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Decontamination of multiple heavy metals-containing effluents through microbial biotechnology

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HIGHLIGHTS

- More than 97 % of heavy metals removal was achieved by the proposed biological methods.
- Camellia oleifera cake degradation promoted by *B. cereus* created an anoxic
- environment that supports SRB.
- Both of SRB and *B. cereus* functioned as decomposers for Camellia oleifera cake and sweepers for heavy metals.
- Camellia oleifera cake was either as sorbent or culture medium for bacteria.
- Biomass particulates could be reused for metal biosorption to reduce the cost of production.

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ABSTRACT

To decontaminate heavy metal-containing waste water, a microbial biotechnology was developed by using the synergy between *Sulfate reducing bacteria* (SRB), *Bacillus cereus* (*B. cereus*) and Camellia oleifera cake (COC). In this process the COC degradation assisted by *B.cereus*, created an anoxic environment and provided energy and nutrition for SRB. Both of *B. cereus* and SRB played significant roles through biosorption, bioaccumulation and biosurfactant production. Meanwhile, a flotation technology commonly used in many effluent treatments has been led into this system for increasing the efficiency as well. After desorption and regeneration with acid and deionized water, the biosorbents could be reused to adsorb metal ions. 97% of heavy metals removal was achieved by the proposed technology. For multiple heavy metals-containing solutions, the capacities are in the order of $Cd^{2+} > Zn^{2+} > Cu^{2+}$.

1. Introduction

Global industrialization, mechanization and various natural processes have led to the continuous accumulation of heavy metals in the environment, which raises threats to public health and the biosphere. The high concentrations of heavy metals in the environment also contribute to several life-threatening diseases, including cancer and cardiovascular diseases [1]. Various approaches such as coagulation, precipitation, ion exchange, solvent extraction and membrane processing, have been employed to clear heavy metal ions from wastewater [2]. Most of these physical remediation strategies are extremely expensive in terms of energy and reagent consumption, and frequently make the water inappropriate for agriculture. Therefore, it is urgent to develop a lower cost, green-clean technology.

As some naturally or genetically modified microbes possess the capacity to chelate or decompose various hazardous materials and hence provide better strategies to combat toxic pollutants, researchers frequently deploy them to eliminate hazardous materials, such as heavy metals [3]. For example, they can alter heavy metals from one oxidative state or organic complex to another [4]. The microbe-based renovation relies on the resistance of the employed microorganisms to the metals [5]. Microbes perform the rectification of heavy metals through mainly three different processes: biosorption, bioaccumulation and biosurfactant production. Biosorption and bioaccumulation are processes by which the microbe, or biomass, bind to and concentrate heavy metals and pollutants from the environment [6]. Biosorption relies on the isolation of heavy metals by the moieties of biosorbent cell surfaces such as those found in fungi/yeast, algae and bacteria [7]. In biosorp-

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tion, heavy metals are adsorbed onto the adsorber's vuggy surface in amounts that rely on the components and kinetic equilibrium of the cellular surface. This is a passive metabolic process not required for energy and respiration. On the other hand, bioaccumulation is an active metabolic process that depends on energy and respiration [8]. Under optimal operational conditions, the candidate microbes were characterized by tolerance to high concentrations of various heavy metals [9]. Among them, *sulfate-reducing bacteria* (SRB) have the potential to efficiently remediate heavy metals-contaminated waste water, in which the produced sulfide can react with heavy metal ions to form insoluble precipitates [10].

Biosurfactants or surface-active agents are matters that vary the prevailing conditions of surfaces through adsorption leading to lower surface tension between liquids or between a liquid and a solid [11]. They are generally classified as high-molecular weight polymers that firmly tie up solid surfaces as well as low-molecular weight molecules that reduce surface and interfacial tensions [12]. Although they have been generally applied in the rectification of organic pollutants, biosurfactants are able to eliminate and remediate heavy metal ions such as Cd^{2+}, Pb^{2+}, and Zn^{2+} [13].

Likewise, various utilized biomasses such as industrial wastes (e.g. biomass of *Saccharomyces cerevisiae* from fermentation), the food industry (e.g. corn core, corn stalks, cotton stalks and rice husk), agricultural (e.g. dead *B. sphaericus*), and other polysaccharide materials, are utilized to remove heavy metal ions from wastewater [8]. In China, thousands of tons of residual COCs, that is produced each year mainly from cooking and oil industries, act possibly as economical metal sorbents. Although the adsorptive particles are generally suspended to allow high surface binding sites, the subsequent separation and purification of effluent could be problematic, specifically in the ultra-fine particle size range. Therefore, a flotation technology (solid/liquid separation process) was often adopted as a possible alternative separation process [14].

To establish an efficient technique for the removal of heavy metals from effluent, three biomaterials are utilized in this work: COC, either as sorbent or culture medium for bacteria; SRB and *B. cereus* (isolated from heavy metal co-contaminated wastewater and soil, respectively) as decomposers for COC and sweepers for heavy metals. The treating process is time dependent and the removal capacity increase with contact time. A flotation column is also applied as the subsequent separation method for collecting the metal-laden biomass, and its running parameters are also analyzed in this process. In comparison, this intriguing treatment approach through the synergy of biomass and microbes could significantly contribute to metals removal in the future.

2. Materials and methods

2.1. Materials

After being dried at 60 °C in a vacuum oven, the COC (collected from Zhuzhou City, Hunan, China) were crushed to small pieces in a pulveriser and grounded in a grinder (FFC-45A, motor power: 11 kW, Qingdao Reida Machinery Co. Limited in China). The refined powders were packed in a polyethylene bag and stored at room temperature (20 ± 2 °C) until use. All of the chemicals used in the studies are of analytical grade.

2.2. Extraction and purification of saponin from COC

In order to detect the saponin content, 2 g COCs were degreased with ligroin (70–85 °C) in a soxhlet apparatus for 3 h, and then their organic solvent were volatilized at 50 °C in a rotary evaporator (RE-52A, Yarong Bio-instrument Co., Shanghai, China). The residues were placed in a round-bottom flask and 80% (v/v) ethanol was added at a solvent/solid ratio of 7:1 (w/w). The compound was boiled in a 200 mL beaker by reflux condensation in an oil bath at 85 °C for 3 h and then filtered through filter paper. The filtrate was concentrated at 65 °C in a rotary evaporator and precipitated with acetone at room temperature [15]. The dried precipitate was passed through an AB-8 macro-reticular resin column (10 mm × 300 mm; Sigma-Aldrich, Shanghai, China) and eluted successively with 50% (v/v)ethanol. After these ethanol eluates containing the primary saponins were concentrated at 55 °C in a rotary evaporator, 30% (v/v) acetone was added as a precipitator. The samples were centrifuged for 5 min at 4000 rpm after they were precipitated for overnight stratification. Next, the centrifuged sediment was freeze-dried and stored at room temperature until use [16]. The procedure was repeated three times to obtain sufficient amount of samples for the experiments. These COCs, which were only used to detect their saponin content, were not used to next experiment.

2.3. COC sorption in different pH

A 20 mL aliquot of solution containing heavy metals ions (e.g. 200 mg/L Cd^{2+}, 100 mg/L Cu^{2+}, 100 mg/L Zn^{2+}, respectively.) was treated with 1 g of COC at desired pH (pH 1.0-10.0). The suspension was stirred for pre-selected periods of time using a magnetic stirrer. After centrifugation (15 min, 4000 rpm), the supernatant solution was removed and the heavy metals ions preconcentrated onto COC were then eluted by using 5.0 mL of 1.0 M HCl, while stirring for 5 min. The suspensions were then centrifuged and eluent solutions containing heavy metals ions were removed from the COC. Finally, the metal contents of the resulted solutions were determined by flame atomic absorption spectrometry (FAAS, DFU-202G, Beijing). The percentage of metal ions extracted from the sorbents (E, %) was determined by comparing its concentrations before, C_i (mg/L) and after extraction, C_e (mg/L), as follows [17]:

$$E(\%) = \frac{C_i - C_e}{C_i} \times 100\% \quad (1)$$

2.4. Preparation of B. cereus for reduction and adsorbent

B. cereus, a strain with a higher heavy metal-tolerant ability, was also isolated from heavy metals-contaminated camellia oleifera culture soil in Zhuzhou. In order to acclimatize the new experiment situation, the bacteria were continually cultured by gradually increasing the concentration of heavy metal ions in the media. As a gram-positive facultative aerobic, spore-forming, and rod-shaped bacterium, it grew well on a petri dish containing agar medium (10.0 g peptone, 5.0 g extract and 10.0 g sodium chloride per liter) at 37 °C and pH 7.2 ± 0.2. After they were inoculated into 50 mL sterile medium in a 250 mL conical flask and cultured on a shaker at 150 rpm and 37 °C for 24 h, the bacteria were inoculated onto the plates, continually cultured for another 24 h, and then stored at 4 °C for further experiments.

2.5. Preparation of SRB for reduction

The activated sludges and water samples containing SRB were collected from some sewage canals in Zhuzhou as well. Based on a standard procedure [18], SRB were cultured in closed infusion bottles, in which the contents of the medium were: 500 mg KH_2PO_4, 1000 mg NH_4Cl, 500 mg Na_2SO_4,100 mg $CaCl_2$, 2000 mg $MgSO_4{\cdot}7H_2O$, 4 mg $CH_3CH(OH)COONa$ and 1000 mg yeast extract per liter at 37 °C and pH 7.0 ± 0.2. The acclimated SRB with high metal tolerance were also obtained by gradually increasing the concentration of Cd^{2+}, $Cu^{2+}$and Zn^{2+} in the media, respectively. Their activities were monitored for 48 h by recording the optical den-