Enzymatic Generation of Hydrogen Peroxide and Gluconic Acid Chelate for Chloro-Organic Destruction by Modified Fenton Reaction

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Abstract. Various remediation technologies are being developed for the destruction of groundwater pollutants like 2,4,6–trichlorophenol (TCP) and polychlorinated biphenyls (PCBs). This paper is focused on the oxidative dechlorination of TCP by chelate-based Fenton reaction near neutral pH using enzymatically generated hydrogen peroxide and chelate gluconic acid. For the application of Fenton reaction in in-situ remediation, its successful operation near neutral pH is essential. Hence, Fenton reaction is carried out in the presence of chelates that sequester ferrous and ferric ions, making its operation near neutral pH possible. The on-site simultaneous production of hydrogen peroxide and a non-toxic chelate for in-situ remediation of toxic organics by Fenton reaction is highly desirable. For this purpose, well-known enzyme glucose oxidase (GOx), and inexpensive reactants such as glucose and air were used. The study involved developing the correct protocols for the simultaneous enzymatic generation of hydrogen peroxide and chelate gluconic acid for the dechlorination of chlorinated organics by Fenton reaction. The oxidation of glucose was carried out using the enzyme in free and immobilized forms. The rate of production of hydrogen peroxide was determined for the homogeneous and heterogeneous systems, and was used to estimate the time required for complete consumption of glucose during the process, thus avoiding any traces of glucose in the Fenton reaction. The Fenton reaction was carried out at varying ratios of gluconic acid:Fe and under different pH conditions, and their effect on the decomposition of TCP and H_2O_2 was studied. The reaction rate was modeled in terms of the oxidation of parent compound TCP and decomposition of H_2O_2.
Keywords: Enzyme glucose oxidase, hydrogen peroxide, gluconic acid, chelate, Fenton reaction, pH, immobilization.

Introduction & Background: The remediation of chlorinated groundwater pollutants like trichloroethylene (TCE), TCP and PCBs has been achieved by the reductive technique using zerovalent metals like Fe[1], Zn, and bimetallics such as Fe/Pd, and corrinoids like Vitamin B_{12}[2]. The reductive route has been found to be slower as compared to various oxidative techniques. Fenton reaction is a powerful oxidation tool and has been widely studied[3,4] for its utilization in destroying organic pollutants at a fast rate. Fenton reaction is the combination of ferrous ion and hydrogen peroxide to produce OH• by the following reaction[5]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet} \quad \text{.....1}$$

The standard Fenton reaction, however, requires acidic pH environment, because of the precipitation of $Fe(OH)_3$ at neutral pH, and the regeneration of Fe^{2+} by the reaction of Fe^{3+} with H_2O_2 under acidic conditions. For in-situ remediation application, successful operation of Fenton reaction near neutral pH is essential. The use of a chelate has been known to make the operation of this reaction near neutral pH possible because of its capability to maintain ferric ions in solution and to control the rate of formation of free radicals.

In order to simultaneously generate hydrogen peroxide and a non-toxic chelate for their application in in-situ remediation of chlorinated organics by Fenton reaction, we have used a well-known enzyme glucose oxidase (GOx), and inexpensive reactants such as glucose and air. The specific objectives of our study involving the coupling of glucose oxidase reaction and Fenton chemistry were: (i) to quantify the rate of production of hydrogen peroxide and gluconic acid by the oxidation of glucose using both homogeneous and immobilized GOx, (ii) to achieve the dechlorination of model compound TCP by Fenton reaction near neutral pH using enzymatically generated hydrogen peroxide and chelate gluconic acid, (iii) to experimentally establish and to model the rate of dechlorination in terms of the oxidation of parent compound TCP, decomposition of H_2O_2, and the amount of chloride generated. Figure 1 represents the research strategy involved in the coupling of the two reactions, and proposes that the gluconic acid formed as a result of oxidation of glucose will act as a chelate in Fenton reaction.

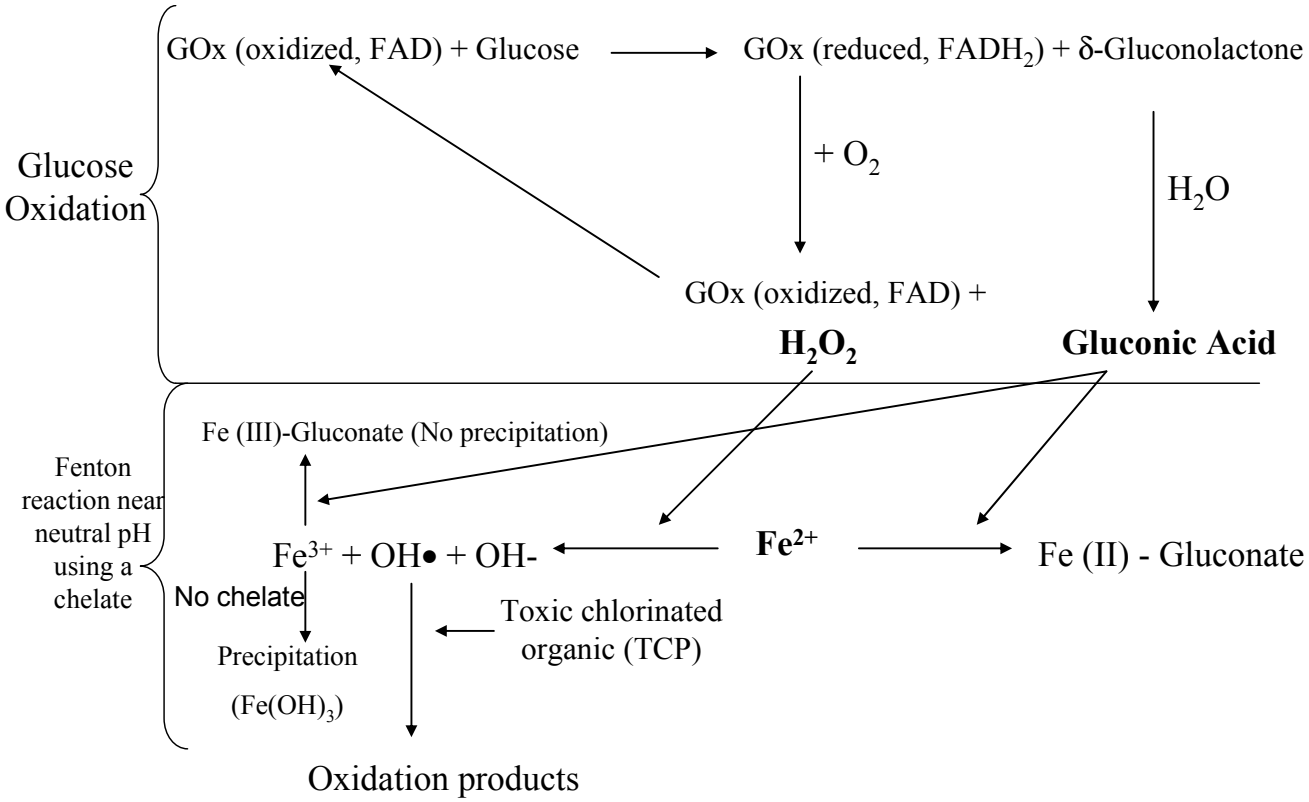

Figure 1. Schematic representation of the coupling of glucose oxidation reaction with Fenton reaction for dechlorination of chlorinated organic compounds

Our study involved two different approaches to couple the two reactions. Figure 2 depicts these two procedures. Specifically, the glucose oxidation has been carried out in homogeneous and heterogeneous reaction systems - both requiring different separation strategies for further application in Fenton reaction. The free glucose oxidase reaction requires separation of the enzyme using an ultrafiltration membrane. In this case, the enzyme activity is not retained for later generation of hydrogen peroxide and gluconic acid. The use of a membrane to separate the enzyme before initiating Fenton reaction adds an extra step to the process. The use of immobilized enzyme is believed to offset these drawbacks, as the use of immobilized enzyme would avoid the step of separation and also the immobilized glucose

oxidase is known to retain activity longer than the free enzyme thus allowing reuse of the enzyme. The pH and temperature stability of glucose oxidase is also known to improve after immobilization.

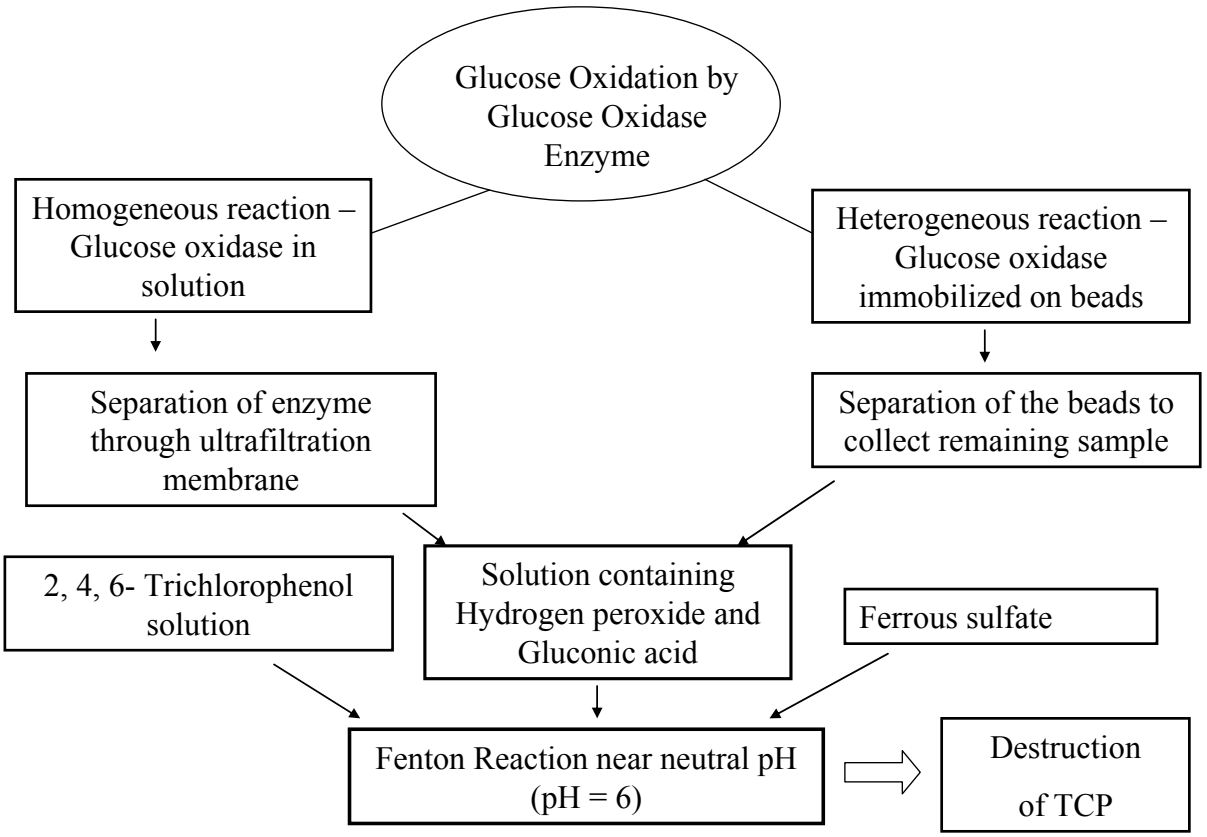

Figure 2. Schematic depicting research strategy for using various modes of enzymatically generated hydrogen peroxide and chelate gluconic acid in Fenton reaction for dechlorination of chlorinated organics

Materials & Methods: The enzyme glucose oxidase (EC 1.1.3.4.) from *Aspergillus niger*, was obtained from Sigma. β - D (+) Glucose, D-gluconic acid (50 % (w/w), solution in water), and periodic acid (minimum 99%) were also supplied by Sigma. Titanium (IV) oxysulfate (99.99%, 15 wt. % solution in dilute H_2SO_4) and glutaric dialdehyde (50 wt. % solution in water) were purchased from Aldrich. Hydrogen peroxide (30 %), sodium hydroxide (0.25 N, 1.0 N), sulfuric acid (0.25 N, 1.0 N), 2, 4, 6- TCP, methylene chloride (Optima grade, suitable for analytical purposes), and ferrous sulfate were obtained from Fisher Scientific.

All the experiments were conducted in a mixed batch reactor at room temperature (23-26^0 C) and were done in duplicate, unless otherwise stated. The kinetics of glucose oxidation was studied by the rate of formation of hydrogen peroxide. Hydrogen peroxide was analyzed by an established colorimetric method involving the formation of a yellow titanium (IV) peroxo complex which was scanned at 407 nm using a UV-Vis spectrophotometer. The unknown concentrations of TCP in the reaction samples were determined using EPA method 8207 on a Saturn 2200 GC/MS equipped with a Varian CP-3800 Gas Chromatograph autosampler and a DB5-ms column. The concentration of chloride ions in the reaction solution was determined using ion chromatography system (ICS 2500) and thermo-orion ion selective electrode.

Results & Discussion: The results of the experiments are discussed in different sections. The generation of gluconic acid and hydrogen peroxide using glucose oxidase is first discussed in detail, followed by the application of the same to Fenton reaction for the dechlorination of TCP.

Kinetics of oxidation of glucose by free and immobilized enzyme: The immobilization of glucose oxidase was carried out by covalent attachment on various polymeric supports such as polystyrene beads, silica beads, and nylon membrane. The binding on hydrazide-functionalized polystyrene beads was achieved by random and site-directed immobilization methods. The enzyme was bound to silica beads by interaction with the epoxide groups on the support, while it was bound to the nylon membrane by the interaction between amine group of enzyme and acyl anhydride groups of the membrane. The activity of the bound enzyme was determined by the rate of oxidation of glucose obtained by using the bound enzyme. Table 1 below shows the relative activity obtained by the different binding methods, when compared to the homogeneous enzyme.

Table 1. Immobilization of enzyme glucose oxidase on various polymeric supports: Relative activity (Activity studies were carried out using 0.075 M glucose, at pH 6, at a temperature of $23\text{-}26^0$ C, and under constant supply of air)

Polymeric support	Type of attachment (Functional group on support-Enzyme functional group)	Relative activity (%), compared to homogeneous enzyme
Polystyrene beads	Random (Aldehyde-amine)	19.05 ± 0.7
Polystyrene beads	Site-directed (Hydrazide-Carboxylic acid)	90.85 ± 2.8
Silica beads	Random (Epoxy-amine)	42.36 ± 3.7
Nylon membrane	Random (Acyl Anhydride-amine)	13.48 ± 2.1

The observed loss of activity of the enzyme bound by random attachment as opposed to site-directed attachment can be attributed to the loss of active amine groups of lysine residues for covalent binding with the aldehyde groups of the support. Such covalent binding of the amine groups of lysine residues on the surface of the enzyme is avoided in site-directed immobilization, as it makes use of the carbohydrate residues for binding, thus resulting in a very low loss of activity after immobilization[6]. Similar loss in activity was obtained while using the membrane. However, while using silica beads, higher relative activity was obtained even by random attachment. This is estimated to be the result of hydrophilicity of the silica beads.

Fenton reaction using enzymatically generated gluconic acid and hydrogen peroxide: The hypothesis behind carrying out this experiment was that, the chelating agent gluconic acid produced by glucose oxidation would prevent precipitation of ferric hydroxide near neutral pH during Fenton reaction. The oxidation of glucose was carried out such that nearly complete conversion would be obtained before separation. The results of the experiment suggested that the rate of dechlorination of TCP diminished immensely after initial 10 minutes (figure not shown). This is estimated to happen due to the decomposition of H_2O_2

present in the system. In order to verify our hypothesis, additional H_2O_2 (50 mM) was added at the end of 2 h, and complete dechlorination (100% chloride formation) was indeed achieved within the next 10 minutes. This suggests that the added H_2O_2 reacted with the free ferrous ions in the solution, resulting in the formation of free radicals, which led to the oxidation of TCP. It was also observed that ferric hydroxide did not precipitate during the reaction time which indicates chelating ability of gluconic acid near neutral pH for carrying out the Fenton reaction.

The dechlorination of TCP was also carried out at varying ratios of gluconic acid:Fe at pH 6. Figure 3 shows the results of these experiments in terms of decomposition of TCP with time.

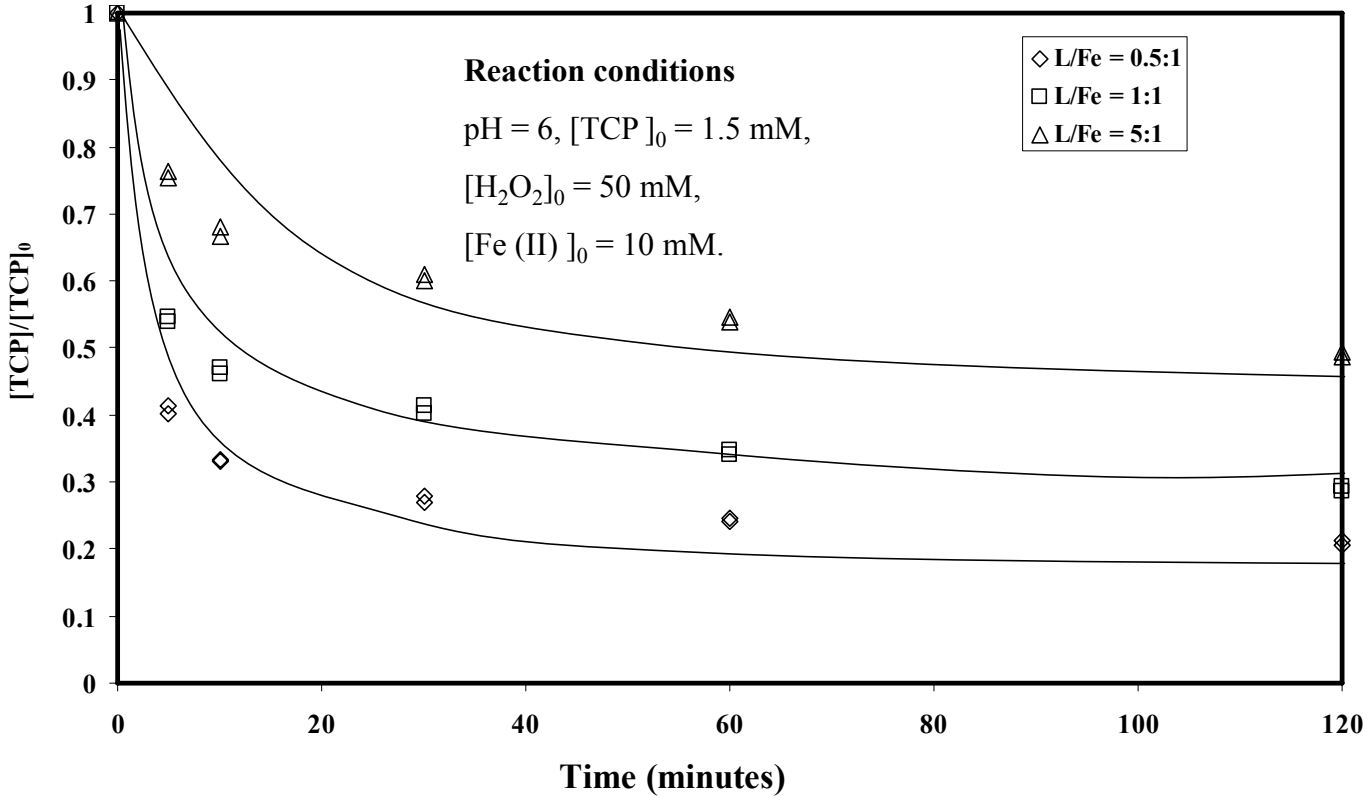

Figure 3. TCP dechlorination profiles under different chelate to iron ratios for Fe^{2+}+H_2O_2+gluconate+TCP system[6]

The simultaneous decay of H_2O_2 was also studied, and the rate of reaction was modeled in terms of the decomposition of TCP and H_2O_2, and relevant kinetic parameters determined for varying ratios of gluconic acid:Fe[7].

The effect of free radicals on gluconic acid was also investigated. The analysis of gluconic acid was established using Ion Chromatography system (ICS), and it was analyzed as gluconate anions. The study was conducted by introducing following different solutions into the ICS: (i) 50 mM gluconic acid, (ii) 50 mM gluconic acid + 10 mM ferrous sulfate, (iii) 50 mM gluconic acid + 10 mM ferrous sulfate + 50 mM hydrogen peroxide, and (iv) 50 mM gluconic acid + 10 mM ferrous sulfate + 50 mM hydrogen peroxide + 1.5 mM TCP. Each of the samples was analyzed for its gluconate anion content, and by comparison between the chromatograms obtained from samples (i) and (iii), it was concluded that 5.3 mM gluconic acid remained as gluconate anions in the solution, even after two hours of reaction with free radicals. The

presence of TCP (sample (iv)) did not have any effect on the degradation of gluconic acid by free radicals.

Conclusions: Our study demonstrated the use of hydrogen peroxide and chelate gluconic acid generated by enzymatic glucose oxidation for the successful operation of Fenton reaction near neutral pH. The oxidation of glucose using glucose oxidase was carried out using free and immobilized forms of the enzyme glucose oxidase to determine the kinetic parameters for these systems. The rate of production of hydrogen peroxide, and thus the rate of consumption of glucose, was determined. Site-directed immobilization of GOx on polystyrene beads was successfully achieved, and the bound enzyme exhibited high percentage of retained activity. Immobilization of GOx allows easy recovery of the enzyme for repeated use. The calculations performed to evaluate the effect of pH on the chelation of ferrous and ferric ions with gluconic acid suggest that gluconic acid indeed chelates to ferrous and ferric ions, and can be used to carry out Fenton reaction near neutral pH. The chelate-based Fenton reaction was carried out using enzymatically produced hydrogen peroxide and chelate gluconic acid, and also using commercial hydrogen peroxide and gluconic acid under similar conditions. No precipitation was observed during the process, suggesting the chelation of ferric ions with gluconic acid. The dechlorination of TCP was carried out at varying ratios of gluconate:Fe and under different pH conditions. The chelate concentration was not found to have a major impact on the rate of dechlorination at pH 4, because of weak chelation of ferrous ions with gluconic acid at that pH.

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References:

1) Matheson, L.J., Tratnyek, P.G., Reductive Dehalogenation of Chlorinated Methanes by Iron Metal. *Environmental Science and Technology*, **28** (1994) 2045-2053.
2) Ahuja, D.K., Gavalas, V.G., Bachas, L.G., Bhattacharyya, D., Aqueous-Phase Dechlorination of Toxic Chloroethylenes by Vitamin B_{12} Cobalt Center: Conventional and Polypyrrole Film-Based Electrochemical Studies. *Industrial and Engineering Chemistry Research*, **43** (2004) 1049-1055.
3) Teel, A.L., Warberg, C.R., Atkinson, D.A., Watts, R.J., Comparison of Mineral and Soluble Iron Fenton's Catalysts for the Treatment of Trichloroethylene. *Water Research*, **35** (2001) 977-984.
4) Huston, P.L., Pignatello, J.J., Degradation of Selected Pesticide Active Ingredients and Commercial Formulations in Water by the Photo-Assisted Fenton Reaction. *Water Research*, **33** (1999) 1238-1246.
5) Walling, C., Fenton's Reagent Revisited. *Accounts of Chemical Research*, **8** (1975) 125-131.
6) Ahuja, D.K., Bachas, L.G., Bhattacharyya, D. Enzymatic generation of H_2O_2 and gluconic acid for Trichlorophenol Dechlorination by Fenton Reaction. *Journal of Chemical Technology and Biotechnology*, Under Review.
7) Ahuja, Deepak K., Bachas, L.G., Bhattacharyya, D. Dechlorination of 2,4,6-trichlorophenol by modified Fenton reaction using enzymatically generated hydrogen peroxide and gluconic acid. *Abstracts of Papers, 229th ACS National Meeting*, March 13-17, 2005.