



## Shewanella mixed culture system and its application progress

Jing Zhu<sup>1</sup>, Yaowen Li<sup>2</sup>, Danni Ding<sup>1</sup>, Guowei Zhang<sup>1</sup>, Danya Mei<sup>1</sup>, Jie Li<sup>1</sup>, Xinyu Wang<sup>1</sup> and Qian Li<sup>1,\*</sup>

<sup>1</sup> School of Environmental and Safety Engineering, Jiangsu University, Zhenjiang 212013, Jiangsu, China

<sup>2</sup> Shanghai Tongqu Engineering Consulting Co., Ltd., Shanghai 200082, Shanghai, China

**SUMMARY:** *In this paper, the advantages of the Shewanella mixed culture system in terms of carbon source utilization range, pollutant degradation performance, and bioelectricity generation performance were reviewed. The impacts of environmental conditions and strain types on the Shewanella mixed culture system were systematically analyzed, the mechanism of action of the Shewanella mixed bacterial system was comprehensively summarized, and the research progress in its applications such as environmental pollution remediation and bioelectricity generation was thoroughly presented. The aim is to provide support for further systematic research and practical application of the Shewanella mixed culture system.*

**KEYWORDS:** *Shewanellas; Mixed culture system; Carbon source utilization; Biological electricity generation; Environmental pollution remediation*

## 1 Introduction

Shewanella is a typical electrogenic bacterium with a broad growth temperature range and strong tolerance to pH levels [1]. It produces fewer toxins during its metabolic processes. Notably, in anaerobic environments, Shewanella can utilize various electron acceptors, including dyes, thiosulfate, dimethyl sulfoxide, metal ions (such as Fe<sup>3+</sup>, Mn<sup>4+</sup>), and electrodes, for respiration [2]. Shewanella has broad application prospects in the field of environmental pollution bioremediation and biomass power generation. However, its disadvantage is that it cannot utilize large organic carbon sources such as cellulose, lignin, and starch [3]. The metabolic process mainly relies on small organic carbon sources such as lactic acid, pyruvic acid, and amino acids as electron donors. Lactic acid has been proven to be its optimal carbon source [4], but high priced carbon sources significantly increase the actual application cost of Shewanella. Additionally, while Shewanella has broad-spectrum degradation capabilities for pollutants, it performs poorly on some hard-to-degrade pollutants, such as Sudan red and Congo red, which somewhat diminishes its effectiveness in environmental pollution control. Therefore, research is underway to transition from pure Shewanella cultures to mixed cultures. By constructing mixed bacterial systems, Shewanella can be co-cultured with other strains under the same environmental conditions [5]. This interdependence and mutual constraints among Shewanella and other bacterial species may lead to complex relationships, such as mutual benefit, competition, and antagonism [6, 7]. If the metabolic pathways and functions of different strains complement and synergize, exhibiting co-metabolic effects, then a mixed microbial system can significantly enhance performance [8]

\*liq1232025@163.com

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without the need for complex DNA in vitro recombination, thus saving time and reducing costs. Moreover, by using co-cultured strains (such as fungi) to break down large organic molecules in agricultural waste, *Shewanella* can utilize the small organic molecules produced as a carbon source for its metabolic activities, eliminating the need for externally added small organic carbon sources. This broadens its carbon source utilization range and reduces the cost of carbon sources, undoubtedly opening up broader prospects for *Shewanella* biotechnology applications.

Therefore, this paper reviews the research results of *Shewanella* mixed bacterial system from four aspects: the mechanism of action, advantages, construction and application progress in the fields of environmental pollution remediation and bioelectricity generation, in order to provide a reference for the in-depth systematic research and practical engineering application of *Shewanella* mixed bacterial system.

## 2 The mechanism of action of the Shiva mixed bacterial system

The mechanism of action of the Shivei mixed microbial system is highly complex, involving the formation of ecological relationships between microbial species, such as mutualism, cross-feeding, and metabolic product interactions (including symbiosis, mutualism, antagonism, and competition), as well as mechanisms of information exchange such as quorum sensing and metabolite-mediated communication. Additionally, it includes spatial structures and ecological niche allocation mechanisms, such as spatial stratification and competition (see Figure 1).

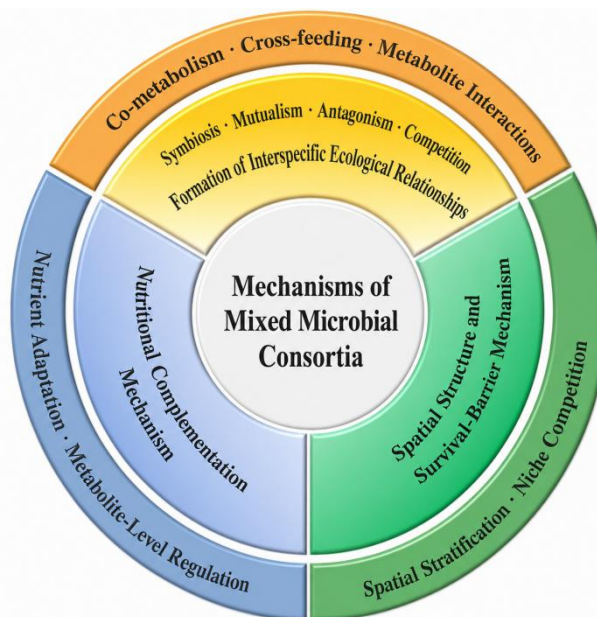


Figure 1: Mechanism of action of Shivas mixed bacterial system

### 2.1 Mechanism of inter-plant ecological relationship formation

There are complex and diverse ecological interactions between microbial communities. In addition to fierce competition for nutrient substrates, coexisting microbial communities can also regulate their survival status and metabolic behavior by secreting extracellular metabolites for interspecies interactions. Strains can construct differentiated ecological relationships such as symbiosis, competition, and antagonism through cross feeding, co metabolism, and metabolite interactions [9], which can not only regulate the synthesis efficiency of target metabolites, but

also induce the synthesis of new secondary metabolites.

Currently, the mechanisms for forming mutualistic symbiotic relationships are primarily attributed to cross-feeding and co-metabolism. In the cross-feeding mechanism, microorganisms form a 'metabolic relay' network through division of labor, collectively degrading complex substrates. Primary decomposers break down large molecules into intermediate products, which are then gradually transformed into simpler compounds by secondary converters. The final metabolic steps are completed by terminal microorganisms. For example, lignin-degrading bacteria convert lignin into intermediate metabolites, which serve as a carbon source for *Shewanella*. In turn, *Shewanella* improves the environment through its metabolic activities, forming a mutually beneficial cycle [10]. In the four-bacteria system (cyanobacteria-lactobacillus-shewanella-geobacter), cyanobacteria use light energy to synthesize sucrose from carbon dioxide, lactobacillus converts sucrose into lactic acid, shewanella and geobacter relay oxidize lactic acid to CO<sub>2</sub>, and generate electricity through extracellular electron transfer, forming a complete 'metabolic relay' network [11]. The co-metabolism mechanism involves microbial communities working together to complete complex substrate degradation processes that individual species cannot accomplish independently. For example, in the co-cultivation system of *Gloeophyllum trabeum* and *S. oneidensis* MR-1, *G. trabeum* breaks down starch into ethanol, providing a usable carbon source for *S. oneidensis* MR-1, which uses ethanol as both energy and a carbon source to degrade dyes [12]. This forms a tight metabolic coupling relationship between the two microorganisms, overcoming the metabolic limitation of a single species being unable to simultaneously perform starch degradation and dye degradation. This co-metabolic mode not only realizes the efficient utilization of complex substrates, but also expands the ecological function of microbial communities, showing important application value in environmental pollution control and other fields.

The competitive mechanism between microorganisms usually involves three aspects: nutritional competition, spatial competition, and metabolite mediated antagonism. *Shewanella oneidensis* MR-1 and *Citrobacter freundii* An1 both use iron oxide as an electron acceptor, and there is obvious nutritional competition between the two. *Shewanella* MR-1 has a stronger competitive advantage and can increase the power generation of mixed microbial communities to six times that of a single strain inoculation system [13]. *Shewanella* can secrete iron carriers, which chelate iron ions in the environment to block the iron dependent metabolic pathway of pathogenic *Vibrio* in shrimp. At the spatial competition level, *Pseudomonas fluorescens* and *Streptococcus* Baltic can form a symbiotic relationship in the early stage of biofilm formation. *Pseudomonas fluorescens* dominates the biofilm microenvironment by regulating biomass accumulation and polysaccharide synthesis [14]. In the antagonistic effect mediated by metabolites, *Bacillus subtilis* BS08 can produce surfactants that can specifically damage the cell membrane integrity of *Bacillus subtilis* and yellow croaker spoilage bacteria. Among them, the 24-hour growth inhibition rate of *Bacillus cereus* can reach 67.2% [15].

## 2.2 Information exchange mechanism

In the *Shewanella* mixed microbial system, communication between microorganisms is generally believed to be mainly achieved through quorum sensing and metabolite mediated signaling mechanisms, which can effectively coordinate the metabolic activities and physiological functions of microorganisms. *Shewanella* can synthesize and sense specific signaling molecules such as AI-2 and DKPs, which can dynamically regulate group behavior. For example, *Shewanella* from the Baltic Sea can promote the formation of biofilms and the expression of decay related genes in rotifers through highly active AI-2 and DKPs signaling

molecules [16]. In marine ecosystems, *Shewanella* regulates the composition and structure of biofilms through quorum sensing, which in turn affects the attachment and metamorphosis processes of thick shelled mussel larvae [17]. Metabolic signal transduction involves *Shewanella* using metabolites as information molecules to regulate the physiological activities of the population. For example, *S. oneidensis* MR-1 can regulate the synthesis of ATP and NADH through a cytochrome mediated electron transfer chain, thereby affecting the metabolic pathway of CO<sub>2</sub> reduction to formic acid. The expression changes of key genes (such as *mtr* B/C and *fdhA1*) can increase formic acid production by 5.59 times compared to wild-type strains [18].

### 2.3 Spatial structure and ecological niche allocation

In mixed microbial communities, *Shewanella* exhibits unique spatial organization patterns and niche allocation strategies: (1) in terms of spatial organization. *Shewanella* can form a specific biofilm layered structure, in which the outer layer cells of the biofilm can dominate metal reduction and various redox reactions through their unique cytochrome network; The inner layer cells focus on basic metabolic activities, ensuring the stability and integrity of the biofilm structure [19]. Normally, *Shewanella* occupies the outer layer of the biofilm and dominates the extracellular electron transfer (EET) process, while other microorganisms in the community maintain the stability of the inner ecological niche of the biofilm through metabolic interactions. (2) In terms of ecological niche allocation. *Shewanella* occupies a dominant position through various competitive strategies. In the microbial fuel cell system co cultivated with *Shewanella oneidensis* MR-1 and *Citrobacter freundii*, *Shewanella oneidensis* MR-1 has an advantage in niche competition due to its stronger lactate utilization ability, metabolic activity, and biofilm formation ability. In the fish preservation system, *Shewanella* can quickly colonize on the surface of fish and dominate the competition with microorganisms such as *Pseudomonas* due to its excellent surface adhesion ability and environmental adaptability [20]. Future research can further explore the interaction mechanisms between *Shewanella* and different microorganisms to optimize its application in bioremediation, energy conversion, and food preservation.

## 3 Advantages of the 2 Shivas mixed bacterial system

Studies have shown that by constructing a mixed bacterial system of *Shewanella* and other bacteria, it can not only broaden the range of carbon source utilization, so that *Shewanella* can use macromolecular organic carbon sources, but also improve the performance of pollutant degradation and bioelectricity production.

### 3.1 Expand the range of carbon source utilization

In pure culture conditions, *Shewanella* cannot utilize large organic molecules such as glycerol, corn stalks, and starch as carbon sources for metabolism. However, the mixed microbial system of *Shewanella* and *Klebsiella pneumoniae* J2B can not only produce electricity from glycerol [21-23] but also generate electricity successfully using corn stalks as a carbon source for microbial fuel cells [24-26]. The mixed microbial system of *Shewanella* and *Bacillus licheniformis* can produce electricity biologically using xylan as a carbon source for microbial fuel cells [27]. Additionally, in the mixed microbial system of *Trichoderma reesei* and *Shewanella*, *Shewanella* can efficiently degrade methyl orange dye using starch as a carbon source [28, 29]. Therefore, when *Shewanella* is mixed with other strains, it not only eliminates

its dependence on small organic carbon sources but also significantly reduces the cost of carbon sources for its applications.

In a mixed microbial system, *Shewanella* can use large molecules like glycerol, corn stalks, and starch as carbon sources for pollutant degradation and bioelectricity production because the co-cultured strains can break down these large molecules into smaller [30-32]-molecules such as lactic acid and pyruvic acid, which serve as carbon sources for *Shewanella*'s pollutant degradation and bioelectricity production. For example, Li et al. [33] studied the carbon source utilization mechanism in a mixed microbial system composed of *Shewanella oneidensis* and *K. pneumoniae*. They found that in the *K. pneumoniae* mixed microbial system, where the ethanol dehydrogenase gene and phosphotransacetylase gene were knocked out, *Shewanella*'s bioelectricity production efficiency was significantly improved. This is because the knockout of these genes blocked *K. pneumoniae*'s ethanol and acetic acid production pathways, accelerating the decomposition of corn stalks into lactic acid, which provided more lactic acid carbon sources for *Shewanella*, thus enhancing its bioelectricity production efficiency. This also indicates that the carbon source preference of *Shewanella* in the mixed microbial system remains unchanged [34]. To enhance the performance of the *Shewanella* in mixed microbial systems, it is essential to improve the ability of co-cultured strains to convert large molecular organic carbon sources (such as straw) into smaller molecular carbon sources. This requires a deep understanding of the decomposition pathways and mechanisms of large molecular organic matter within these systems. However, there is currently limited research in this area, and the methods used are primarily focused on product detection. Further, a more comprehensive approach is needed to study the biological transformation mechanisms of large molecular organic matter in mixed microbial systems, to better facilitate the practical application of this technology [35].

### 3.2 Improve the degradation performance of pollutants

The *Shewanella* mixed bacterial system not only efficiently utilizes macromolecular organic matter as a carbon source, but also demonstrates excellent performance in the field of pollutant degradation. For example, Li et al. co cultured *Shewanella oneidensis* FJAT-2478 with *Lactobacillus plantarum* FJAT-7926, significantly improving the decolorization efficiency of methyl orange and greatly enhancing the degradation efficiency of pollutants. Liu et al. [36] developed a mixed bacterial system of *S. putrefaciens* CN32 and *B. circulans* BWL1061, which significantly increased the decolorization rate of Sudan red I from less than 30.0% in pure *S. putrefaciens* CN32 to 90.2%. Wang et al. [37] found that pure *S. oneidensis* cannot degrade Congo red, whereas the mixed bacterial system of *S. oneidensis* and *Pseudomonas putida* can successfully degrade Congo red within 72 hours. In addition to its high efficiency in dye degradation, the *Shewanella* mixed bacterial system also excels in nitrogen removal. Research by He et al. [38] found that when *S. oneidensis* MR-1 is co-cultured with denitrifying microorganisms, the total nitrogen removal rate increases from 56.7% to 74.7%, and nitrite levels decrease significantly from 9.90mg/L to 0.02mg/L. Additionally, Cholnam et al. demonstrated a 1.7-fold increase in the degradation rate of 2,4-dichloronitrobenzene by constructing a mixed microbial community of *Shewanella* and microorganisms derived from activated sludge, further highlighting the significant advantages of the *Shewanella* mixed microbial system in pollutant degradation.

In the *Shewanella* mixed bacterial system, the enhanced degradation performance of *Shewanella* pollutants is primarily attributed to the synergistic effects between the mixed strains. This synergy not only promotes the growth of the strains but also enhances the activity of functional enzymes. For instance, in the mixed bacterial system of putrefactive *Shewanella* and

*Bacillus circulans*, the two strains work together to degrade azo dyes. *Bacillus subtilis* can efficiently degrade azo dyes through its own synthesis of azo reductase, NADH-DCIP reductase, and laccase; *Shewanella* can act as an electron acceptor and participate in the degradation process of azo dyes through non-specific bioreduction. These two different degradation pathways form a synergistic effect, significantly improving the degradation efficiency of pollutants. However, the degradation mechanism of this system is complex and has not been fully elucidated yet. Further systematic research is needed to clarify its molecular mechanism and regulatory rules.

### 3.3 Improve the bioelectricity production performance

In microbial fuel cell (MFC) systems using pure cultures of *Shewanella*, the mixed *Shewanella* system demonstrated enhanced power generation and a shorter power generation time. For instance, in the mixed *Shewanella* and *B. licheniformis* system, the power generation capability increased from 530.42 mW/m<sup>3</sup> in pure culture to 1.29×10<sup>4</sup> mW/m<sup>3</sup>[39]. In the mixed *Shewanella* and *Clostridium beijerinckii* system, the power generation increased from 48 mW/m<sup>3</sup> in pure culture to 87 mW/m<sup>3</sup>[40]. The current generation time in the mixed *Shewanella* and *Escherichia coli* MFC system was reduced by 3 hours compared to the pure culture MFC system, indicating the potential of mixed systems to shorten the power generation cycle. In the mixed *Shewanella* and *Saccharomyces cerevisiae* [41], *Citrobacter freundii* An1[42], or *P. putida* [43] MFC systems, the power generation capabilities were 1.7 times, 6 times, and 3 times those of the pure culture systems, respectively, further highlighting the advantages of mixed systems in improving power generation performance.

The improvement of *Shewanella*'s power generation performance in mixed microbial systems is mainly due to the following two aspects: (1) at the metabolic activity level. The mixed microbial system significantly expands the substrate utilization range, providing *Shewanella* with a directly utilizable carbon source and energy, thereby promoting electron generation and providing sufficient electron sources for power generation. (2) At the level of electronic transfer. The strains within the community secrete extracellular polymers, which can assist *Shewanella* in colonizing on the electrode surface and forming a stable biofilm, effectively reducing energy loss during electron transfer and shortening the power generation cycle. Although the mixed microbial system of *Shewanella* has shown significant advantages in electrogenic performance, further research is needed to understand the metabolic pathways and the specific mechanisms of biofilm formation within the mixed microbial system.

## 4 Key factors in the construction of Shiva's mixed bacterial system

The performance of the Shivali mixed bacterial system varies with the co-cultured bacterial strains, pH value, temperature, salinity and toxic substances. In order to construct a good Shivali mixed bacterial system, it is necessary to consider the key factors such as environmental factors and the selection of bacterial strains.

### 4.1 Selection of co-cultured strains

Generally, the properties of different strains vary significantly, making strain selection crucial when constructing a *Shewanella* mixed bacterial system. It is essential to select strains that are compatible and can grow together. Research has shown that *Shewanella* can form mixed bacterial systems with various anaerobic (facultative) bacteria, such as *Lactococcus lactis*

(lactic acid bacteria), *Lactobacillus plantarum* (plant lactic acid bacteria), *Escherichia coli* (enterobacteria), *Citrobacter freundii* (Freydson citrobacter), and *Klebsiella pneumoniae* (*Klebsiella pneumoniae*). These mixed bacterial systems, formed by *Shewanella* and these bacteria, can work synergistically in material exchange and electron transfer.

Further research has found that *Shewanella* can be co cultured with fungi. The fungi used in co culture, which have been reported in previous studies, mainly include brewing yeast and *Beauveria bassiana*, fully utilize the core advantages of fungi in the degradation of macromolecular organic compounds such as lignin and cellulose. They can degrade macromolecular organic compounds such as lignin and cellulose that *Shewanella* finds difficult to utilize into small molecule carbon sources for *Shewanella* to utilize. This not only broadens *Shewanella*'s carbon source utilization range, but also significantly improves its metabolic activity and overall functional performance.

Moreover, microalgae are also potential candidates for co-cultivation with *Shewanella* in the future. Microalgae and bacteria have maintained a long-term symbiotic relationship in nature. Although early studies suggested that bacteria were detrimental to microalgae cultivation, recent research has shown that they mutually enhance each other's growth. For instance, Zhang et al.'s experiments demonstrated that when *E. coli* co-cultured with *Aurantiochytrium* sp. SW1, this system not only promoted microalgae growth but also increased the production of their metabolic products. Xie et al. successfully constructed an algal-bacterial symbiotic wastewater treatment system that efficiently removed nitrogen and phosphorus from wastewater. Although there are few reports on the co-cultivation of *Shewanella* with microalgae, other bacterial-microalgae co-cultivations have yielded promising results, providing valuable insights for constructing *Shewanella*-microalgae co-cultivation systems. In particular, cyanobacteria, due to their unique photosynthetic mechanisms, can accumulate organic and inorganic substances. Bahar et al.'s research has shown that the monosaccharides, oligosaccharides, and amino acids produced from the decomposition of cyanobacteria can be anaerobically utilized by *S. oneidensis* MR-1, offering new ideas for constructing *Shewanella* mixed bacterial systems.

## 4.2 Optimization control of environmental condition factors

Environmental factors include pH value, temperature, salinity and toxic substances, among which the influence of pH value and temperature is the most significant. The research on the influence of environmental factors mainly focuses on these two aspects.

Temperature is a critical factor affecting microbial activity, as the growth rate, metabolic activities, and enzyme synthesis of microorganisms are all influenced by environmental temperature. Therefore, maintaining an appropriate temperature is essential for the growth and reproduction of microorganisms. Liu et al. found that at temperatures ranging from 10°C to 30°C, the decolorization rate of *Serratia marcescens* on active black 5 increases with rising temperature, reaching 87.6% at 30°C. However, when the temperature increased to 40 °C, the dye decolorization rate of *Serratia marcescens* decreased to 31.9%, indicating a significant impact of temperature on its dye degradation ability. Hu et al. constructed a mixed microbial system of *Shewanella oneidensis* MR-1 and *Gloeophyllum trabeum* (abbreviated as *G. trabeum*), which achieved a dye decolorization rate of over 97.0% at 30 °C, but significantly decreased to 36.0% when the temperature dropped to 15 °C. In addition, for mixed microbial systems, the importance of temperature optimization is more prominent. Different types of microorganisms have their own unique optimal growth temperatures, and the setting of temperature conditions should fully consider the physiological and metabolic characteristics of each strain in the system to ensure that all strains are in a suitable growth environment.

PH value, like temperature, is also a key environmental factor affecting microbial growth

and reproduction. For example, the study by Xiu *et al.* showed that when the environmental pH value is in the range of 5.5-7.0, the growth rate of *Proteus mirabilis* remains stable, while when the pH value increases to 7.0-8.0, the growth rate of the strain will sharply decrease. The study by Zhang *et al.* found that after co culturing *Proteus mirabilis* and *Clostridium acetobutylicum* for 16 hours in an environment with pH=5.0, the activity of *Proteus mirabilis* was completely lost and could not be restored. The core reason is that there is a significant difference in the pH adaptation range of the two strains, and *Clostridium acetobutylicum* secretes organic acids, causing the environmental pH to drop below 5.0, exceeding the suitable growth range of *Proteus mirabilis* and leading to its inactivation. Therefore, when constructing a mixed microbial system with *Proteus mirabilis* as the core, it is necessary to focus on the pH adaptation characteristics of different strains, ensure that the physiological metabolism of each strain is in the best state, and maintain the ecological balance between strains. In natural environments such as soil and water, pH value also significantly affects the form and toxicity intensity of pollutants. The content of exchangeable heavy metals such as Cd, Zn, Pb in soil environment is significantly negatively correlated with soil pH value.

Moreover, for high-salt wastewater, the impact of salinity on the *Shewanella* mixed microbial system cannot be overlooked. Different microorganisms have varying salt tolerance; some thrive in high-salt environments, while others perform better in low-salt conditions. For complex wastewater, it is also important to consider the toxic effects of harmful components.

To sum up, in the construction of Shiva's mixed bacterial system, it is necessary to comprehensively consider the physiological characteristics of different strains, the optimal growth conditions of different strains and the control of environmental salinity and toxic substances, so that different strains in the system can form a good relationship of mutual growth and symbiosis, rather than a competitive and antagonistic relationship.

## **5 Application progress of Shiva's mixed bacterial system**

The advantages of the Shivali mixed bacterial system are mainly reflected in carbon source utilization, pollutant degradation performance and bioelectricity production performance. Therefore, the current research on the application of the Shivali mixed bacterial system mainly focuses on environmental pollution remediation and bioelectricity production. The following is a summary of the research results on the application of the Shivali mixed bacterial system in these two aspects.

### **5.1 Application in environmental pollution remediation**

The application of the *Shewanella* mixed bacterial system in environmental pollution remediation primarily focuses on wastewater treatment. Current research has demonstrated that this system excels in treating dye wastewater, nitrogen-containing wastewater, and organic wastewater. For dye wastewater, such as Sudan Red I, the mixed bacterial system constructed by *S. oneidensis* MR-1 and *B. circulans* BWL1061 can achieve efficient degradation. Methyl Orange can be effectively degraded using the mixed bacterial system formed by *S. oneidensis* MR-1 and *L. plantarum*. Congo red can also be efficiently degraded through a mixed bacterial system constructed with odorless *Pseudomonas*. For example, when *Shewanella* MR-1 strain is co cultured with anaerobic microbial communities, the degradation efficiency of 2,4-dichloronitrobenzene can be significantly improved. In the field of nitrogen-containing wastewater treatment, He *et al.* optimized the electron transfer process within the system by introducing *Shewanella* into the denitrifying microbial community, thereby significantly improving nitrogen removal efficiency; Wu *et al.* constructed a mixed bacterial system of

denitrifying bacteria and *Shewanella oneidensis* MR-1, which significantly improved the denitrification rate of Cr (VI) - and nitrate containing wastewater systems by promoting electron production and transfer in denitrifying bacteria.

In addition, the *Shewanella* mixed bacterial system has significant potential for application in soil pollution remediation. It can effectively reduce heavy metal content in soil, improve soil physicochemical properties, and efficiently degrade various heavy metal pollutants such as iron, cadmium, and lead. For example, when high-order bacteria form a mixed microbial community with *Shewanella* and *Clostridium*, they can reduce heavy metal ions in the soil to  $S^{2-}$  through specific metabolic pathways, thereby forming stable sulfide precipitates and achieving efficient removal of such heavy metals, significantly reducing the degree of soil heavy metal pollution.

Although the *Shewanella* mixed bacterial system has made some progress in the field of environmental pollution remediation, its application scope is still relatively limited. Therefore, it is necessary to expand its application research scope, such as the degradation performance of anthraquinone compounds, antibiotics and environmental hormones in wastewater, and further increase the application research in the remediation of soil heavy metal pollution.

## 5.2 Application in the field of biological electricity generation

Currently, global energy shortages and the rapid increase in traditional energy consumption have made biopower generation technology a focal point. As a typical electricity producing microorganism, *Shewanella* bacteria have the core advantage of forming a stable biofilm on the surface of the electrode anode, which significantly improves the energy conversion efficiency of microbial fuel cells (MFCs). Currently, research on *Shewanella*'s hybrid cultivation system focuses on optimizing its power generation performance, which is manifested in better power generation performance and shorter power generation cycles. For example, when *Shewanella oneidensis* MR-1 was co cultured with *Clostridium perfringens*, *Pseudomonas putida*, and *Bacillus licheniformis*, the power generation performance of the constructed hybrid microbial fuel cell was significantly improved; When *Shewanella oneidensis* MR-1 is co cultured with *Clostridium beijerinckii* and *Klebsiella pneumoniae* J2B, it can achieve stable power generation for microbial fuel cells that cannot generate electricity when cultured alone; When co cultured with *Escherichia coli*, it can not only shorten the power generation cycle of microbial fuel cells, but also effectively improve their energy conversion efficiency.

The core advantage of the *Shewanella* mixed bacterial system in the field of bioelectric production is that it can significantly improve the power generation performance of microbial fuel cells (MFCs), while effectively reducing energy consumption and environmental pollution, which meets the development needs of low-carbon and environmental protection. This system can effectively promote the development of bioelectric production towards high efficiency, energy conservation, and environmental protection by optimizing the microbial community structure and enhancing electronic transfer efficiency. However, this technology still faces multiple technical challenges: (1) optimizing the combination ratio of bacterial strains to ensure synergistic effects between different strains; (2) Need to enhance the stability of biofilm and reduce issues such as biofilm detachment and degradation; (3) Efficient utilization of nutrients such as carbon sources in domestic sewage is necessary to achieve resource recycling, further reduce operating costs, and improve the economic and practical efficiency of the system.

## 6 Conclusion

The *Shewanella* mixed microbial system has shown great potential in environmental pollution remediation and biomass power generation, but still faces many urgent challenges: (1) the

interaction mechanism between microorganisms in the mixed system is extremely complex, and if not properly regulated, it can easily lead to a decrease in system stability, functional abnormalities, or even complete failure. (2) The construction of mixed microbial systems requires full consideration of the growth adaptability of different strains, and there are significant differences in the environmental adaptation range between *Shewanella* and other microorganisms such as cellulose degrading bacteria and fungi, which greatly increases the difficulty of optimizing cultivation parameters. (3) The co metabolism mechanism of recalcitrant pollutants has not been fully elucidated, which to some extent limits the application effectiveness of this system in complex pollution scenarios. (4) How to achieve large-scale and efficient cultivation of mixed microorganisms while maintaining long-term stable metabolic activity of the system remains the core bottleneck for the industrial scale promotion of this technology.

Despite the challenges mentioned above, the *Shewanella* mixed microbial system can efficiently decompose large molecular organic matter in agricultural waste by introducing cellulose degrading bacteria or fungi, converting it into small molecular carbon sources that can be utilized by *Shewanella*, effectively reducing the cost of carbon source usage. In the field of pollutant degradation, the synergistic effect of co metabolism in mixed microbial systems can effectively overcome the limitations of a single strain in the degradation of difficult to degrade dyes, significantly improving the decolorization efficiency and degradation thoroughness of azo dyes. In the field of biological power generation, mixed microbial systems can achieve the functional integration of power generating strains and substrate degrading strains, improve the electron transfer efficiency of microbial fuel cells (MFCs), and enhance the utilization rate of substrates, thereby significantly improving the battery output power.

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## References

- [1] Xiu Yanhui, Guo Quanyou, Jiang Chaojun. The effects of pH, water activity, and NaCl on the growth/non-growth thresholds and growth kinetics parameters of *Clostridium perfringens* [J]. *Modern Food Science & Technology*, 2016,32(6):156-162,199.
- [2] Sturm G, Richter K, Doetsch A, Heide H, Louro RO, Gescher J. A dynamic periplasmic electron transfer network enables respiratory flexibility beyond a thermodynamic regulatory regime[J]. *The ISME Journal*, 2015, 9(8): 1802-1811.
- [3] Rodionov D A, Yang C, Li X, Rodionova IA, Wang Y, Obraztsova AY, Zagnitko OP, Overbeek R, Romine MF, Reed S, Fredrickson JK. Genomic encyclopedia of sugar utilization pathways in the *Shewanella* genus[J]. *BMC Genomics*, 2010, 11(1): 494.
- [4] Li Q, Feng X L, LI T T, et al. Anaerobic decolorization and detoxification of cationic red X-GRL by *Shewanella oneidensis* MR-1[J]. *Environmental Technology*, 2018, 39(18): 2382-2389.
- [5] Zhang Jie. Enhanced butanol fermentation system by co-culturing *Clostridium acetobutylicum* and *Shewanella* [D]. Dalian: Dalian University of Technology, 2020.

- [6] Faust K, Raes J. Microbial interactions: from networks to models[J]. *Nature Reviews Microbiology*, 2012, 10(8): 538-550.
- [7] Hu Jingli, Hua Tingyu, Li Shanshan, Yan Wei. Advances in Aerobic Microbial Degradation of MTBE Complex Pollution in the Environment [J]. *Journal of Central South University (Natural Science Edition)*, 2024,55(09):3257-3269.
- [8] Woyke T, Teeling H, Ivanova NN, Huntemann M, Richter M, Gloeckner FO, Boffelli D, Anderson IJ, Barry KW, Shapiro HJ, Szeto E. Symbiosis insights through metagenomic analysis of a microbial consortium[J]. *Nature*, 2006, 443(7114): 950-955.
- [9] Xu S, Li M, Hu Z, Shao Y, Ying J, Zhang H. The potential use of fungal co-culture strategy for discovery of new secondary metabolites[J]. *Microorganisms*, 2023, 11(2): 464.
- [10] Peng QN, Zhao C, Wang XP, Cheng KL, Wang CC, Xu XH. Modeling bacterial interactions uncovers the importance of outliers in the coastal lignin-degrading consortium[J]. *Nature Communications*, 2025, 16(1): 639.
- [11] Zhu HW, Xu LR, Luan GD, Zhan T, Kang ZP, Li CL, Lu XF, Zhang XL, Zhu ZG, Zhang YP. A miniaturized bionic ocean-battery mimicking the structure of marine microbial ecosystems[J]. *Nature Communications*, 2023, 13(1): 5608.
- [12] Hu Rui. Study on the synergistic degradation of dye wastewater by electroactive bacteria and fungi co-cultured with starch as carbon source [D]. Jiangsu: Jiangsu University, 2021.
- [13] Xiao Y, Chen G, Chen Z, Bai R, Zhao B, Tian X, Wu Y, Zhou X, Zhao F. Interspecific competition by non-exoelectrogenic *Citrobacter freundii* An1 boosts bioelectricity generation of exoelectrogenic *Shewanella oneidensis* MR-1[J]. *Biosensors and Bioelectronics*, 2021, 194: 113614.
- [14] Zhu J, Yan Y, Wang Y, Qu D. Competitive interaction on dual-species biofilm formation by spoilage bacteria, *Shewanella baltica* and *Pseudomonas fluorescens*[J]. *Journal of Applied Microbiology*, 2019, 126(4): 1175-1186.
- [15] Zhang Wen, Ni Li, Chen Yajing, Ye Xiuyun. *Bacillus subtilis* BS08 inhibits spoilage bacteria of yellow croaker and optimizes the culture medium [J]. *Chinese Journal of Food Science*, 2013,13(08):102-109.
- [16] Li Can, Zhang Yuchen, Sun Lijun, Wang Yaling, Liang Meijing, Hu Hanqiao, Xu Defeng, Liu Ying. Isolation and identification of dominant bacteria and measurement of quorum sensing signaling molecules in the spoilage of *Pandalus persicariae* [J]. *Food Science*, 2013,13 (08):102-109.
- [17] Zhang Liwei, Hou Mingyi, Tao Yu, Yang Jinlong, Liang Xiao. The impact of the marine *Serratia marcescens* population on biofilm formation and the attachment metamorphosis of thick-shelled mussels [J]. *Microbiology Bulletin*, 2024,51 (09):3422-3437.
- [18] Li YX, Luo QL, Su JY, Dong GW, Cao MF, Wang YP Metabolic regulation of *Shewanella oneidensis* for microbial electrosynthesis: From extracellular to intracellular[J]. *Metabolic Engineering*, 2023, 80: 1-11.

- [19] Teal TK, Lies DP, Wold BJ, Newman DK. Spatiometabolic Stratification of *Shewanella oneidensis* Biofilms[J]. *Applied and Environmental Microbiology*, 2006, 72 (11):7324-7330.
- [20] L. Gram, J. Melchiorson. Interaction between fish spoilage bacteria *Pseudomonas* sp. and *Shewanella putrefaciens* in fish extracts and on fish tissue[J].*Journal of Applied Bacteriology*, 1996,80(6).
- [21] Rosenbaum M A, Berger, C, Schmitz S, Uhlig R. Microbial electrosynthesis I: pure and defined mixed culture engineering[J]. *Advances in biochemical engineering/biotechnology*, 2017: 181-202.
- [22] Li Y, Liu G, Shi H. Expansion of carbon source utilization range of *Shewanella oneidensis* for efficient azo dye wastewater treatment through co-culture with *Lactobacillus plantarum*[J]. *Archives of Microbiology*, 2023, 205(8): 297.
- [23] Yang Y, Wu Y, Hu Y, Cao Y, Poh CL, Cao B, Song H. Engineering Electrode-Attached Microbial Consortia for High-Performance Xylose-Fed Microbial Fuel Cell[J]. *ACS Catalysis*, 2015, 5(11): 6937-6945.
- [24] Kim C, Song Y E, Lee CR, Jeon BH, Kim JR. Glycerol-fed microbial fuel cell with a co-culture of *Shewanella oneidensis* MR-1 and *Klebsiella pneumoniae* J2B[J]. *Journal of Industrial Microbiology and Biotechnology*, 2016, 43(10): 1397-1403.
- [25] Lin T, Bai X, Hu Y, Li B, Yuan YJ, Song H, Yang Y, Wang J. Synthetic *Saccharomyces cerevisiae* - *Shewanella oneidensis* consortium enables glucose-fed high-performance microbial fuel cell[J]. *AIChE Journal*, 2017, 63(6): 1830-1838.
- [26] Zhang MY, Xu XR, Zhao RP, Huang C, Song YD, Zhao ZT, Zhao YB, Ren XJ, Zhao XH. Mechanism of enhanced microalgal biomass and lipid accumulation through symbiosis between a highly succinic acid-producing strain of *Escherichia coli* SUC and *Aurantiochytrium* sp. SW1[J]. *Bioresource Technology*, 2024, 394: 130232.
- [27] Xie P, Wang QS, QU WY, Chen X, Feng YJ, Ma J, Ren NQ, Ho SH. Revealing real impact of microalgae on seasonal dynamics of bacterial community in a pilot-scale microalgal-bacterial consortium system[J]. *Water Research*, 2025, 274: 123145.
- [28] Baniasadi B, Vahabzadeh F. The performance of a cyanobacterial biomass-based microbial fuel cell (MFC) inoculated with *Shewanella oneidensis* MR-1[J]. *Journal of Environmental Chemical Engineering*, 2021, 9(6): 106338.
- [29] Angelova B, Avramova T, Stefanova L, Mutafov S. Temperature effect on bacterial azo bond reduction kinetics: an Arrhenius plot analysis[J]. *Biodegradation*, 2008, 19(3): 387-393.
- [30] Liu J, Fan L, Yin W, Zhang S, Su X, Lin H, Yu H, Jiang Z, Sun F. Anaerobic biodegradation of azo dye reactive black 5 by a novel strain *Shewanella* sp. SR1: Pathway and mechanisms[J]. *Journal of Environmental Management*, 2023, 347: 119073.
- [31] Thorn G J, King J R, Jabbari S. pH-induced gene regulation of solvent production by *Clostridium acetobutylicum* in continuous culture: Parameter estimation and sporulation

- modelling[J]. *Mathematical Biosciences*, 2013, 241(2): 149-166.
- [32] Wang Haifeng, Tian Yi, Ye Jing, Wang Yujing, Liu Wanrong. pH The Impact of Values on the Form of Heavy Metal Elements and Suggestions for Pollution Prevention and Control [A]. See: China Environmental Science Society, Central South University, Central South University of Forestry and Technology, Hunan Agricultural University. Proceedings of the 10th Symposium on Heavy Metal Pollution Prevention and Control Technology and Risk Assessment [C]. Changsha: China Environmental Science Society, 2020:88-90.
- [33] Li F, An X, Wu D, Xu J, Chen Y, Li W, Cao Y, Guo X, Lin X, Li C, Liu S. Engineering Microbial Consortia for High-Performance Cellulosic Hydrolyzates-Fed Microbial Fuel Cells[J]. *Frontiers in Microbiology*, 2019, 10: 409.
- [34] Kumar A, Pandit S, Sharma K, Mathuriya AS, Prasad R. Evaluation of bamboo derived biochar as anode catalyst in microbial fuel cell for xylan degradation utilizing microbial co-culture[J]. *Bioresource Technology*, 2023, 390: 129857.
- [35] Ji Q, Liu G, Zhou J, Wang J, Jin R, Lv H. Removal of water-insoluble Sudan dyes by *Shewanella oneidensis* MR-1[J]. *Bioresource Technology*, 2012, 114: 144-148.
- [36] Liu W, You Y, Sun D, Wang S, Zhu J, Liu C. Decolorization and detoxification of water-insoluble Sudan dye by *Shewanella putrefaciens* CN32 co-cultured with *Bacillus circulans* BWL1061[J]. *Ecotoxicology and Environmental Safety*, 2018, 166: 11-17.
- [37] Wang VB, Chua SL, Cai Z, Sivakumar K, Zhang Q, Kjelleberg S, Cao B, Loo SC, Yang L. A stable synergistic microbial consortium for simultaneous azo dye removal and bioelectricity generation[J]. *Bioresource Technology*, 2014, 155: 71-76.
- [38] He L, He X, Fan X, Shi S, Yang T, Li H, Zhou J. Accelerating denitrification and mitigating nitrite accumulation by multiple electron transfer pathways between *Shewanella oneidensis* MR-1 and denitrifying microbial community[J]. *Bioresource Technology*, 2023, 368: 128336.
- [39] Ri C, Li F, Mun H, Liu L, Tang J. Ball-milled Fe<sup>0</sup>/FeS<sub>2</sub> enhanced interaction of *Shewanella oneidensis* MR-1 with anaerobic microbial community: Impact on 2,4-dichloronitrobenzene reduction and methane yield[J]. *Chemical Engineering Journal*, 2023, 452: 139086.
- [40] Fapetu Segun. Enhancing energy recovery from industrial wastewater using microbial fuel cells[D]. London: University of Westminster, 2018.
- [41] Zeng J, Banerjee A, Kim J, Deng Y, Chapman TW, Daniel R, Sarpeshkar R. A novel bioelectronic reporter system in living cells tested with a synthetic biological comparator[J]. *Scientific Reports*, 2019, 9(1): 7275.
- [42] Wu M, Xu Y, Zhao C, Huang H, Liu C, Duan X, Zhang X, Zhao G, Chen Y. Efficient nitrate and Cr(VI) removal by denitrifier: The mechanism of *S. oneidensis* MR-1 promoting electron production, transportation and consumption[J]. *Journal of Hazardous Materials*, 2024, 469: 133675.
- [43] Gao Yu, Liu Yuchen, Guo Xiaofang, Ji Li, Zhang Guixiang, Zhang Zhehai, Xia Hongli,

He Wenfeng, and Zhang Boyuan. The effect of sulfate-reducing bacteria on the passivation of heavy metals in alkaline and acidic farmland soils and its mechanism [J]. *Environmental Science*, 2022,43(12):5789-5797.