

Multi-way Degradation and Process Optimization of Phenol from Simulated Wastewater System

ABSTRACT

This research was based on the comparative study between microbial, enzymatic and photocatalytic phenol degradation. Different experiments were carried out under three distinct methodologies that sought to examine which method is more feasible between them through various aspects. For the microbial study, *E-coli* was used for phenol degradation at an optimum condition of *E-coli*. In the enzymatic study, peroxidase was extracted from soybean seed hulls, and it was purified. The purified peroxidase enzyme was applied in phenolic solution at neutral pH. The H₂O₂/UV/TiO₂ scheme was adopted in the photocatalytic treatment of phenol. Maximum phenol degradation was observed in photocatalysis. From this comparative study, a microbial method was found to be more time consuming and an enzymatic method require more steps to perform the experiment while photocatalysis took less time with a more feasible results.

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Keywords: Comparative study, microbial treatment, enzymatic treatment, photocatalytic treatment, first-order reaction kinetics

1. INTRODUCTION

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Recently, considerable attention has been received by many researchers on biodegradation of aromatic compounds due to their toxicity. Among them, phenol and its derivatives are a standard compound in wastewater of many industries such as oil refineries [1], coal refining, petroleum, textiles and pharmaceuticals [2]. It is quite known that the toxicity of phenols towards the whole environment is high and thus has been incorporated in the list of pollutants by the U.S. Environmental Protection Agency [3]. Many researchers are engaged in research on phenol degradation by diverse techniques and methods. The attention is that to investigate which technology will be most feasible, eco-friendly, cost-effective and time saving is the primary goal of the present investigation. The present study compares three methods viz. microbial degradation, enzymatic degradation and photocatalytic degradation.

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Until today, many investigators have reported numerous types of microorganisms that remove phenol from wastewater. From the literature reviewed, some microorganisms can consume phenol as a sole source of carbon and energy. These bacterial species include *Streptococcus epidermis* [4], *Escherichia coli*, *Micrococcus sp.*, *Brucella sp.* [5], *Bacillus subtilis*, *Pseudomonas putida*, *Acinetobacter calcoaceticus*, *Bacillus subtilis* [6-8] and *Streptococcus sp.* [8].

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Besides, enzymes are applied in biodegradation study of the phenol. Enzymes play a vital role in phenol biodegradation reactions as a biocatalyst [9]. These enzymes include Peroxidase, Chloroperoxidase, Lignin peroxidase, Mn-peroxidase [9] and catalase [10] that isolated from specific plants viz. soybean [11], horseradish, radish [12], and their materials

40 such as seeds [13], leaves [14], stem [15], roots [16]. Tyrosinase and Laccase [9] are
41 obtained from different fungal species.

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43 In recent years, photocatalysis has been developed in wastewater treatment. In this
44 technique, some photocatalysts and their chemically modified transformations were
45 employed for the photodegradation of toxic compounds. The TiO₂ and ZnO were broadly
46 tested as photocatalyst used in this technique [17-20]. Many researchers increase the
47 efficiency of a catalyst by doping with metals such as Ag, Fe, Pr, Co, V under various
48 illumination systems [21]. Some researchers synthesized bimetallic or trimetallic
49 transformations for degradation study [22].

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51 Here, we focus on all related aspects or parameters to select a better, efficient, cost-effective
52 and feasible degradation technique. From the overall primary study, we use *E. coli* for the
53 microbial study while peroxidase extracted from soybean seed hulls and selected for the
54 further process of phenol degradation. Alike we introduced single TiO₂ nanoparticles in
55 phenolic wastewater under both UV and Solar light.

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57 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

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59 2.1 Materials

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61 All analytical grade and HPLC grade chemicals were purchased from Fisher scientific and
62 Himedia, Mumbai, India. Milli-Q water used for chemical preparations obtained from Milli-Q
63 make of Schimadzu, Japan. *E-coli* microbial culture was given by my friend. Soybean seeds
64 were collected from agricultural fields and washed thoroughly with distilled water.

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66 2.2 Microbial Methodology

67 *E-coli* bacterial culture was grown on slants of nutrient agar medium for further microbial
68 phenol degradation study and stored at 4°C until further use. Then the minimal salt medium
69 was prepared as (g/L) Na₂HPO₄ 33.9, KH₂PO₄ 15, NH₄Cl 5, NaCl 2.5, 2 ml of MgSO₄ 0.1 M
70 and 0.1 ml of CaCl₂ 1 M per liter for actual degrading study [4]. Four consecutive same
71 interval different concentrations of phenolic wastewater were prepared in the range between
72 250 mg/L to 1000 mg/L in phosphate buffer with pH 7.0. The reaction mixture containing only
73 MSM media and phenol that was used as a control mixture in the microbial study. Similarly,
74 bacterial inoculum was added to the control mixture for further phenol degradation study.
75 Experiments were carried out in a 250 ml conical flask containing 50 ml of MSM media with
76 phenol concentration of above-given range. The mixture was incubated at room temperature
77 (37°C ± 2) on the shaker (100 rpm). Samples were collected and tested at every 24 h time
78 interval for five days.

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80 The samples were centrifuged, and the remaining phenol concentration determined
81 quantitatively by direct UV-visible spectrophotometric method [23]. Optical density was
82 measured at λ_{max} = 269 nm. Remaining concentration of phenol (%) was calculated using
83 following formula:

$$\% \text{ Phenol degradation} = \frac{\text{Absorbance of sample}}{\text{Slope phenol degradation (by graph)}} \dots \dots \dots (1)$$

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85 2.3 Enzymatic Methodology

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87 The experimental procedures of SBP extraction and purification followed with some
88 modifications reported by Liu *et al.* 2005. The fresh soybean seed hulls was weighed and

89 washed with milli-Q water. These cleaned seeds were soaked in milli-Q water overnight. The
90 soaked seeds were smashed and blended with 500 ml milli-Q water for 10 to 15 min. Then
91 the homogenized mixture was filtered through cheesecloth and after that filtrate of
92 cheesecloth centrifuged at 10,000 rpm for 20 min at 4°C. The collected supernatant was rich
93 in proteins.

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95 The SBP purification process was performed as reported in Liu *et al.* 2005. The process
96 included three steps. The first step was acetone-ammonium sulphate cooperation
97 precipitation. It comprised both acetone and ammonium sulphate precipitation
98 simultaneously. The volume of acetone taken 0.3 fold of the original amount and solid
99 ammonium sulphate added to form up to 45% saturation. This combination was placed in a
100 refrigerator for 2 h. After that, the mixture was centrifuged for 15 min at 5000 to 7000 rpm.
101 The supernatant and precipitant were collected separately. This 45% saturation was
102 continued to 75% saturation by adding solid ammonium sulphate again with 0.3 fold acetone
103 in the supernatant. The mixture was centrifuged for 15 min at 5000 to 7000 rpm. Only one
104 condition followed that the acetone was pre-stored in a refrigerator and that cooled acetone
105 was added under a cold atmosphere in all our experimental sets. The resulted precipitants
106 were dissolved in milli-Q water to get primary purified SBP. The second step consisted of
107 acetone precipitation alone. The volume of acetone mixed as 1.4 fold separately into the
108 primary purified SBP. The mixture was centrifuged for 15 min at 5000 to 7000 rpm. The
109 resulted precipitant was dissolved in milli-Q water to get secondary purified SBP. The third
110 step included only zinc sulphate precipitation. Before introducing zinc sulphate into the
111 enzyme solutions, the pH was adjusted to eight by HCl or NaOH and then 1.0 mol L⁻¹ zinc
112 sulphate solution was mixed to form 0.015 mol/l zinc concentration. The mixture was
113 centrifuged for 15 min at 5000 to 7000 rpm. Lastly, the supernatant was collected and
114 denoted as highly purified SBP enzyme solution [24].

115
116 Enzyme assay and protein content were examined after each purification step by the
117 procedures described by Kolhe *et al.* 2015 [13]. The Reinheitszahl (RZ) values were
118 assayed after each purification steps. The purified SBP stored at 4°C until the further use of
119 the enzyme. Different concentrations of phenolic wastewater were prepared in the range
120 between 250 mg/L to 1000 mg/L in phosphate buffer with pH 7.0. The reaction mixture
121 contained 50 ml phenolic wastewater, 30 per cent H₂O₂ and enzyme solution. Analyze the
122 initial phenol concentration. The sample was collected after every 1h to examine the residual
123 phenol.

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125 The remaining phenol concentration of each sample was determined quantitatively by the
126 direct UV-visible spectrophotometric method at phenol λ_{max} . The remaining concentration of
127 phenol (%) was calculated by formula 1.

128 129 **2.4 Photocatalytic Methodology**

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131 The third methodology opted for photocatalytic degradation of phenol. In this study, TiO₂
132 nanoparticles were used as the photocatalyst while 11 watts of UV lamp was used as
133 illumination for energy. Various concentrations of phenolic wastewater were prepared in the
134 range between 250 mg/L to 1000 mg/L. The pH range kept as 2, 4, 6, 8 and 10 and adjusted
135 with 0.1 M HCl and 0.1 M NaOH solutions. The retention time was 10 h, but samples were
136 collected at every 1h time interval. The reaction mixture contained 50 ml phenolic solution,
137 30% H₂O₂ and TiO₂ nanoparticles. Analyze the initial phenol concentration. The sample was
138 collected after every 1h to examine the residual phenol.

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140 The remaining phenol concentration of each sample was determined quantitatively by the
141 direct UV-visible spectrophotometric method at phenol λ_{max} . The residual concentration of

142 phenol (%) was calculated by formula 1. The first and second order kinetics study were
143 evaluated from graphs of log concentration versus irradiation time [25].

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145 3. RESULTS AND DISCUSSION

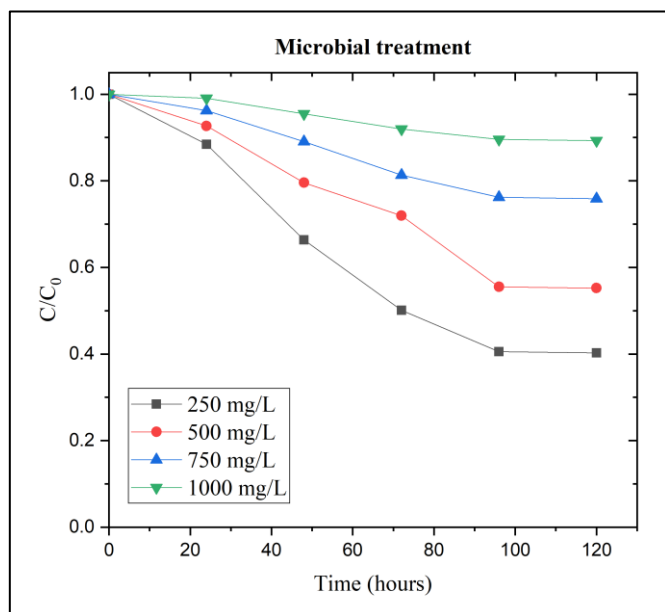
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147 3.1 Microbial Treatment

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149 The phenol degradation performance of *E-coli* strain was examined for different phenol
150 concentrations viz. 250 mg/L, 500 mg/L, 750 mg/L and 1000 mg/L at various time intervals.
151 The per cent phenol degradation was derived based on residual phenol concentration.
152 Figure 1 shows the effect of phenol concentration indicating that 60.07% phenol degradation
153 was observed at 250 mg/L phenolic concentration at neutral pH after 96 h. As the phenolic
154 concentration increases the phenol degradation decreases. Hence, only 11.75% phenol
155 degradation was observed at 1000 mg/L phenolic concentration at neutral pH after 96 h.
156 Reshma et al. 2014 also used *E-coli* treatment on phenolic wastewater. They obtained 100%
157 phenol degradation for 10 mg/L phenolic solution. We had only 60.07% phenol degradation
158 because 250 mg/L concentration was much more than 10 mg/L concentration. Some
159 bacterial strain may have died at this high phenolic concentration; hence, the *E-coli* bacterial
160 strain did not achieved 100% phenol degradation.

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163

164 **Fig. 1. Phenol degradation by microbial treatment for different concentration of the**
165 **phenol**

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167 3.2 Enzymatic Treatment

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169 The SBP was extracted from soybean seed hulls by blending it for 10 to 15 min. During the
170 blending of soybean seed hulls, the blended material was lightly warmed, but this was
171 not essential because the SBP activity persisted up to 75°C [11]. A volume of the original
172 enzyme solution was recorded as 530 ml. Table 1 shows the enzyme purification steps and
173 their characteristics. A product of the last purification step having 71.01% recovery and 1.12
174 RZ value which is near about 1.32 RZ value reported in Liu et al. [24]. This enzyme
175 purification method is more comfortable and cost-effective than other purification methods

176 because it is merely based on only precipitation technique. Total volume, total activity, %
177 recovery, protein content, specific activity, fold purification and RZ value for each step are
178 shown in table 1.

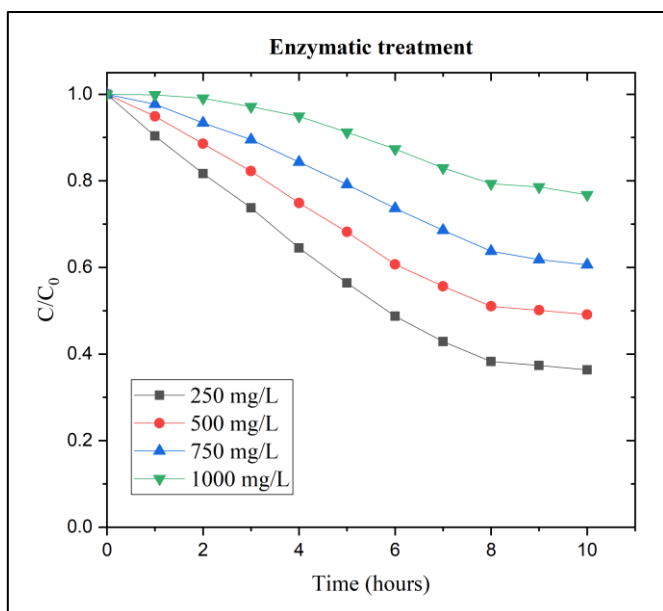
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Table 1. Purification steps and their characterization of SBP

Steps	Total Volume (ml)	Total Activity (U/ml)	Recovery (%)	Protein Content (mg/ml)	Specific Activity (U/mg)	Fold Purification	RZ value
Original enzyme solution	530	6.091	100	2.325	2.62	1	0.19
Acetone-ammonium sulphate cooperation precipitation	100	5.451	89.49	0.847	6.44	2.46	0.47
Acetone precipitation	10	4.847	79.58	0.461	10.51	4.01	0.83
Zinc sulphate precipitation	10	4.325	71.01	0.257	16.83	6.42	1.12

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This purified SBP was introduced in various phenol concentrations viz. 250 mg/L, 500 mg/L, 750 mg/L and 1000 mg/L at various time intervals to evaluate the phenol degradation. The per cent phenol degradation was determined based on residual phenol concentration. Figure 2 on effects of phenol concentration shows that 62.31% phenol degradation was obtained in 250 mg/L phenolic concentration at neutral pH after 8 h. As in microbial treatment, here also it was observed that as phenol concentration increases the phenol degradation decreases. Hence, only 21.82% phenol degradation was observed in 1000 mg/L phenolic concentration at neutral pH after 8 h but this 21.82% phenol degradation is more as compared to microbial treatment. Pradeep *et al.* also gave a treatment of SBP on phenolic wastewater. They obtained 72% phenol degradation of 100 mg/L phenolic solution. We had 62.31% phenol degradation in 250 mg/L concentration, which was more.



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197 **Fig. 2. Phenol degradation by enzymatic treatment for different concentration of the**
198 **phenol**

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200 **3.3 Photocatalytic Treatment**

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202 **3.3.1 Effect of pH condition**

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204 Some properties of photocatalysts are highly pH dependent. Hence phenol degradation at
205 different pH **was** carried out under UV light. In this treatment, TiO₂ nanoparticles were used
206 as a photocatalyst. These nanoparticles **were** introduced at different pH (2-10) conditions to
207 examine the phenol degradation. It is clearly seen that in figure 3, the basic conditions are
208 unfavorable while acidic conditions are favorable for the photocatalytic degradation of
209 phenol. In acidic medium, from pH 2 to pH 6 phenol degradation increases and after pH 6 **it**
210 **decreases**. The higher phenol degradation was observed with 63.08% at pH 6. The optimal
211 pH condition was found **to be** acidic.

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213 Phenol has a pKa value of 9.95 and can be charged positively or negatively under the pH
214 range studied; i.e., the attraction and interaction between both photocatalyst and phenol will
215 be diverse with the solution pH. Moreover, as the pKa value of phenol is 9.95, it has negative
216 charge above pH 9.95 ≈ 10 and referred as phenolate anions but the conversion of
217 phenolate **anions commences** when solution pH in between 6 to 8 [26]. Conversely, in highly
218 acidic condition phenol gets a positive charge while in weak acidic and neutral condition
219 phenol molecules exist primarily in their non-ionic form. Additionally, the maximum OH[•]
220 radicals are produced in the pH range of 6 to 7 [27], due to this reason rate of phenol
221 degradation is higher in this pH range. These hydroxyl **radicals are** formed from some
222 photocatalytic oxidative and reductive reactions. They have a capacity to directly break **down**
223 **an aromatic** ring of phenol molecule and transmute them into the final products **which** are
224 CO₂ and H₂O through various intermediates, because they are extremely strong, non-
225 selective oxidants [28].

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227 **3.3.2 Effect of catalyst load**

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229 To examine the effect of TiO₂ nanocatalyst dosing on the phenol degradation, several
230 experiments were carried out at catalyst loading from 1 to 4 g/L with 250 mg/L pollutant
231 concentration. Figure 3 indicates that the increase in the amount of nanocatalyst loading
232 also increases the rate of phenol degradation up to a particular catalyst dose of 3 g/L. This
233 increased rate of degradation may be due to the higher surface area. Nevertheless, after 3
234 g/L amount of catalyst loading the degradation rate starts declining. As the catalyst load
235 increases, the experimental solution becomes turbid and resulting in UV rays getting
236 scattered leading to a decrease in reaction rate [29]. The maximum phenol degradation at 3
237 g/L of catalysts dose was considered as an optimum condition for further study.

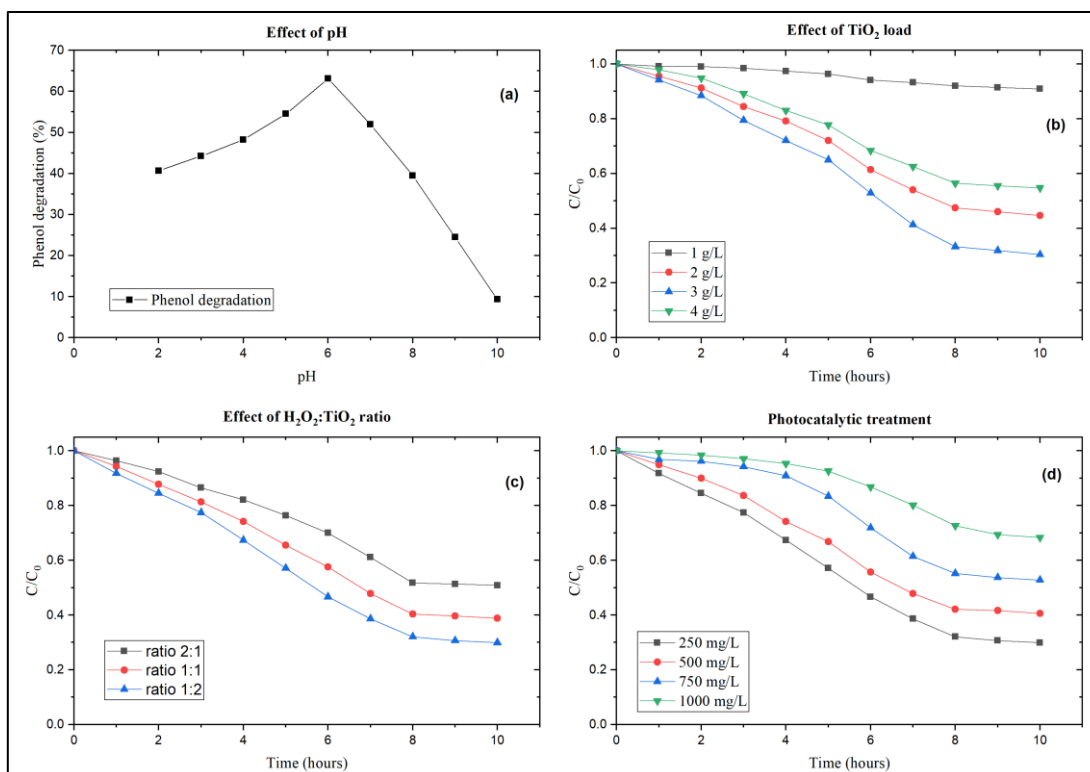
238 239 **3.3.3 Effect of H₂O₂ and TiO₂ ratio**

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241 An oxidizing agent is another aspect of the photocatalytic oxidation process. Other
242 experimental sets were performed for the study of the impact of various rates between H₂O₂
243 and catalyst load as 2:1, 1:1 and 1:2. Figure 4 shows that a maximum phenol degradation
244 was recorded at 1:2 ratio. It happens obviously because half the quantity of H₂O₂ as on
245 catalyst dose was enough for phenol degradation. The H₂O₂ used only an oxidizing agent in
246 a reaction medium. There is no use of a double quantity of H₂O₂ in the reaction mixture.
247 Because in an excess amount of H₂O₂ reacts with those hydroxyl radicals which are
248 responsible for degrading the pollutant molecule [30]. While the same quantities of H₂O₂ and
249 catalyst load, also not well for the degradation because there is no sufficient amount of
250 catalyst in the mixture. This phenomenon was reported earlier in 2001 by Ghaly *et al.*

251 252 **3.3.4 Effect of phenol concentration**

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254 TiO₂ nanoparticles applied in various phenol concentrations viz. 250 mg/L, 500 mg/L, 750
255 mg/L and 1000 mg/L at various time intervals to evaluate the phenol degradation. The per
256 cent phenol degradation was determined based on residual phenol concentration. As initial
257 phenol concentration increases, the rate of phenol degradation decreases from 250 mg/L to
258 1000 mg/L. This happens due to the competitive adsorption on the active sites of
259 photocatalyst between the hydroxide radicals and phenol molecules [31]. Figure 4 on effect
260 of phenol concentration shows that 68.39% phenol degradation obtained in 250 mg/L
261 phenolic concentration at neutral pH after 8 h. As in microbial treatment, here also seen that
262 the phenolic concentration increases the phenol degradation decreases. Hence, only 28.46
263 % phenol degradation observed in 1000 mg/L phenolic concentration at neutral pH after 8 h,
264 but this 28.46% phenol degradation is more than in microbial treatment. Pradeep et al. also
265 gave a treatment of SBP on phenolic wastewater. They obtained 72% phenol degradation of
266 100 mg/L phenolic solution. We had 68.39% phenol degradation in 250 mg/L concentration,
267 which was more.

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Fig. 3. (a) Phenol degradation at various pH conditions, (b) Effect of TiO₂ nanoparticles loading on phenol degradation, (c) Effect of H₂O₂:TiO₂ nanoparticle ratio on phenol degradation and (d) Effect of different phenolic concentration on phenol degradation under UV light

3.3.5 Degradation rate kinetics

278 The kinetic study of photodegradation of phenol was investigated for UV/H₂O₂/TiO₂ system.
279 A model with a higher value of correlation coefficient (R²) considered as more applicable.
280 The equation for first and second order kinetics is shown below.

281 First order reaction kinetics: $\log(qe - qt) = \log(qe) - \left(\frac{K_f}{2.303}\right) t$ (2)

282 Second order reaction kinetics: $\left(\frac{t}{qt}\right) = \left(\frac{1}{K_s qe^2}\right) + \left(\frac{1}{qe}\right) t$ (3)

283 Where q_e and q_t are the amounts of phenol degradation (mg g⁻¹) at equilibrium time and at
284 time t (min), respectively. K_f is the rate constant of first-order reaction (min⁻¹) which can be
285 obtained from the slope of log (qe-qt) versus time plot. Also, a rate constant of pseudo-
286 second-order K_s reaction (g mg⁻¹ min) can be obtained from t/qt versus t plot. For the phenol,
287 first-order reaction kinetic was fitted than second-order reaction kinetics first order having a
288 maximum value of R². Besides the apparent first-order rate constants decreased with the
289 increase of initial phenol concentrations [32]. Hence, kinetic constant based on phenol
290 degradation by UV calculated for a first-order reaction. Table no. 2 shows a description of
291 first-order reaction kinetics.

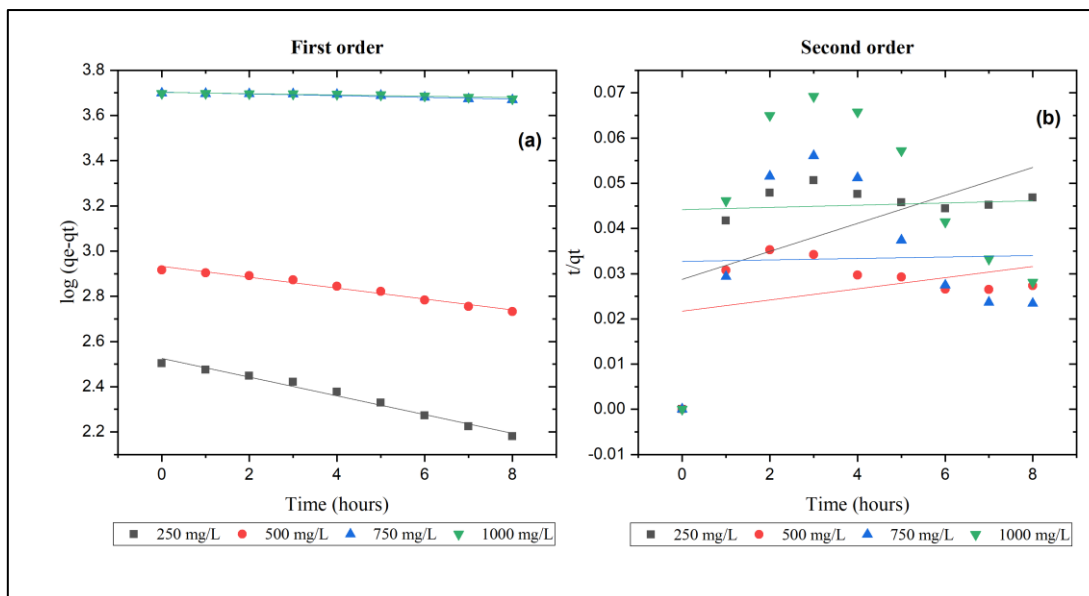
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Table 2. Description of first-order reaction kinetics

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Substrate	Concentration (mg/L)	K (min ⁻¹)	R ²
Phenol	250	0.0953	0.9838
	500	0.0555	0.9793
	750	0.0088	0.8960
	1000	0.0067	0.8546

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298 **Fig. 4. Phenol degradation corresponds to the (a) first-order and (b) second-order**
 299 **model for 250, mg/L, 500 mg/L, 750 mg/L and 1000 mg/L**

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4. CONCLUSION

This study adopted three methodologies which were microbial, enzymatic and photocatalytic treatments of phenol for the degradation. Microbial treatment gave 60.07%, enzymatic treatment gives 62.31%, and photocatalytic treatment gives 68.39% phenol degradation in 250 g/L phenolic concentration. All treatments gave approximately the same phenol degradation, but each treatment has some advantages as well as some disadvantages. About 60.07% phenol degradation achieved under 96 h in microbial treatment whereas 62.31% and 68.39% phenol degradation takes place under 8 h in enzymatic and photocatalytic treatment. Based on the time parameter, microbial treatment is a very time-consuming method for phenol degradation while the other methods are less time-consuming.

In enzymatic treatment, additional one-step is required for phenol degradation. That step was enzyme purification. Enzyme purification method was adopted in this study, and that the purified enzyme used as a catalyst. An enzymatic treatment did not show significant phenol degradation even after purified enzyme was introduced in the reaction mixture. In phenol degradation follow another one-step and degrade the phenol which is not much more. Therefore, this enzymatic treatment is not a feasible method for phenol degradation.

A remaining method is a photocatalytic degradation. It requires less time, no need for extra steps. The maximum phenol degradation achieved in this photocatalytic method was 68.39%. The whole photocatalytic degradation was performed under acidic condition, this is

323 one thing which is noticeable. However, there is no need of extra handling of that acidic
324 medium. Overall, from the comparative study of all the three methods reported in this study,
325 the photocatalytic process is efficient for phenol degradation than others.
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328 **COMPETING INTERESTS**

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330 Authors have declared that no competing interests exist.
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