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Extraction of sludge protein enhanced by electron beam irradiation and calcium oxide

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ABSTRACT

In order to enhance the extraction yield of sludge protein, electron beam irradiation (EBI) pretreatment in the presence of calcium oxide was investigated. The influences of the moisture content of sludge, dose and calcium oxide additive amount on the extraction yield have been studied. The experimental conditions were optimized by response surface methodology. The optimized conditions for the extraction process of sludge protein are as follows: dose of 30.83 kGy, moisture content of 90.56%, and calcium oxide additive amount of 0.31 mg/mL. Under the optimal conditions, the protein extraction yield can reach 88.67%. Regression analysis with R^2 value of 0.9964 showed a satisfactory correlation between the predicted values and experimental data. Based on the mechanism and feasibility analysis, the EBI/calcium oxide method was effective and feasible to improve the extraction yield of sludge protein.

1. Introduction

In wastewater treatment processes, large amount of waste activated sludge (WAS) has been produced. It has been reported that the outputs of WAS in China have increased to 35 million metric tons (moisture content: 80%) in 2016 [1]. However, over 80% of WAS has not been disposed and treated of safely and effectively in China [2]. WAS has been a severe problem because of its tremendous environmental risk and high cost for disposal and treatment [3]. Therefore, the question of how to exploit eco-friendly utilization and management technologies of WAS has caused extensive concern.

Dewatering is usually the primary step for energy utilization and final disposal of WAS [4]. Extracellular polymeric substances (EPS), which represent 60–80% of total WAS mass [5], exert certain influences on many characteristics of WAS and serve as well-known barriers to the WAS dewatering. EPS usually consists of useful organic matters such as polysaccharides, proteins, enzymes, and nucleic acids [6]. Proteins have been estimated to represent about 50% of the dry weight of EPS [7]. Therefore, the sludge protein has showed a great potential as a low cost biological nutrient [8], in which both high nutrient recovery and WAS treatment are accomplished in the meantime.

Accordingly, over the past decades, many endeavours have been made to break EPS and recover protein. For instance, ultrasonic pretreatment [9], sono-thermal pretreatment [10], microwave irradiation [11], mechanical disintegration [12], Fenton oxidation [13], enzymes pretreatment [14], and other methods have been widely used for EPS degradation. However, the enormous energy consumption and/or serious secondary pollution risks have created bottlenecks against the extension of these methodologies.

Irradiation pretreatment method (especially electron beam irradiation) is considered to be one of the most promising pretreatment method because of its prominence for WAS disintegration, WAS flocs destruction and EPS solubilization [4,15,16]. Irradiation method can effectively change polymer properties and induce changes in the microstructure and solubility of EPS [4]. Furthermore, this method also has many other advantages as below: mild temperature, short treatment time, and less undesirable inhibitory byproducts than chemical pretreatment methods *etc*. [15]. In addition, Xie et al. (2014) has reported that EPS was solubilized more significantly in an alkaline environment than that of in the other surroundings [17]. High solid sludge was disintegrated by sodium hydroxide (pH = 13), and the solubilization rate of organic matters was obviously enhanced [18]. Hydrothermal alkaline pretreatment of sludge at 140 °C with calcium oxide showed a protein recovery rate of 61.37% [8]. However, enhancing protein extraction efficiency from WAS using electron beam irradiation-calcium oxide pretreatment method has not been reported.

Therefore, the aim of this study was to study the effects of electron beam irradiation-calcium oxide method on protein extraction efficiency

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Nomenclature

c_0	Protein content of the WAS sample (g)
c_1	Protein content of the filtrate (g)
CCD	Central composite experimental design
EPS	Extracellular polymeric substances
m_0	Mass of the WAS sample (g)
m_1	Mass of the filtrate (g)
RSM	Response surface methodology
WAS	Waste activated sludge
X_1	Dose (kGy)
X_2	Moisture content (%)
X_3	Calcium oxide additive amount (mg/mL)
Y	Protein extraction efficiency (%)

of WAS. The effects of dose, moisture content of WAS, and calcium oxide additive amount on extraction efficiency of sludge protein by central composite experimental design and response surface methodology under the operation of electron beam irradiation-calcium oxide were analyzed systematically. The results provided a mathematical model for optimizing the extraction process of sludge protein and determined the optimum conditions. Finally, a feasibility analysis for the protein extraction process was discussed.

2. Materials and methods

2.1. Materials

WAS was collected from the municipal waste water treatment plant of Yulin (China). Main characteristics of WAS are shown in Table 1. Calcium oxide (analytical pure).

2.2. Experimental section

In order to study the effect of calcium oxide and electron beam irradiation on extraction efficiency of WAS protein, three experimental programs were designed as follows: electron beam irradiation-calcium oxide, calcium oxide alone and electron beam irradiation alone. Above all, moisture contents of the WAS samples were adjusted by moving supernatant or diluting with ultrapure water, then 300 ml of the preparative WAS was placed in a 500 ml glass bottle. The experiment was initiated by adding the preset amount of calcium oxide (0 to 0.4 mg/ml) and stirred until well-mixed, then the glass bottle was sealed. EBI experiment of the sludge sample was conducted at the electron beam facility with 1.5 MeV of electrons energy and 6 m/min conveyor stream velocity (Changchun Yi Fu Irradiation Accelerator Ltd., China). The sample was placed in a Pyrex tray (the width: 2.5–5.5 cm, and about 320 g each absorbed dose). The selected doses were in the range from 0 to 40 kGy. Dosimetric control was performed by CTA FTR125® and cellulose triacetate. Finally, the glass bottle was cooled to room temperature in a water bath. Soluble protein in the supernatant was obtained by centrifuging the sludge sample at 3000 r/min for 15 min at room temperatures of 21–25 ℃. Supernatant was dried at 35 ℃ for 1 week into protein powder. Fig. 1 shows the experimental schematic.

In order to investigate the effects of calcium oxide and electron beam irradiation on protein extraction efficiency of WAS, a series of experiments was performed under various dose, moisture content of WAS, and calcium oxide additive amount. Total nitrogen measurements by Kjeldahl method (GB/T5009.5-2003) was undertaken for indirect protein determination of filtrate. Protein extraction yield was determined by Eq. (1) [19]:

$$Y = \frac{m_1 \times c_1}{m_0 \times c_0} \times 100\ \% \quad (1)$$

Where Y is protein extraction efficiency; c_0 and c_1 is protein content of the WAS sample and filtrate, %, respectively; m_0 and m_1 is mass of the WAS sample and filtrate, g, respectively.

2.3. SDS-PAGE and SEM analyses

SDS-PAGE was performed based on the modified method of Laemmli [20]. Protein solution was centrifuged at 3000 x g for 10 min. Sludge protein solution (2.5 mg/mL) was mixed 1:1 with Lammeli buffer (25% glycerol, 2% SDS, 0.01%bromophenol blue, 62.5 mM Tris-HCl, pH 6.8) to a final concentration of 1 mg/mL. Electrophoresis buffer contained 0.2 M glycine, 0.1% SDS, and 20 mM Tris-HCl at pH 8.3. SDS-PAGE was run with 4 ℃ running buffer at room temperature. Protein solutions were loaded onto gels and run at 100 V for 40 min using a Mini-Protean III cell (Bio-Rad) and PowerPac Basic power supply. Gels were stained using a colloidal blue stain kit followed by destaining for 3–5 h. Protein molecular weight was analyzed using the software Gel-Pro analyzer.

The structure variations of the WAS were analyzed by a scanning electron microscope (SEM, Quanta-600, FEI) before and after disintegration.

2.4. Data analysis

Response surface methodology (RSM) was widely used to optimize a multivariable system [21]. In this work, the RSM and central composite experimental design (CCD) were used to optimize the protein extraction process and study effects of variables. The three parameters (dose, moisture content of WAS, and calcium oxide additive amount) were selected. The effects of these parameters on the extraction efficiency of WAS protein were discussed. Table 2 shows the ranges and levels of the parameters. The three-factor and five-level of CCD include 20 experimental points. Table 3 shows response surface experimental design.

The Design-Expert software (version 8.0.6) was applied for a statistical analysis of experimental data and to produce the response surface. The standard deviations of all measurements were less than 5%, and the results were analyzed using the SAS 9.0 and Origin8.0 software.

3. Results and discussion

3.1. Single-factor experimental analysis

In this research, the influences of three key parameters on the protein extraction efficiency were picked out for investigation. The effect of dose on the extraction efficiency was studied from 0 to 40 kGy, with other fixed extraction conditions as follows: moisture content, 90%; and calcium oxide additive amount, 0.3 mg/mL. As shown in Fig. 2a, the extraction efficiency increased quickly as the dose ascended from 0 to 30 kGy and then, the efficiency significantly decreased along with increase of dose. Xie et al. (2014) showed that the microorganisms from WAS were disintegrated by irradiation [17]. As is well known, proteins are both important parts of microorganisms, and proteins are released into sludge solution after disintegration of sludge cells. With the irradiation dose increasing, the rate of degradation increased and then the release and diffusion of proteins into water increased greatly.

Table 1
Main characteristics in WAS.

Sludge source	pH	Moisture content (%)	Protein content (%)	
			wet basis	dry basis
Yuyang zone	8.9	89. 4226	4.8391	33.9768