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Bioleaching of iron from fly ash using a novel isolated *Acidithiobacillus ferrooxidans* strain and evaluation of catalytic role of leached iron in the Fenton's oxidation of Cephelaxin

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Iron is the sole energy source for the acidophilic bacterium *Acidithiobacillus ferrooxidans*. Feeding indirect iron source to this bacteria results in leaching of iron from complex minerals. In this study fly ash, a waste is fed to the isolated bacteria under stress condition and is made to recover the traces of iron present in the fly ash for its application as a Fenton's catalyst to degrade Cephalexin. The investigation evaluates the leaching potential of a novel isolated strain *Acidithiobacillus ferrooxidans* BMSNITK17 in leaching iron from fly ash. About 89 mg/L of iron is recovered within the initial five days of inoculation. It is observed that the rate of metabolism of bacteria is very slow with fly ash as source. Catalytic efficiency of recovered iron was investigated to degrade Cephalexin, a major waste found in pharmaceutical and hospital discharge. About 87.98% of Cephalexin is degraded in first two hours with COD reduction of 74.21%. Reaction follows pseudo-first order kinetics with rate constant 0.017/min.

Keywords: Bioleaching, *Acidithiobacillus ferrooxidans*, fly ash, Fenton's process, Cephalexin.

Introduction

Effluent from pharmaceutical industries and hospital are of more concern to the environmentalist for its toxic and hazardous compounds which pose direct threat to the environment on its disposal[1]. Many of these compounds sustain the degradation even at the final stage of treatment and manage to persist for a long period in the environment posing hazard to life. Cephalexin is a group of cephalosporin drug synthesized from penicillin sulfoxide by its ring expansion[2]. This antibiotic drug has its application in the treatment of wide variety of disease. In spite of its beneficial use Cephalexin may cause diahhrea, nausea, skin irritation etc. On its way to environment through household drug usage, pharmaceutical and hospital discharge these are resistant for biological degradation[3–5]. Hence biological methods to treat Cephalexin fails in this concern[6]. Advance oxidation is the chemical method of treatment which is effective for the wide variety of organic pollutants with short duration of treatment. Among advance oxidation process Fenton's oxidation is of very much interest to the environmentalists. Fenton's oxidation to degrade Cephalexin has been tried by few researchers[7,8].

Resource recovery from the waste and zero discharge is the topic of concern that has been in research from decades. Fly ash has its application as reinforcement in concrete and soil studies giving rise to core strength to materials. Environmental concern towards fly ash has been neglected for many decades. Fly ash by virtue of its composition contains silicon dioxide, aluminium oxide, magnesium oxide and iron oxide. Iron content of fly ash is comparatively very low marking its content up to 10–15% of its overall composition[9,10]. Fenton's oxidation in the treatment of water and wastewater has got importance for its efficient degradation capability of vast number of organic pollutants. Use of iron as a catalyst reduces the treatment time and increases the efficiency[11–14]. Iron from the fly ash was recovered by some of the researchers using conventional methods[55]. Valeev and co-workers succeeded in the recovery of aluminium and iron by magnetic separation and carbon floatation technique[14]. Elomaa and others claims acid leaching is more suitable in the recovery of iron from fly ash and also they found no iron is leached out with ethanol leaching[16,17]. Carbon thermal reduction is again found to be effective in iron recovery from fly ash with an account up to 89%[18]. Bioleaching of iron from fly ash was studied by few researchers. It is said that bioleaching is effective in the recovery of nickel, lead, cobalt and other heavy metals than iron[19,20]. Commercially available iron is being used in the treatment practice increases the treatment cost and sludge generation. Replacement for commercial iron including other divalent cat ions and natural iron extracted from available laterite soil has been tried by many researchers for its efficient application as Fenton's catalyst[21–24]. However research on the recovery of iron from industrial waste such as fly ash for its application as Fenton's catalyst has not been carried out so far. The present study focus on leaching of iron content from the fly ash using an isolated strain of acidophilic bacteria *Acidithiobacillus ferrooxidans* BMSNITK17 and evaluation of its potential in iron leaching from the fly ash. The investigation also deals with the application of leached fly ash iron as a catalyst in the Fenton's oxidation to degrade Cephalexin, a pharmaceutical waste.

Material and methods:

Bioleaching of iron from fly ash:

Fly ash used in the study was collected from the nearby industry, Karnataka, India. The bioleaching studies were carried out in modified 9K media with no ferrous iron supplement to which fly ash was added to makeup definite volume of mixture[25]. 10 ml of an isolated bacterial strain *Acidithio bacillus ferrooxidans* BMSNITK17 (Accession No. MG27180) inoculum of 1.0×10^7 cells/ml was added further to this mixture to initiate the leaching studies[26]. Studies were conducted for different pulp density and different initial pH to study the effect of fly ash loading and pH on leaching. All the experiments were conducted in dual with sterile conical flasks on incubator shaker.

Bioleached fly ash iron catalyzed Fenton's oxidation of selective herbicides:

Assessment of catalytic efficiency of leached iron was carried out. Synthetic Cephalexin solution with initial concentration of 150 mg/L was prepared in the laboratory. The pH was adjusted to 3 using 1 *N* H_2SO_4 prior to the study. Different dosage of leached iron was added at incremental rate (16 mg/L, 18 mg/L, 20 mg/L) aftermath H_2O_2 was added at different dosage (160 mg/L, 180 mg/L, 200 mg/L) to study the combined effect on the process. Samples were drawn at regular intervals for analysis. During sampling, each time 1 ml of sodium thiosulphate was added to arrest the reaction[27]. All the experimental analysis was conducted in triplicates.

Scanning Electron Microscopic (SEM) analysis:

Morphological feature of fly ash before and after bioleaching was studied with SEM. Change in mineralogical composition during the leaching was studied. The sample was mounted on aluminum stub using double sided carbon tape, sputter-coated with gold and visualized using a S-3400N scanning electron microscope (Hitachi, Japan).

Analytical procedure:

Cephalexin concentration was measured with UV-Vis spectrophotometer. Chemical oxygen demand (COD) measurement was by colorimetric method as per 5220D of Standard Methods for Examination of Water and Wastewater[28]. The H_2O_2 consumption was measured using UV-Vis spectrophotometer[29]. Concentration of ferric iron was measured by potassium thiocynate method using UV-Spectrophotometer (Systronics make, AU-2701)[30]. The pH was monitored by digital pH meter (HANNA make).

Results and discussion

Bioleaching of iron from fly ash:

Bioleaching of traces iron present in the fly ash was investigated varying initial pH and pulp density at a shake flask speed of 180 rpm. Fly ash by virtue contains high amount of carbon, silica, and aluminium with traces of iron. Fig. 2 represents the EDS data before and after bioleaching which gives the comparison of fresh and bioleached fly ash. In the present study at a pulp density of 2.5% maximum iron dissolution of 89.405 mg/L was observed in 5 days. With increase in pulp density to 5% and 10% significant iron dissolution has not been found whereas at 1% pulp density the bioleaching of iron was very slow. Since fly ash contains fine grading particles increase in pulp density might limits the gas transfer resulting in the death of cells due to oxygen deficiency[31,32]. Halt of bacterial oxidation indicates that the pulp density less than 2.5% is not suitable for bacterial metabolic activity to occur due to constraint in the iron availability. Fig. 4 represents the iron dissolution rate at different pulp densities and at different initial pH. Maximum iron dissolution was found at pH 2.5. Bacterial strain employed in the present study was evaluated for its bioleaching ability at different conditions with lateritic soil (Data not shown) in which the optimum pH range found was 2.5–3.0. Maximum iron leaching found in the present study supports that the high activity of bacterial strains at pH 2.5. Bacterial growth is usually inhibited at pH less than 1.5[33,37]. In addition to this the redox potential observed were high during first four days later on decrease in redox potential indicates the inhibition of bacterial oxidation. Fig. 5 dissipates the variation of redox potential and corresponding pH at different pulp densities. It is observed that with pulp density 1% and 10% the redox potential dropped than the initial redox value observed. With the pulp density 2.5% maximum redox potential of 580 mV was observed with corresponding final pH dropped to 2.2. The redox potential value drops with a rise in pH indicating that shift of biooxidation to hydrolysis. Fig. 1 represents the SEM images of fresh and bioleached fly ash. Fig. 3 dissipates the XRD data of fresh and bioleached fly ash. The structural and compositional changes in fresh and bioleached fly ash can be observed with these data. SEM images of fresh fly ash appear to be circular nodule with smooth surface whereas the changes in the structure are observed with a fly ash subjected to bacterial action. Fig. 2 and Table 1 dissipate EDS data of fresh and bioleached fly ash indicating elemental compositions.

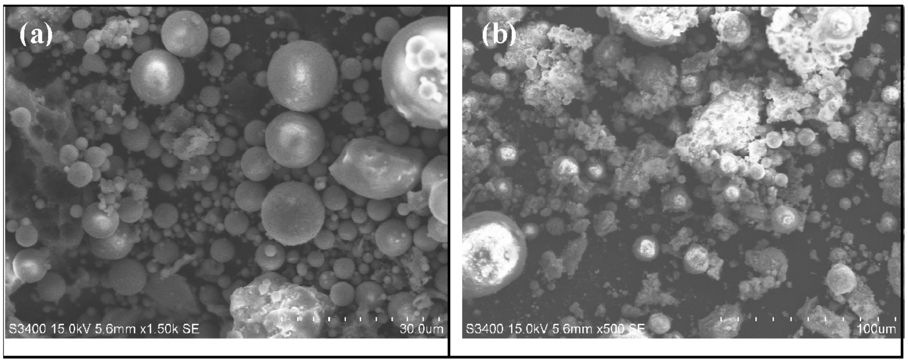

Fig. 1. SEM images showing fly ash morphology: (a) before leaching and (b) after bioleaching.

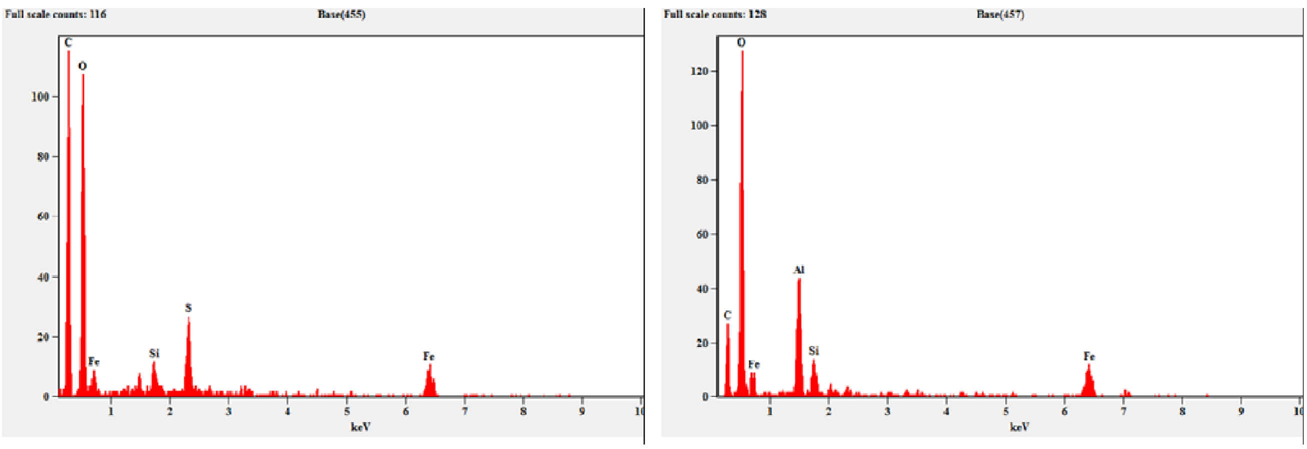

Fig. 2. EDS spectra showing elemental compositions of fly ash: (a) before leaching and (b) after bioleaching.

Table 1. Major elemental composition of fresh and bioleached fly ash

Elements	Fresh fly ash (% wt)	Bioleached fly ash (% wt)
Carbon	32.21	56.13
Silicon	48.96	35.82
Iron	10	5.41
Sulfur	Nil	1.87

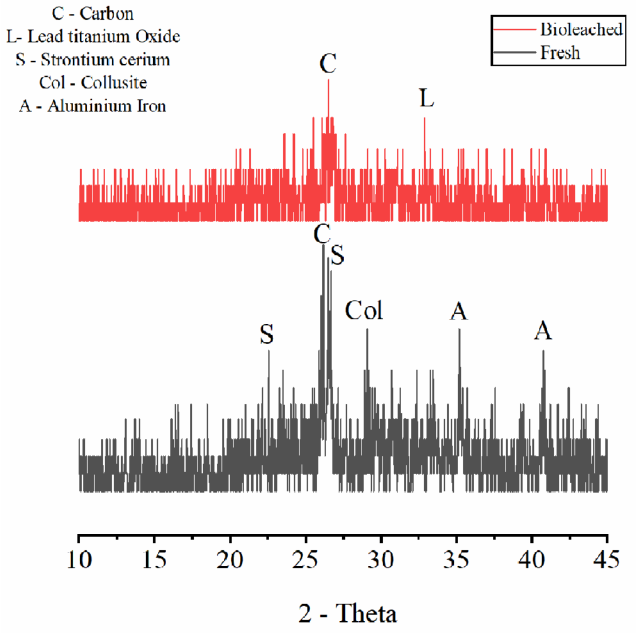

Fig. 3. XRD pattern of fly ash before and after bioleaching.

Iron present in the fly ash is reduced by 5% on weight basis after bioleaching. The XRD data of fresh fly ash shows the several broad sharp peaks at 2θ (26.58, 29.17, 22.54, 36.12 42.12) indicating the presence of carbon, aluminium, iron, strontium, cerium and colusite (PDF No. 03-065-6212; 00-045-0982; 00-022-0903; 01-073-1666). The XRD data of bioleached fly ash indicates the peak with respect to carbon remains as it is with new peak appears to be lead titanium oxide (PDF No. 01-076-0993) was observed at 2θ (33.95). It can be interpreted that the peak referring colusite contains zinc, titanium, lead, copper, iron and molybdate. Due to bacterial action these metals might have been leached out forming lead titanium oxide. Inhibition of bacterial activity within five days is attributed to heavy metals present in the fly ash which might have induced toxic effects on the metabolic activity of bacterial cells.

Catalytic degradation of Cephalexin by bioleached fly ash iron:

Iron leached biologically from the fly ash was evaluated for its catalytic role in the degradation of Cephalexin by Fenton's oxidation process. Initial target pollutant of 150 mg/L of Cephalexin was subjected to the investigation[38,39]. Initially with hydrogen peroxide and target pollutant negligible degradation was observed within two hours of treatment. Again the investigation was carried out with leached iron alone which does not yield any leaching. Degradation of target com-

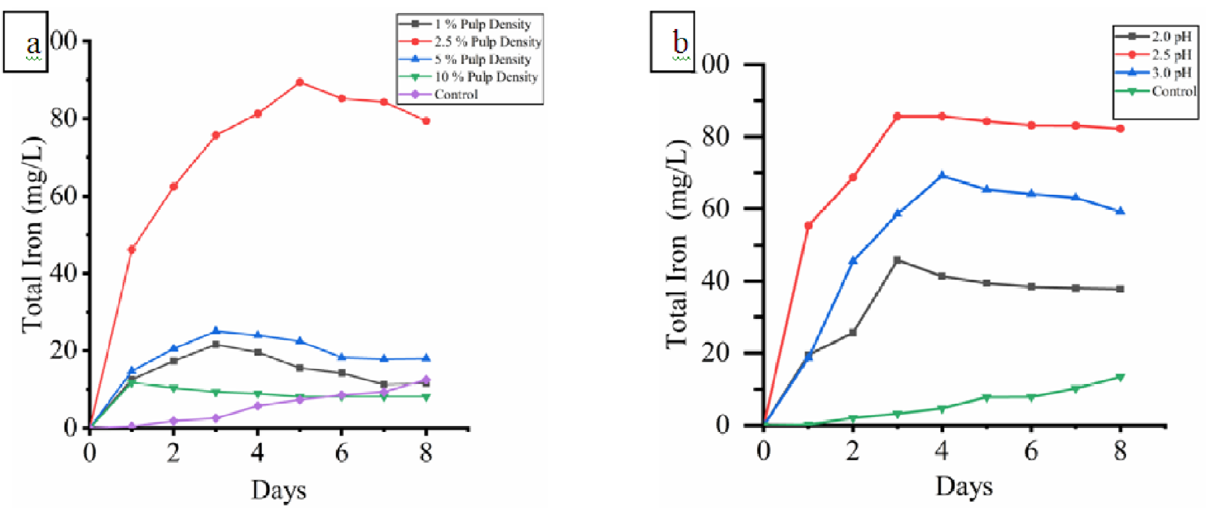

Fig. 4. Iron dissolution on bioleaching under different (a) pulp density and (b) initial pH.

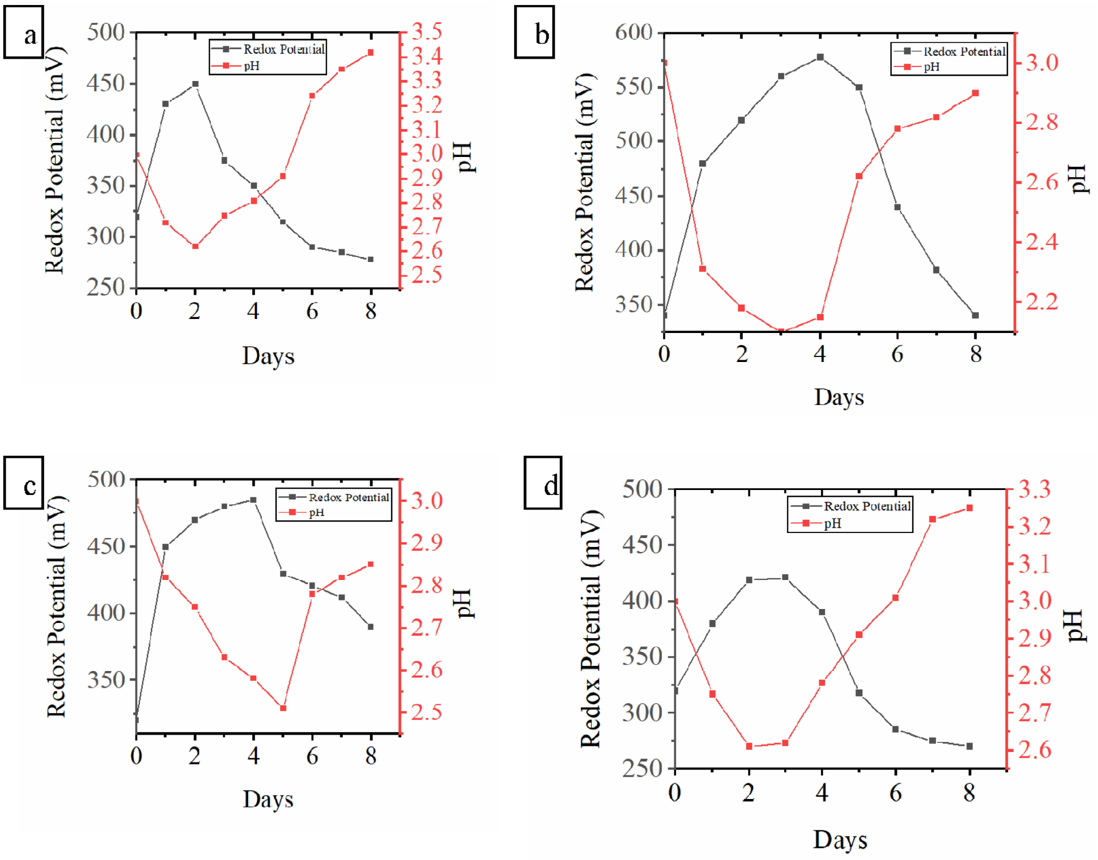

Fig. 5. Variation of redox potential and pH at different pulp densities on experimental investigation: (a) 1% PD, (b) 2.5% PD, (c) 5% PD and (d) 10% PD.

pound up to 87.98% was observed within 2 h of treatment when both leached iron and hydrogen peroxide were used. This confirms the catalytic role of leached iron in the degradation of Cephalexin. Bansal and Verma[8] observed 89% of Cephalexin degradation and claims it is the synergetic effect of combined photo catalysis and photo Fenton's reaction responsible for the degradation at low pH[8]. Al-Musawi and his team observed overall 90% degradation within 60 min by sono Fenton's treatment[7]. Variation in H_2O_2 dosage with leached iron dosage to study its effect on degradation was carried out and is graphically dissipated in Fig. 6(a,b,c). No significant increase in the degradation rate was observed on increase in H_2O_2 dosage above 160 mg/L. This marks the limits for H_2O_2 dosage for the target pollutant with initial concentration of 150 mg/L. Above this dosage dissipation of H_2O_2 in the process may cause scavenging effect instead of active involvement in the degradation process and also due to no reduction in the induction period of process on H_2O_2 increase[40,41]. Again with increase in extracted iron dosage from 18 mg/L to 20 mg/L significant degradation was not observed. This indicates more iron dosage than 18 mg/L does not involve actively in the degradation of target compound. This might be due to addition of more iron dosage reacts with the hydroxyl radicals hindering its role in the target compound degradation. The experimentation was carried out in an acidic pH 3 which marks the favorable condition for Fenton's oxidation to occur. During the process slight pH changes was observed (Data not shown). Overall COD removal of 74.21% indicates the better oxidation during the process. Degradation of Cephalexin against time at optimum dosage of iron

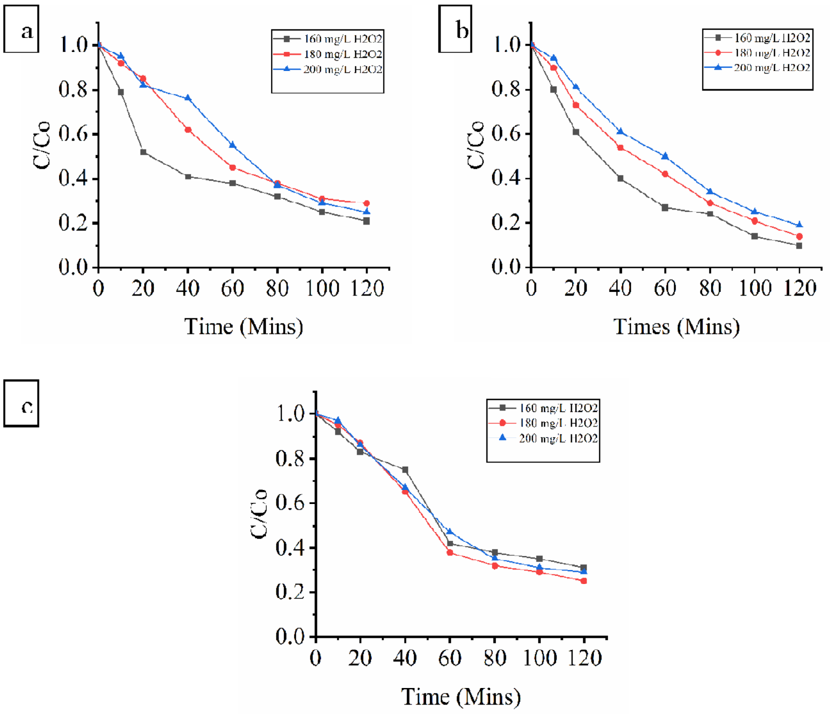

Fig. 6. Effect of H_2O_2 concentration on the process at leached iron dosage of (a) 16 mg/L, (b) 18 mg/L and (c) 20 mg/L.

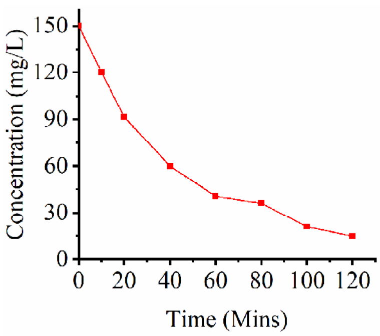

Fig. 7. Degradation of Cephalaxin at leached iron dosage of 18 mg/L and H_2O_2 dosage of 160 mg/L.

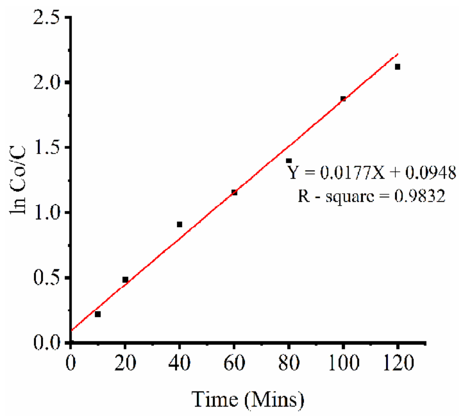

Fig. 8. Pseudo-first order kinetic linear fit.

dosage and hydrogen peroxide is given in Fig. 7. The overall reaction follows pseudo-first order kinetics with the linear fit shown in Fig. 8.

Conclusions

Traces of iron present in the fly ash was recovered biologically using an isolated bacterial strain *Acidithiobacillus* *ferrooxidans* BMSNITK17 investigating its potential to leach out the iron from fly ash. It is observed that the bacterial strain has successful involvement in the recovery of iron from the fly ash. Hindrance in the leaching process observed is due to limitation in the gas transfer which inhibits the bacterial activity and also to the heavy metals associated with fly ash by its composition. Leached iron has its catalytic role in the degradation of Cephelaxin, a major pharmaceutical waste thereby reducing the catalyst cost in the treatment process. The reaction follows pseudo-first order kinetic with curve best fit.

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