Harnessing the Potential of GOD-catalysed reactions: A Breakthrough Approach for Enhancing Biological Wastewater Treatment

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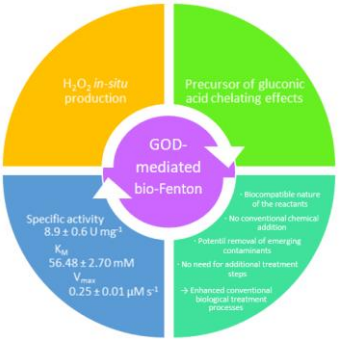

Conventional wastewater treatment faces challenges in removing recalcitrant contaminants. This research explores an innovative approach to address these challenges using glucose oxidase (GOD) as a precursor for *in-situ* production of H_2O_2 and gluconic acid. Michaelis-Menten kinetics highlight the production rates of 14.9 µM·min^{-1} active even after 48h of reaction. This approach not only highlights the eco-friendly nature of the reactants but also mitigates the reliance on conventional chemical additives by leveraging the natural presence of iron and glucose in wastewater. GOD-mediated bio-Fenton can be a feasible alternative with high potential to improve the efficiency of WWTPs on the removal of emerging contaminants, reduce the need for additional treatment steps, and address concerns related to environmental impact of emerging contaminants.

Introduction

Biological reactors play a pivotal role in wastewater treatment, effectively targeting organic matter. However, challenges persist in eliminating recalcitrant compounds (*i.e.* pharmaceutical drugs, personal care products, pesticides) [1]. Characterized by exceptional chemical stability, reactions involved in biodegradation are very slow. Consequently, Wastewater Treatment Plants (WWTPs) are *hotspots* for the spread of these pollutants into the environment. Addressing this issue requires upgrading WWTPs or implementing tertiary treatment methods to enhance effluent quality [2].

Advanced Oxidation Processes (AOPs) have been widely investigated and successful strategies have made these processes more attractive and effective under different conditions (matrices, operation, etc.) [2]. However, from an engineering standpoint, cost-effectiveness is a major concern, and high costs can hinder the application of AOPs.

Bio-Fenton, an innovative alternative to Fenton reaction, could ideally complement conventional biological treatment. This approach is a sustainable process for *in-situ* production of hydrogen peroxide (H_2O_2) through enzyme-catalysed reactions. Specifically, glucose oxidase (GOD) catalyses the oxidation of D-glucose to gluconic acid, simultaneously generating H_2O_2 as a by-product while using molecular oxygen as an electron acceptor (Eq. 1) [3].

$$C_6H_{12}O_6 + H_2O + O_2 \xrightarrow{GOD} C_6H_{12}O_7 + H_2O_2 \quad (1)$$

Resultant H_2O_2 can directly act as an oxidizing agent for organic and inorganic molecules. However, in the presence of a source of iron ions, H_2O_2 may act as a supply for Fenton (Eq. 2) or Fenton-like reaction (Eq. 3) [3].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + HO^- \quad (2)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO\bullet + H^+ \quad (3)$$

The highly reactive radicals formed in the process can be effective in the removal of hazardous organic and inorganic pollutants from wastewater [2].

Despite the promising advantages of bio-Fenton, its application in wastewater treatment is a relatively recent trend, with research mainly directed on dye effluent treatment. In addition, existing studies often overlook fundamental enzyme kinetic parameters. Furthermore, these studies usually apply free-state enzymes under unrealistically high activities [4]. Thus, this study represents a pioneering effort to improve conventional biological treatment by investigation immobilized GOD, commonly employed in the food industry. The kinetics investigation aims to uncover the potential application of GOD-catalysed reactions in real-world WWTPs.

Material and Methods

The Michaelis-Menten kinetics constants for GOD activity was measured based on the enzymatic reduction of benzoquinone to hydroquinone. The reaction catalysed by GOD (0.05 g·L^{-1}) in the presence of different glucose concentrations (1.5 mM to 1 M) was performed at pH 5.0 and 25°C. GOD activity was measured spectrophotometrically by the increase in the absorbance of the product formed (*i.e.* hydroquinone - ε_{290nm} = 2.31 mM^{-1}·cm^{-1}), recorded minute by minute up to 48h of reaction. The A_{290nm} = f(t) function (Eq. 4) is utilized to find the kinetics parameters and the enzymatic activity [5].

$$GOD_{activity} = \frac{\left(\Delta A_{290nm}/_{t}\right).Volume\ of\ reaction}{l_{(1\ cm)}.\varepsilon_{290nm}.Volume\ of\ GOD} \quad (4)$$

Results and Discussion

The analysis of the enzyme activity allowed for the determination of the specific GOD activity, defined

as the amount of enzyme per min that oxidizes 1µM of glucose to gluconic acid and hydroquinone in the presence of benzoquinone as an electron acceptor, at pH 5.0 and at 25°C [5].

An enzyme activity of 8.9 ± 0.6 $U \cdot mg^{-1}$ was found for a constant glucose concentration (100mM), and a range of GOD concentrations from 0.01 to 1 $g \cdot L^{-1}$. Although free-state enzymes, with specific activity in the range of 450 to 1000 $U \cdot mg^{-1}$ are usually applied in bio-Fenton studies [6], some practical perspectives must be considered. For instance, free-state enzymes are usually unstable, with a possible denaturation at higher H_2O_2 concentrations presenting a short half-life (30 min at 37°C) and has an additional enzyme denaturation step after treatment. Moreover, losses of enzymes cause additional operational costs [4,6]. Despite the decreased enzymatic activity, immobilized GOD showed great stability, with activity even after 48h of reaction. Besides, the slow and continuous supply of H_2O_2 might alleviate the self-quenching of •OH radicals by a high concentration of H_2O_2 [7]

Furthermore, the investigation of enzyme activities as a function of glucose concentration (Table 1) led to a K_M (Michaelis–Menten constant) value of 56.5 ± 2.7 mM and a maximum velocity (V_{max}) at 0.25 ± 0.01 $\mu M \cdot s^{-1}$. In addition, the dependence of the reaction rate by concentration of the total active enzyme (v/E_t) *vs.* glucose concentration (Figure 1) resulted in high correlation (R^2 = 0.998) according to Michaelis–Menten equation. Accordingly, the K_{cat} (first-order rate constant) obtained is 0.79 ± 0.01 s^{-1}, with a K_{cat} K_M^{-1} resulting number of 0.01 $mM^{-1} \cdot s^{-1}$.

Table 1. Average GOD-catalysed reaction velocity (V_{med}) observed as a function of glucose concentration.

[Glucose] (mM)	1000	800	600	400	200	100	50	25	12.5	6	3	1.5
V_{med} ($\mu M \cdot s^{-1}$)	0.235	0.233	0.227	0.219	0.192	0.154	0.118	0.073	0.047	0.034	0.011	0.000 [a]

[a] no enzymatic activity was observed.

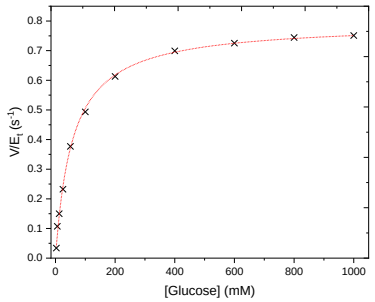

Figure 1. Plot of v/E_t versus [Glucose] according to the Michaelis–Menten equation.

The K_{cat} number indicates the efficacy and affinity of GOD for the glucose substrate at an oxidation energy level of 0.01 $mM^{-1} \cdot s^{-1}$. The K_M value is close to the values reported in the literature [8,9]. Furthermore, the *in-situ* production achieves 14.9 µM of H_2O_2 per minute (*i.e.* 0.5 $mg \cdot L^{-1}$) with an activity time higher than 240 min. Typically, H_2O_2 additions ranged from 10-160 $mg \cdot L^{-1}$ with a reaction time ranging from 30-240 min [2]. Besides that, different *in-situ* H_2O_2 production systems achieve lower production rates. For instance, the use of graphite as an electrode material in electrocatalysis achieved only 4.8 10^{-5} $\mu M \cdot min^{-1}$ and the use of graphitic carbon nitride as a non-metallic semiconductor material used for the photochemical activation of O_2 achieves 2.83 $\mu M \cdot min^{-1}$ [10].

Therefore, the GOD-catalysed reaction can be considered as a continuously supply of H_2O_2 which in the presence of Fe^{2+} enables the Fenton reaction. However, although the use of Fe^{2+} allows for a faster Fenton reaction ($k_1 \approx 70$ $M^{-1} \cdot s^{-1}$), an optimal working pH around 2.8 due to the solubility of Fe^{2+} is a limitation [11]. Nevertheless, GOD-mediated bio-Fenton also produces gluconic acid, an organic chelating agent, which allows the Fenton reaction to occur over a wider pH range since gluconic acid acts as an organic ligand facilitating Fe^{3+}/Fe^{2+} cycling [7]. Furthermore, although Fenton-like reaction has slower kinetics ($K_3 \approx$ 0.001-0.01 $M^{-1} \cdot s^{-1}$) [11], some WWTPs add $FeCl_3$ to improve phosphorus precipitation [12]. Leveraging the natural presence of ferric ions in wastewater further promotes the sustainability of the treatment system, reducing the need for external chemicals.

Conclusions

GOD-assisted bio-Fenton has great potential as a biocatalysis-based AOP for bioremediation strategy aiming to improve the removal of recalcitrant compounds in WWTPs. The mechanism has the potential to enhance biological systems for degrading emerging contaminants by integrating microbial systems with bio-Fenton reaction. Nonetheless, additional research is required to increase reaction efficiency under environmental conditions and to optimize iron sources.

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