



Performance study of open channel reactor on AO7 decolorization using glucose oxidase/TiO₂/polyurethane under UV–vis LED



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ABSTRACT

An open channel photo-bioreactor irradiated with visible and UVA light-emitting diode (UVA–vis LED) was evaluated in a recirculated mode. The novel process was composed of TiO₂ nanoparticles/polyurethane (PU) and glucose oxidase (GOx)/TiO₂/PU. The ability of LEDs as a light source in decolorization of acid orange 7 (AO7) was investigated using the mentioned simultaneous method. Furthermore, decolorization of AO7 was studied under various conditions including different feed flow rates and glucose concentrations in the photo-bioreactor. The results demonstrated that application of GOx/TiO₂/PU photo-biocatalyst under UVA-LED and Blue-LED was technically feasible due to high percentage (>99%) of decolorization in 45 and 90 min with recycling rate of 10 mL/min, respectively.

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1. Introduction

Globally, environmental pollution has become a threatening menace to the survival of life on the Earth. Among all the pollution-causing effects, waste water released from different segments of the society is one of the most primary causes. The colored water caused by the presence of dyes can block both sunlight penetration and oxygen dissolution, which are essential for aquatic life [1]. Removal of dyes from waste water has been given much attention in the last few decades by adopting different effective traditional technologies, including physical, chemical, and biological treatments and also the techniques, based on high energy UV light, such as advanced oxidation processes (AOPs) [2,3]. TiO₂ nano particle, as one of the well-known nano-materials, has great promise in environmental pollution control due to its high photocatalytic activity, stability, and almost harmless nature. The AOP with TiO₂ nanoparticles as a photo catalyst can degrade complex structures with free radical attack, which produces less poisonous products. However, at present, TiO₂ nanoparticle is still difficult to be freely applied in practice on the account of its lack of reusability, which results in cost and environmental pollution [4]. Sponge-type polyurethane (PU) was selected as a carrier of photo catalyst to inhibit catalyst separation. PU as a carrier had merits of high

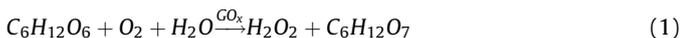
porosity, low cost, commercial supply, and ease of usage, reusability and replacement. Despite these advantages, only a few studies have been used PU as a carrier [5]. Additionally, use of TiO₂ nanoparticles in LED photo reactor has increasingly attracted researchers' attention due to its several merits, such as monochromatic light, long lifetime (more than 100,000 h in comparison with 8000 h lifetime of Hg lamps), energy efficiency, flexible configuration, and small footprint (no Hg waste) [6]. Decomposition of chemicals such as o-cresol, aniline by TiO₂ nanoparticles, and bisphenol-A by N₂ doped TiO₂ were reported and decolorization of dyes by TiO₂ nanoparticles such as Reactive Red 22, methyl orange, and Rhodamine B were studied under LED lamps [7–12].

The second most extensively used way to treat waste water is biological. This method is a relatively inexpensive way to remove pollutants and accounts for “green technology” [13]. The main disadvantage of the biological method is related to its long treatment period [14,15].

Recently, hybrid methods using coupling photochemical and biochemical treatment have been highly focused on and proven to be more efficient for degradation of pollutants [16]. These newborn methods are categorized into sequential and simultaneous manners. Simultaneous coupling is preferred to sequential one due to diminishing operation time, a serious and critical problem in water treatment, which leads to noticeable decrease in cost. To achieve an efficient coupling process, enzymes are preferred to microorganisms due to having higher efficiency, convenience of storage and handling [17–19]. Glucose oxidase (GO_x) is one of the

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enzymes that has attracted great interest because of its robustness and stability [20]. It catalyzes inexpensive reactants (glucose and air) into hydrogen peroxide, which is an oxidant added to AOPs to improve pollutant removal efficiency (Eq. (1)):



Therefore, the objective of this article was to produce *in-situ* hydrogen peroxide by GO_x in open channel circulating photo-bioreactor for AO7 decolorization under LED lamps. To the best knowledge of the present authors, simultaneously photochemical (TiO_2/PU) and enzymatic (GO_x) decolorization of acid orange 7 (AO7) under UVA-LEDs and Blue-LEDs have not been reported. Furthermore, kinetic study of AO7 decolorization was done. The results revealed competitive role of photocatalytic-enzymatic process under LED lamps in decolorization of AO7. Photo-bioreactor parameters (feed flow rate, glucose concentration) were optimized to obtain good understanding of effective operation parameters in the photo-bioreactor.

2. Materials and methods

2.1. Chemicals

TiO_2 nanoparticles (commercial Degussa P25) was a mixed phase containing 80% anatase and 20% rutile with average crystal size of 21 nm and specific surface area (analyzed by BET) of 50 m²/g. Methanol (99.9%) was used to prepare TiO_2 suspension; glucose oxidase (EC 1.1.3.4, from *Aspergillus niger*), β -D-glucose, KI (99.99%), NaOH (99.99%), potassium hydrogen phthalate (KHP), and ammonium molybdate tetrahydrate ($Mo(VI)\cdot 4H_2O$) were obtained from Sigma-Aldrich. Hydrogen peroxide (30% (v/v)) was purchased from Merck AG. Acid orange 7 (AO7, ShimiBoyakh-saz Co. (Iran)) solutions were prepared by dissolving pigment in distilled water.

2.2. $GO_x/TiO_2/PU$ preparation method

The photo-biocatalyst was prepared in two sequential steps. In the first step, an specific amount of TiO_2 nanoparticles was suspended in 23 mL of methanol and sonicated for 15 min by Sonoplus Ultrasonic Homogenizer HD 2200 (Germany). The solution was poured into pre-treated PU, which was formerly submerged in NaOH solution (0.1 N). It was then allowed to dry at 70–80 °C. To accomplish better attachment, TiO_2/PU was covered with aluminum foil and kept at mentioned temperature for 5 h. The coated PU by TiO_2 nanoparticles was thoroughly washed with distilled water twice to remove weakly attached particles. The amount of trapped TiO_2 nanoparticles could be calculated by subtracting dry weight of TiO_2/PU from dry weight of PU. The ratio of immobilization was calculated by Eq. (2). TiO_2 immobilization ratio was >95% with about 0.250 g of TiO_2 nanoparticles.

Ratio of TiO_2 immobilization

$$= \frac{\text{Initial weight of } TiO_2 - \text{entrapped weight of } TiO_2}{\text{Initial weight of } TiO_2} \quad (2)$$

Diverse strategies have been used for immobilization of GO_x [21]. The method of physical adsorption for GO_x immobilization was selected due to less enzyme denaturation, high activity, and the ease of procedure [22]. For the second step of photo-biocatalyst preparation, the enzyme solution with concentration of 0.1 mg/mL in phosphate buffer with a pH value of 6.15 was prepared; studies have shown that more or less concentration of GO_x than 0.1 mg/mL leads to lower enzymatic activity [23]. The enzyme solution was poured into the prepared porous photo catalyst, and the mixture

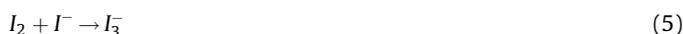
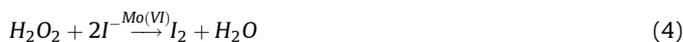
was incubated at speed rate of 170 rpm for 1 h at 37 °C. This time was enough to allow the enzyme to reach equilibrium in porous photo catalyst. After immobilization of the enzyme, the supernatant was separated and kept at 0–4 °C to study GO_x immobilization on TiO_2/PU . The amount of immobilized GO_x was determined by measuring the concentration of free enzyme in the supernatant by a UV-vis spectrophotometer (1700 UV-vis Shimadzu, Japan) and using a calibration curve, which was constructed with different known concentration of GO_x solution. The amount of immobilized GO_x was obtained by Eq. (3). Under the mentioned conditions, more than 85% of the enzyme was immobilized.

$$\text{Immobilized } GO_x = \left(1 - \frac{C}{C_0}\right) \times V \quad (3)$$

where C_0 and C are initial GO_x concentration in the solution and supernatant, respectively. V is total volume of the solution.

2.3. GO_x activity measurement

Glucose oxidase, as a catalyst, oxidizes β -D-glucose to β -D-glucono- δ -lactone and *in-situ* hydrogen peroxide (Eq. (1)). A spectrophotometric technique was used to measure activity of GO_x in the presence of glucose, in which generation of *in-situ* hydrogen peroxide was measured. In the current study, the method was based on the formation of I_3^- to determine the concentration of H_2O_2 according to Eq. (4)–(5) [24]. In this technique, 100–200 μ l of the enzyme solution (or supernatant solution) was mixed with glucose solution (20 mM). The mixture was incubated for 1 min, and the equiweight (with weight ratio of 1:1) of solution A (33 g of KI, 1 g of NaOH, 0.1 g of ammonium molybdate tetrahydrate) and B (10 g of KHP) were immediately added to the mixture of glucose and enzyme. Once the whole mixture was incubated, a sample was taken in a spectrophotometric cell. The amount of I_3^- present in the mixture was determined by a UV-vis spectrophotometer, equipped with a computer for data acquisition at the wavelength that the mixture showed maximum adsorption ($\lambda_{max} = 351$ nm). The concentration of hydrogen peroxide could be determined by a standard calibration curve. One unit of GO_x activity (U) was defined as the amount of the catalyst required for the production of 1 μ mol H_2O_2 in 1 min at 25 °C.



2.4. LED photo-bioreactor configuration

The rectangular open channel photo-bioreactor and schematic diagram of the process are shown in Fig. 1. The channel had length of 12 cm, inner width of 4 cm, and height of 4 cm. The highly porous photo-biocatalyst with TiO_2/PU ratio of 0.4 was fixed at the bottom of the photo-bioreactor. The photo-bioreactor was equipped with a thermometer at inlet and outlet and sampling valve at the outlet. The dye solution was recycled by means of a peristaltic pump (Heidolph, Germany) at constant rate. Furthermore, the holding volume in the reactor, tubes and pump was 70 mL. Two sets of experiments were conducted under two kinds of LED lamps (UVA-LEDs and Blue ones). By switching on the LED lamps, the photocatalytic-enzymatic process started.

For the first sets of the experiments, the UVA-LEDs (*p-n* junction devices made of indium gallium nitride (InGaN), SUNRISE LED Co., Iran) were utilized. The UVA-LED lamps had diameter of 5 mm and peak emission wavelength between 390 and 395 nm. Directivity of these LEDs was 20° for 50% of total irradiation energy. Light intensities were 3–3.5 mW·cm⁻² at 20 mA. Twenty-two numbers of UVA-LED in the array of 2 × 11 were adhered to the

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