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Selective Biosorption of Precious Metals by Cell-surface Engineered Microalgae

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Biosorptive recovery of precious metals (PMs) has gained attention in industry due to its potential low cost, high efficiency and environmentally compatibility. This study aims to use highly selective engineered microalgae species as biosorbents for recovery of PMs. Microalgae proliferate in both freshwater and marine environments unlike macroalgae found at coast lines. Microalgae have showed great potential as biosorbents owing to their high binding affinity, low nutrient requirements and local availability. Previous research was undertaken by sourcing microalgae from a eutrophic dam in Hartbeespoort, South Africa. The results from tested microalgae showed a relatively higher sorption capacity for lanthanum (La), thallium (Tl) and cadmium (Cd). Among the microalga sorbents, the self-flocculating microalgae have the ability to aggregate together and form flocs, which can facilitate their gravity sedimentation for biomass recovery. This technology offers a more cost-effective and energy-saving method than conventional separation methods such as centrifugation and filtration. This study attempts to combine the cell-surface engineering with the self-flocculating microalga sorbents, which would be a high efficient potential method to selective biosorption of target PM from multi-metal solutions.

1. Introduction

Precious metals (PMs) are rare and widely used in jewellery finish, dentistry, automobiles, electronics due to their unique physical and chemical properties such as pleasing appearance, tarnish resistance, excellent catalysis, among others (Mack, 2008). PMs refer to 8 elements including gold (Au), silver (Ag), platinum (Pt), palladium (Pd), rhodium (Rh), iridium (Ir), ruthenium (Ru) and osmium (Os), which are limited in their nature resources (Won et al., 2014). Cost-effective method for recovery of PMs from secondary sources such as industrial wastewater has recently attracted attention. Several conventional methods were employed to remove and recover heavy metals and PMs from solutions, including chemical precipitation, ion exchange, electrochemical methods and membrane processes. However, the aforementioned methods suffer from a high operation cost hence the need to develop alternative separation technologies especially for the treatment of lower metal concentrations ≤ 100 mg/L. Biosorption is a passive process where metals in solution are bound by chemical sites naturally present and functional on the dead biomass (Volesky, 2007). This process has been considered as an alternative technology for uptake of heavy metals or PMs from high volume and low concentration wastewaters (Won et al., 2014).

Microalgae are microscopic photosynthetic organisms both found in marine and freshwater environments, with the ability to discriminate non-essential heavy metals from essential ones for their growth (Suresh Kumar et al., 2015). Over the last two decades, researchers have focused more attention on the removal of heavy metals by brown algae which were distinguished to possess higher uptake capacity compared with red and green algae (He and Chen, 2014). However, large-scale application of brown algae can only be practical in the vicinity of sea coastlines; in contrast, microalgae proliferate everywhere in both freshwater and marine environments (Birungi, 2015). Monteiro et al. (2012) have proposed microalgae are promising biosorbents mainly due to the constituents of their cell wall with high uptake capacity, which could compare favourably with other microbe biosorbents like fungi, bacteria and yeast. In addition, algae have some advantages over other

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microorganisms as they do not require the specific nutrients and oxygen, and they are suitable for aerobic and anaerobic systems (Suresh Kumar et al., 2015).

Won et al. (2014) summarized a great diversity of biosorbents used for PMs biosorption. Compared to commercial adsorbents such as activated carbons and ion-exchange resins, the waste biosorbents showed low binding capacity. Therefore, research interest has been shifted to focus on improving the binding capacity of biosorbents through various surface modification methods. One such method is using genetic or protein engineering to produce engineered organisms with higher adsorption capacity for metal remediation (Bae et al., 2001). In nature, microorganisms survived in harsh conditions have evolved resistance and regulation mechanisms to confront the high level of toxic metals. The overexpression of various metal-binding proteins, e.g. metallothioneins (MTs), phytochelatins (PCs) and metalloregulatory proteins, evolved by microbes inside or on the surface of microbe cell is expected to improve the binding capacity and selectivity towards specific metal ions (Yang et al., 2015). A new possible strategy has been developed to overcome the present limitations of separating microalga biosorbents from diluted solutions and the lack of selectivity for target metals. In this study, an economic and efficient potential method was developed to improve the selective adsorption of target PM from multi-metal solutions.

2. Potential of green microalgae as biosorbents

Previous research was undertaken by sourcing microalgae from a eutrophic dam in Hartbeespoort dam, South Africa, which was aimed at investigating the biosorption potential of microalgae from freshwater habitat. The pure algal species were identified using molecular methods and found to have 95 - 98 % identities to, Desmodesmus multivariabilis, Chloroidium saccharophilum, Scenedesmus acuminutus and Stichococcus bacillaris. Chlamydomonas reinhardtii and Chlorella vulgaris were purchased from the culture centre for algae and protozoa (CCAP) for comparison purposes.

The adsorption studies on La, Tl and Cd were carried out in 250 mL conical flasks which were stirred on a magnetic stirrer at a constant speed of 350 rpm and room temperature. In all the algal biomass tested, the adsorption experiments were carried out for 6 - 24 h, times more than required for equilibrium, Table 1. A high q_{max} and lower b implies sorbents with the highest sorption capacity regardless of the recovery rate. Also a higher q_{max} and a higher b implies sorbents with both a high sorption and recovery rate at low equilibrium concentration (Birungi and Chirwa, 2015b). D. multivariabilis was found to have a relatively high uptake at q_{max} of 100 mg/g and a high recovery rate at b of 4.55 L/g for La (Birungi and Chirwa, 2014), Table 2. All the tested algal species had significant q_{max} in a range of 830 - 1,000 mg/g which showed excellent sorption capacities to Tl (Birungi and Chirwa, 2015a), Table 2. In the adsorption study of Cd (Birungi and Chirwa, 2015b), S. bacillaris was found to be the best adsorbent with both a higher q_{max} of 125 mg/g and higher b of 0.049 L/g, Table 2. All the green microalgae tested from the eutrophic dam as waste biomass showed potential for removal and recovery of heavy metals (Tl and Cd) and rare earth elements (La) in single metallic system.

Table 1: The conditions of equilibrium studies on biosorption of La, Tl, and Cd using tested microalgae

Tested metals	Biomass dosage (mg)	Initial concentration (mg/L)	pH	Contact time (h)	Reference
La	50	15 - 150	6	24	(Birungi and Chirwa, 2014)
Tl	50	15 - 500	6	6	(Birungi and Chirwa, 2015a)
Cd	50	15 - 150	5.5	24	(Birungi and Chirwa, 2015b)

Table 2: Langmuir model constants for biosorption of La, Tl, and Cd using tested microalgae

Alga species	Tested metals					
	La		Tl		Cd	
	q_{max} (mg/g)	b (L/g)	q_{max} (mg/g)	b (L/g)	q_{max} (mg/g)	b (L/g)
Stichococcus bacillaris	51.02	4.56	833.33	0.293	125	0.049
Desmodesmus multivariabilis	100	4.55	909.09	0.524	48.54	0.06
Scenedesmus acuminutus	111.1	0.120	833.33	0.290	-	-
Chloroidium saccharophilum	129.87	0.142	1,000	1.667	200	0.0095
Chlorella vulgaris	74.60	0.178	1,000	1.111	-	-
Chlamydomonas reinhardtii	142.86	0.25	1,000	1.667	-	-

3. Genetically engineered microbes as biosorbents

There are considerable works published in the literature on genetically-engineered biosorbents for removal of heavy metals by expressing the functional proteins inside or on the surface of microbe cells especially bacteria (Escherichia coli), yeast (Saccharomyces cerevisiae) and a few on microalgae (Chlamydomonas reinhardtii), Table 3. The recombinant strains expressing the metal-binding proteins/peptides showed higher binding capacity than the wild strains. Whereas, it is difficult to recover the metal species binding with functional proteins expressed inside microbe cells unless breaking the cells, and thus making the recombinant cells non-reusable. For easy recovery of metal ions, the metal-binding protein expressed on the cell surface acts as an alternative method to intracellular accumulation (Won et al., 2014). However, little information has been available on engineered microorganisms as biosorbents for PMs recovery, especially surface engineered microbes.

4. Limitations and strategies

Microalgae, possessing high binding affinity to different metals, are attracting more attention in recovery of PMs. However, there are two major practical limitations of microalgae as biosorbents in commercial applications including: a) the difficulty of separating metal-laden microalga cells from diluted solutions; b) the lack of selectivity for target metals.

Table 3: Genetically engineered microbes as biosorbents for heavy metal remediation

Microorganism	Metal-binding Proteins/peptides	Target metal	Location	Binding capacity (dry weight) Engineered cells	Wild cells	Reference
Bacteria						
Pseudomonas putida	MT	Cd^{2+}	Intercellular	28.9 nmol/mg	10.4 nmol/mg	(Valls et al., 2000)
Escherichia coli	Hg transport system and MT	Hg^{2+}	Intercellular	26.8 mg/g	17.75 mg/g	(Deng and Wilson, 2001)
Rhodopseudomonas palustris	Hg transport system and MT	Hg^{2+}	Intercellular	75 mg/g	22 mg/g	(Deng and Jia, 2011)
Escherichia coli	Ni transport system and MT	Ni^{2+}	Intercellular	9.89 mg/g	1.52 mg/g	(Deng et al., 2003)
Escherichia coli	Human hepatic MT	As(III)	Intercellular	0.3196 mg/g	0.0763 mg/g	(Su et al., 2009)
Caulobacter crescentus	6His	Cd^{2+}	Cell surface	16.0 mg/g	11.6 mg/g	(Patel et al., 2010)
Escherichia coli	Fish MT	Hg^{2+}	Cell surface	5.6 mg/g	3.0 mg/g	(Lin et al., 2010)
Escherichia coli	Hexa-His	Cd^{2+}, Ni^{2+}	Cell surface	Cd^{2+}:656.2 nmol/mg Ni^{2+}:276.5 nmol/mg	Cd^{2+}:276 nmol/mg Ni^{2+}:80 nmol/mg	(Saffar et al., 2007)
Yeast						
Saccharomyces cerevisiae	MT	Cd^{2+}	Cell surface	27.1 nmol/mg	-	(Ueda, 2003)
Saccharomyces cerevisiae	Hexa-His	Cd^{2+}	Cell surface	16.6 nmol/mg	-	(Ueda, 2003)
Saccharomyces cerevisiae	Hexa-His	Cu^{2+}, Ni^{2+}	Cell surface	bind eightfold more Cu^{2+}, Ni^{2+} than wild-type cells		(Kuroda et al., 2001)
Microalgae						
Chlamydomonas reinhardtii	MT	Cd^{2+}	Intercellular	0.43 mg/g	0.24 mg/g	(Cai et al., 1999)
Chlamydomonas reinhardtii	Pyrroline-5-carboxylate synthetase (P5CS)	Cd^{2+}	Intercellular	bind fourfold more Cd^{2+} than wild-type cells		(Siripornadulsil et al., 2002)

4.1 Self-flocculating microalgae

Due to the small size of microalga cells (2 - 20 μm) and strong negative charge on cell surface (Barros et al., 2015), the economical harvesting/separation of microalgae from large volume solutions poses a formidable challenge for the industry commercialization. This can be avoided by the use of immobilized microalgae, but the mass transfer limitation and additional process cost restrict their practical application (Moreno-Garrido, 2008).The commonly used separation methods like centrifugation and filtration (Volesky, 2007) are energy-intensive and costly for large-scale application, which are only widely used in laboratory-scale biosorption processes.

Bio-flocculation, has been considered as the most efficient, economical, and technologically feasible method to harvest/separate microalgae from diluted solutions (Zhou et al., 2015). Recently, several self-flocculating microalgae, such as Ettlia texensis SAG79.80, Scenedesmus obliquus AS-6-1 and Chlorella vulgaris JSC-7 (Zhou et al., 2015) demonstrated bio-flocculating properties. These self-flocculating microalgae with the ability to aggregate together and form flocs can facilitate their gravity sedimentation for separation from aqueous solutions, which is more economical and energy saving than centrifugation and filtration. Comparing with other flocculation methods, no additional chemical agents are needed for separating the self-flocculating microalgae from diluted solutions. Zhang et al. (2016) demonstrated that the self-flocculating microalgae could be an efficient, economical and environmentally-friendly biosorbents for heavy metals removal. However, there is limited work on the recovery of PMs using the self-flocculating microalgae as biosorbents.

4.2 Cell-surface engineering

The common failure of adsorption processes is the lack of selectivity for the target metals (Birungi, 2015). The aim of genetic modification of biosorbents is to improve the sorption capacity for target-metal species, without regard to selectivity (Yang et al., 2015). As mentioned above, it is feasible to construct the engineered microbes with high binding capacity and selectivity towards specific metal ions by overexpression of functional protein on cell surface. Such a method called cell-surface engineering or surface-display technique, which enables the metal-binding proteins expressed on the cell surface by fusing the interest proteins/peptides (passenger proteins) with anchoring proteins (carry proteins). The secretory vesicles encompassing the passenger and carry proteins pass through the cell membrane and anchor the passenger proteins to the surface of cell wall (Kuroda and Ueda, 2011).

In the surface-display system, the bound metal species on the cell surface could be readily recovered by the mild eluent without cell disruption and thus makes it feasible to repeatedly use the cell-based biosorbents. Also, surface binding is a metabolism-independent biosorption process, so non-viable cells can also be used as whole-cell biosorbents (Yang et al., 2015).

The MerR family of proteins is a group of bacterial transcriptional regulators that control the concentrations of metal ions, radical, and antibiotics inside their cells. Checa et al. (2007) found the GolS, a new member of the MerR family from Salmonella, directly controls the expression of at least two transcriptional units responsible for selective recognition of gold ions. This metalloregulator protein GolS evolved by bacteria to sense and survive under high levels of gold ions has the most attractive ability to bind Au^{+} while discriminating Cu^{+}. Based on the understanding of selective recognition of metal ions by metalloregulatory proteins and genetic organization of GolS (Chen and He, 2008), it is conceivable to recognize and bind the target PM from multi-metal solutions by displaying the protein GolS on microalga cells surface, Figure 1.

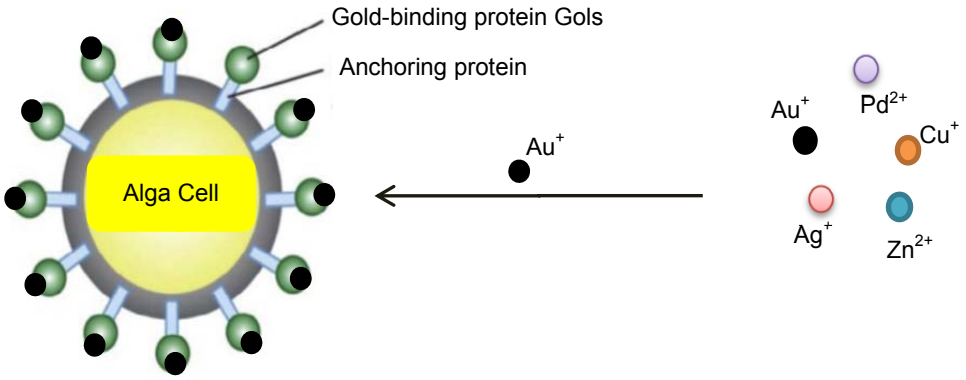

Figure 1: Schematic diagram of selective biosorption of Au^{+} by gold-binding protein Gols fused with the cell wall anchoring protein

5. Conclusions

A new possible strategy has been developed to overcome the present limitations of separating microalga biosorbents from diluted solutions and the lack of selectivity for target metals. By utilising cell-surface engineering technology and bio-flocculating properties, the self-flocculating microalgae are able to recognize and bind the target metal ions and separate from aqueous solutions by cost-effective sedimentation. Further studies are needed to investigate the potential of selective recovery of target PMs from multi-metal solutions by combining the cell-surface engineering with the self-flocculating microalga sorbents.

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