Composite nanolayer photocatalyst-biocatalyst *Rhodococcus erythropolis* R1 for desulfurization of dibenzothiophene

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Abstract

A nanolayer of composite and *Rhodococcus erythropolis* biocatalyst was studied for the first time for desulfurization of dibenzothiophene as a model sulfur compound and its performance was compared with that of composite and *R. erythropolis* alone. The nanolayer of composite was synthesized by sol-gel method from ferrous oxalate and zinc oxalate precursors coated on glass by spin coating technique. The structural and morphological properties of the coated sample were characterized by means of X-ray diffraction (XRD), atomic force microscopy (AFM), and field emission scanning electron microscopy (FESEM). The cell debris and crude cell extract enzyme in the presence of zinc ferrit nanolayer was used for the degradation of dibenzothiophene as a model sulfur compound. Dibenzothiophene was degraded to a large extent after 5 min UV irradiation and biocatalytic treatment of enzymes were extracted from *R. erythropolis* R1.

Key words: Composite, Nanolayer, Dibenzothiophene, Biocatalyst, Desulfurization

Highlights

- Combination of zinc ferrite nanolayer and *Rhodococcus erythropolis* as biocatalyst
- Synergic effect for enhanced nanolayer photocatalytic activity under visible light
- High efficiency of dibenzothiophene desulfurization by nanolayer-*Rhodococcus* biocatalyst
Introduction

Fossil fuels have sulfur organic compounds and their combustion release sulfur pollutants into the atmosphere. Sulfur level in diesel oil is 5000 ppm which must be reduced to less than 10-15 ppm (1 & 2). The reduction of the sulfur content in oil is usually carried out by desulfurization. Dibenzothiophene (DBT) has been used as a compound model to study biodesulfurization. DBT is sulfur heterocyclic compound that exists in fossil fuel and is known as an environmental pollutant. There are some reports that microorganisms can degrade DBT in the environment (3-5). DBT may cause a potential health risk in the environment due to its mutagenic and carcinogenic potentiality (6). The natural biological degradation of DBT is difficult which may be due to its hydrophobicity (7). The extracellular enzymes and their catalytic actions produce water-soluble metabolites, which prompt the degradation of DBT. To eliminate sulfur from DBT, biodesulfurization can be used in which microorganisms are able to eliminate sulfur from DBT. Enzymes are used to produce low sulfur content gasoline and diesel oil fractions. Rhodococcus erythropolis is a Gram-positive bacterium that are able to extract sulfur from DBT (8). The excellent photocatalytic activity for degradation of organic pollutants is achieved by the photocatalysts like TiO$_2$ or ZnO. The photocatalytic activity can be improved by the use of coupled semiconductors in which two types of energy-level systems play an important role in achieving charge separation (9-11). Coupling of different semiconductor oxides can reduce the band gap, extending the absorbance range to visible region leading to electron–hole pair separation under irradiation and consequently achieving a higher photocatalytic activity for degradation of organic pollutants (12). The photocatalyst-enzyme coupled system for artificial photosynthesis process has been reported for solar energy conversion for the synthesis of organic chemicals or fuel (13). Choudhury and co-workers have reported a photocatalyst/ enzyme-coupled artificial photosynthesis system that harnesses solar energy through the combination of photocatalysis and biocatalysis (14). A new potentially promising visible-light driven photobioreactor synthesizes fine chemical via photobiocatalysis is reported by generating NADH in a non-enzymatic light-driven process and coupling it to the enzymatic dark reaction catalysis (15). A new photocatalyst has been used as a biomimetic catalyst to degrade Rhodamine B in aqueous solution (16). Improving the photocatalytic properties of rayon fibers containing a titanium dioxide photocatalyst through enzymatic treatment has been reported by Takahashi et al (17). Little work has been developed about photocatalyst/ enzyme coupled systems. Considering another approach to assist the catalytic action of the enzyme, the possibility to enhance the oxidation by the use of a photocatalyst is proposed. This system could be implemented in the desulfurization of sulfur containing fossil fuels. There are no reports in which zinc
ferrite nanolayer photocatalyst/biocatalyst *Rhodococcus erythropolis* R1 is used for desulfurization processes.

In continuation of our research in nanocomposite thin film (18), here we report for the first time the desulfurization of DBT by a coupled zinc ferrite nanolayer photocatalyst/biocatalyst *R. erythropolis* R1 was investigated for desulfurization of DBT. For this study nanolayer of zinc ferrite was synthesized, coated on glass and structural and morphological properties were characterized by means of XRD, DRS, AFM, and FESEM. The cell debris and crude cell extract enzyme from *R. erythropolis* R1 in the presence of zinc ferrite nanolayer was used for the degradation of DBT as a model sulfur compound.

**Material and Method**

**Fabrication of zinc ferrite nanolayer and characterization:** Iron nitrate nonahydrate, 2.45 g added into zinc sol (zinc acetate monohydrate, 3.1 g as precursor was dissolved in the mixture of isopropyl alcohol, 15 mL as a solvent, and mono ethanolamine, 0.86 mL) with vigorous stirring to obtain a uniform sol. The sol was aged for 2 h at ambient temperature in a closed vessel. The mixture of zinc sol and iron sol with molar ratios of 1:1 was prepared by mixing the solutions and spin coating on glass in air. The produced solids were thermally treated in air at 450°C to give ZnFe$_2$O$_4$ nanolayer coated on glass. The borosilicate glass substrate was coated using spin coating method (Spin Coater, Modern Technology Development Institue, Iran). The crystallization of titania thin film was studied by XRD analysis using a DB. DBT content was analyzed by a UV–vis spectrophotometer (Shimadzu, Japan, UV-160A). Morphology was analyzed by using a FE-SEM, Hitachi, model S-4160. A C26 DME atomic force microscope (AFM) was used to examine the surface morphology.

**Crude enzyme extracts preparation:** *R. erythropolis* R1, a biocatalyst with desulfurization ability was grown on basal salt medium (BSM) containing DBT (0.3 mM) as the sole sulfur source (500 ml medium in 2 L Erlenmeyer flask Meyer) and glucose (10 gL$^{-1}$) as the sole carbon source, incubated for 80 h at 30°C on a shaker (8). The cells were harvested by ultracentrifuge (model Mistral 41) at 4000 rpm for 20 min then the precipitate washed twice with phosphate buffered saline (pH 7), centrifuged and resuspended in the same buffer to get absorption of 250 at 600 nm. A treatment of lysozyme (10 mg mL$^{-1}$) and incubation at 37°C for two hours was useful to break the cells. After pretreatment, the cells were centrifuged at 4°C in 3400 rpm, and 20 g of wet weight of cells were suspended in 40 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 10% of glycerol, 1 mM dithiothreitol (DTT). Phenyl methyl sulfonyl fluoride (PMSF) buffer was used to prevent protease activities, 1 mM of PMSF solution was added and freeze the suspension. The cells and enzyme extract were prepared using 10 cycles of 30 sec rest intervals on ice by ultrasonication (Hielscher UP200S, 24 KHz, 200W). Broken cell suspension was centrifuged in a refrigerated unit (Apndrvf Model 5810R).
with 12000 rpm for 10 min at 4°C. Supernatant was separated from the cell debris and the soluble intracellular enzyme extracts were analyzed for protein content using the Bradford method. The cell debris and crude cell extract enzyme were tested for sulfur removal of DBT (19 & 20).

Desulfurization of dibenzothiophene by coupled zinc ferrite nanolayer photocatalyst/biocatalyst *R. erythropolis* R1: 100 mM potassium phosphate buffer (pH 7.4) containing 0.3 mM DBT and crude enzyme extract was added in a sterile Petri dish (10 cm$^3$), then nonolayer slide merged in the suspension in the Petri dish and incubated at 30°C. To investigate the effect of light on the reaction, the mentioned Petri dishes were incubated in the presence of light and at dark with nanolayer. In a first experiment nanolayer and DBT buffer were kept under UV irradiation for 5 min followed by addition of enzyme and incubated at 30°C for 15 min.

**Results**

X-ray and FESEM analysis: The phase and purity of the zinc ferrite nanolayer was examined by XRD pattern and is shown in Fig. 1. The patterns were indexed to the cubic spinel structure belonging to zinc ferrite (22) having lattice constant $a = 8.442$ Å. All of the peaks match well with Bragg reflections of the standard spinel structure (space group Fd3hm). Three main peaks were observed with orientations along the (3 1 1), (4 0 0) and (5 1 1) directions at $2\theta = 35^\circ$, $43^\circ$ and $52^\circ$, respectively, in all the films. Absence of any peak from zinc oxide, ferrous oxide or any other oxide phases confirmed the formation of phase pure zinc ferrite. The zinc ferrite nanostructure has a high degree of crystallinity. The average grain size is found to be about 40 nm, which is in good agreement with the result obtained by FESEM (Fig. 2). In zinc ferrite Zn ions are four coordinated and Fe ions six coordinated by nearest oxygen anions (21-23). The particle size for each of the samples was calculated using the Scherrer equation and are in good agreement with FESEM values (Fig. 2) (24-27). The average crystallite size (D) of the films was estimated from XRD data using the Scherrer formula $D = \frac{0.9\lambda}{\beta \cos \theta}$, where $\lambda$ (1.540 Å) is the wavelength of the used x-rays, $\beta$ is the broadening of the diffraction line measured at the half maximum intensity in radians and $\theta$ is the angle of diffraction (28 & 29).

AFM studies: The surface morphology of the zinc ferrite nanolayer was analyzed using AFM. Fig. 3 shows a two dimensional AFM image of the zinc ferrite nanolayer grown on glass substrate. It can be seen that the zinc ferrite nanolayer exhibits a dense microstructure compared.
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Fig. 1- XRD patterns of zinc ferrite nanolayer annealed at 500°C

Fig. 2- FESEM image for zinc ferrite nanolayer annealed at 500°C
Fig. 3- Two-dimensional AFM image of the zinc ferrite nanolayer deposited on glass at 500 °C.

Fig. 4- Comparison of photocatalytic and enzymatic degradation in desulfurization of dibenzothiophene by zinc ferrite nanolayer and intracellular enzymes coated on glass at different conditions; LA, uncoated glass slide only; ZFA, glass slide coated with zinc ferrite nanolayer; ZFB, glass slide coated with zinc ferrite nanolayer and enzyme; ZFC, glass slide coated with zinc ferrite nanolayer and UV irradiation; ZFD, glass slide coated with zinc ferrite nanolayer, enzyme and UV irradiation; ZFE, glass slide coated with zinc ferrite nanolayer at dark.
Degradation of DBT: The degradations of DBT by zinc ferrite nanolayer coated on glass and intracellular enzymes were monitored at various time intervals. The results showed that DBT was degraded rapidly after 5 min (Fig. 4). Zinc ferrite nanolayer was grown on glass substrate and intracellular enzymes were decomposed most of the DBT in a very short time. The effect of zinc ferrite nanolayer grown on glass substrate in UV light on the degradation of DBT was investigated and the results indicated that initial DBT concentration was degraded as 89% (Fig. 4, ZFD). This indicated that the photocatalytic-biodegradation occurred (30 & 31). As shown in Fig. 4, degradation of DBT was increased sharply to about 89% using zinc ferrite nanolayer photocatalyst coated on glass and intracellular enzymes (32-35). Removal of DBT by simultaneous photocatalytic-enzymatic treatment of DBT with zinc ferrite nanolayer-R. erythropolis biocatalyst was significantly increased as compared with the immobilized zinc ferrite or R. erythropolis biocatalyst alone. The high efficiency of the coupled degradation process provided a novel strategy for degradation of DBT (27, 36-38).

Discussion and Conclusion
Zinc ferrite has been frequently investigated as visible light active nanocomposite photocatalyst. In this study the nanolayer of zinc ferrite was synthesized by sol-gel method coated on glass by spin coating technique. The structural and morphological properties of the coated sample were characterized by means of XRD, AFM and FESEM. A combination of zinc ferrite nanolayer and R. erythropolis as biocatalyst demonstrated the synergic effect for enhanced photocatalytic activity under visible light in comparison with zinc ferrite nanolayer and R. erythropolis biocatalyst alone. Various spectroscopic characterization methods confirmed the visible light absorption of zinc ferrite nanolayer. The coupled zinc ferrite nanolayer-R. erythropolis biocatalyst showed high efficiency and promising applications in desulfurization of petroleum.

Acknowledgements
This work has been financially supported by Iran National Science Foundation: INSF (Project no. 90008170) and the University of Isfahan.

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فناوری زیست محیطی ایران، سال اول، شماره 2، پاییز و زمستان 1394

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چکیده
در پژوهش حاضر، برای تخمین برای نانونیا کامپوزیت و بیوکاتالیست رودوکوکوس اریتروپولیس R1، سولفورزدایی از دی بنزوتیوفن به عنوان ترکیب مدل سولفور مطالعه و کارآیی آن با کامپوزیت و رودوکوکوس اریتروپولیس گزارش می‌شود. نانونیا کامپوزیت به وسیله روش sol-gel از پری کورسراه از اگزالت فر و اگزالت روش سنتی داده و با روش اسپین کوپینگ روش شیشه پوشش داده شده است. برای تولید نمونه پوشش داده شده با روش اشکاری (XRD)، میکروسکوب بیونی اتومیک (AFM)، و میکروسکوب الکترونی (FESEM) بررسی شده است. لاشه‌های سلولی و عصاره خام آنیمی سلول در حضور نانونیا فریت- روي برای تجزیه دی بنزوتیوفن به عنوان ترکیب مدل گوردی به کار برده شده است. در نمونه‌های پس از 5 دقیقه تایش اشعه UV و تماشای بیوکاتالیست آزمایش استخراج شده از رودوکوکوس اریتروپولیس به میزان بالایی تجزیه شده است.

واژه‌های کلیدی: کامپوزیت، نانونیا، دی بنزوتیوفن، بیوکاتالیست، سولفورزدایی

*نوسنده مسئول مکاتبات
تاریخ دریافت: 92/06/07- تاریخ پذیرش: 92/08/12