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INVITED REVIEW PAPER

A Novel Strategy for the Microbial Removal of Heavy Metals: Cell-surface Display of Peptides

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Abstract Cell surface engineering is a rapidly developing technology of microorganism that achieves modification of cell surface function by joining external functional peptides with surface anchoring proteins, for example, Outer Member Protein (OMP). On account of these proteins possessing metal responsive motifs, they can be specifically used for metal adsorption and dissociation. To elucidate the problems caused by heavy metals and develop various technologies for their removel or recovery, various metal-binding proteins/peptides fused on microorganism cell surface have been applied as novel methods. During the past few years, bacterial cell surface display strategy has received growing attention for their availability to eliminate heavy metals. In this paper, the existing problems, progress, and suggestions for furture of the peptide displaying system are summarized.

Keywords: wastewater treatment, biosorption, cell-surface display, bacteria, yeast, algae

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1. Introduction

Intensive industrialization has led to emission of various toxic compounds into the surrounding areas, which causes environmental contamination [1]. Aside from natural geological weathering, the enrichment of heavy metals in aquatic environments is mainly due to human industrial activities, such as metallurgical industry, chemical industry, and alloy preparation [2]. In general, heavy metals in industry treatment indicates those poisonous metals even at low concentrations, and normally refers to two main categories: toxic metals including Mercury (Hg), Lead (Pb), Zinc (Zn), Stannum (Sn), Nickel (Ni), Cadmium (Cd), Chromium (Cr), Manganese (Mn), Copper (Cu), Iron (Fe), and Barium(Ba); and common precious metals including Palladium (Pd), Platinum (Pt), Silver (Ag), Lithium (Li), Titanium (Ti), Molybdenum (Mo), and Cesium (Cs) [3]. Several heavy metals are essential for biological activities, but the safety value should be strictly controlled [4,5]. Unlike organic contaminants, precious metals and toxic heavy metals are generally known to be non-biodegradable, and they need to be detoxified through absorption, enrichment, and removal from wastewater.

Several studies have been carried out for the development of efficacious heavy metal removal methods, including chemical precipitation, physical and chemical adsorption, ions exchange and electrochemical methods, but their applications are limited due to some disadvantages, such as secondary pollution, inefficiency in low-concentration wastewater treatment, low regeneration efficiency, high energy requirement, and investment cost. Based on variable structure and function of microbial cell surface, cell surface display technology has attracted most attention and is applied in biosorbent, biosensor, antibody production, and biocatalyst in recent years. Taken together, various biosorption

methods for removing heavy metals have been developed along with recent advances in molecular biology, which enable biochemical and genetic characterization of the interactions between microorganisms and heavy metals [6].

2. Biosorption of Heavy Metals on the Cell Surface

Biological adsorption of heavy metals generally consists of two processes: the first stage is physical adsorption of metal ions in water; the second stage is active metabolic adsorption, depending on the chemical structure and composition of bio-adsorbents [7]. Compared with conventional chemical processes, which lower heavy metals and are easily poisoned by organic matters, biological adsorption methods have several advantages, including low operating cost due to easy desorption and recyclability, validity in removing heavy metals with low concentrations, availability of a wide range of microorganisms as bio-adsorbents, and applicability to conditions of low pH and high temperature [8].

The mechanism of heavy metal adsorption includes intracellular, extracellular, and cell-surface adsorption (Fig. 1) [3]. Intracellular adsorption happens when heavy metal ions and traces of refractory organic molecules are transferred to some organelles to form precipitates or other biological accumulations. Extracellular adsorption is mediated by the secretion of extracellular polysaccharide (EPSs), such as glycoprotein, lipopolysaccharides, and soluble peptides [9]. The heavy metal ions can be adsorbed by interaction with negatively charged groups on EPSs or functional groups of peptides/proteins on the cell wall [10]. Among above biological adsorption methods, cell-surface adsorption is considered to be the most promising technology for heavy metals adsorption. Next, the biosorption is introduced from three aspects according to the classification of usable microbial species.

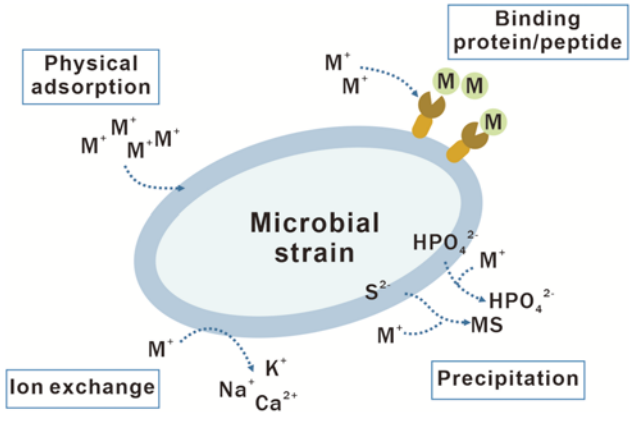

Fig. 1. Various mechanisms of microbial adsorption of heavy metals, including physical adsorption, ion exchange, protein binding, and precipitation.

2.1. Fungi

Metal adsorption capacity is mainly decided by amount of peptidoglycans on the cell wall. Fungal cell has a thick cell wall containing chitin, glucan, and mannan, which interact with metal ions in various ways, including mobilization and immobilization, sorption, and uptake [11]. These characters make fungi a good candidate for heavy metal adsorption. Based on several advantages of fungi, many heavy metal adsorption studies have been carried out using *Rhizopus*, *Mucor rouxii*, *Aspergillus*, *Penicillum*, *Trichoderma*, *Agaricus*, *Suillus bovinus*, and *Phanerochaete chrysosporium* [12].

Recently, uranyl ions recovery fungi was successfully constructed to absorb uranyl ions from aqueous solutions [13]. The result shows that fungal isolates rather than bacteria were dominant in selectively binding uranyl ions by NikRm mutant protein. Fungi represent a great potential for pollutant remediation, since they are able to endure various environmental stresses, such as nutritional deficiency, desiccation, or high metal contenting. Santelli *et al*. explored oxidation process of Mn(II) under the function of bacterial and fungal [14]. Two manganese oxidizing fungi are found to be *Plectosphaerella cucumerina* and *Stilbella aciculosa*. Ambarish *et al*. revealed that Pb^{2+} and Cd^{2+} in aqueous environments can be removed by *Fusarium oxysporum* [15].

2.2. Phage and viruses

Proteins or peptides expressed by foreign DNA fragments are normally fused within the coat protein of bacteriophage. Lim *et al*. suggested that metal mineralization on virion surface acts on chemisorption sites of sulfhydryl groups [16]. They also investigated the effects of pH and temperature conditions on Zn^{2+} adsorption ability. Zn^{2+} adsorption stayed at the minimum level when exposed to an acidic environment and increased with the rise of pH until 7.5; a pH beyond which interferes with the adsorption process because the precipitation of zinc hydroxide starts to occur. As temperature rose from 2°C to 50°C, the approximate maximum adsorption capacity of tobacco mosaic virus (TMV1Cys) increased aming to Au(III) and Pd(II). Franchi *et al*. tested effects of Cu^{2+} and Hg^{2+} on gene transfer via clay-phage PBSl complexes [17]. They found that in the presence of Cu^{2+} (mM) and Hg^{2+} (mM), free phage decreased, but clay-associated phage remained essentially constant. These results indicate that bacteriophage activity can be negatively affected by heavy metals but increased through its adsorption by clay minerals. This result provides ideas for the study of metals adsorption using phage.

2.3. Algae

Algae are considered as convenient biosorbent materials because they do not need special treatment (excluding acid or base modification). In the actual decontamination industry, their applications are 84.6% more than fungi and bacteria, and 15.3% more than other biomasses. These advantages are summarized in two reviews by Kanchana *et al.* and Sweetly *et al.* [18,19]. Microalgae are easy to culture and do not generate toxic sludge. They have good binding affinity benefit from specific surface with negative charge. They perform well even at low-contaminant-concentration environment. All of the above advantages make algae suitable for different scales remediation applications. Microalgae have been developed a broad prospect in dealing with heavy metals (extracellular and intracellular), especially suitability for practical application in wastewater bioremediation [20].

Rayson *et al.* did research about *Datura innoxia* and proved superiority of non-living algae, including high targeting, rapid binding, and high capacity toward heavy metals [21]. They also proclaimed that living fungal cells are unfavorable biosorbents directly used for heavy metal. Vela *et al*. disclosed that some common green algae (*Chlorella*, *Scenedesmus*, and *Cladophora*), or the consortia of them and cyanobacteria (*Spirulina*, *Oscillatoria*, and *Anabaena*) can be used for Zn, Cu, and Mn elimination [22]. Brinza *et al*. mentioned the particular use of various microalgae, such as *Chlamydomonas reinhardtii*, *Chlorella salina*, *Spirulina platensis*, and *Stichococcus bacillaris* [23]. Behnke *et al.* found that proteins ISIP1_To and ISIP2 were pivotal in detection and absorption of iron on algae cell surface [24]. The peptide molecules on the surface of these algae bind with heavy metals to form organometallic complexes inside the vacuoles, which effectively controls the cytoplasmic concentration of heavy metal ions. Jalil *et al.* studied the influence factors of biosorption capacity of *Chlorella coloniales* including reaction time and initial metal concentration. Heavy metal biosorption ability increased from 30 h to 100 h then smoothly decreased. *Chlorella coloniales* showed maximum efficiencies for the removal of Cr, Cd, Co, Fe, and As were 97.8, 97.05, 95.15, 98.6, and 96.5% respectively at high concentrations, as well as for Cr, Co, Fe, and As bioaccumulation at low concentrations [25].

3. Cell-surface Display of Recombinant Peptides

Surface display technique, which displays the fusion protein of a short protein or peptide (target protein) and microbial outer membrane protein (OMP, carrier protein) through genetic engineering technology, has already been established for several decades. Charbit *et al*. and Freudl *et al.* published the earliest reports about CSD by fusion of external proteins/peptides with carrier proteins [26,27]. A number of surface display systems and gene fusion strategies since then have been constructed in succession.

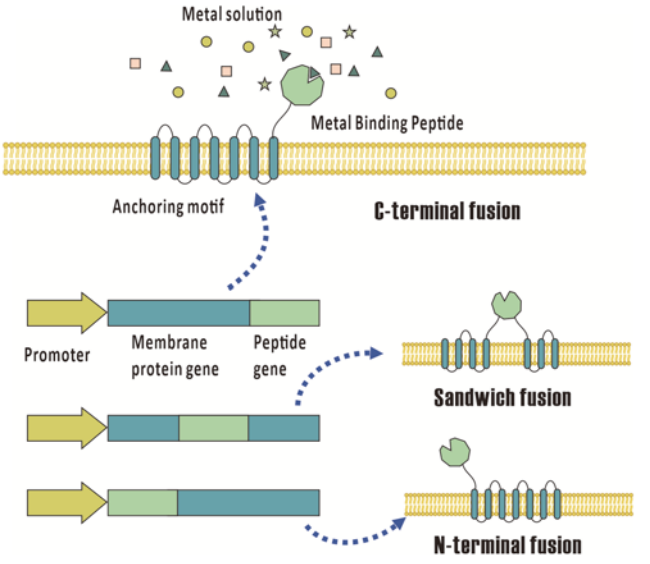

Fig. 2. Procedure of microbial cell surface engineering towards metal adsorption, including plasmid recombination, metal-binding proteins/peptides display, and metal adsorption. The yellow layer represents the cell membrane. The green polygons represent metal binding peptides.

Among all of the biological adsorption methods, CSD has attracted more attention over the years. A better adsorption effect of metal ions can be achieved by CSD because the accumulation of metals in most microorganisms tends to occur on the cell surface rapidly. The procedure of microbial CSD toward metal adsorption is shown in Fig. 2, including plasmid recombination, proteins/peptides display, and metal adsorption. To date, various successfully displayed metal-binding proteins/peptides have been developed and verified to have good combination effects (Table 1).

Comparing the adsorption methods, the adsorption based on CSD has several advantages over intracellular and extracellular adsorption: (i) easy preparation, the cells prepared by cell cultivation do not need further protein purification and concentration procedures; (ii) no need for cell disruption; (iii) easy reutilization of bio-adsorbents; and (iv) selective and stable adsorption of target metal ions.

Cell-surface strategy has also been used in diverse other applications, such as biocatalysts, live vaccines, and high-throughput screening application *et al.* In all aspects there are four keywords for CSD: carrier protein, passenger protein, host strain, and environmental conditions.

Table 1. Cell surface display systems that have been successfully displayed on microbials

Strain	Metal-binding peptide/protein	Binding site	Target metal	Reference
Escherichia coli and *Providencia vermicola*	EC 20	Sulfhydryl sites	Pb and Pt	Tan *et al*. [9]
Escherichia coli	SynHMB and type VI secretory system (T6SS) cluster	OmpA	Cd and Pb	Zhu *et al*. [37]
Escherichia coli BL21	PbrR, PbrR691, and PbrD	N-domain of ice-nucleation protein	Pb	Jia *et al*. [32]
Gram-positive bacteria	Siderophore-binding proteins (SBP)	OMR, BtuB, BtuF	Fe	Endicott *et al*. [63]
Escherichia coli	CueR	Prolipoprotein (Lpp)	Cu	Wang *et al*. [34]
Escherichia coli	IscA	Lpp-OmpA	Cu, Ni, Cd, Pb, As, Co, and Hg	Jiang *et al*. [33]
Escherichia coli	CysLysCysLysCysLysCys (CL)	INP-N	Hg	Liu *et al*. [35]
Bacillus subtilis	His_{12}	CotE	Ni, Pb	Kim *et al*. [44]
Yeast	SsoFe2	α-agglutinin	Fe	Cruz-Teran *et al*. [49]
Escherichia coli	PbrR and PbBD	Lpp-OmpA	Pb	Hui *et al*. [50]
Caulobacter crescentus	Lanthanide binding tags (LBTs)	S-layer	Tb	Park *et al*. [66]
Saccharomyces cerevisiae	CP2 peptide	α-agglutinin	Cd	Yang *et al*. [51]
Escherichia coli	PbrR	N-terminal of OmpA	Pb	Wei *et al*. [52]
Yeast	α-agglutinin	INP	U	Matano *et al*. [48]
Escherichia coli	Lead-binding peptide	OmpCt	Pb, Cu	Nguyen *et al*. [39]
Saccharomyces cerevisiae	ModE mutant protein(T163Y)	α-agglutinin	W, Mo	Kuroda *et al*. [29]
Caulobacter crescentus	Synthetic phytochelatins	LgA β-domain	Pb, Zn, Cu, Cd, Ni, Mn, Co	Biondo *et al*. [65]
Escherichia coli	Zinc binding peptide (ZBP) and copper binding peptide (CBP)	OmpC	Zn,Cu	Ravikumar *et al*. [53]
Escherichia coli	Zinc binding protein	OmpC	Zn	Ravikumar *et al*. [37]
Caulobacter crescentus	Hexa-His	RsaA	Cd	Patel *et al*. [54]
Saccharomyces cerevisiae	NP peptides with CXXEEmotif	α-agglutinin	Pb	Kotrba *et al*. [55]

3.1. Carrier protein

A qualified carrier protein should meet specific functional requirements, including passability through cell membrane along with the premature fusion protein; binding stability with fusion proteins on cell surface through its strong anchoring structure; permitting insertion or fusion with compatible sequences; and maintaining resistance to attacks by corresponding proteases in physiological environment or culture medium [28]. It is worth noting that different carriers can change the physiological environment around strains, for example, combined Outer Membrane Proteins may bring out cell growth defects and membrane growth instability. Due to the possible direct facing to the surrounding medium, it is necessary to find out the extracellular region of carrier protein. For this, several ways, such as homology comparison and hydropathy profile calculation through predictive algorithm or computer programs can be used.

3.2. Passenger protein

The passenger protein is synthesized in the cell, after transported across the membrane it will be fixed on the cell surface. Its activity directly determines the performance of the cell surface display. The passenger protein should be foldable, and the position of its active center should not be too close to the fusion site. Some structural characteristics such as disulfide bond and amino acid electronegativity also affect its function.

3.3. Host strain

A good host should have good compatibility with other foreign proteins and low protease degradation activity for smooth production of fusion proteins. It also should possess rigid cell surface instead of frail outer membrane for easy cultivation without cell lysis. Therefore, gram-positive bacteria are considered to be more suitable for whole cell adsorbents than gram-negative bacteria. *Bacillus* and *Staphylococcus* strains are popular gram-positive bacteria in most previous studies. Among other types of micro-organisms than bacteria, *Saccharomyces cerevisiae* and algae are often used as good tools for loading the combined structures [29].

3.4. Environmental conditions

Generally, a pH between 3 and 8 is considered favorable, because metal ions exist in the hydroxide form in solutions with high pH, and the adsorption process cannot be carried out. Moreover, bacteria behave better in a low pH environment, so exploring microbes that are adapted to low pH

environments is important.

Controlling the cell culture time to save costs is also important, because the adsorptive amount does not reach adsorption saturation after the equilibrium stage. It was reported that the over-abundance of adsorption material can generate competition among adsorption groups, and over-decentralization of adsorption material can cause insufficient adsorption. The coexisting ions can occupy the adsorption sites, thus decreasing the adsorption efficiency of the target ion [30].

4. Application of CSD on Heavy Metal Adsorption

Metal ions can be adsorbed on the cell surface through coordinating with a number of functional groups in peptides or proteins displayed on the cell wall, for instance, carboxyl, hydroxyl, amino, sulfhydryl, guanidine, and imidazole. Wang *et al*. analyzed the adsorption performance of the cell membrane with Cu^{2+} in *Pseudomonas* (*Pseudomonas putida* 5-x) [31]. The cell walls were estimated to play main functions in this process, with a contribution of about 40-90%.

Modification of cell-surface properties makes specificity binding with metals become possible, due to the proteins containing metal-responsive motifs fusing with anchoring proteins on the cell surface. In the past few decades, viruses, phage, algae, land plants (or their products), and especially bacteria (or bacterial exopolysaccharides) and fungi were applied for their availability to eliminate heavy metals.

4.1. Bacteria

Jia *et al.* displayed three proteins on the cell surface of *Escherichia coli* BL21 to realize *in situ* remediation of Pb^{2+}-contaminated water or soil. The maximum Pb^{2+} adsorption of engineering strains displayed proteins PbrR, PbrR691, and PbrD were 942.1, 754.3, and 864.8 μmol/g cell respectively. Seed germination test and *Nicotiana benthamiana* growth test proved the detoxification effects as well [32]. Jiang *et al.* constructed a fusion protein vector of Pet-Lpp-OmpA-IscA with prelipoprotein Lpp, membrane protein OmpA and iron-sulfur assembly protein IscA, which was then transferred in *E. coli* BL21. The IscA surfaced-displayed strain showed multiple absorption capacity of heavy metals Cu, Ni, Cd, Pb, As, Co, and Hg, with up to five times higher absorption capacity than control strain [33]. Wang *et al.* developed a dual-functional whole cell senser/absorbent for copper pollution in water. A copper-sensing element and a copper-adsorbing element were fused in temperature inducible plasmid pBV220 and then were transformed into an engineered *E. coli* strain. Gene copA and gene cueO were knocked out in the engineered *E. coli* strain in advance. The CueR surface-displayed strain showed improved and stable adsorption ability for copper ions in aqueous solutions. What's more, the adsorption-desorption cycles could be controlled via pH and temperature regulation, which make it convenient for regeneration of functional strain [34]. Liu *et al.* modified a kind of Hg^{2+}-binding peptide with sequence of CysLysCysLysCysLysCys (CL) to selectively adsorb mercury ions and reduce Hg^{2+} accumulation in fish. The modified protein was anchored on N-terminal region ice nucleation protein and transformed into *E. coli* DH5α. Approximately 95% of mercury ions was caught by combined strains from surrounding pollutions, which was four-fold higher than that of the control strains. Then the transformed strains were fed to *Carassius auratus* and colonize fish intestine. The results showed that total mercury accumulation of engineered bacteria-fed *C. auratus* was significantly less (51.1%) when compared with the no fed-group, which indicated that the engineered *E. coli* protect the fish from ingesting Hg^{2+}-polluted food [35]. Ravikumar *et al*. developed a kind of cell-surface-engineered bacteria which efficiently sensed and adsorbed extracellular zinc without additional induction system [36]. In this study, the genomic region containing the 237 bp *zraP-zraS* intergenic region was amplified from *E. coli* genomic DNA, plasmid pZGFP1 and the *gfp* gene encoding GFP was amplified from plasmid pPROBE-NT0 and was ligated with plasmid pUHup1 to construct pZGFP1 (sensing reporter) in which the GFP reporter protein was under the control of a *zraP* promoter. Zinc binding peptide (HYQHNTHHPSRW) was anchored on the N-terminus of truncated outer membrane protein C ($OmpC_t$) on *E. coli* XL1-Blue. This engineered zinc-sensing system detected zinc ions sensitively under low concentrations to 0.001 mM without pollution risk (Zinc pollution limits stipulated by Environmental Protection Agency is 0.030 mM). And this system accumulated substantially higher amounts of zinc than other reported bacterial surface display systems. The development of the pZGFP1 reporter in conjunction with bioremediation efforts can be a breakthrough in monitoring and removing toxic zinc contamination in aquatic environments. Zhu *et al.* introduced a functional genetic bacteria cell system coordinating with magnetic nanoparticles for heavy metals adsorption [37]. In this study, metal binding peptide SynHMB and magnetic nanoparticles synthesized by a co-precipitation method was anchored on the cell surface to form biotic complex. This co-assemblies system showed high absorption efficiency with higher than 90% from 50 mg/L of Cd^{2+} and 50 mg/L of Pb^{2+}. Ravikumar *et al*. studied a bacterial system of copper adsorption constructed by chimeric OmpC and cusC promoters (PcusC) [38]. The combined strain increased adsorption of Cu^{2+} to more than 4-5 times. C_t level of RNA samples from cultured *E. coli*,

which reflects the transcriptional efficiency of cusC, was found to increase with elevated Cu^{2+} concentration. Especially, induction of 1 mM of copper caused six-fold increase of transcriptional level compared with 0.1 mM. This research reflected a positive relationship between induced quantity of metal-binding peptides and metal concentration. Nguyen *et al*. introduced a highly specific lead-binding system [39]. This study anchored peptide ThrAsnThrLeuSerAsnAsn on outer membrane protein C (OmpCt) under control of the arabinose promoter. They expressed the peptide sequence TNTLSNN to bind lead ions and the recombinant cells successfully implemented a 5 times higher selective removal of lead than Ni^{2+}, Co^{2+}, and Cu^{2+}. Adsorption capacity and selectivity of Pb^{2+} was simultaneously strengthened than the wild strain.

Maruthamuthu *et al*. first reported copper adsorption system in *E. coli* through microbial CSD technology [40]. Three copper binding peptides, NAKHHPR, NRWHHLE, and SPHHGGW were cloned to C-terminus of OmpC at loop8 into the BL21 (DE3) strain respectively. Among these three binding peptides, SPHHGGW showed the best binding peptide and its dimeric peptide was constructed for adsorption evaluation in wastewater samples and copper ions containing textile dye wastewater. This dimeric peptide adsorption system (543.27 lmol/g DCW) possess better ability than monomeric peptide (243.53 lmol/g DCW). This system also showed a selective adsorption of copper ions, rather than lead, chromium, and cobalt. Ferri *et al*. firstly reported an efficient display of external autotransporter protein antigen 43 in cyanobacteria [41]. The antigen 43 from *E. coli* was anchored on cyanobacterial outer membrane protein *Synechococcus* sp. PCC 7942 SomA, then this recombined protein was displayed in cyanobacteria. This is a big progress compared with previous realization of only a fraction of the protein. This may lead to an effective display applied in aggregation, mobilization of toxic compounds on special surfaces, and extracellular bioconversion. Hinc *et al*. introduced recombinant spores of *Bacillus subtilis* for Ni (II) remediation [42]. The protein CotB on spore surface was fused with histidine residues, and the recombinant CotB18His spore was proved statistically more efficient than wild type spores. Interestingly, Taguchi experimental design analysis implied that pH and temperature are not influence factors aiming to adsorption efficiency, the amount of spores is unique factor among all three normal experimental conditions. Kim *et al.* inserted His12 (double histidine 6 tag) at the C-terminal of CotE protein, then this recombinant spore DB104 (pCotE-His12) was applied for Ni^{2+} and Cd^{2+} adsorption. The results showed 24% higher amount of Ni^{2+} adsorption and 2.5 times of Cd^{2+} adsorption compared to the wild strain [43].

4.2. Yeast

The rigid cell wall, feasible folding and glycosylation make yeast a high-quality platform for protein production, and a suitable host strain for CSD well. Yeast is particularly effective as a host when the protein is required to be prepared in the natural form [44]. Born better than bacteria and phage, yeast is equipped with natural modification systems of eukaryotic secretory pathways. Various histidine/cysteine-rich peptides have been anchored on surface of the yeast. CP2, HP3, CadR, and NikR peptides were proved to be able to chelate well with Zn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}. The functional yeast with acid tolerance was also developed [45].

Tao *et al*. displayed the redesigned CadR from *P. putida* on the surface of *S. cerevisiae* [46]. The modified strain showed a 5.7-times higher affinity toward Cd^{2+} and better adsorption toward Zn^{2+} and Pb^{2+} than the wild strain. Kuroda *et al*. developed a mutant ModE protein at the oxyanion-binding site which is closely related to chelation mechanism [47]. Restraining the mutant ModE on the yeast cell surface enabled a good adsorpion of 75% MoO_4^{2-} and 10% WO_4^{2-} in the mixture of molybdate and tungstate. Nishitani *et al.* discussed influence of C-terminal domain and N-terminal domain of ModE for molybdate adsorption [29]. They found that adsorption of molybdate by N-terminal absence peptides on DNA binding domain is higher than that of full-length ModE displaying peptides. What's more, more than 50% adsorbed molybdate was found to be desorped by papain that act on the C-terminal domain of ModE. These phenomena indicate that the C-terminal domain of ModE plays a key role in adsorption process and deletion of the N-terminal domain enhanced the binding ability on the contrary. Kuroda *et al*. fused NikRm protein from *S. cerevisiae*, two metal-binding domains (MBDs) with yeast-agglutinin as anchor domain on the yeast cell surface [13]. The NikRm-MBD-displaying yeast cell is effective for fast recovery of uranyl ions under concentrated conditions. Matano *et al*. constructed cellulase-displaying *S. cerevisiae* system for ethanol production, and developed an energy saving fermentation process through reuse of the the yeast cells [42]. This research provided an idea to save cost of cell cultivation according to different preferences of environmental conditions, which enhance the economic feasibility in industry. Nishitani *et al.* reported that the CXXEE motif is sensitive to low pH. As a result, its Pb^{2+} binding efficiency at pH 5.5 is 4 times higher than that at pH 4 [29]. In contrast, the ModE protein is sensitive to high pH [48]. These consequences indicated that biosorption efficiency are limited to pH condition.

Some other new technologies may also provide benefits to industries, such as packed-bed column systems and waste bacteria recovery from the fermentation industry.

Furthermore, standard technology and equipment even have been established for production, such as wastewater treatment system based on *B. subtilis*. However, the control and optimization of bioremediation processes constitute a complex system due to many factors. Several barriers come from continuing problems related to proteins and peptides, such as difficulty of small-sized peptides, accuracy and efficiency in simultaneous display of multiple peptides, and high-throughput peptide library screening. Other factors include the existing microbial population that is capable of degrading the pollutants, available contaminant population, and environmental conditions (temperature, pH, other electron acceptors, and nutrients). Therefore, it is also necessary to apply this biotechnology to real-world ecological experiments and settings. The earlier applied proteins and their applications are listed in Table 1 [49-55].

4.3. Combination of cell surface display technology and *in situ* physical methods

In situ remediation of heavy metals in soil has been extensively studied, but there are few researches on *in situ* metal removal in water environment because physico-chemical properties of the aquatic bodies extremely affect the performances of biosorbents. Mishra *et al.* [56] summarized current methods about decontamination/ removal of metals in industrial processes, and pointed out that precipitation and bioleaching [57] could be useful technologies rather than phytoremediation [58,59], which have been applied broadly in solid system remediation. What's more, few novel researches reported *in-situ* treatments for heavy metals by sewage includeing immobilization technology [60] and biofuel cell [61]. Li *et al.* proposed new ideas of ClO_2 treatment to reduce lysis effect of NaOH to *erythrocytes*, *E. coli* and *M. rouxii* lysis [62]. They found that erythrocyte membrane proteins were altered and vesicles decreased in sodium hydroxide (NaOH) aqueous solution after ClO_2 treatment, which can be seen as a new approach to improve mechanical stability of native micro-organism cells. As a result, ClO_2 could be a promising auxiliary for effective biosorption of metal ions. The above methods are believed to be the good beginning to achieve efficient and economical heavy metal removal through combination of cell surface diaplay technology and physical biosorption technologies.

5. Conclusion

Biosorption is a promising technology that highlights the fabrication of economical, convenient, and active materials in wastewater purification. Cell-surface adsorption has advantages due to decisive structures, such as no requirement for cell disruption, recyclability, and reusability, and specificity adsorption of target metal ions. Nowadays, more and more new functional proteins are discovered, and a growing number of surface display systems are developed. Therefore, recombinant microorganism including recombinant bacterial system through fused peptide display is still potential in novel wastewater treatment process based on their specificity. However, it is endless to thoroughly understand the membrane protein channel, search for new passenger proteins and display-anchor protein systems. As well as to examine the properties, including stability, activity, and specificity. As expression systems and interdisciplinary approaches become increasingly broader, solutions will be provided for technical hurdles. For example, Endicott *et al.* designed a new model and found that siderophore-binding protein (SBP) might act as acceptors of ferric iron in a Gram-negative bacterial iron shuttle. The SBP-bound ferric siderophore complex can absorb and go through extracellular siderophores. This newly discovered siderophore-dependent ferrichelatases revealed that metabolite translocation across bacterial membranes is still largely unexplored [63]. Peng *et al.* study the mechanism of *Ochrobactrum MT180101* as a membrane bioreactor in electroplating wastewater. The results proved that O*chrobactrum MT180101* was a copper-resistant bacterium with complicated mechanism of generating protein on surface, participating in enzyme-mediated biotransformation, extracellular chelation, and bio-transporting [64]. Binodo *et al*. described a synthetic phytochelatin, which showed good affinity to metal ions due to its strong promoter without external inducers [65]. In a study from Park *et al*., the engineered bacterium *Caulobacter crescentus* can be applied for rare-earth element adsorption through high-density cell-surface display technology [66]. Won *et al*. introduced a new direction of drawing into unnatural amino acids cooperating with peptides [67]. Microbial surface display will undoubtedly contribute to metal pollution control and environmental protection, thereby dedicate to the higher degree of technologically inclined and industrialized world.

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