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Visible light photocatalytic inactivation of *Escherichia coli* by natural pyrite assisted by oxalate at neutral pH

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ABSTRACT

Escherichia coli is the main microbial indicator in water. The enhancement of visible light photocatalytic inactivation of bacteria using oxalate – natural pyrite was evaluated in order to prevent development of disinfection byproducts and cost-effective operation. Influences of operating parameters include light intensity, pyrite dosage, particle size, oxalate concentration; pH, O_2, H_2O_2 and inorganic ions were evaluated in a batch environment. Increase of light intensity, O_2 flow, H_2O_2 concentration, smaller particle size and increased pyrite dosage boosted the inactivation rate of *E. coli* through more generation of reactive spices (RSs). Oxalate increased inactivation rate of *E. coli* via increasing release of iron, preventing formation of oxy layer on pyrite surface, high quantum yields of Fe^{+3} – organic ligand, enhancing permeability of *E. coli* membrane, increasing pH range close to neutral and visible light region. The scavenging tests confirmed participation of OH_b, OH_s and $\cdot O_2^-$ as dominant RSs in the system. The influence of inorganic ions on inactivation rate of *E. coli* was obtained as $F^- > HCO_3^- > Ca^{+2} > NO_3^- > Cl^- > SO_4^{-2}$. The regrowth of *E. coli* was not observed in the optimum condition. This work suggests that oxalate – natural pyrite complex under light-emitting diode visible light can be a proper alternative for disinfection of natural water.

1. Introduction

Microbial contamination of water is a worldwide problem that has led to serious public health concerns such as typhoid fever, cholera, amoebiasis and etc. among consumers [1,2]. The World Health Organization (WHO) reported that over 80% of all diseases are due to lack of access to safe water [3]. *Escherichia coli* (*E. coli*) is an important indicator of water-borne diseases among the total coliform bacteria [4,5]. Presence of *E. coli* in water indicates health risks of other bacteria and viruses [6,7]. Conventional disinfection methods such as chlorination and ozonation are the most widely used approaches in disinfecting water [5,8]. Despite the effectiveness of these methods, the reaction with organic materials can produce a wide range of disinfection byproducts (DBPs) [9,10]. Most of DBPs including nitrogenous side products (HANs and HNMs) have serious adverse effects on human health such as carcinogenesis, mutagenesis and abortions [11]. In order to reduce of these drawbacks, the photocatalytic disinfection process has been proposed to be used as a proper alternative for water disinfection [12]. Natural photocatalysts excited by visible light (VL) can be a more potent potential for usage in the disinfection process [13]. Natural Pyrite (NP) is a widely available natural photocatalyst which can produce hydrogen peroxide and hydroxyl radicals in presence of water [14]. However, hydrogen peroxide and reactive species (RSs) generation is limited at near natural pH due to formation of oxide layer on the pyrite surface, blocking functional groups and lack of releasing iron ions [15].

Also, pyrite has some photocatalytic properties include great optical property suitable band gap (Eg direct −1.03 eV; Eg indirect −0.95 eV), high absorption coefficient (105 cm^{-1} for $h\nu > 1.3$ eV) and appropriate minority carrier diffusion length (100–1000 nm) which makes it competitive in many photoelectric devices [16].

Organic ligands lead to enhancement of photocatalytic activity of pyrite through avoiding formation of oxide layer and increasing iron solubility [17]. Organic ligands also can form ferrous ions from ferric – chelate complexes via Ligand to Metal Charge Transfer (LMCT) mechanism, which finally leads to production of ligand radicals and RSs [18]. Organic ligands must be non-toxic for environmental application, exist in high amounts in aqueous solutions and react with hydroxyl radical less [17]. Accordingly, oxalate was selected as an organic ligand which has a high iron solubility at neutral pH conditions and high photochemical activity in natural waters under sunlight [19,20].

Moreover, oxalate is a naturally occurring compound in the aqueous solution and produced by plants, fungi and some bacteria [21]. In the previous studies, ferrioxalate was used for removing contaminants (e.g. bacteria, antibiotic, organic pollutants and etc.) [22–26]. A high

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concentration of iron salts is required to generating higher levels of hydroxyl radicals in the photo-Fenton process. Increased concentration of iron salts competes with the hydroxyl radical itself leading to diminished rate of bacteria inactivation [18]. In contrast to iron salts, pyrite regularly increases the RSs generation through continuous release of ferrous ions from catalyst surface, leading to controlled formation rate of RSs [27]. A visible light-emitting diode (LED) lamp was used as lighting source due to energy-saving and more specific wavelength compared to other light sources [19]. This study was carried out to evaluate the visible light photocatalytic inactivation of *Escherichia coli* by natural pyrite assisted by oxalate at neutral pH.

2. Materials and methods

2.1. Chemicals and reagents

The NP was obtained from university of Tehran, Department of Mine Engineering, crushed by machine and milled, ultra-sonicated in ethanol (95%) for 5 min and washed with nitric acid (1 M). Afterwards, it was rinsed several times with deionized water and then with ethanol, and finally dried at 30 °C [27]. All NP particles were ground into powder and then sieved through mesh 200 (74 μm). Oxalic acid (Catalogue Number 8161440050), an organic ligand, was purchased from Merck with the highest purity, and directly used in the experiments with no further purification [28]. The pH was adjusted by Sodium hydroxide (NaOH, 98%) and hydrochloric acid (HCl, 36.5%).

2.2. Bacterial strains and growth media

E. coli ATCC 25922 was used as the target bacterium and provided by Pasteur Institute of Iran. The *E. coli* was inoculated into 50 mL of Nutrient Broth (No. 1, for microbiology-Sigma-Aldrich) and incubated at 37 °C for 18 h in order to achieve a stationary phase. Afterwards, cells were harvested by centrifugation at 3000 rpm for 15 min. Then bacterial pellet was washed with sterilized saline (0.9% NaCl) solution two times in the centrifuge apparatus. The final cell density was adjusted to 1×10^7 cell mL^{-1} via 0.5 Mac Farland standards [29,30].

2.3. Bacterial inactivation kinetics

Kinetic inactivation of bacteria was investigated according to first order kinetics proposed by Chicks-Watson model (Eq. 1).

$$N_t = N_0 e^{-kt} \quad (1)$$

where, N_0 is the density of *E. coli* as determined by viable count prior to irradiation, N_t is density of *E. coli* as determined by viable count after irradiation, t is the irradiation time (at a constant light flux) and k is the first order inactivation rate. Accordingly, the data was expressed over time (cfu mL^{-1} vs. time) and fitted into the log-linear-regression to determine the inactivation rate (k_{obs} (min^{-1})). The fitting was conducted using GInaFIT software suggested by Geeraerd et al. [31]. Finally, k_{obs} was used to compare inactivation between the different treatments tested.

2.4. Photocatalytic inactivation of bacteria under visible light

A total of 100 mL sterilized saline solution containing 0.3 g NP and oxalate (0. 5 mM) was homogenized by sonication at 35 kHz for 1 min with a sonicator (BANDELIN electronic, Germany). Thereafter, the bacterial colony was transferred to a photocatalytic reactor and initial cell density was adjusted to 10^7 cfu mL^{-1}.

The photocatalytic reactor containing the suspension was exposed to VL irradiation (400–600 nm), provided by LED lamp (λ_{max} = 450 nm).

One hundred μL aliquots of the sample was collected during certain intervals within the inactivation time and diluted to 10-fold concentration gradient serially with a saline solution. Then, 0.1 mL of the diluted sample was immediately spread onto the Nutrient Agar plates (Lab M, Lancashire, UK). Then, the plates were incubated at 37 °C for 24 h for the enumeration of cell survival in cfu mL^{-1}.

Various control experiments (without NP, oxalate and light) were conducted in order to evaluate of NP, oxalate and light effects on the bacteria inactivation. Moreover, the influence of O_2 (1–4 L/min) and H_2O_2 (0–2 mM) under VL photocatalytic condition was investigated for better understanding the role of RSs in the bacteria inactivation [2, 32].

2.5. Regrowth ability of bacteria

The regrowth ability of bacteria to perform self-repairing was investigated after inactivation process to evaluate the residual effect of the system. For this purpose, the photocatalyst was recollected and the supernatant was transferred to a new flask and incubated in darkness at room temperature for 24 h to determine the number of viable cells through viable plate count method.

2.6. The effect of inorganic ions on the photocatalytic process

Natural water contains different inorganic ions which can affect photocatalytic disinfection process. Therefore, HCO_3^-, F^-, NO_3^-, SO_4^-, Cl^- and Ca^{+2} ions were selected to simulate inorganic ions commonly exist in natural water [33,34].

2.7. Determination of the cell membrane damage

Cell membranes damage was evaluated by scanning electron microscope (SEM) technique (SEM, PHILIPS, S360, and Mv2300) before and after the photocatalytic inactivation of *E. coli* in order to observe the cell destruction directly [13].

2.8. Scavengers study to evaluate the inactivation mechanism

Scavenger studies were evaluated to detect the dominant RSs responsible for bacterial inactivation. Accordingly, *tert* – buthanol (0.5 mmol/L) and Cr(VI) (0.05 mmol/L) were applied as the specific scavengers of $\cdot OH_b$, e^- and $O_2^{\cdot-}$ respectively. In addition, KI (0.5 mmol/L) was used as scavenger of the h^+ and $\cdot OH_s$ [13,35,36].

2.9. Iron and H_2O_2 measurement

The total iron was analyzed by atomic absorption spectroscopy (GBC System 5000, Australia) [37]. H_2O_2 concentration was measured at 510 nm by a spectrophotometer (DR6000, HACH, Germany) based on DPD HRP-catalyzed oxidation with a detection level of 0.2 μg/L [38].

3. Results and discussion

3.1. Optical spectra of Fe-Oxalate complex

Fig. 1 depicts the spectral properties of Fe-Oxalate. The maximum absorption band was 450 nm for Fe-Oxalate. The result revealed that the maximum adsorption for Fe-Oxalate complex lies on UV–vis region (300–450 nm).

3.2. Photocatalytic bacterial inactivation

3.2.1. The effect of visible light (VL) intensity

Pyrite is a semiconductor with a small gap band (0.95 eV) which can be excited by visible light and generate photoinduced electron/hole pairs. Fig. 2 illustrates the effect of VL intensity on bacterial inactivation.