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Design and development of an integrated treatment system for pharmaceutical waste with toxicological study

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ABSTRACT

In the present study, sole adsorption, Fenton's oxidation, ozone treatment and biochemical treatment by *Alcaligenes feacalis (A. feacalis)* and *Exiguobacterium aurantiacum* (*E. aurantiacum*) and combination of the above treatments have been studied. It was observed that Fenton's treatment is efficient for degradation of Pantoprazole in any concentration pantaprazole. In Fenton's process, the degradation obtained 56.77% at pH 3.5 in an hour and in ozone treatment, 36% removal was achieved in 8 min for 600 ppm concentration. The microbial treatment is proved to be a good one but in the lower range of pantoprazole about 200 mg/L. The degradation of pantoprazole was achieved 58.40% and 60.08% by using *A. feacalis and E. aurantiacum* respectively in 72 h. Combination treatment has been designed, and the system worked efficiently. In the first step either Fenton's treatment, activated carbon or ozone treatment were performed followed by biochemical treatment. The maximum percentage removal of pantoprozole was observed 90.17%, 81.57%, and 71.07% respectively. The process also proves that it is partially cost effective, energy saving and environmentally safe operation.

Keywords: Wastewater; Pharmaceutical; Pantoprazole; Integrated wastewater treatment; Toxicological study

1. Introduction

The pharmaceutical industry is one of the important life-saving industries in India, which earns large foreign exchange through the medicine export to USA, EU, Russia [1]. The Indian pharmaceutical industries becoming a great contributor of delivering drugs in the world [2]. Pantoprazole is a proton-pump inhibitor (PPI), which inhibits gastric acid secretion. This is used in the treatment of peptic ulcers, gastro-oesophageal reflux diseases etc. [3]. Pantoprazole may cause long-term adverse effects in the aquatic environment [4]. The worldwide production and consumption of medicines provide a continuous release of these substances or their metabolites to the environment through industrial wastewater and domestic sewage [5]. The occurrence and fate of emerging micro-pollutants like pharmaceuticals are under attracted considerable attention in recent years. An extensive variability of these compounds (e.g. antibiotics, analgesics, anti-inflammatories, antiepileptics, hypnotics) has been reported to be present in aquatic systems worldwide [6–12]. The impact of pharmaceutical on the ecosystem is behavioral alteration in aquatic living beings are reported [13]. This rising concern of the pharmaceutical wastes in the aquatic environment is due to their potential impacts on the aqueous ecosystems and eventually human health [14–16]. Moreover, the additive effects with other micro-pollutants have been observed. Thus the release of these bioactive pharmaceutical compounds has to stop totally or has to degrade it for safe disposal. There are several treatment technologies has been reported in the literature, like advance oxidation

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process by Fenton's reagents [17], adsorption [18], ozone treatment [19], electrocoagulation [20], membrane filtration [21], biochemical treatment [22] and combination of treatments [23,24]. But adsorption and membrane filtration process are costly and problem for sludge disposal. Similarly, advance oxidation process by Fenton's treatment produce huge amount of sludge as sole process and ozone treatment is costly [22,25]. On the other hand, biological treatment is time-consuming. Thus no single process is unique for the treatment of any individual wastewater. Therefore the present study was designed to assess the sole process of adsorption, ozone treatment, Fenton's treatment and biological treatment and combination of these treatments in degradation of pantoprazole from wastewater.

2. Materials and methods

2.1. Materials

Pantoprazole sodium ($C_{16}H_{14}F_2N_3NaO_4S$) [Fig. 1], hydrogen peroxide (H_2O_2, 30%, density 1.11 kg/L, Merck, India), ferrous sulfate ($FeSO_4$,$7H_2O$), sulfuric acid (1 M), sodium hydroxide (5 M), ozone, were used. All reagents used in this study are of analytical reagent grade and used without further purification.

2.2. Waste water sample preparation

Samples of different concentrations have been prepared by mixing pantoprazole sodium ($C_{16}H_{14}F_2N_3NaO_4S$) in double distilled water in different concentrations. The pH of the samples was adjusted with sulfuric acid (1 M), sodium hydroxide (5 M) as per the experimental condition.

2.3. Pantoprazole wastewater treatment

2.3.1. Fenton's oxidation treatment process

In the present study, the treatment of pantoprazole solution conducted with Fenton's reagents in batch mode. In this experiments, Fenton's treatments were conducted in batch reactors taking 100 ml of 650 ppm pantoprazole wastewater sample in 250 ml of conical flasks with 4 ml/L H_2O_2 and 1.5 g/L $FeSO_4$ in ambient temperature (25°C) for 60 min. Fenton's reagents works well at acidic pH [22,26]. The pH was set at a value of 3.5 [22]. The pH of the wastewater was adjusted with H_2SO_4 (1 M) and NaOH (5 M). The experiment has been done with varying dosage of reagents ($FeSO_4$:H_2O_2). The ratio of H_2O_2/$FeSO_4$ played a vital role in controlling the efficiency of the treatment [24,27].

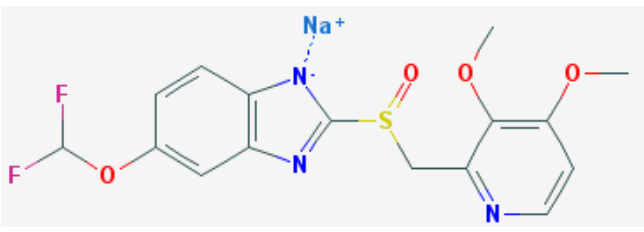

Fig. 1. Structure of pantoprazole.

2.3.2. Ozone treatment process

This experiment has been performed in the laboratory by taking 100 ml pantoprazole wastewater sample in a 500 ml conical flask. The simulated pantoprazole wastewater was used as a primary effluent having concentration varied from 400–600 ppm in batch mode. The sample was treated with ozone by generating the ozone in a generator (OZ Air, 15 M 5 g/h) at a rate of 5.2 g/h for 8 min. Treated wastewater samples were collected and properly marked according to time. Optical density was measured using spectrophotometer (Thermo scientific evaluation 201, UV2300 Techcom Germany) at a wavelength of 291 nm.

2.3.3. Biological treatment process

Biological treatment of pantoprazole was performed in a batch process taking 100 ml wastewater in 250 ml conical. The pantoprazole concentration was varied from 400 to 600 ppm. The microbial culture of *A. feacalis* and *E. aurantiacum* in log phase was used to treat the pantoprazole in the aqueous medium. This biochemical treatment was performed in a batch mode with 10% of acclimatized *A. feacalis* and *E. aurantiacum* inoculum. Acclimatized *A. feacalis* and *E. aurantiacum* cells were harvested by centrifugation (Sigma laborzentrifugen; 2K30) at 6000 rpm for 10 min. Bacterial monocultures cells pellets were added to the reaction medium at pH-7 and incubated at 37°C in an incubator shaker with 150 rpm. At the end of each experiment time interval, samples were collected, for different time intervals it was centrifuged (Sigma Laborzentrifugen; 2K30) filtered and the supernatant was analyzed for measurement of pantoprazole concentration in the reaction medium. The corresponding control sets were maintained with deactivated (boiled) microbes. The result shown is the mean values of triplicate experimental sets. Operating parameters temperature, pH and inoculum size were used pre-optimized conditions.

2.3.4. Adsorption treatment process

In the physical treatment experiment, the adsorption study has been done using commercially available activated carbon. 4 g/L of activated carbon were added to each of the samples of varying pantoprazole concentration of 400–600 ppm. The 100 ml samples were prepared in a 250 ml conical flask. The samples were kept into a shaker incubator (Daihan labtech: LSI:3016R) at 150 rpm and 35°C temperature was maintained. pH of the sample was found to be neutral. The samples were run for 120 min. All the experimental samples were collected and stored for analysis. The samples were first filtered using a filter paper to remove the activated carbon. Optical density was measured by a spectrophotometer (Thermo scientific, Evaluation 201 UV2300 Techcom Germany) at a wavelength of 291 nm.

2.4. Integrated treatment process

2.4.1 Fenton's oxidation and biological integrated treatment process

The combined effect of Fenton's treatment followed by biochemical treatment with *A. feacalis* and *E. aurantiacum*

bacteria were studied in the present study. At first 100 ml of 600 ppm pantoprazole containing wastewater was treated with Fenton's reagents consisting of 2 ml/L H_2O_2 + 0.75 g/L $FeSO_4$ for 60 min at 35°C and pH 3.5, Soon after the Fenton's treatment over pH was adjusted to 7.0, and biological treatment was executed by adding *A. feacalis* and *E. aurantiacum* from 10 ml matured growth culture of the microbes in two different reactors. This reaction system was incubated at 37°C in an incubator shaker with 150 rpm for 72 h. The samples were collected, centrifuged (Sigma Laborzentrifugen; 2K30) and filtered for the analysis of total pantoprazole degradations.

2.4.2 Adsorption and biological integrated treatment process

The treatment of pantoprazole of 600 ppm has been studied in activated carbon treatment system and subsequently microbial treatment by *A. feacalis* and *E. aurantiacum* has been performed. At the first 100 ml sample from 600 ppm concentration, pantoprazole wastewater was treated by adding 2 g/L of commercial activated carbon in a 250 ml conical flask at temperature 37°C in an incubator shaker with 150 rpm for 2 h. Soon after the adsorption study pH was adjusted to 7.0 and biological treatment has been commenced by adding *A. feacalis* and *E. aurantiacum* in the pantoprazole containing wastewater into two different reactors for 72 h and temperature 37°C in an incubator shaker with 150 rpm. Samples were filtered and stored for analysis.

2.4.3. Ozone and biological integrated treatment process

In the investigation 100 ml wastewater sample were taken of 600 ppm concentration of pantoprazole by keeping in shaker incubator for 2 h. Ozone treatment was performed by generating ozone in an ozone generator by 3.2 g/h for 8 min. Soon after the ozone treatment, biological treatment was performed using *A. feacalis* and *E. aurantiacum* at pH 7.0 and temperature 37°C in an incubator shaker with 150 rpm.

2.5. Spectrophotometric analysis

Spectrophotometer (Scientific Evolution 201 UV2300 Techcom Germany) was used to measure the concentration of the samples for measurement of pantoprazole in the treated, and untreated sample and GC (Agilent 7890B)-MS (Agilent 5977) was utilized for pantoprazole concentration in the samples and degraded products by Fenton's treatment.

2.6. Toxicity assay

It is difficult to destroy all the harmful chemicals present in wastewater completely. Though treatment reduces it to a great extent yet some pollutants may remain which can contribute to the toxicity of the wastewater and non-degraded products may contribute too. So it is desirable to check the toxicity of the treated sample natural aquatic stream before discharging into the stream. The toxicity test also indicates the effectiveness of the treatment in the degradation of harmful wastes. In the present study, *Vigna radiate* has been used to study the toxicological effects of pantoprazole at different concentrations [28]. Root meristem of *Vigna radiata* was exposed to samples for 5 d. Seeds were surface sterilized with 0.1% $HgCl_2$ and 70% ethyl alcohol for the prevention of surface fungal/bacterial contamination in petri dish experiment. Selected healthy seeds of the same size were soaked in pantoprazole solution of different concentration along with distilled water for 3 h. The seeds were then transferred to the petri dish with cotton bed followed by incubation at room temperature for 5 d. On the 6th day, the percent of germination was calculated on the basis of seed germination.

2.7. Effect of pantoprazole on DNA

In the study a fluorescence spectrophotometer (Cary eclipse fluorescence spectrophotometer; Agilent technologies; G9800A) was utilized for the quantification. Sample preparation has been done with 8 µL ethidium bromide and 80 µL hs-DNA. Determinately the sample was making up the total volume up to 4 ml utilizing pantoprazole integrated treated wastewater. After that, the sample incubated at 34°C for 30 min. Conclusively fluorescent intensity and result obtained.

3. Results and discussion

3.1. Pantoprazole wastewater degradation by Fenton's oxidation treatment

Fenton's oxidation treatment is a well-established method for treating wastewater from years [29]. The treatment of pantoprazole wastewater has been treated with Fenton's reagents in the present study. The pantoprazole concentration was taken 650 ppm and 4 ml/L H_2O_2 with 1.5 g/L $FeSO_4$ in a 250 ml conical flask. It was observed that 56.77% of pantoprazole removal was achieved in 60 min in this set of experiment. This is due to the fact that, in the first step of reaction $FeSO_4$ reacted with H_2O_2 and for hydroxyl radical ($OH^{\bullet}$) presented in Eq. (1), This $OH^{\bullet}$ radicals are a strong oxidant. It can oxidize any type of waste materials unselectively and mineralized [Eqs. (2), (3)].

$$H_2O_2 + FeSO_4 \longrightarrow Fe_2(SO_4)_3 + OH^{\cdot} \quad (1)$$

$$^{\cdot}RH + OH^{\cdot} \longrightarrow R^{\cdot} + H_2O + BP \quad (2)$$

$$R^{\cdot} + OH^{\cdot} \longrightarrow DegradedProducts + H_2O) \quad (3)$$

The pH of the system was maintained at 3.5. In literature, several studies [22,24,27,30,31] indicates that acidic pH is required for better formation of $OH^{\bullet}$ generation which is the key component for waste degradation but pH less than 2.0 generates less formation of $OH^{\bullet}$. Due to the formation of ferric hydroxo complex which retardate $OH^{\bullet}$ formation on the other hand pH above 4.5, another type of complexion is formed which is stop the $OH^{\bullet}$ formation and eventually waste degradation. Similarly, for temperature, ambient temperature (40°C) was considered for the experiment. Different scientist studies suggest that ambient temperature is

suitable for Fenton's treatment. But some studies demanded that 50–60°C is the optimum for the waste treatment by Fenton's reagents [32]. With increasing temperature reaction rate constant has been increased by Arrhenius rule, $k = k_0 e^{-E_a/RT}$ or $\ln k = \ln k_0 - E_a/RT$ which indicate that with increasing temperature, the reaction rate will also have increased. But for the present study treatment of waste by Fenton's reagents has been performed in ambient temperature. The fact behind that is the $H_2O_2 + FeSO_4$ reacts very fast, produce OH• radical and heat (exothermic reaction), which helps to increase the temperature rise of the reaction medium about 60–70°C and satisfy the two thoughts regarding optimum temperature for Fenton's treatment [22]. From the different literature it is also observed that with employing higher Fe^{2+} and H_2O_2 lower in the oxidation reaction, percentage removal of waste has been enhanced, but the proper ratio of H_2O_2 and $FeSO_4$ is very important to have maximum waste removal using minimum chemicals used [27]. The ratio of H_2O_2:$FeSO_4$ has been chosen from a preliminary study and a published report [22,23]. In the present study, the pantoprazole degradation has been studied with time and presented in Fig. 2. From the figure, it reveals that initially the degradation is very sharp and with time it is decreasing and often an hour the degradation is eventually negligible. This indicates, that the reaction of Fenton's reagents is very fast and within 60 min all H_2O_2 has reacted with $FeSO_4$ and produce OH•. This OH• radical has been utilized for pantoprazole degradation in the present study. The degradation achieved about 56.77% in the present dosage and condition. The disappearance of pantoprazole molecule has been studied concerning time, and the data has been used to study the kinetics of pantoprazole degradation. The following differential equation has been used for the kinetic study.

$$-\frac{dCp}{dt} = k\left(C_p\right)^n \tag{4}$$

$$\ln\left(-\frac{dCp}{dt}\right) = \ln k + n\ln C_p \tag{5}$$

Plotting $\ln\left(-\frac{dCp}{dt}\right)$ vs $\ln C_p$ and we have $k = 1.27$ mg/L·min, which indicate that Fenton's oxidation is a very fast reaction and thus it can generate OH• radical quickly OH• radical indiscriminately degrade the components present in the reaction system. The overall rate constant has been calculated in the present study. The exponent 'n' indicates the order of the reaction. In this waste degradation, three steps reaction has been considered. First step reaction due to very first reaction other two steps are not rate limiting. So 'n' value has been calculated and obtained 1.27, which indicate the reaction is closely 1st order in nature.

3.2. Effect of pH in Fenton's oxidation

pH plays a great role in Fenton's reagents mediated treatment system [33]. The OH• formation depends on system pH and eventually the % degradation. If the pH above 6.0 there is a chance of formation of ferric hydroxo complexes which subsequently form $[Fe(OH)_4]$ at basic pH and retardate the formation of OH• radical at attributed to the less waste degradation. At pH below 3.0, the waste degradation decline due to the fact that in this pH range formation of complex species $[Fe(H_2O)_6]^{2+}$ has been reported. This $[Fe(H_2O)_6]^{2+}$ perhaps reacts more slowly with peroxide in comparison to $[Fe(OH)(H_2O)_5]^{2+}$. Peroxide might get solvated in the presence of high concentration of H^+ ion to form a stable oxonium ion $[H_3O_2]^+$. Thus reactivity of Fe^{2+} ion is reduced substantially in the presence of oxonium ion. Therefore pH 3.5 was chosen for the treatment of pantoprazole solution.

3.3. Pantoprazole wastewater degradation by ozone treatment

Ozone treatment is an attractive technology today in the treatment of different industrial [34], domestic [35] and hospital waste [36] based on its O• generation property. The sample pantoprazole pollutant has been treated by ozone at a rate of 5.2 g/h at ambient temperature and varying the concentration of the pantoprazole sample; Ozone treatment reduces 35%, 29%, 36% of 400 ppm, 500 ppm, 600 ppm of pantoprazole pollutants sample respectively shown in Fig. 3. The percentage removal of pollutant decreases with increase in the concentration of pollutant for the same time of operation under ozone diffusion process because the mass transfer diffusional resistance increases towards the diffusion in ozone diffuser with an increase in the concentration of the pollutant pantoprazole.

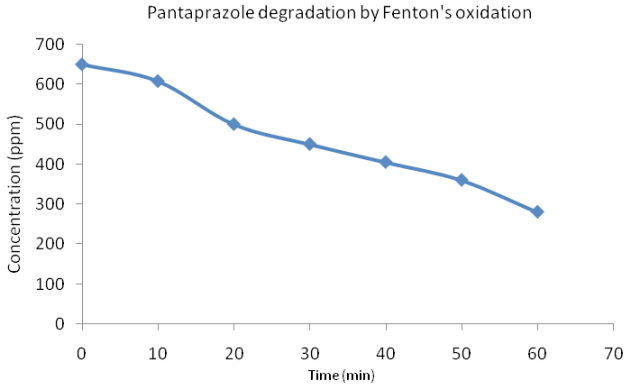

Fig. 2. Degradation of pantoprazole by Fenton's oxidation.

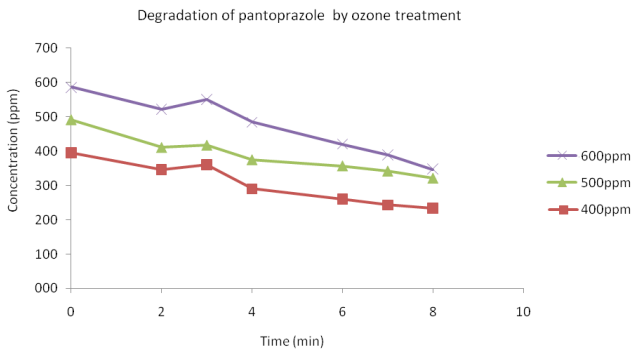

Fig. 3. Comparative study on degradation of pantoprazole by ozone treatment.

3.4. Degradation by biological treatment

Microbes can do wonder if it is used properly. Different studies reveal that, these technologies has been successfully utilized to degrade wastewater in textile industry [27], pharmaceutical industry [37], leather industry [22], food industry [38], petrochemical industry [39], etc. In the present study *A. feacalis* and isolated from coke oven waste and *E. aurantiacum* Isolated from paddy field has been studied. The results in Figs. 4 and 5 reveal that the bacteria *A. feacalis* and *E. aurantiacum* could reduce pantoprazole up to 58.4% and 60.08% respectively in 72 h in comparison with 4.36% in the control set. The pattern of degradation and growth of microbes is clearly indicating its involvement in pantoprazole degradation. The degradation potential of *A. feacalis* and *E. aurantiacum* can be explained on the basis of facts the bacteria can oxidize benzene ring directly due to the presence of enzyme phenol hydroxylase, catechol 1,2-dioxygenase and 2,3-dioxygenase [40]. This fact may be attributed to the degradation of the benzene ring of pantoprazole as a sole carbon source for the cellular growth maintenance and reproduction of *A. feacalis and E. aurantiacum*. In this study, The degradation of pantoprazole (predicted) is assumed that *A. feacalis* and *E. aurantiacum* oxidize pantoprazole and break the benzene ring by enzymatic action to produce organic radical R according to the reaction as :

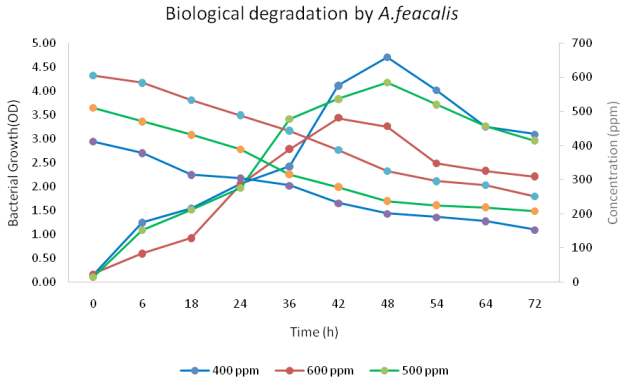

Fig. 4 . Comparative study of pantoprazole removal by bacterial (*A. faecalis)* degradation.

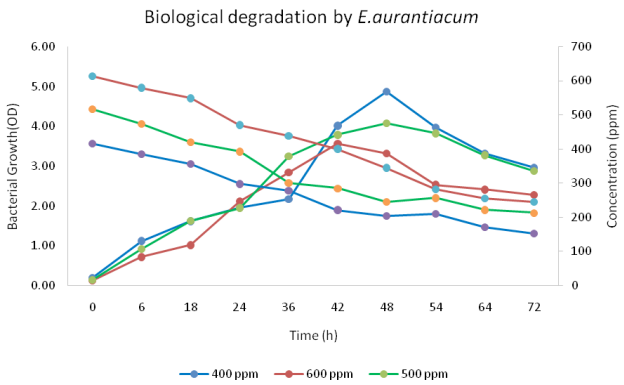

Fig. 5 . Comparative study of pantoprazole removal by bacterial (*E. aurantiacum*) degradation.

$$A.\ faecalis/\ E.\ aurantiacum + P_n + O_2 \rightarrow R^{\bullet} + H_2O + \quad (6)$$

P_n = Pantoprazole, $R^{\bullet}$= Intermediate product.

The component $R^{\bullet}$ gets oxidized by the release of an electron from $R^{\bullet}$ radical to R, according to the following reaction [22].

$$A.\ faecalis\ /\ E.\ aurantiacum\ s + R^{\bullet} \rightarrow P + NMC \quad (7)$$

NMC = More number of microbial cells. Another probable path ways is,

$$R^{\bullet} + A.\ faecalis\ /\ E.\ aurantiacum \rightarrow R^{+} + BP + NMC$$

BP = some other product

$$R^{+} + H_2O \rightarrow P + H^{+}$$

P = degraded small simple carbon product like CO_2, CO etc.

The percentage removal of pollutant also decreases with increase in the concentration of pollutant for same period of time because with increasing concentration, toxicity enhanced in the system. Therefore bacterial growth rate in 600 ppm and 500 ppm have been observed lower than in 400 ppm.

3.5. Pantoprazole wastewater degradation by activated carbon adsorption treatment process

In the present study adsorption by activated carbon is used as a proven technique for wastewater degradation. As described in Fig. 6, it was found that activated carbon 4 g/L reduces 85.65%, 83.72%, 82.84% of 400 ppm, 500 ppm, 600 ppm of pantoprazole respectively. The percentage removal of pantoprazole pollutant increases with time and dose of activated carbon is being used because the pores of activated carbon is being occupied by more pollutant pantoprazole and it engenders a mass transfer resistance towards diffusion in activated carbon pores with an increase in the concentration of pollutants. There are two methods that have been applied for the determination of state of equilibrium of adsorption system by Langmuir adsorption iso-

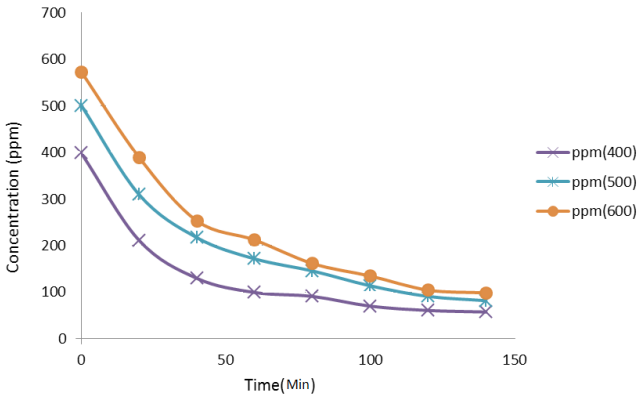

Fig. 6. Comparative study on degradation of pantaprazole by adsorption treatment.

therm (R^2 = 0.9981) (Fig. 7) and Freundlich isotherm model (R^2 = 0.8411) (Fig. 8). In Langmuir isotherm Q_e (mg of adsorbate/g of adsorbent) is equal to 46.22 and Rl (dimensionless separation parameter) = 0.01032 was obtained for 1000 ppm and in Freundlich isotherm the value of n and k_f were observed 6.31 and 19.20 respectively. The evaluated value of Rl determines whether the isotherm is unfavorable (Rl > 1), linear (Rl = 1), favorable (0 < Rl < 1) or irreversible (Rl = 0). Therefore the Langmuir isotherm is favorable.

3.6. Integrated treatment

3.6.1. Pantoprazole wastewater degradation by Fenton's oxidation and biological integrated treatment process

The sole advanced oxidation process by Fenton's treatment is costly and, consequential harm is high due to huge sludge generation (4.40 g/L) and other toxic ingredients formation [22,41]. To overcome from these problems for an individual process, an integral treatment process has been designed. In the 100 ml solution of 600 ppm pantoprazole, a particular concentration of H_2O_2 (2 ml/L) and $FeSO_4$ (0.75 g/L) was added at a rotating condition of 100 rpm at 35°C and pH 3.5. Soon to the Fenton's treatment, biological treatment by *A. feacalis* and *E. aurantiacum* for three days (72 h) has been done at pH 7.0 and temperature 37°C. The result showed an additive effect on pantoprazole degradation. The result has been presented in Figs. 9 and 10. It clearly indicates the Fenton's treatment has enhanced the biodegradability of the waste [37]. Thus total removal of pantoprazole has been reached to 90.17% and 87.44% by *A. feacalis* and *E. aurantiacum* respectively other way an individual biological treatment by *A. feacalis* and *E. aurantiacum* were capable of degrading only 55.48% and 60.08% respectively of pantoprazole removal. Thus it can be said that Fenton's pretreatment to the pantoprazole stimulates the waste reduction by *A. feacalis* and *E. aurantiacum* is two times compared the sole biological treatment by *A. feacalis* and *E. aurantiacum* for three days. On the other hand compared to the sole Fenton's treatment the degradation can be achieved up to 56.77%. The integrated treatment shows an additive effect on overall pantoprazole treatment. It can also be said that cost of the treatment with sole Fenton's treatment to achieve 90% of pantoprazole removal is being reduced by using a lower dosage of Fenton's reagents (H_2O_2+$FeSO_4$) in the combination with biological process. Moreover sludge generation will be reduced in combined study (1.9 g/L). Thus it can be said that the integral treatment is better than the sole Fenton's and the biological process by *A. feacalis* and *E. aurantiacum* to treatment the pharmaceutical waste pantoprazole.

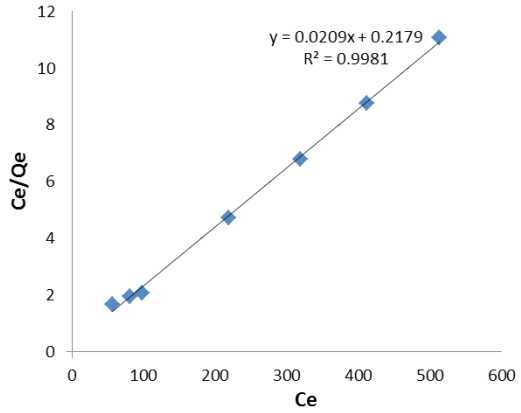

Fig. 7. Langmuir isotherm model curve.

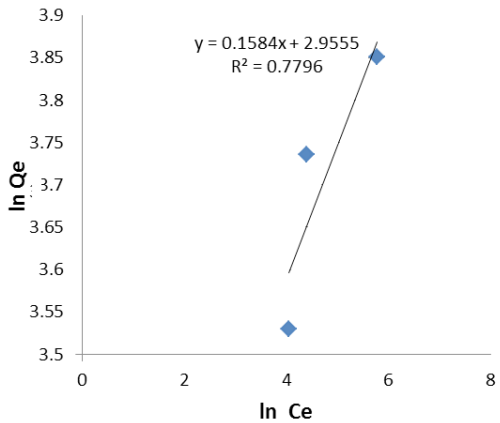

Fig. 8. Freundlich isotherm model.

3.6.2. Pantoprazole wastewater degradation by adsorption and biological integrated treatment process:

The sole adsorption by activated carbon treatment of pantoprazole wastewater has been studied. Due to the high cost of commercial activated carbon and disadvantage such as spent adsorbent could be come into hazourdous waste, this process is not much promising. To come over from that situation. The present study has been set up to combine the biological treatment and adsorption [42,43]. The integral treatment has been designed with a 100 ml solution of 600 ppm pantoprazole. After sample preparation, 2 g/L activated carbon has been added into the sample and placed in incubator shaker. Soon after the treatment and filtration, the sample has been inoculated with *A. feacalis* and *E. aurantiacum* bacteria in two different batch reactors. The result shown in Figs. 11 and 12 that the integral treatment has been removed about 81.57% and 77.14% of pantopra-

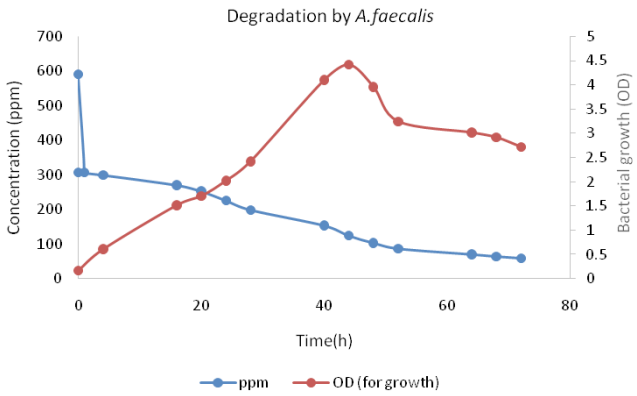

Fig. 9. Degradation of pantoprazole by Fenton's oxidation and biological integrated treatment using bacteria *A. faecalis*.

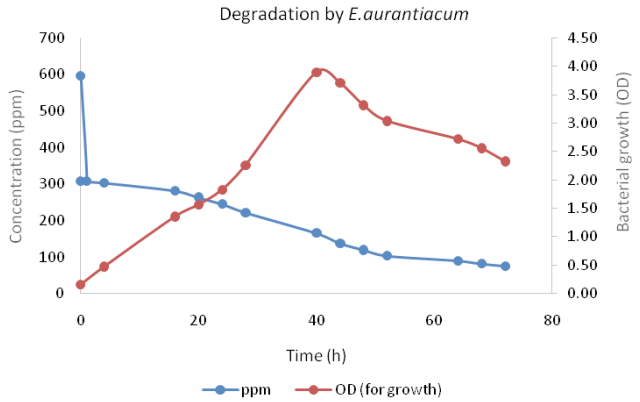

Fig. 10. Degradation of pantoprazole by Fenton's oxidation and biological integrated treatment using bacteria *E. aurantiacum*.

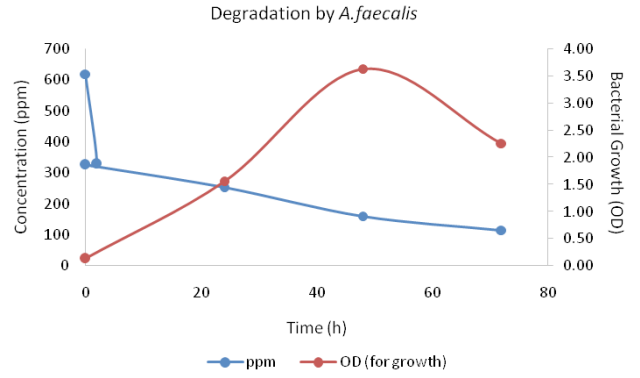

Fig. 11. Degradation of pantoprazole by adsorption through activated carbon with biological integrated treatment using bacteria *A. faecalis*.

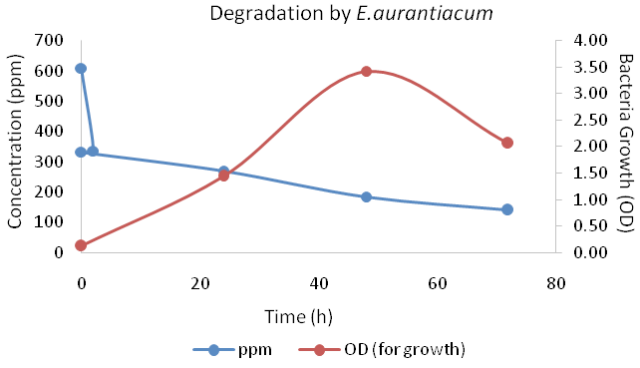

Fig. 12. Degradation of pantoprazole by adsorption through activated carbon with biological integrated treatment using bacteria *E. aurantiacum*.

zole respectively. Whereas the sole biological treatment by *A. feacalis* and *E. aurantiacum* reduced 58.40% and 60.08% of waste. Sole activated carbon adsorption treatment removed 82.84% of pantoprazole pollutants with a higher dose of activated carbon (4 g/L). Hence it can be said that the combined treatment is more cost effective and efficient with less consequential harm.

3.6.3. Pantoprazole wastewater degradation by ozone and biological integrated treatment process

The sole ozone treatment process by the ozone generator can be costly and harmful to the operator (human beings) as it can cause nausea, headache, etc. Pantaprazole removal rate additionally not much promising. Similarly, the biological treatment alone is time-consuming process. To overcome this problem an integral process has been set up. In the setup, a 100 ml solution of 600 ppm pantoprazole has been treated in ozone generator with a rate of 3.2 g/h at ambient temperature. After that *A. feacalis* and *E. aurantiacum* bacterial treatment has been studied for three days (72 h) at pH 7 and temperature 37°C. The result showed a synergetic effect in pantoprazole removal; presented in Figs. 13 and 14, clearly indicate that the combined treatment can enhance the biodegradability of the pantoprazole by removal of waste has reached up to 71.07% and 70.98% respectively by *A. feacalis* and *E. aurantiacum*. The sole bacterial treatment for pantoprazole shows that 58.40% and 60.08% respectively by *A. feacalis* and *E. aurantiacum* of pantoprazole degradation has been archived in three days. This is due to the fact that in the integrated treatment, the preliminary degradation by ozone treatment, the chemical structure of pantaprazole may have changed, and it became more degradable to bacteria and as a result, the degradation percentage has been increased. The integrated treatment shows a result much promising in all aspects.

3.7. Toxicity assay (Effect of pantoprazole)

Toxicity assay is a proven method to measure the toxic effect of the treated pollutants [44]. Results from the root and shoot growth inhibition study are depicted in Fig. 15 and Table 1. A root growth inhibition of nearly 56% and shoot growth inhibition of nearly 51% has been seen for a pantoprazole concentration of 10 ppm while 1 ppm showed 13.22% and 10.24% inhibition respectively. It can be seen from the results, and root growth is more hampered than shoot.

3.8. Effect of pantoprazole on DNA

3.8.1. Result on DNA study of Fenton's with biological integration treatment process

The fluorescence spectroscopic technique was used to determine the type of interaction that exists between pantoprazole with Hs-DNA. Emission scan was performed for incubated samples by fixing excitation wavelength as 471 nm (Fig. 16). Control was taken to be distilled water containing DNA and EtBr dye. The concentration of DNA and EtBr was fixed as 0.2 mM and 0.02 mM. Peak was observed for control at 609 nm. Quenching was observed for the sample before Fenton's treatment, this might be possibly due to the competition existing between EtBr and pantoprazole molecules. The pantoprazole has intercalated between base pairs and released some EtBr molecules away from DNA;. this results in a decrease in fluorescence intensity. Quenching was found to be maximum for sample underwent Fenton's treatment.

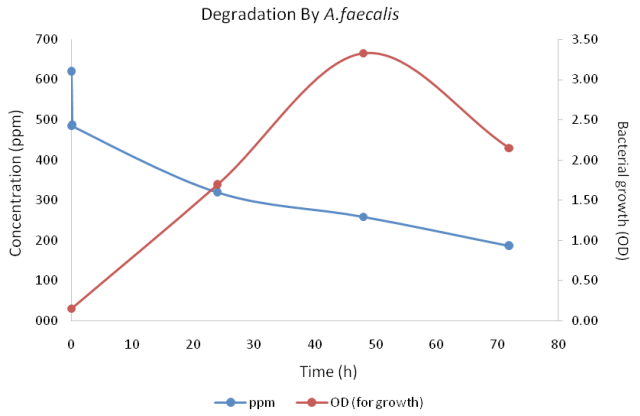

Fig. 13. Degradation of pantoprazole by Ozone and biological integrated treatment using bacteria *A. faecalis*.

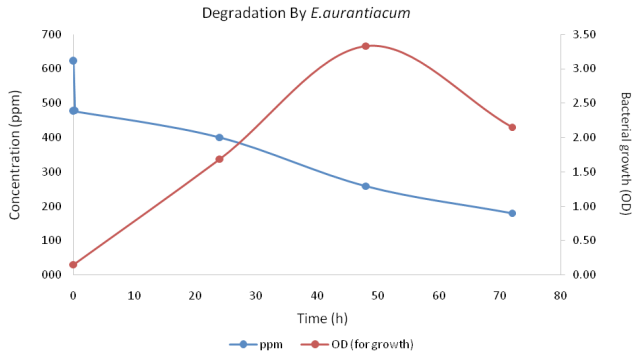

Fig. 14. Degradation of pantoprazole by ozone and biological integrated treatment using bacteria *E. aurantiacum*.

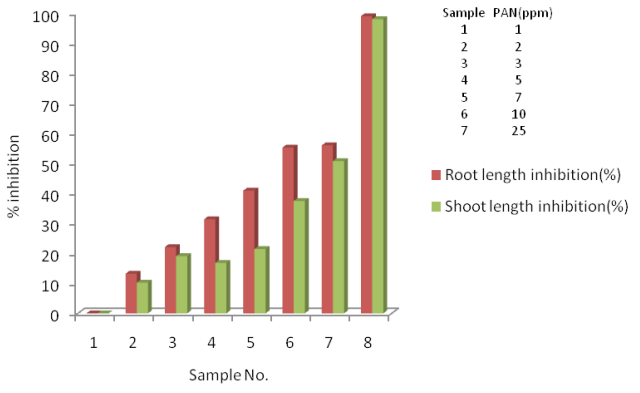

Fig. 15. Growth inhibition study of pantoprazole on *Vigna radiate*.

Fenton's treatment involves the generation of $OH^{\bullet}$ radicals from $FeSO_4$ and H_2O_2; $OH^{\bullet}$ is a powerful oxidizer. It breaks the complex structure of pantoprazole into large number of small molecules. Small molecules can interact with DNA by intercalation, electrostatic interaction and groove binding resulting in the release of large number of EtBr molecules out from DNA helix. Nutrient broth control was prepared and scanned since all the bacterial treated samples contain nutrient broth as one of its major

Table 1
Toxicity analysis study

Sample	Root length inhibition (%)	Shoot length inhibition (%)
Control	0	0
1	13.22	10.24
2	22.07	19.05
3	31.33	16.81
5	40.89	21.40
7	55.24	37.45
10	55.95	50.72

constituents. The intensity of nutrient broth control is found to be very much lesser than control containing distilled water. Nutrient broth is a complex media containing many components such as peptone etc.; they can also interact with DNA. The samples treated with *A. faecalis* and *E. aurantiacum* for 72 h showed more quenching relative to nutrient broth control. This indicates that the bacterial degradation of pantoprazole (and small molecules produced by Fenton's treatment) results in increased production of molecules that can intercalate into DNA. Also, bacteria utilize the media and also excrete toxic compounds. The present study with combination treatment shows much less toxicological effect according to Fig. 16, whereas the sole Fenton's treatment shows a more toxic effect in the study.

3.8.2. Result on DNA study of ozone treatment with biological treatment integrated process

Control was made with distilled water containing only DNA and dye, and no pollutant. Maximum intensity was visualized at 609 nm by keeping excitation wavelength at 471 nm and performing emission scan (Fig. 17). Intensity was reduced to half the control for the sample before ozone treatment. This indicates that the sample contains molecules which have the ability to compete with EtBr. Quenching results due to release of EtBr from DNA as the pantoprazole intercalates into the region between base pairs of DNA. Ozone treatment has resulted in an increase in fluorescence intensity. This might be due to the oxidation of pantoprazole by oxygen radical $O^{\bullet}$. Carboxylic acids can be produced as end products of ozone degradation. Carboxylic acids being negatively charged will get repulsive attraction from negatively charged oxygen atom of phosphate diester backbone. So the interaction between DNA and degraded products would be decreased, indicated by rise a in intensity. Bacterial treatment of ozone degraded sample by *A. faecalis* might result in the metabolism of carboxylic acid molecules into acyl CoA which gets utilized by the *A. faecalis* in the kreb cycle. Interactions between hs-DNA and degraded products do not vary much in intensity. Bacterial treatment by newly isolated *E. aurantiacum* strain converts the ozone degraded products into small molecules which were capable of interacting with hs-DNA. The result indicates a considerable decrease in intensity. The toxicological

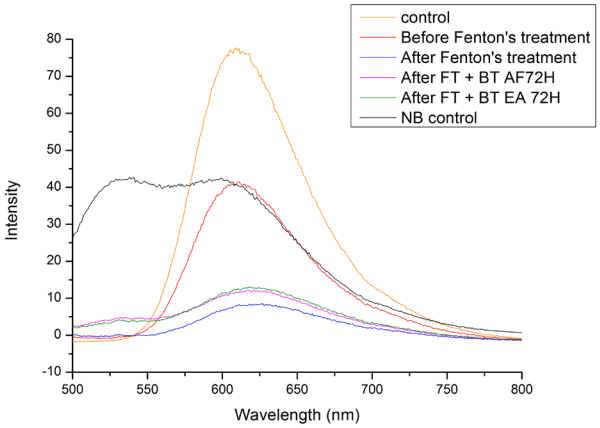

Fig. 16. Toxicological study by fluorescence spectroscopy (Fenton's reaction and bacterial integrated treatment).

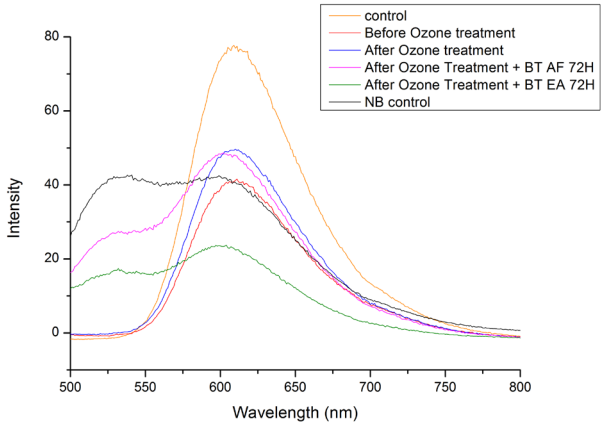

Fig. 17. Toxicological study by using fluorescence spectroscopy (Ozone and bacterial integrated treatment).

effect is much lower in combination treatment compared to sole ozone treatment, which is much promising and environment-friendly.

3.8.3. Result on DNA study of adsorption treatment with biological treatment combination

Control was made with distilled water containing only DNA and dye, and no pollutant. Only activated carbon adsorption treatment degraded 82.80 % of pantoprazole pollutants in the simulated wastewater. It was observed that the intensity of the sample before ozone treatment reduced to nearly half of the control sample. Activated carbon treatment of the sample showed an increase in the fluorescence intensity. The pores of the activated carbon become loaded with the pantoprazole waste, entrapping the pollutants and thus decreasing the concentration of the pollutants but the toxicity of the sample does not decrease. Activated carbon treatment and subsequent filtering achieved more degradation of pollutants by further biological treatment. The biological treatment of the sample also leads to reduce cost efficiency as well as environmental relief due to less toxicity. Bacterial treatment of

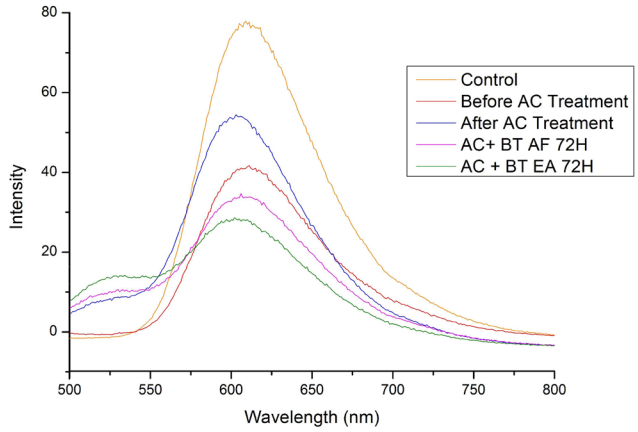

Fig. 18. Toxicological study by using fluorescence spectroscopy (Adsorption and bacterial integrated treatment).

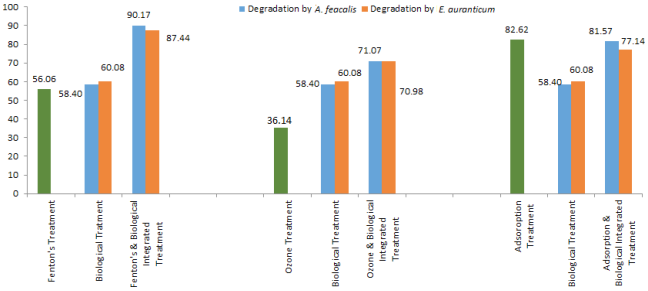

Fig. 19. Comparative study on removal of pantoprazole from waste.

activated carbon treated pantoprazole wastewater sample by *A. faecalis* displayed some significant drop in the pollutant levels as the intensity dropped from that of the activated carbon treated sample. Bacterial treatment by *E. aurantiacum* strain further breaks down the activated carbon treated products into smaller molecules which were capable of interacting with hs-DNA. The result indicates a considerable decrease in intensity (Fig. 18). It can be concluded that even though the sole adsorption process can reduce a huge percentage of pantoprazole concentration, but the toxicity effect has increased by the same. Similarly, after integrated treatment, the toxicological effect has been decreased significantly as per Fig. 18 which will be more suitable to nature.

4. Conclusion

In the present study all the treatment systems have shown their the potential to degrade pantoprazole in the lower molecule to save the environment but having some elimination in the individual case. In integrated treatment system altogether provides significant benefit over each of the independent system. Treatment of pantoprazole wastewater by integrated treatment shows additional effects of pantoprazole removal (Fig. 19). A toxicological study also reveals that the toxicity has been reduced to a greater extent for safe disposal of treated pantoprazole containing waste-

water to the environment. Ultimately integrated process looks like efficient, cost-effective, environmentally safe and less energy consuming process.

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Conflict of Interest

None

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