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Bioleaching of Al from spent fluid catalytic cracking catalyst using Aspergillus species

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ABSTRACT

Bioleaching uses biodegradable organic acids, thereby making the process environmental friendly as compared to chemical leaching. In this work, bioleaching of aluminium (Al) metal from spent catalyst was investigated by using three *Aspergillus* strains (*A. niger, A. foetidus, and A. carbonarius*). Bioleaching was performed in batch culture mode at different loading densities of spent catalyst (i.e., 0.4%, 0.8% and 1.2% (w/v)). The highest Al leaching efficiency of 88.43% was obtained at 0.8% ((w/v)) catalyst loading using *A. foetidus*, further increase in the catalyst loading decreased the efficiency. In addition to this, molasses was used as a carbon source (low-cost) at various concentrations for bioleaching of spent catalyst and the results were found to be significant at 40 g/L sugar concertation with 60% bioleaching efficiency. Overall, this study indicates that *A. foetidus* have the potential for leaching of Al from spent catalysts. Therefore, present research findings suggested that, instead of using mineral acids, organic acids (biodegradable) usage for metal leaching process is highly reliable and eco-friendly as well.
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1. Introduction

Solid waste is the primary problem being faced by today's world. It is produced from different sources like household, biomedical and industrial, which are hazardous to the environment [1]. Among these, waste material derived from petroleum industries shows the adverse effect on environment. Over the years, landfilling and incineration processes have been considered to be the most commonly used methods to manage solid wastes [2]. These conventional methods have wide range of disadvantages such as landfilling which hampers the underground water quality and contaminate the soil as well. Whereas, incineration emits greenhouse gases which are inducers of global warming. Therefore, a greener route i.e. bioleaching is being considered for extraction of value-added chemicals from wastes [3]. The mechanism of bioleaching includes the transformation of solid compounds present in the waste materials into soluble elements by the microorganism's derived organic acids. These soluble elements are then recovered and reused. Basically, it is an interaction between the metal and microorganisms [3].

Spent catalyst is one of the most encountered wastes produced from petroleum industry [4]. Generally, when the activity of the catalyst declines below the acceptable level, it can be regenerated and reused, but regeneration is not always possible. After a few cycles of regeneration and reuse, the catalyst activity decreases to below the acceptable levels and further regeneration may not be economically feasible. These catalysts are known as spent catalysts and are discarded as solid waste. About 4×10^8 kg of spent catalysts are being generated annually on a global scale. In 1993, the world-wide quantity of spent hydro-processing catalysts was estimated to be 5×10^7 kg/year [5]. Some of the spent catalysts apart from being classified as hazardous materials contain valuable metals such as platinum, molybdenum, cobalt, and vanadium etc. Recovery of these metals can be achieved by leaching process using either mineral acids or organic acids. Utilization of organic acids such as citric acid, oxalic acid, and gluconic acid is more advantageous over mineral acids since they are biodegradable and eco-friendly. Usage of mineral acids (Sulfuric acid, Nitric acid, and Hydrochloric acid) for leaching process generates huge amount of potentially hazardous waste and gaseous emission [6]. Therefore, utilizing the organic acids which are biologically synthesized by a wide variety of microbial strains could be helpful for leaching of metals in an ecofriendly manner. As a part of microbial metabolism, microorganisms preliminarily utilizes the carbohydrates and produces organic acids [7]. These organic acids can be further assimilated by microorganisms itself after completion of

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carbohydrate content in the bioleaching medium. Therefore, disposal of bioleaching medium after extraction of metals may not have any toxic effect on eco-system. Therefore, bioleaching has been predominantly considered as an alternative process over the conventional chemical leaching for removal and extraction of metals and it is also cheaper and environmentally friendly. Bacteria and fungus are the most widely used microorganisms for the bioleaching of metals [8–10]. The most commonly used bacteria for effective metal solubilization are *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* which belong to the *Bacillus* genus. In addition to this, some of the species belong to *Penicillium* and *Aspergillus,* which are also well explored fungal strains for bioleaching studies.

The main objective of this present study is to evaluate the best bioleaching performing strain amongst three different *Aspergillus* species (*A. niger, A. foetidus and A. carbonarius*) for the removal of aluminum (Al) from spent catalyst. Two different carbon sources namely sucrose and molasses were used as substrates for bioleaching process. In this process, different spent catalyst loadings (0.4%, 0.8%, and 1.2% (w/v)), sugars (sucrose, glucose, and fructose) consumption and organic acids (citric acid) production were comprehensively investigated with corresponding bioleaching efficiencies.

2. Materials and methods

2.1. Chemicals

Citric acid anhydrous (99.7%), Sulfuric acid (97%), Nitric acid (70%), Acetone (99.8%), AAS standards, Sucrose, Sodium nitrate (99.8%), Magnesium sulfateheptahydrate (99.8%), Potassium chloride (99%), Potassium dihydrogen phosphate (99.5%) were purchased from Sigma-Aldrich, Bangalore (India).

2.2. Bioleaching (Fungal strain and growth conditions)

Aspergillus niger (AS-282), Aspergillus foetidus (AS-508) and Aspergillus carbonarius (AS-621) were procured from MTCC as a freeze-dried culture. These fungal strains were sub-cultured on potato dextrose agar (PDA) plates and kept in an incubator for 5 days at 30 °C. Sterile distilled water (which contains 0.1% Tween 80 act as a surfactant) was used to recover the spores. For the preparation of fungal seed culture, 1 mL of spore suspension was added to 100 mL of sucrose medium which contains (g/L): sucrose: 80 g, $NaNO_3$: 1.5 g, KH_2PO_4: 0.5 g, $MgSO_4.7H_20$: 0.025 g, KCl: 0.025 g, yeast extract: 1.6 g. The cultures were maintained at 30 °C in an incubator shaker at 120 rpm. Further 1 mL of the 2 days old seed culture was counted under an optical microscope using a hemocytometer. The spore suspension was then diluted to attain 1×10^7 spores/mL and then added into 100 mL of sucrose medium in 250 mL Erlenmeyer flasks. The two-step bioleaching was performed (where the sterile catalyst was added after two days of cultivation time) at different loadings of spent catalyst i.e., 0.4%, 0.8%, and 1.2% (w/v). To establish a comparative study between conventional and non-conventional carbon source, HPLC grade sucrose and sucrose from molasses (ranging from 40 g/L – 80 g/L) was used for bioleaching experiments.

The initial pH of bioleaching medium was maintained at 5.5 and incubated for 20 days at 30 °C with 120 rpm. Further 2 mL of sample was withdrawn at regular interval of 5 days for the analysis of pH, metal concentration (by Atomic absorption spectroscopy), sugar consumption and organic acids production (by using High performance liquid chromatography).

2.3. Analytical methods

2.3.1. Characterization of catalyst

The particle size distribution was determined using a particle size analyzer (Beckman Coulter, Delsa Nano C, UK) which measures particle size distribution of suitably dispersed field of particles in the range of 0.02–2000 μm. The particle size analyzer was used to find the average size of spent catalyst. The specific surface area of a spent catalyst was determined by Brunauer–Emmett–Teller (BET) method (Quantachrome Corporation, Autosorb-IQ MP and the USA). The morphological changes of the catalysts were observed with a Scanning Electron Microscope (SEM) (Zeiss, Sigma, Germany). The sample was spread on a metallic stub using carbon tape and coated with gold. Image analysis was conducted at an accelerating voltage of 8–10 kV, and under high vacuum.

2.3.2. Analysis of concentration of sugars and organic acids

The concentration of sucrose, glucose, fructose along with the biologically produced citric acids were determined using high-performance liquid chromatography (Varian-210, The Netherlands) with MetaCarb-87 H column at UV wavelength of 210 nm for the organic acids and RI detector (355, Varian) for sugars. The column temperature was maintained at 30 °C and 0.013 N sulfuric acid was used as mobile phase with a flow rate of 0.5 mL/min. Samples were filtered through 0.2 μm syringe filter prior to HPLC analysis, to avoid clogging by fine particles in the sample.

2.3.3. Analysis of Al composition

Al analysis was performed using Atomic Absorption Spectroscopy (AAS) (Varian, AA240, and the Netherlands) at the wavelength of 309.3 nm for Aluminum. The multi-element standard at 1000 mg/L was used to prepare the calibration standards and a drop of 0.1 M nitric acid was added. Samples obtained from bioleaching medium was filtered through 0.2 μm nylon membrane

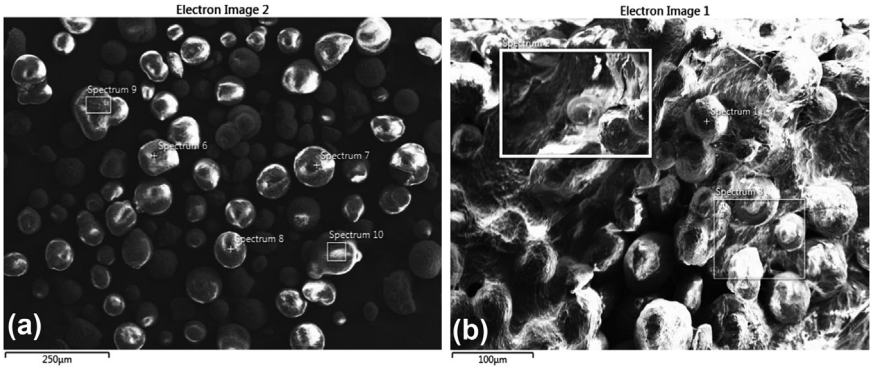

Fig. 1. SEM image of spent FCC catalyst a) before bioleaching, b) after bioleachin.

syringe filter before the analysis. The percentage of Al extraction in bioleaching was calculated based on the initial Al concentration obtained through AAS analysis of spent catalyst sample (Eq. (1)).

$$\% \text{ Al extraction} = \frac{\textit{Final metal concentration in the bioleached medium}}{\textit{Initial metal concentration present in the spent catalyst}} \times 100 \quad (1)$$

3. Results and discussion

3.1. Characterization of the catalyst

3.1.1. Particle size, surface area, and morphology

The mean particle diameter of spent and bioleached FCC catalyst was found to be 68.05 μm and 54.56 μm respectively. The higher mean particle diameter of spent catalyst could be because of the deposition of coke and other metal contaminants. Bioleaching resulted in approximately 20% reduction in the mean particle diameter, as the coke deposits and metal contaminants were leached during bioleaching [3]. BET analysis showed that the specific surface area, total pore volume and average pore diameter of spent catalyst were 125.56 m^2/g, 20.74 cm^3/g, and 6.6 nm, respectively. Particle size analyzer showed that the particle size of the catalyst was less than 200 μm in all cases, and 90% of the total volume consisted of particles smaller than 150 μm. The SEM photomicrograph of the spent catalyst showed that the particles were almost spherical (Fig. 1a) with considerable variation in particle size. However, cracks were observed after the bioleaching of spent catalyst which could be due to the effect of bioleaching process [11].

3.2. Bioleaching

3.2.1. Growth study of A. niger, A. foetidus and A. carbonarius

In order to determine the optimum period for the addition of a spent catalyst, each *Aspergillus* strains such as *A. niger, A. foetidus and A. carbonarius* were inoculated into sucrose medium for 6 days at identical culture conditions. This study revealed that, due to the activity of invertase enzyme, complete hydrolysis of sucrose was observed after 24 h of the incubation period. The rate of sugar consumption was found to be higher after 48 h of incubation period (shown in Supplementary Table 1), which indicated that, *Aspergillus* strains were in active growth phase after 48 h. Therefore, spent catalyst was added to the bioleaching medium after 48 h of the incubation period [12]. Along with this, during 6 days incubation period, primary metabolites like, citric acid, oxalic acid, and gluconic acids were also produced. Citric acid was found to be the predominant organic acid present in the bioleaching medium than oxalic acid, and gluconic acids., *A. niger*, *A. foetidus* and *A. Carbonarius* produce 1.9 g/L, 2.6 g/L and 2.2 g/L of citric acid (Fig. 2), respectively.

3.2.2. Bioleaching of spent catalyst using sucrose as carbon source by two-step method

Sugars consumption, citric acid production and bioleaching efficiency profiles of *Aspergillus* strains have been investigated by two-step bioleaching process conducted at a various pulp density of 0.4%, 0.8% and 1.2% (w/v) spent catalyst. During the bioleaching process, citric acid production was initiated after 48 h of the incubation period for all *Aspergillus* species. The highest citric acid production was observed at 15 days of incubation time (Fig. 3). The Al leaching efficiency was increased during this period (Fig. 4), where consecutive drop in pH was observed (Fig. 5).

Sucrose was completely hydrolyzed by *Aspergillus* strains before the addition of spent catalyst to the culture medium. The citric acid concentration increased along with simultaneous decrease in the glucose and fructose concentrations. Glucose consumption was found to be faster than fructose (Fig. 5). The total sugars consumption rate was found to be higher at 5 days incubation period, thereafter, lower sugars consumption rate was observed (Shown in Supplementary Table 1). Increase in citric acid concentration and decrease in medium pH was observed until 15 days of incubation time, thereafter, decrease in citric acid concentration and increase in media pH was observed [3]. These

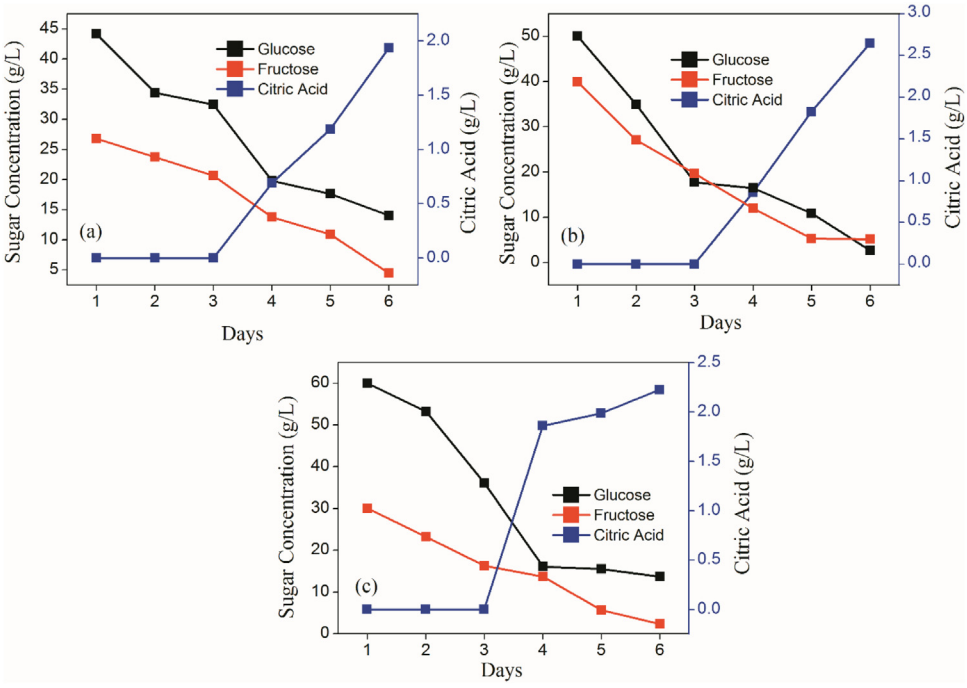

Fig. 2. Sugar consumption and citric acid production profile of three different strains of (a) *A. niger* (282), (b) *A. foetidus* (508), and (c) *A. carbonarius* (621).

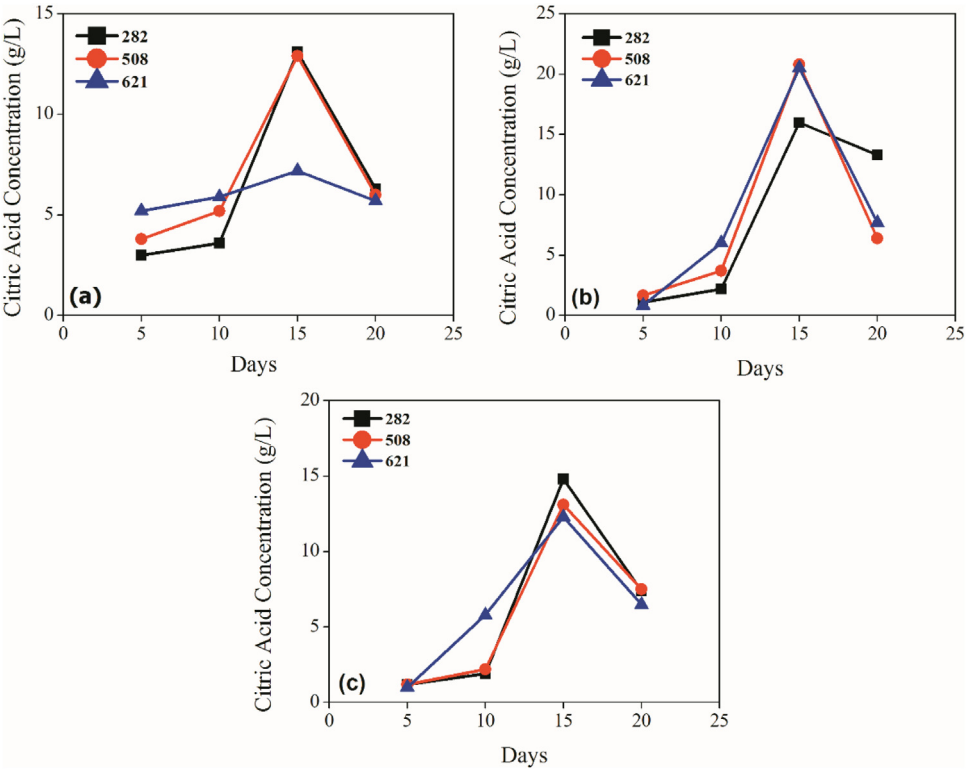

Fig. 3. Concentration of citric acid during bioleaching of spent FCC catalyst at different catalyst loading (a) 0.4% (w/v), (b) 0.8% (w/v), and (c) 1.2% (w/v).

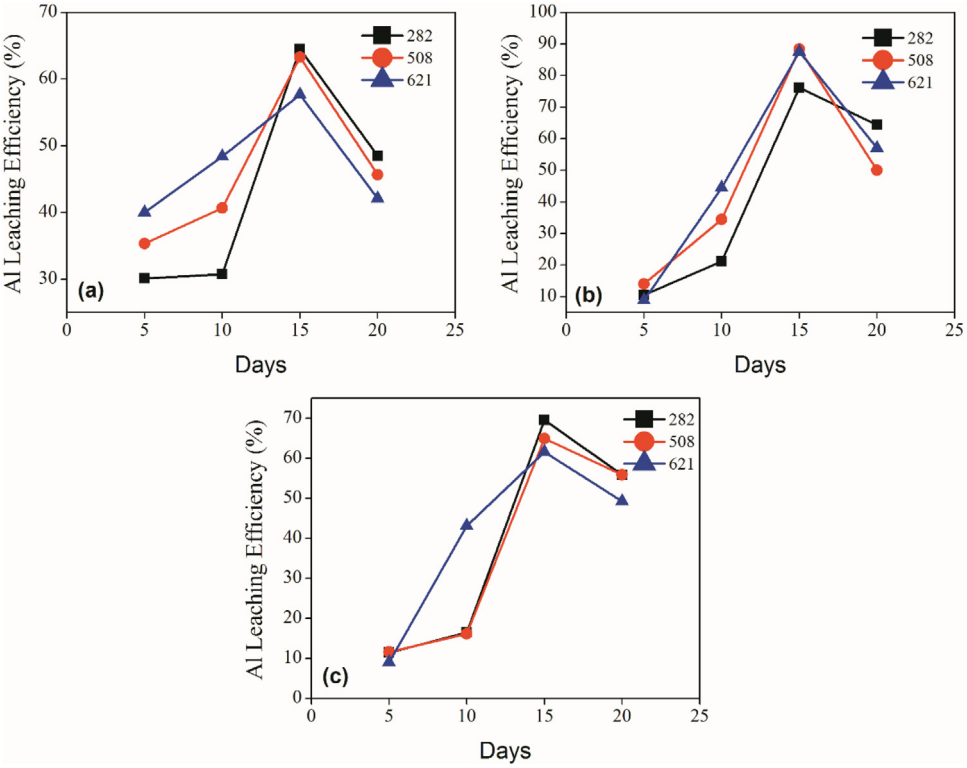

Fig. 4. Al leaching efficiency during bioleaching of spent FCC catalyst at different catalyst loading (a) 0.4% (w/v), (b) 0.8% (w/v), and (c) 1.2% (w/v).

similar patterns were observed in all three fungal strains (Fig. 3) and (Fig. 5). The percentage metal leaching efficiency was found to be parallel with time and reached maximum at the end of 15th day incubation period followed by declining phase (Fig. 4). This phenomenon indicated that *Aspergillus* strains utilized sugars (glucose and fructose) as the carbon source for producing citric acid which effectively acidifies the leaching medium. According to the literature, acidic nature of the medium is favorable for metal

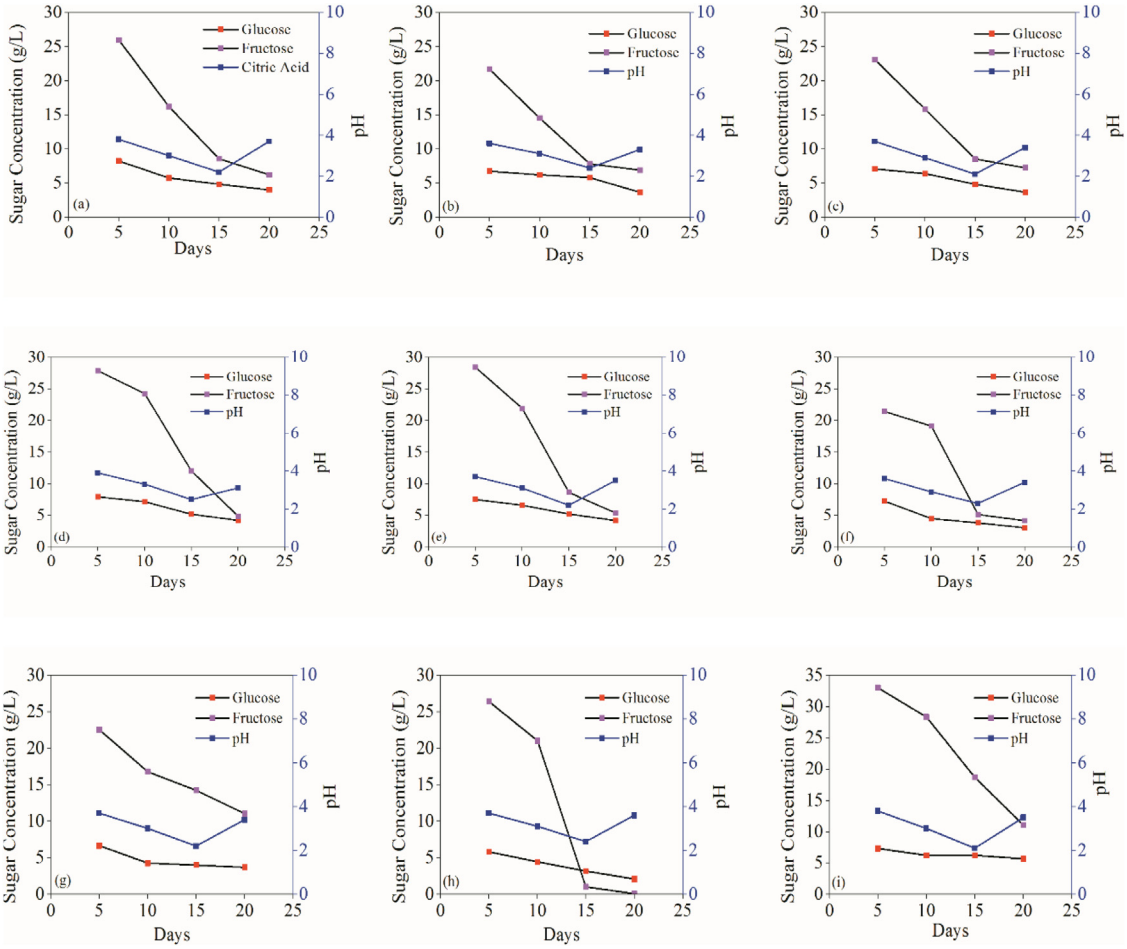

Fig. 5. Sugar consumption profile and change in pH during bioleaching of spent FCC catalyst by different *Aspergillus* strains at different catalyst loading (a) AS-282-0.4% (w/v), (b) AS-282-0.8% (w/v), (c) AS-282-1.2% (w/v), (d) AS-508-0.4% (w/v), (e) AS-508-0.8% (w/v), (f) AS-508-1.2% (w/v), (g) AS-621-0.4% (w/v), (h) AS-621-0.8% (w/v) and (i) AS-621-1.2% (w/v) *AS: *Aspergillus* strain.

leaching from any substance [13]. Hence, pH played a vital role during the metal leaching process. It is also evident from Fig. 3 that increase in the citric acid concentration leads to decrease in the medium pH, which gradually increases the percentage of Al leaching with respect to time (Fig. 4). On the other hand, increase in the medium pH and decrease in citric acid concentration along with Al leaching was observed after 15 days incubation time. According to the literature, after the completion of sugars consumption, *Aspergillus* strains utilizes citric acid as a carbon source for their growth [14] which eventually increases the pH of the bioleaching medium and decreases the metal solubility [15]. It has been known that solubility of metals increased with a decrease in solution pH, whereas, the solubility of metals was decreased by increasing the solution pH and found to precipitate at neutral pH [15]. Based on this theory, on decreasing the medium pH, precipitation of Al could be initiated. In this present study, the solubility of Al in the medium was comparatively lower than that of 15th day incubation period. A study conducted by Deshavath et al. [16] observed that pH of fermentation medium increased during the consumption of organic acids, and the product formation was still unknown. It is evident from Fig. 3, particularly at 15 to 20 days incubation period, the citric acid concentration decreases from 13.1 g/L to 6.3 g/L, medium pH increases from 2.2 to 3.7 and Al leaching efficiency decreases from 64.5 to 48.5%. The decrease in Al leaching efficiency was mainly due to the increase in pH that leads to precipitation of Al which was unfortunately not visualized due to the fungal biomass along with residual spent catalyst present in the bioleaching medium. However, this similar trend was observed during bioleaching process with all *Aspergillus* strains.

3.2.3. Effect of catalyst loading on bioleaching efficiency

Al leaching efficiency was increased upon addition of catalyst from 0.4 to 0.8% (w/v) followed by sharp decreasing while increasing the concentration upto 1.2% (w/v). Therefore, an optimum spent catalyst leaching was attained at 0.8% (w/v) of solid loading. Similar pattern was observed in all *Aspergillus* strains. It is evident from Fig. 3; that comparatively high concentration of citric acid was observed at 15th day of incubation time with 0.8% (w/v) catalyst loading which eventually exhibits the highest Al leaching (Fig. 4). The catalyst loading at 1.2% (w/v) initiates the microbial inhibition [3] which could be observed by sugar consumption and citric acid production rates (Shown in Supplementary Table 1). Primarily, microbial inhibition along with the presence of few heavy metals lead to lowers the utilization of sugars which subsequently lower the citric acid production and resulted in lower leaching efficiency.

However, among the *Aspergillus species, A. foetidus* strain showed better results for Al leaching from spent catalyst. As shown in the Fig. 3, citric acid production was competitively higher in *Aspergillus foetidus* cultivated medium. According to the previous literature reports, *Aspergillus* strains have the ability to produce significant levels of citric acid for effective bioleaching process [17–19]. The order of citric acid production along with the

percentage of Al leaching after 15 days was: AS 621 < AS 282< AS 508.

Finally, it was observed that Al leaching efficiency increased with increasing the citric acid concentration. This phenomenon indicated that the biologically produced organic acids played a vital role in the bioleaching process. The Al extraction efficiency was the highest (88.43%) with *Aspergillus foetidus* strain at 15th day of incubation time. The decrease in Al leaching efficiency beyond the maximum was probably due to increasing the pH of bioleaching medium by citric acid consumption which leads to decrease in the solubility of Al in the medium.

3.2.4. Bioleaching efficiency with molasses as a carbon source

Bioleaching was also studied with molasses (sugar) as a carbon source for the comparative study with the commercially available sucrose. According to the HPLC analysis, one mL of molasses contains 260 mg of sucrose, 90 mg of glucose, 70 mg of fructose. Apart from this, the aforementioned data suggested that *Aspergillus foetidus* showed highest Al leaching efficiency at 0.8% (w/v) catalyst loading. Therefore, further studies were conducted with *Aspergillus foetidus* at the similar catalyst loading (0.8% (w/v)) by varying the sugar concentrations i.e., 40, 60 and 80 g/L. During the bioleaching process, the Al leaching efficiencies gradually decreased with increasing the molasses concentration. Citric acid production on 5th day with 40 g/L total sugar concentration was found to be 4.7 g/L whereas, only 3.2 g/L and 1.8 g/L citric acid were produced at 60 and 80 g/L sugars concentrations as depicted in (Fig. 6). Additionally, the highest Al leaching efficiency of 59.5% was also observed at 40 g/L sugar loading, whereas, 52.0% and 37.7% Al leaching was obtained at 60 and 80 g/L sugar loading respectively at the end of the 15th day (Fig. 7). Therefore, decreasing trend of Al leaching efficiency was attained by increasing the molasses loading. In general, the density of molasses was noted to be 1.4 kg/L [20]. Consequently, by increasing the molasses loading, viscosity of the bioleaching medium increased which decreases the aeration to the microbes, thus microbial growth eventually decreased. However, a considerable amount (59.47%) of Al leaching efficiency was attained by using molasses as a carbon source. On comparing with pure sucrose as a carbon source, 0.3-fold lower Al leaching efficacy was attained by using molasses as a sole carbon source. Thus it is confirmed that sucrose is the best carbon source for bioleaching media.

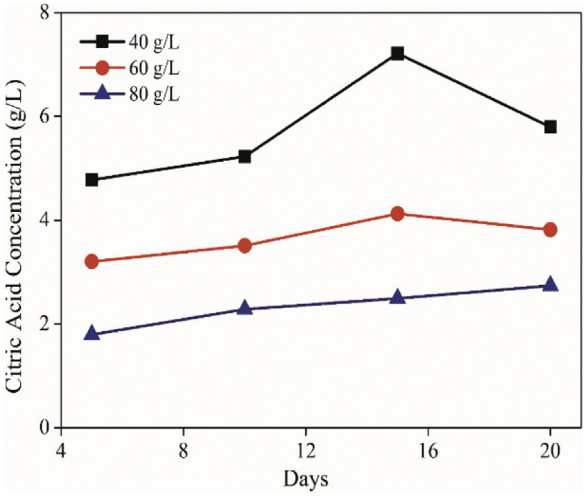

Fig. 6. Concentration of citric acid during bioleaching of spent FCC catalyst at 0.8% (w/v) catalyst loading using molasses as a carbon source.

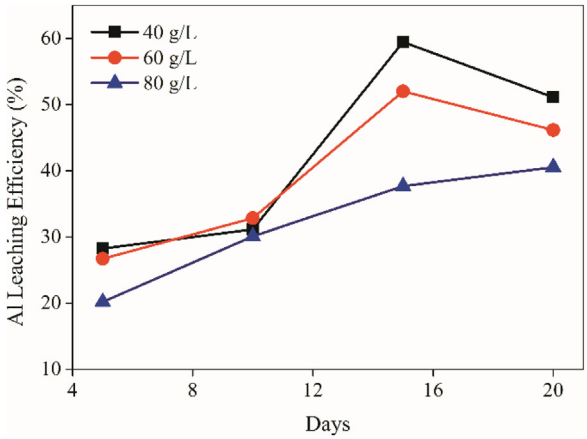

Fig. 7. Al leaching efficiency during bioleaching of spent FCC catalyst at 0.8% (w/v) catalyst loading using molasses as carbon source.

4. Conclusions

Bioleaching of spent fluid catalytic cracking catalyst was investigated using different*Aspergillus* strains with varying loading densities (0.4, 0.8 and 1.2% (w/v)). The optimum Al bioleaching efficiency of 88.43% was found at 0.8% (w/v) catalyst loading with *Aspergillus foetidus*. Based on these results, bioleaching was also performed with molasses to establish a comparison between conventional and non-conventional carbon source. The results are found to be promising, around 60% bioleaching efficiency was achieved at 40 g/L sugar concentration. HPLC analysis revealed that, among all the organic acids (Biogenically produced by *Aspergillus* strains), citric acid concentration was found to be highest and played vital role in the Al leaching process. Therefore, present research findings suggested that, instead of using mineral acids, organic acids (biodegradable) usage for Al leaching process is highly reliable and eco-friendly as well.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2019.e00349.

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