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Reduction of ethylenediaminetetraacetic acid iron(III) by *Klebsiella* sp. FD-3 immobilized on iron(II, III) oxide poly (styrene-glycidyl methacrylate) magnetic porous microspheres: Effects of inorganic compounds and kinetic study of effective diffusion in porous media

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HIGHLIGHTS

- Magnetic porous microspheres were introduced to immobilize *Klebsiella* sp. FD-3.
- Immobilized FD-3 exhibited good reduction activity and tolerance to inhibitors.
- The intraparticle diffusion is negligible and bioreduction is the rate-limiting step.

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ABSTRACT

Fe_3O_4 poly (styrene-glycidyl methacrylate) magnetic porous microspheres (MPPMs) were introduced to immobilize *Klebsiella* sp. FD-3, an iron-reducing bacterium applied to reduce Fe(III)EDTA. The effects of potential inhibitors (S^{2-}, SO_3^{2-}, NO_3^-, NO_2^- and Fe(II)EDTA-NO) on Fe(III)EDTA reduction were investigated. S^{2-} reacted with Fe(III)EDTA as an electron-shuttling compound and enhanced the reduction. But Fe(III)EDTA reduction was inhibited by SO_3^{2-} and Fe(II)EDTA-NO due to their toxic to microorganisms. Low concentrations of NO_3^- and NO_2^- accelerated Fe(III)EDTA reduction, but high concentrations inhibited the reduction, whether by free or immobilized FD-3. The immobilized FD-3 performed better than freely-suspended style. The substrate mass transfer and diffusion kinetics in the porous microspheres were calculated. The value of Thiele modulus and effectiveness factors showed that the intraparticle diffusion was fairly small and neglected in this carrier. Fe(III)EDTA reduction fitted first-order model at low Fe(III)EDTA concentration, and changed to zero-order model at high concentrations.

1. Introduction

The usage of fossil fuels has been increasing in step with economic growth. However, nitrogen oxides (NO_x), generated by the combustion of fossil fuel, cause ozone layer destruction, global warming and bring harm to human health. NO_x removal from fuel combustion is becoming a serious problem in air pollution control (De Richter and Caillol, 2011; Yu et al., 2012).

Fe(II)EDTA (EDTA: ethylenediaminetetraacetate) absorption combined with microbial reduction had been proposed and researched in recent years (Li et al., 2006; Zhang et al., 2007, 2008). There are some unfavorable problems by free bacteria because of the inhibition of microbial Fe(III)EDTA reduction with the components in the scrubbing solution. Our group has proposed a two-stage bio-reduction system to regenerate the scrubbing solution using immobilized microorganisms, in which, Fe(II)EDTA-NO and other nitrogen containing compounds (NO_3^- and NO_2^-) are reduced to Fe(II)EDTA and N_2 by immobilized denitrifying bacteria at the first stage, then Fe(III)EDTA is reduced to Fe(II)EDTA by immobilized iron-reducing bacteria at the second stage. Therefore, the inhibition of Fe(III)EDTA reduction with Fe(II)EDTA-NO, NO_3^-, and NO_2^- can be alleviated, and NO_x removal efficiency is improved. According to our previous research, NO_x removal efficiency kept at about 95% using immobilized microorganisms during 18 days continuous operation, in the case of free microorganisms, the value decreased to 85% after 5 days, and then the experiment was stopped due to the stuffing of the microorganisms

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Nomenclature

D_{es}	substrate diffusivity, cm^2/s	r	position in particle, μm
K_0	half-saturation constant for zero-order, h^{-1}	v_{max}	maximum specific consumption rate, h^{-1}
K_1	half-saturation constant for first-order, mmol/(L h)	ϕ	Thiele modulus
R	particle diameter, μm	η	effectiveness factor

in the carrier (Zhou et al., 2013b). Moreover, the conventional carriers are easy to swell and also limit the substrate mass transfer.

Recently, magnetic carrier has shown significant attraction for bacterial immobilization due to low mass transfer resistance and easy operation (Bayramoğlu and Arıca, 2008; Tang et al., 2013). In our previous study (Wang et al., 2013), Fe_3O_4 poly (styrene-glycidyl methacrylate) magnetic porous microspheres (MPPMs), prepared via surfactant reverse micelles and emulsion polymerization method, were introduced to immobilize an iron-reducing bacterium (FD-3). The immobilized FD-3 performed better in Fe(III)EDTA reduction than free FD-3 under unfavorable pH and temperature. However, Fe(II)EDTA-NO, as well as small amount of NO_2^-, NO_3^- and sulfide, formed in the scrubber solution during absorption, may inhibit Fe(III)EDTA reduction in the second stage (Li et al., 2011; Zhang et al., 2009). So, the tolerance of the immobilized FD-3 to these inhibitors needs to be clarified, which is useful for the scrubbing solution regeneration and NO_x removal process design. Furthermore, evaluation of effective diffusion and intrinsic kinetic has not been investigated.

Since the substrate is consumed and formed to the other products by the immobilized bacteria, the substrate must diffuse from the bulk of the solution to some internal location where reaction occurs. This entire scenario is inverted for the products. In this situation, one must consider the intraparticle diffusion processes as well as external mass transfer. Many studies had been conducted for this purpose with immobilized cell or enzyme (Dizge and Tansel, 2010; Do, 1983). Generally, Thiele modulus and effectiveness factor are used to determine diffusion influence and rate-limiting step after immobilization (Bandhyopadhyay et al., 2001; Barranco-Florido et al., 2001). It was pointed out that the effective diffusion was positive correlation with Thiele modulus (Valdes-Parada and Alvarez-Ramirez, 2010). The diffusion transfer in porous media was weakly affected by homogeneous chemical reaction. There were some reports on modeling of the immobilized system, but few on the magnetic porous microspheres immobilized bacteria.

The objective of this study is to investigate the effects of S^{2-}, SO_3^{2-}, NO_3^-, NO_2^-, and Fe(II)EDTA-NO on Fe(III)EDTA reduction, and to develop a kinetic model to evaluate the intrinsic kinetics of Fe(III)EDTA reduction using immobilized FD-3.

2. Methods

2.1. Materials

Glycidyl methacrylate (GMA, 98% grade) was obtained from Aladdin Chemistry Co., Ltd. Styrene (St, AR), divinyl benzene (DVB, 45% grade), hexadecane (HD, AR) and benzoyl peroxide (BPO, CP) were purchased from Tianjin Guangfu Fine Chemical Research Institute. St and DVB were extracted with NaOH solution to remove inhibitors before used. Sodium dodecyl sulfate (SDS, AR), polyvinyl alcohol (PVA-124, degree of polymerization 1700 ± 50), hydroquinone (HQ, AR) and oleic acid (OA, AR) were purchased from Xilong Chemical Co., Ltd. Sorbitan monooleate (Span80, CR) was purchased from Shanghai Qinxi Co., Ltd. Ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous chloride ($FeCl_2 \cdot 4H_2O$), disodium ethylenediaminetetraacetate (Na_2EDTA), sodium sulfate (Na_2SO_4), ammonia ($NH_3 \cdot H_2O$), sodium sulfite (Na_2SO_3), sodium nitrite ($NaNO_2$), sodium nitrate ($NaNO_3$), sodium sulfide (Na_2S) and other reagents were analytical grade.

2.2. Microorganism, media, and preparation of the immobilized FD-3 bacteria

Klebsiella sp. FD-3, a strictly anaerobic bacterium, was isolated from the cultivated mixed culture in our laboratory (Zhou et al., 2013a). The preparation of Fe(III)EDTA solution, phosphate buffer solution, the basal medium, and the cultivation of FD-3 were reported in our previous study (Wang et al., 2013). Fe(II)EDTA-NO solution was prepared by bubbling NO through Fe(II)EDTA solution until full breakthrough of NO was observed in the sparging vessel effluent. Then, the achieved solution was kept in glass serum vials under N_2 protection from oxidation (Li et al., 2007).

FD-3 was covalent immobilized on Fe_3O_4 poly (St-GMA) magnetic porous microspheres (MPPMs). The MPPMs were synthesized via the improved surfactant reverse micelles and emulsion polymerization method, in which, the Fe_3O_4 nanoparticle was modified with OA according to our previous report (Wang et al., 2013). The optimum immobilization conditions were determined as 5 mg/mL microspheres, 0.14 mg/mL FD-3 and 3 day of immobilization time.

2.3. Fe(III)EDTA reduction

Experiments of Fe(III)EDTA reduction were carried out in 100 mL serum bottles at 40 °C shaking at 140 rpm. The bottles contained 60 mL medium, 10 mmol/L Fe(III)EDTA and 8.4 mg (dry weight) of free FD-3 or 0.3 g of MPPMs with 8.4 mg (dry weight) of immobilized FD-3. One comparison experiment was set by adding 0.3 g of blank MPPMs without bacteria, and the other was set without any MPPMs and bacteria. The pH of the medium was adjusted to 7.0 ± 0.1 using 0.1 mol/L HCl or NaOH. The headspace was replaced with nitrogen gas to assure anaerobic conditions. Fe(III)EDTA concentration was determined at regular time intervals. To explore the effects of S^{2-}, SO_3^{2-}, NO_2^-, NO_3^-, and Fe(II)EDTA-NO on Fe(III)EDTA reduction, different concentrations of Na_2S (0, 0.5, 1, 2, 4 mmol/L), Na_2SO_3 (0, 0.5, 1, 2, 4 mmol/L), $NaNO_2$ (0, 0.5, 1, 2, 4,8 mmol/L), $NaNO_3$ (0, 0.5, 1, 2, 4, 8 mmol/L) and Fe(II)EDTA-NO (0, 3, 4, 6 mmol/L) were added respectively to the medium.

2.4. Analytical methods

Ferrous ion and total iron concentrations in the solution were determined using a modified 1, 10-phenanthroline colorimetric method at 510 nm (UV-PC spectrophotometer, Mapada Co., Ltd., Shanghai, China) (Wang et al., 2013). The ferrous ion calibration curve was obtained using ferrous ion standard solutions having EDTA concentrations equal to those used in the reduction experiments. Fe(III) concentration was calculated as the difference between the total iron and ferrous ion concentrations. Fe(II)EDTA-NO concentration was determined by a standard curve for magnitude of spectrophotometric absorbance responses of