have enabled the immobilization of bacteria in distinct compartments created by hydrogels (bioinks) composed of biocompatible materials such as hyaluronic acid and κ-carrageenan. These 3D-printed functional microbial communities constitute optimal environments for substance exchange and promote effective bacterial attachment through quorum sensing, thereby helping microorganisms resist hypersaline stress [88]. This approach offers significant potential for improving HSOW treatment by strengthening microbial resilience and promoting efficient bioremediation processes in hypersaline environments.

A biocompatible ink named Flink, which contains high concentrations of salt, has been shown to enhance the phenol degradation rate of *Pseudomonas putida* using direct ink writing [89]. In contrast to conventional surface-adhering counterparts, embedded bacteria exhibit heightened resistance to adverse environmental conditions, such as salinity and toxicity [90]. Certain bacterial metabolites, including compatible solutes, diffuse through the interior of 3D-printed structures, boosting the halotolerance of other bacteria in the community and allowing the metabolites (e.g., betaine, proline) as metabolic substrates. When AnoxKaldnes™ (K5) carriers and 3D-printed biological carriers in continuous flow-activated sludge systems were applied to treat petrochemical wastewater characterized by high salinity, 3D-printed carriers achieved better organic removal than K5 carriers [91], highlighting the significant potential for enhancing HSOW treatment.

Moreover, advancements in bio-ink technology are crucial for increasing the halotolerance of 3D-printed functional microbial communities. A glucose-modified dendritic hydrogel for immobilizing bacteria in bio-ink has been developed. This bio-ink gels spontaneously in the presence of Na^+ or K^+ ions, eliminating the need for additional conditions and making the gel suitable for hypersaline wastewater applications [92]. Future research endeavors could leverage computer-aided design software to refine carrier models with customized structures and pores, thereby optimizing the surface area and the microenvironment for microbial growth while strategically distributing microbial communities. Integrating 3D bioprinting with functional communities and biocompatible inks provides a promising, sustainable solution for enhancing microbial halotolerance and advancing HSOW treatment technologies.

4.3. External energy intervention enhances microbial halotolerance

4.3.1. Electrical intervention

Utilizing external energy to strengthen the halotolerance of microbial communities is a well-established technique. Low-voltage stimulation (typically not exceeding 2 V) can significantly improve the halotolerant adaptation mechanisms of microbial communities (Fig. 6). These mechanisms include enriching halotolerant microorganisms, improving ion transport, and upregulating stress-related genes, all of which contribute to increased treatment efficiency. For instance, applying 1.5-V external energy to a sequencing batch reactor treating HSOW (3.5% NaCl) increases the total nitrogen (TN) removal rate by 28%, achieving a rate of 0.65 kg m^{-3} d^{-1} compared to the control group without electrical stimulation [93]. Similarly, applying 1.2 V to an upstream anaerobic membrane reactor keeps the COD removal rate at 93% with up to 5% NaCl, significantly higher than the 53% COD removal rate observed in the unstimulated group (Table 1) [94]. These improvements can be attributed to the ability of electrical stimulation to enhance ion transport, boost microbial metabolism, and promote the expression of key genes involved in halotolerance.

The key mechanisms through which electrical intervention enhances microbial halotolerance are: (1) *Enrichment of halotolerant microorganisms*. Electrical stimulation selectively enriches halotolerant microorganisms capable of degrading refractory compounds. For example, in a BES operating at 0–4% salinity, the abundance of *Halobacterium* sp. increases from 24.2% to 66.4% under electrical stimulation, whereas unstimulated groups show the opposite trend [95]. This selective enrichment is driven by stimulating cellular processes that promote microbial halotolerance. (2) *Ion transport and stress-response regulation*. The application of low-voltage electrical stimulation alters the membrane permeability potential of microbial cells, facilitating the movement of ions such as Na^+ and K^+. This outcome improves the microbial ability to regulate osmotic pressure in hypersaline environments. Electrical stimulation also upregulates the expression of betaine transporter proteins (e.g., *proX*) and salt-in strategy genes (e.g., *trkA*, *trkH*), which are crucial for maintaining cellular integrity

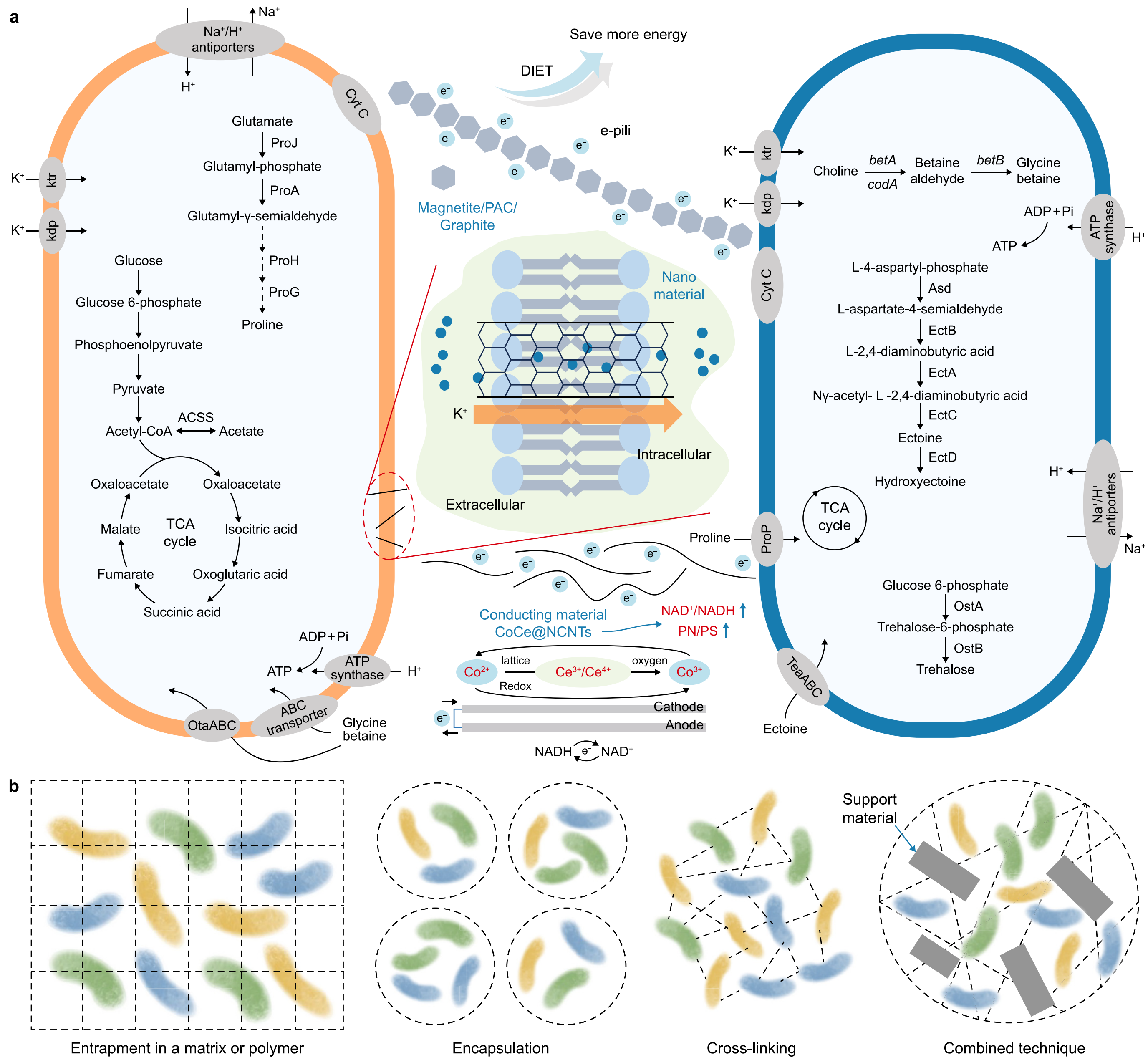

Fig. 5. Enhancing microbial halotolerance using functional materials. **a**, Conductive materials promote direct interspecies electron transfer (DIET), thus conserving microorganisms' energy levels. Carbon nanotubes (CNTs) facilitate transmembrane K^+ transport, while nitrogen-doped carbon nanotube catalysts (CoCe@NCNTs) increase nicotinamide adenine dinucleotide and its reduced counterpart (NAD^+/NADH) and protein-to-polysaccharide (PN/PS) ratios, enhancing microbial metabolic activity in hypersaline environments. **b**, Microbial cell immobilization techniques, including entrapment in a matrix or polymer, encapsulation, cross-linking, and combined techniques, further enhance a microbe's ability to adapt to stressful environments. TCA cycle: Tricarboxylic acid cycle; ATP: Adenosine triphosphate; ABC transporter: ATP-binding cassette transporter.

under saline stress [25]. Electrical stimulation can affect the membrane potential of transporters by altering the charge movement, leading to the accumulation of transient protons on the outer surface [96]. This process promotes the production of Na^+ and K^+ transporters, thereby improving microbial halotolerance [97]. Furthermore, electrical intervention reduces the intracellular reactive oxygen species (ROS) levels, preventing oxidative damage and enhancing microbial survival [98]. (3) *EPS production*. Electrical stimulation augments the secretion of EPSs, which play a crucial role in microbial adhesion, biofilm formation, and protection against environmental stressors. For example, applying 0.2 V to a BES elevates EPS concentration (39.4 mg L^{-1}) compared to the control group (33.4 mg L^{-1}) [99]. This increase in EPS production improves microbial halotolerance by facilitating microbial attachment to bioreactor surfaces and providing a protective matrix for microorganisms under high salinity. (4) *Gene regulation and cellular stress response*. Electrical stimulation can also impact gene regulation related to microbial stress responses. In electric field-coupled membrane bioreactors (EMBRs), electric fields upregulate genes involved in the synthesis of compatible solutes, such as betaine (*betA*) and glutamate (*gltB*), and transport genes (*proV* and *proW*) while downregulating genes involved in reducing membrane permeability (*phoQ* and *bmpA*) (Table 1) [98]. This regulation helps microorganisms cope better with osmotic stress and improves treatment efficiency. Electrical stimulation offers a multifaceted approach to addressing the challenges posed by high salinity. Therefore, future research should focus on elucidating the underlying enhanced mechanisms and exploring the scalability of this technology in practical engineering applications.

4.3.2. Magnetic intervention

Static magnetic field (SMF) technology enhances microbial growth and enzymatic activity without requiring additional energy inputs (Fig. 6) [100,101]. A 50-mT SMF boosts EPS secretion by microbial communities and enriches halotolerant bacteria (e.g., *Xanthomarina*, *Thauera*, *Pseudofulvimonas*, *Azoarcus*), thus improving the efficiency of aerobic granular sludge in saline wastewater treatment and resulting in COD and TN removal rates of 100% and 72.9%, respectively (Table 1) [102]. Furthermore, a 95-mT SMF enhances the decolorization ability of *Candida tropicalis* SYF-1 toward Acid Red B by increasing the halotolerance of yeast SYF-1 through the regulation of cell membrane components and significant upregulation of cell-wall-related genes (e.g., chitin synthase) [103]. Similarly, a 206-mT SMF enhances the halotolerance of *Pichia occidentalis* A2 by promoting glycerol synthesis and accumulation while regulating cell-wall composition [104]. In anaerobic digestion reactors, adding magnetite improves the treatment of high-salt food waste primarily through its impact on quorum sensing. Magnetite, along with K^+, aids in microbial resistance to hypersaline conditions by regulating membrane transport proteins and enhancing biofilm formation [105]. Additionally, iron oxide magnetic nanoparticles (IOMNPs) can absorb bile acids that inhibit the growth of halophilic bacteria in the culture medium (Table 1), thereby promoting the proliferation of halophilic bacteria (*Halomonas salina*, *Halorubrum halophilum*, and *Halobacillus* sp.) [106]. In summary, integrating magnetic energy enhances bacterial halotolerance through modulation of cell-membrane composition and compatible solute synthesis, representing promising low-carbon technology for HSOW treatment.

4.3.3. Radiation and ultraviolet intervention

Radiation treatment has enhanced microbial halotolerance by inducing mutations in their genomes, producing mutants with improved salt resistance. Radiation affects microbial genomes directly and activates specific gene repair and tolerance mechanisms [107]. This process can increase the synthesis of osmoregulatory substances in response to hypersaline environments. For example, the gamma irradiation of *Halomonas* sp. YJPS3-3 results in a mutant with increased halotolerance, rising from 15% NaCl to 20% NaCl (Table 1). Additionally, a mutant named halo6 shows a significant 11% increase in polyhydroxyalkanoate (PHA) production [108]. PHA acts as a carbon storage compound that strengthens a cell's resistance to high osmotic pressure.

Exposure to controlled ultraviolet (UV) light can induce oxidative stress responses, leading to metabolic changes that enhance cellular adaptability to hypersaline environments [109]. *Lactococcus lactis* subsp., exposed to a 254-nm UV radiation for 0.5 h, produces heat-shock proteins such as GroEL and GroES, whose cross-protective effects improve its ability to resist high osmotic pressure [110]. Additionally, a mutant strain, UV-1, is obtained through the UV mutagenesis of the moderately halophilic bacterium *Halomonas* sp. TTW4 [111]. The highest intracellular betaine content in strain UV-1 reaches 190 μg mg^{-1} compared to 110–150 μg mg^{-1} in the salt-sensitive mutant strain, indicating its significantly

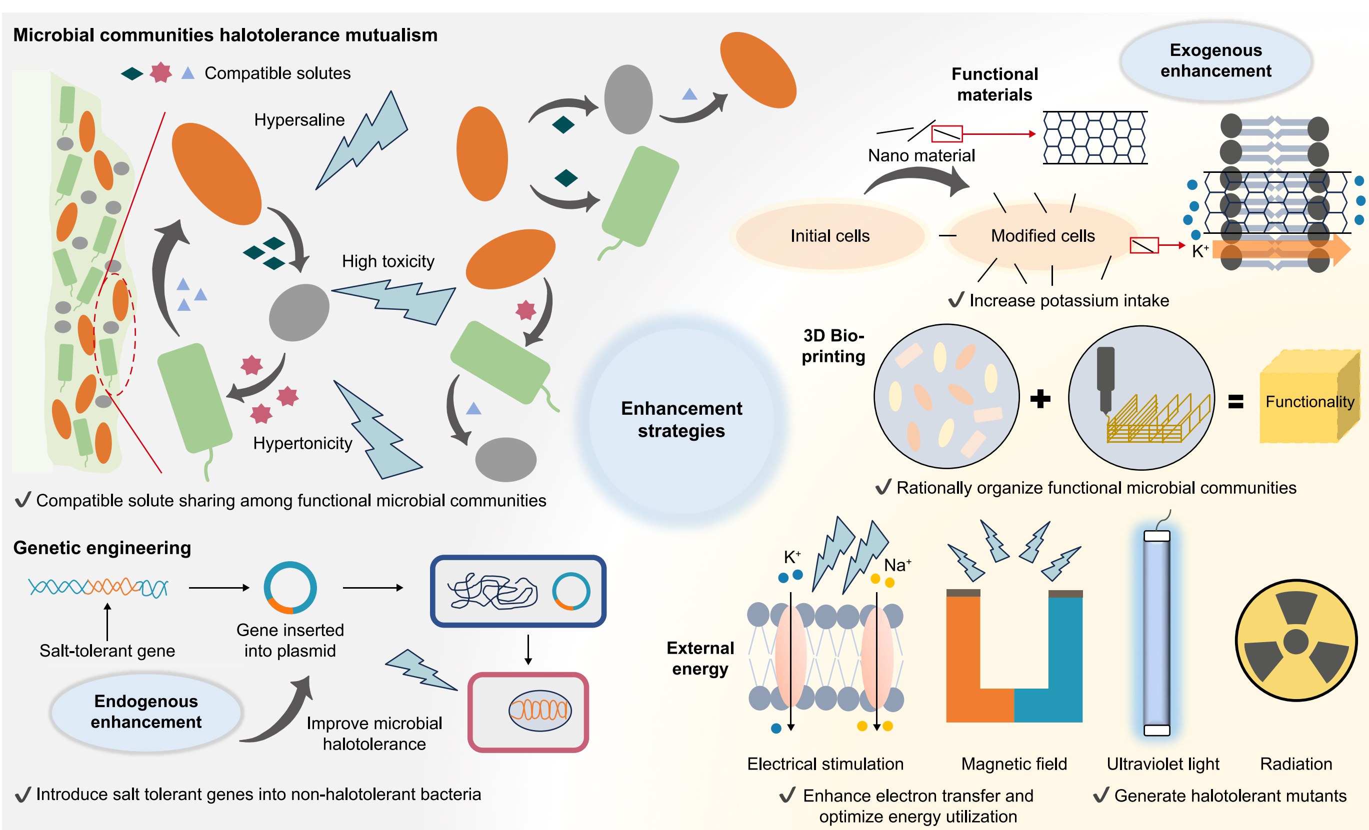

Fig. 6. Strategies for enhancing microbial halotolerance. Endogenous strategies focus on enhancing halotolerance through interbacterial mutualism and genetic engineering of microbial communities, whereas exogenous strategies employ functional materials, 3D bioprinting, and external energy interventions (e.g., low-voltage stimulation and static magnetic fields).

increased halotolerance (Table 1).

Despite the potential of radiation and UV exposure to enhance microbial halotolerance, several limitations remain. High doses can cause excessive and nonselective DNA damage, leading to cell death and decreased microbial viability. Moreover, these treatments' effectiveness varies widely among microbial species, and whether the induced mutations will have long-term stability remains uncertain. Future research should focus on optimizing the dosage and duration of radiation and UV treatments to balance the enhancement of halotolerance with the preservation of microbial viability. Combining these treatments with genetic engineering methods may result in synergistic effects, maximizing the overall efficiency and applicability of microbial halotolerance strategies (Fig. 6).

The strengths, weaknesses, opportunities, and threats (SWOT) associated with both endogenous and exogenous strategies for enhancing microbial halotolerance have been evaluated. The strengths of endogenous methods include leveraging natural stress-response mechanisms, such as the synthesis of compatible solutes and upregulation of stress-related genes [59], ensuring adaptability and ecological safety. Exogenous methods, such as genetic engineering, 3D bioprinting, and supplementation with conductive materials, offer rapid and targeted enhancements to microbial performance. The weaknesses of endogenous strategies lie in their slower adaptation and limited scalability, while exogenous methods may introduce unintended genetic changes or ecological risks. Opportunities include the integration of endogenous and exogenous approaches to synergistically optimize microbial halotolerance and exploring novel materials or technologies to improve process efficiency. Threats involve regulatory barriers, potential horizontal gene transfer risks (e.g., acquiring antibiotic resistance genes [ARGs] by opportunistic pathogens after salt treatment), and public concerns about environmental safety, which could hinder practical implementation. Therefore, a balanced application of both endogenous and exogenous strategies is essential for effectively boosting microbial halotolerance and ensuring its sustainable use in HSOW treatment.

5. Physicochemical–biochemical coupling technologies improve ZLD of HSOW

5.1. Critical role of microbial detoxification and microbial desalination in coupling treatment processes

Microbial detoxification and desalination are pivotal in achieving ZLD in the physicochemical–biochemical coupling treatment of HSOW. The biochemical process mitigates wastewater toxicity by decomposing organic compounds and reducing salinity through the simultaneous removal of carbon, nitrogen, and sulfur [112,113] (Fig. 7). These processes create more favorable conditions for subsequent physicochemical treatments (e.g., AOPs, evaporation crystallization). Microbial detoxification, achieved through the microbial decomposition of recalcitrant organics, is essential for HSOW treatment [114]. Employing both endogenous and exogenous enhancement strategies helps maintain microbial stability under hypersaline stress. By optimizing functional communities, complex organics are degraded into smaller, more manageable molecules, thereby improving the efficiency of biological detoxification and reducing the energy and chemical inputs required for subsequent physicochemical processes [115]. For example, preliminary microbial detoxification significantly reduces the toxicity of organic matter in AOPs, decreases the required oxidant dosage, and increases the mineralization efficiency of low-concentration recalcitrant compounds [116]. Microbial detoxification also minimizes by-product formation, which lowers the risk of secondary pollution and improves the environmental compatibility of the treatment system [117]. The low-energy consumption and high efficiency of the microbial detoxification of HSOW are crucial for achieving low-carbon emission targets. When effectively integrated with physicochemical processes, microbial detoxification reduces resource consumption in wastewater treatment, thereby enhancing the system's economic viability and sustainability.

Salinity reduction is critical for subsequent processes in HSOW treatment. The biological treatment process can exploit sulfate and nitrate reduction processes to be coupled with the biodegradation of recalcitrant organics, which not only improves the removal efficiency of organic pollutants but also reduces the competition for electron acceptors within the system, leading to biological desalination [118]. This process decreases salinity by converting inorganic salts into other solid or gaseous precipitates [119,120]. Previous research has employed a mixed-culture, embedding microbial cell immobilization (EMCI) process to treat nitrate in brine wastes. This method exhibits minimal impact even at salt concentrations as high as 2%, with a yield of approximately 0.36 g of suspended solids produced per gram of nitrate-N removed [88]. Additionally, this reduction in salinity alleviates the negative effects of high salt concentrations on subsequent physicochemical treatments, such as membrane separation or evaporation crystallization, while improving the quality of salt separation and enabling resource recycling. For instance, elemental sulfur recovery from sulfate-containing wastewater reduces the generation of hazardous mixed salts and produces valuable chemicals [121]. By regulating microbial community functions and metabolic pathways, biological desalination effectively decreases inorganic salt concentrations in wastewater, reducing the energy consumption and costs associated with traditional physicochemical processes. This biological desalination process is often accompanied by the degradation and detoxification of organic compounds, a synergy that further enhances the overall efficiency of coupled treatment processes.

5.2. Optimizing physicochemical–biochemical coupling processes for enhanced treatment efficiency and ZLD in HSOW

The current processes of treating HSOW primarily consist of pretreatment and biological and physicochemical treatments. The effective integration of these processes is essential for achieving efficient treatment and ZLD of such wastewater. Among them, the biological treatment process plays a critical role in removing organic matter, yet its effectiveness is often constrained by the hypersaline environment, making bio-enhancement a necessary intervention. Introducing functional microbial agents with halotolerance can significantly improve treatment efficiency, particularly in hypersaline chemical mother liquor, where organic pollutants are relatively uniform [122]. However, the complex composition of industrial wastewater often makes the sole use of biochemical treatment insufficient for effective processing under hypersaline conditions. This situation has led to the urgent need to combine biochemical enhancement with physicochemical technologies to discharge such wastewater in compliance with regulatory standards. Upgrading biological treatment processes involves combining multiple functional strains and leveraging their metabolic interactions to enhance survival and pollutant degradation efficiency under hypersaline stress; this constitutes a key area of research [18]. The high toxicity and recalcitrance of organic pollutants in hypersaline pharmaceutical and chemical wastewater complicate the synergistic transformation of multiple pollutants during biological treatment. Developing a highly salt-tolerant

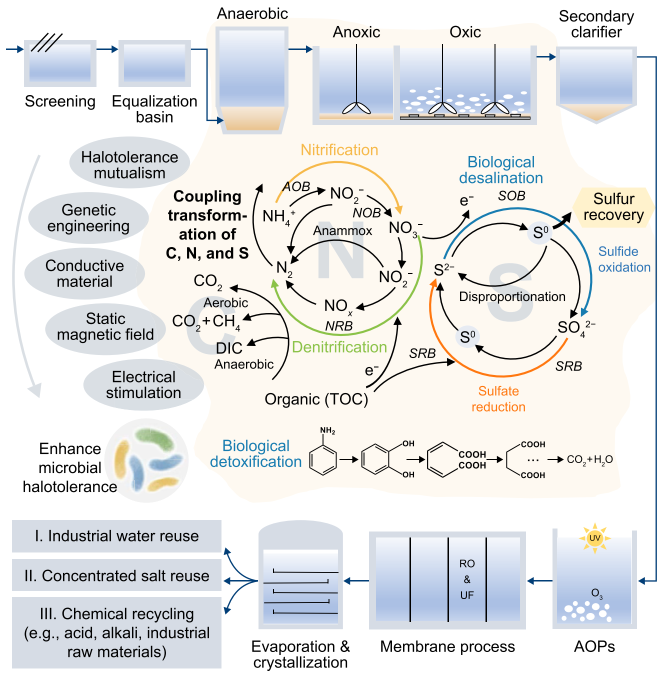

Fig. 7. The application of physicochemical and biochemical coupling technologies to treat hypersaline organic wastewater and achieve zero liquid discharge goals. Biological detoxification is achieved by degrading and transforming refractory organics. Exogenous enhancement methods (e.g., electrical stimulation and the use of conductive materials) improve microbial halotolerance and electron transfer. These methods facilitate the coupling of sulfate and nitrate reduction, which aids in the degradation of recalcitrant organics; promotes the simultaneous removal of carbon, nitrogen, and sulfur; and reduces the impact of hazardous mixed-salt waste in subsequent physicochemical processes. AOPs: advanced oxidation processes; TOC: total organic carbon; DIC: dissolved inorganic carbon; AOB: ammonia-oxidizing bacteria; NOB: nitrite-oxidizing bacteria; NRB: nitrate-reducing bacteria; SOB: sulfur-oxidizing bacteria; SRB: sulfate-reducing bacteria; RO: reverse osmosis; UF: ultrafiltration.

microbial community capable of targeting various pollutants represents a significant technological breakthrough. It is essential to explore microbial resources with high salt and toxin tolerance, analyze their adaptation mechanisms in high-inhibition environments, investigate the correlation between pollutant degradation, on one hand, and transformation efficiency, enzyme activity, and stability under high stress, on the other hand, and uncover the mechanisms for long-term maintenance of microbial functions. These steps are key to constructing effective halotolerant microbial agents. Furthermore, employing functional materials to support microbial colonization and external energy interventions such as electrical stimulation, SMF, radiation, and UV can significantly boost microbial halotolerance and degradation efficiency [102,123]. The effective integration of microbial detoxification and microbial desalination can substantially reduce the toxicity and salinity of HSOW, thereby optimizing the operational conditions of subsequent physicochemical processes.

To further enhance the efficiency of HSOW treatment and achieve ZLD, the integration of AOPs and membrane separation technologies is crucial. AOPs, such as ozonation and photocatalytic oxidation, can be employed to treat residual recalcitrant organic pollutants following biological treatment [124,125]. Coupled with efficient adsorption materials and membrane technologies, such as ultrafiltration (UF) and reverse osmosis (RO), these processes can further remove dissolved organic matter and the remaining concentrated salts [1,126]. After removing organic matter, HSOW is typically treated using methods such as RO and evaporative crystallization to reduce salinity prior to discharge or reuse. The treated wastewater can either be safely discharged into the environment in compliance with regulatory standards or reused for industrial applications, such as cooling processes. Biological treatment effectively reduces the load on subsequent physicochemical processes, allowing AOPs, membrane separation, and evaporation–crystallization technologies to operate more efficiently. Ultimately, this optimized process combination enables the efficient reuse of industrial wastewater and salts and the recovery of valuable chemicals (Fig. 7). Leveraging coupled physicochemical–biochemical coupled technologies has significantly advanced HSOW treatment. This integrated approach reduces energy and chemical consumption and establishes a robust foundation for developing sustainable, low-carbon wastewater treatment models to achieve ZLD.

To quantify the standards for coupled physicochemical–biochemical treatment of HSOW, key performance indicators (KPIs) should be established, focusing on treatment efficiency, energy consumption, and operational costs. Treatment efficiency can be assessed by monitoring the removal of key pollutants, such as COD, total dissolved solids (TDSs), and specific toxic organic compounds, achieving a target removal rate (e.g., ≥90% for COD, ≥85% for TDSs) [10]. Energy consumption should be quantified in terms of specific energy demand, which is the energy required per unit of pollutant removed, targeting the minimum energy input while maintaining high treatment performance. Operational costs can be evaluated by considering the capital expenditures, maintenance expenses, and chemical consumption, aiming for a cost-effective solution that balances performance with economic feasibility. The efficiency of achieving near ZLD should also be quantified by the volume of residual liquid waste generated, with a target reduction of liquid discharge below 5% of the influent volume [117]. Integrating these KPIs would guide optimizing the coupled treatment process, ensuring high efficiency, low energy consumption, and economic sustainability in HSOW treatment.

6. Conclusion and future perspectives

The effective treatment of HSOW presents a formidable challenge for the water treatment industry due to its complex chemical composition and elevated salt concentrations. Inadequate treatment can severely impact aquatic ecosystems, resulting in decreased biodiversity and reduced efficiency in wastewater treatment plants (WWTPs) [127,128]. The application of bio-enhancement technologies in HSOW treatment shows significant promise, and the integration of halotolerant and halophilic bacteria into bioreactors has been widely validated [129,130]. Simultaneously, improving the halotolerance of functional strains through endogenous and exogenous enhancement strategies has emerged as an effective and low-carbon approach to HSOW treatment. The physicochemical–biochemical coupling treatment of HSOW has arisen as a crucial strategy for enhancing treatment efficiency, enabling resource recovery, and ultimately achieving the ZLD goals. The optimized physicochemical technologies (e.g., improved membrane antifouling performance) and biochemical treatments (e.g., increased microbial metabolic activity) complement each other and collectively improve overall HSOW treatment efficiency. Integrating advanced physicochemical and biochemical treatment technologies can also constitute an effective method of achieving resource recovery from wastewater. In hypersaline pharmaceutical wastewater, many toxic pollutants (e.g., antibiotics) possess significant resource recovery potential [131]. Developing innovative technologies that harness this potential for toxicity reduction represents a crucial strategy for carbon emissions minimizations. Additionally, traditional biological processes of treating hypersaline

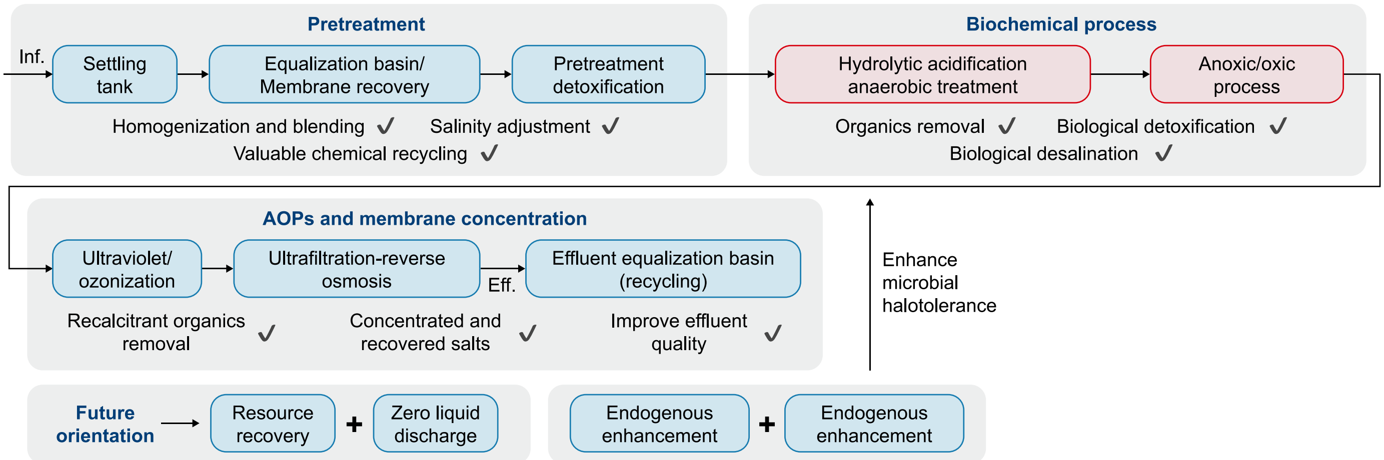

Fig. 8. Process flow and future development strategies for hypersaline organic wastewater treatment. Membrane technology is integrated into the pretreatment process to enable the preliminary recovery of valuable chemicals (e.g., antibiotics). This biochemical process prioritizes biological detoxification and biological desalination, thereby minimizing the impact of organic matter and salinity on subsequent processes. Physicochemical processes (e.g., advanced oxidation processes and membrane concentration) further enhance the removal of recalcitrant organic compounds and salts, while resource recovery is optimized to achieve zero liquid discharge. Inf.: Influent; Eff.: Effluent; AOPs: advanced oxidation processes.

pharmaceutical wastewater primarily target removing carbon and nitrogen pollutants, exhibiting limited salt reduction efficiency [132,133]. Given enterprises' growing emphasis on achieving ZLD for industrial wastewater, advancing biological desulfurization (sulfate reduction) and deep denitrification (nitrate removal) technologies is crucial. For instance, halophilic archaea such as *Haloarcula*, *Halolalin*, and *Halobacterium* play a dominant role in reducing nitrite through denitrification [134]. *Haloferax mediterranei* has been identified as a model denitrifier in high-salinity wastewater [135]. *Halodesulfurarchaeum formicicum* utilizes formate or hydrogen as an electron donor, with elemental sulfur or thiosulfate serving as an electron acceptor in the process [136]. These microorganisms can significantly alleviate the burden on subsequent salt separation stages, minimize the generation of hazardous waste with impurities, and reduce carbon emissions and the overall carbon footprint.

Future research on bio-enhancement technology for HSOW treatment should prioritize some key areas. Optimizing microbial metabolic pathways and physiological characteristics through gene editing and metabolic engineering is crucial for developing salt-tolerant strains capable of efficiently degrading specific pollutants in HSOW. Utilizing synthetic biology techniques to selectively enhance functional microbial communities, improve the decomposition of recalcitrant organics, and boost the biological treatment efficiency of specific pollutants in water is critical for increasing biological performance in hypersaline environments [137]. Developing specialized 3D printing bio-inks for treating hypersaline wastewater and constructing microbial communities with high salt tolerance and effective degradation capabilities are also necessary. Maximizing the synergistic effects of multiple technologies and creating integrated treatment systems are vital steps in this process. Integrating the Internet of Things (IoT) and big data technologies can enable precise control and optimization of HSOW treatment processes. Combining physiochemical enhancements with biochemical processes represents a significant advancement toward achieving cost-effective efficiency improvements [138]. To further enhance the efficacy of biochemical treatment processes, it is imperative to implement targeted separation and recovery of characteristic components from HSOW in chemical industrial parks [139]. Developing specific detoxification pretreatment technologies will improve the biological stress resistance and overall performance of these treatment processes. Scaling up microbial-enhanced technologies for HSOW treatment requires validating microbial robustness under diverse industrial conditions, optimizing large-scale system parameters, and integrating these approaches with existing infrastructure. Economic feasibility, regulatory compliance, and scalability should also be assessed to ensure global market adoption and sustainable, low-carbon wastewater management. While current research has often focused on reducing carbon emissions, achieving ZLD or even negative carbon emissions through energy recovery should be the goal of effective HSOW treatment (Fig. 8). Negative carbon emissions can be achieved through innovative recycling technologies that convert organic matter into energy, thereby offsetting emissions and promoting sustainable development. Ultimately, a replicable and economically viable low-carbon technology system for treating HSOW in industrial parks will be built in the near future. Finally, evaluating the environmental and ecological risks associated with various technological approaches, including the potential transmission of ARGs and alterations in microbial communities affecting ecosystems, is essential for ensuring human and ecological safety [140]. In summary, as technological advancements continue and research progresses, bio-enhancement technology will perform an increasingly crucial role in HSOW treatment, significantly contributing to environmental protection and sustainable development.

CRediT authorship contribution statement

Yan-Qing Zhang: Writing - Original Draft, Methodology, Conceptualization, Investigation, Visualization. **Jing-Long Han:** Supervision, Resources, Writing - Review & Editing. **Hao-Yi Cheng:** Methodology, Writing - Review & Editing. **Hong-Cheng Wang:** Methodology, Writing - Review & Editing. **Tie-Jun Liu:** Writing - Review & Editing, Methodology. **Bin Liang:** Writing - Review & Editing, Supervision, Resources, Funding Acquisition, Methodology. **Ai-Jie Wang:** Writing - Review & Editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

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