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A novel eco-friendly hybrid approach for recovery and reuse of copper from electronic waste

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A B S T R A C T

Amid the plethora of initiatives and regulations targeting the minimization of electronic waste generation and its transboundary shipment, efforts to adopt a sustainable resource conservation from this emerging waste stream are considerably missing. Present study is a pioneer effort to develop a novel two stage biorecvoery process followed by electrochemical treatment to recover copper in its reusable form from waste printed circuit board. An isolated strain *USCT-R010* was employed for the leaching of copper and process parameters (reaction pH, substrate concentration, inoculum size, pulp density, agitation speed) were optimized in order to maximize bioleaching of copper. The leach liquor containing mobilized copper was subjected to purification step where biosorption was carried out using dead biomass of *Aspergillus oryzae* and Baker's Yeast under optimized reaction condition. Desorption was performed using 0.1N HCl to recover pure copper from dead biomass and more than 86% copper was desorbed from both the biosorbents. Electrowinning was carried out at current: 2A and electrolysis time: 150 min to recover 92.7% Cu from eluate. Characterization studies suggested 95.2% purity of recovered copper which was reused as an antibacterial agent against *Escherichia coli*. Utilization of low cost biomaterial for recovery and purification of metals, eco-efficient technology with high level of purity, reusability of recovered copper and environmentally sound practices are the aided incentives associated with this novel approach. The proposed work can be reckoned as successful demonstration of 'Greening the waste' concept in order to generate substantial economic, environmental and social benefits from e-waste.

1. Introduction

'Greening the waste' concept is gaining attention these days in context of electronic waste (e-waste) management due to exponential growth in the generation of e-waste and its hazardous effect on human health and ecosystem. Global e-waste generation is anticipated to reach 130 million metric tons (MMT) in year 2018 from 93.5 MMT in year 2016 at a compound annual growth rate (CAGR) of 17.6% [1]. India has emerged as fifth largest producer of e-waste in the world, discarding 1.8 MMT of e-waste each year and likely to generate 5.2 MMT of e-waste per annum by year 2020 [1]. A worrying increase in the volume of discarded electronics and uncontrolled dumping of these electronic scraps in landfills without any attempt to recycle the materials have led the severe challenges to the non-rigorous management strategies developed so far for solid waste management. E-waste should not be thrown away recklessly into the ecosystem due to its richness in metal values which may leach into soil-water-air continuum and may pose significant environmental footprint due to complex composition of e-waste. Therefore, it would be worth transforming the waste into wealth by retrieving its metal content and conserving the resources.

Recovery of metals from e-waste has been an active area of research in recent years. Various hydrometallurgical processes such as chemical leaching [2–4], acid leaching [5,6], hydrometallurgical etching, ligand assisted leaching [7,8] are employed for metal recovery from e-waste. Chemical leaching leaches out maximum concentration of metals in a rapid and efficient manner, however toxicity of chemicals and environmental concerns inhibit their applicability at industrial scale [9]. Mechanical [10,11] and pyrometallurgical methods [12,13] have also been reported for processing of e-waste. Nevertheless, these methods in spite of being employed in various industrial processes, are not recommended due to intense energy requirement, high economics and release of toxic fumes. It is apparent that prior work has focused primarily on high recovery of metals while economic and environmental issues associated with these conventional technologies have been disregarded. Hence, concomitant increase in resource depletion and substantial environmental footprints trigger the need to develop a novel green technology for metal recovery. Combination of two or more technologies (hybrid strategies) have also been investigated for efficient

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extraction of metals from various contaminated sites [employ14,15], although very few hybrid approaches have been reported yet for extraction of metals from e-waste. In hybrid approaches, different processes are performed in various steps which will complement each other to attain an efficient and improvised method of metal recovery.

A combination of bioleaching as recovery step and biosorption as purification step followed by electrotreatment was employed for the very first time (to the best of our knowledge) to recover copper from waste printed circuit board (WPCB) in its reusable form. The primary objectives of the proposed work are to mitigate the negative environmental effects of toxic chemicals utilized in hydrometallurgical treatment and to conserve valuable materials in a sustainable eco-technie way. A microbial strain *USCT-R010* was isolated from heavy metal contaminated soil and 16S-rDNA sequence analysis was performed for molecular characterization of the strain. Optimization of process parameters was carried out in order to maximize the metal recovery and reusability of the extracted copper was investigated as an antibacterial agent against *Escherichia coli.* Characterization studies were performed to identify changes in morphology and crystalline phases of metal during leaching process which confirmed the formation of jarosite after bioleaching experiments. In addition, purity of recovered copper was also investigated by employing various characterization techniques.

2. Materials and methods

2.1. WPCB processing and characterization

WPCBs of desktop computer were kindly provided by local e-waste collection center, New Delhi (INDIA) to carry out the present study. All the capacitors, resistors and plastic materials were removed manually from WPCB using pliers. The metal plate was shredded into the small pieces ($2 \times 2\,cm^2$), crushed in ball mill and then sieved to form homogenous powder of desired particle size 2810 μm, 1680 μm, 420 μm and 250 μm. The crushed WPCB was washed with ethanol to remove polymer layer and was then oven dried to sterilize for bioleaching experiments. Inductive coupled plasma optical emission spectrometry (ICP-OES) was performed at FICCI research & analysis center, New Delhi to characterize metal content in WPCB. Percentage fraction of various metals in WPCB obtained by ICP-OES analysis are given in Appendix A (Table A.1 in Supplementery information) which suggested presence of 13.3% Cu along with trace amount of other heavy and precious metals in WPCB sample. Scanning electron microscopy (SEM) analysis (SEM-EVO50) was performed at 20 kV to investigate the changes in morphology of WPCB after leaching experiments. Since an electron beam is incident on the samples for SEM analysis, samples were coated with about 20–50 nm thick gold to provide electrical conductivity and structural analysis was performed at a magnification of 5000X. Philips X'pert-1 X-ray diffractometer was employed for X-ray diffraction (XRD) analysis in order to identify the metal phase distribution in WPCB sample and its residues. Analysis was performed at a step size of 0.05° and a count time of 1 s per step over the range $10° < 2\theta < 80°$ to identify crystalline phases before and after leaching [16].

2.2. Isolation and screening of isolated strain USCT-R010

Heavy metal-contaminated soil was collected from an e-waste dumping site in New Delhi to isolate Cu-leaching strain. 2 g of soil sample was suspended in 100 ml of distilled water for 15 min so that the microorganisms get suspended in the water. 1% (v/v) of the heavy metal contaminated soil suspension previously prepared was used to inoculate the initial stock of mixed bacterial culture in an enrichment medium (9K-Fe Medium: 3 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4$. $7H_2O$, 0.1 g/L KCl, 0.01 g/L $Ca(NO_3)_2$, 44.22 g/L $FeSO_4{\cdot}7H_2O$) at pH = 1.5, agitation speed = 120 rpm and reaction temperature = 30 °C for 18 h. Thereafter 4% (v/v) was re-inoculated in fresh medium after every 10 days in order to achieve healthy growth of the isolated Cu leaching strain *USCT-R010* and inactivation of the non-copper leaching bacteria. Plating was done after 3rd transfer in order to minimize the presence of acidophilic heterotrophs. Gelrite-9 K plates were specifically used for identification of strain.

Preliminary characterization was done on the basis of morphology (gram staining) of the isolated strain and furthermore, 16S-rDNA sequence analysis was performed for the molecular characterization. Genomic DNA was extracted from the culture and 16S rDNA amplification was carried out with 8F and 1492R universal primer sequences. The capillary sequencing was done by Abi-3730XL genetic analyzer machine at GCC Biotech (India) Pvt. Ltd., Kolkata (INDIA) and the obtained sequences were subjected to nucleotide–nucleotide BLAST, a tool for checking percentage of similarity with the NCBI database.

2.3. Bioleaching of copper: recovery step

Batch experiments were conducted to investigate optimum reaction conditions for the bioleaching of Cu^{2+} from WPCB using an isolated strain *USCT-R010*. 0.5 g of pre-treated WPCB powder was added to 100 ml of working solutions of Fe-9 K media with 5% (v/v) inoculum. Shaking speed of 120 rpm was provided to the sample by keeping the flasks on an orbital shaker at reaction temperature 303 K for a time period of 96 h. Effect of pH on the bioleaching capacity was investigated at initial pH value ranging from pH = 1.0 to pH = 3.0. No further adjustments were made afterward to maintain solution pH. Effects of process parameters such as media pH, substrate concentration, inoculum size, particle size of crushed WPCB and pulp density on the bioleaching process were studied. Samples were taken at regular time interval and were centrifuged at 5000 rpm for 10 min to remove all the suspended biomass and WPCB powder. All experiments were performed in triplicates. Leached metal concentration in the supernatant was determined with the aid of UV–vis spectrophotometer (Hitachi U-2900). The residue powder obtained after bioleaching experiments was mustard yellow in colour due to formation of Jarosite. Jarosite is a mixture of various monovalent cations, moderate to high ferric ion and sulfate concentrations. The residue was also characterized with the aid of SEM and XRD analysis in order to ensure the formation of Jarosite and extraction of copper. Block diagram of the proposed two stage biorecovery approach is given in Fig. 1. The growth rate of isolated bacteria *USCT-R010* (OD_{600}), copper concentration (OD_{670}) and ferrous concentration (OD_{480}) was analyzed using a UV–vis spectrophotometer in order to identify optimum process parameters for efficient bioleaching.

2.4. Biosorption-desorption of copper: a purification step

Leach liquor obtained from the bioleaching step consist of copper salt ($CuSO_4$) along with other metal salts such as $FeSO_4$, $MgSO_4$ etc, therefore in order to recover pure copper, the leach liquor obtained from the optimized bioleaching conditions was subjected to the biosorption-desorption cycle. Biosorption was performed using dead biomass of *Aspergillus oryzae* (NCIM 1212) and commercially available baker's yeast at conditions previously optimized in our other piece of work (submitted for publication). The pure copper was then desorbed from the biomass using 0.1N HCl solution and obtained in the eluate.

2.5. Electrowinning of eluate

Eluate obtained from desorption process was subjected to electrowinning in order to obtain copper in solid form so that it can be reutilized in other applications. Experiments were conducted in a batch mode of operation at room temperature (25 ± 1 °C) by taking 500 ml of the eluate solution in a glass beaker of capacity 1000 ml. Graphite electrodes (Length: 30 cm, Diameter: 1.5 cm) were used and inter

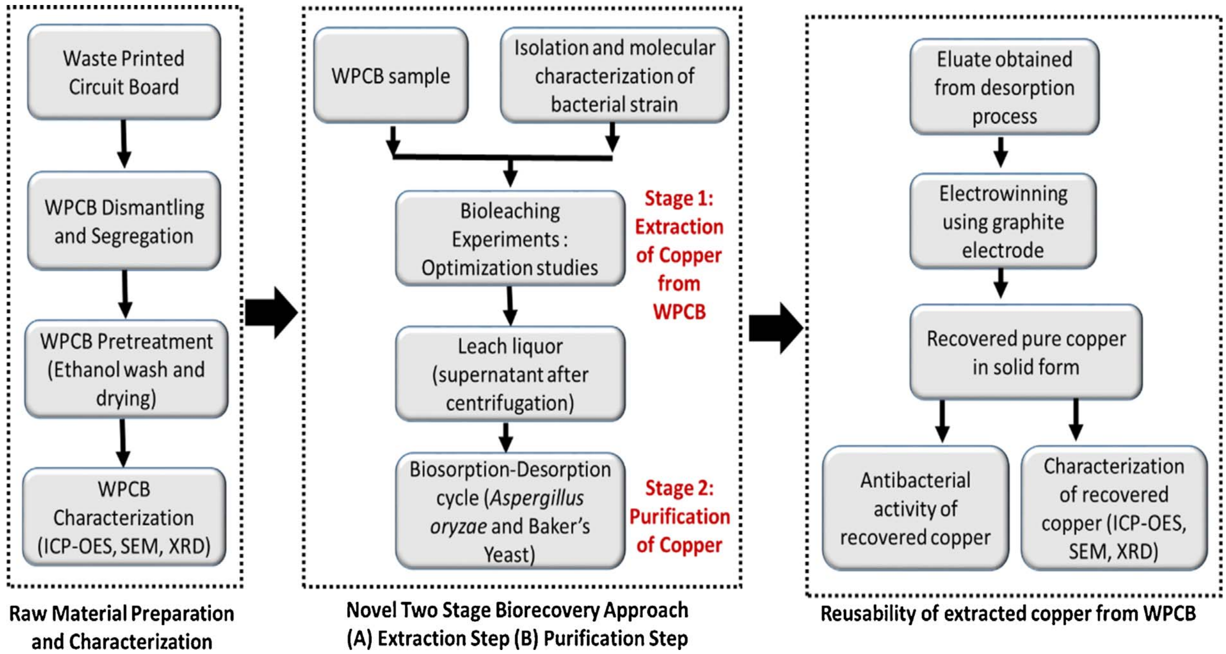

Fig. 1. Block diagram of novel hybrid two stage biorecovery of Cu from WPCB.

electrode distance was maintained at 2 cm. The electrodes were dipped up to 8 cm into the solution and the effective area of the electrodes in the solution was 41.25 cm^2. To sustain uniform concentration, contents of the beaker were agitated by a magnetic stirrer (Labman, India). The electrodes were connected to a DC power supply (Kitheley, China) providing 0–30 V and 0–3A current. Retention time of two hours was given after the completion of the process and the deposition of copper metals was collected from the electrodes. Electrolysis time and current was varied in order to optimize the copper recovery from electrowining experiments.

2.6. Reusability of recovered copper

The antibacterial activity of the recovered copper was studied against the commonly found gram-negative *E. coli* bacteria via well diffusion method. Luria agar plates were made and 1 ml inoculum of *E. coli* was spread on the entire agar surface using a sterile spreader and was left for 2 h to be absorbed. Afterwards, five holes (diameter: 6 mm) were made using a sterile tip and 20 μl of different concentrations of copper (10 g/L, 5 g/L, 2 g/L, 1 g/L) in distilled water was added in respective wells, with distilled water in one of the well as control. The plates were incubated in an incubator at 303 K for 24 h and antibacterial activity was assessed against distilled water control based on diameter of zone of inhibition.

3. Results and discussions

3.1. Screening and characterization of USCT-R010 strain

3.1.1. Morphological characterization

Preliminary characterization was based on morphology of the isolated strain *USCT-R010*. 4% (v/v) inoculum was re-inoculated in fresh medium after every 10 days in order to achieve healthy growth of *USCT-R010*. It was observed that colour of the medium became darker with incubation after 10 days and finally after 3rd transfer, the medium turned reddish brown as shown in Fig. A.1 (Appendix A in Supplementery information) indicating healthier and specific growth of the isolated strain. Confirmatory tests were performed in the laboratory to ensure that the microorganism responsible for turning the medium reddish brown was from *Acidithiobacillus ferrooxidans* genera. *A* small amount of inoculum was added to the gelrite–9 K medium plates on which reddish colonies were obtained after 5–7 days of incubation confirming the presence of iron oxidizing bacteria. Gram staining was also performed and it was observed that the strain *USCT-R010* is gram positive confirming it to be of *A. ferrooxidans* genera.

3.1.2. Molecular characterization

The evolutionary history was inferred using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method. It is an agglomerative (bottom-up) hierarchical clustering method and considered as the simplest distance analysis for constructing trees. The optimal tree with the sum of branch length = 19.61 obtained from UPGMA methods is shown in Fig. A.2 (Appendix A in Supplementery information). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. Phylogenetic analyses were conducted in MEGA4. BLAST comparisons of the sequences to the NBRIP nucleotide database confirmed high sequence similarity (> 99%) of *USCT-R010* to *A. ferrooxidans* strain. Significant copper leaching activity has been reported in literature also for A. *ferrooxidans* genera [17,18], therefore bioleaching experiments were conducted using the isolated strain *USCT-R010.*

Growth kinetics of the *USCT-R010* was investigated and the obtained growth curve is shown in Fig. A.3 (Appendix A in Supplementery information). It can be observed that the isolated strain of *A. ferrooxidans* genera reached the exponential phase of its growth after a lag period of 24 h. A steady phase was reached after almost 10 days of growth therefore a period of 5 days was fixed for optimizing the bioleaching process parameters so that maximum conversion of Fe^{2+} to Fe^{3+} can take along with copper dissolution before the cells reach the steady phase.

3.2. Optimization of the bioleaching process parameters

3.2.1. Effect of solution pH

The pH profile of the bioleaching medium having 1.0 g/100 ml concentration of WPCB was studied with the isolated strain *USCT-R010*. Fig. 2(a) indicates the growth curve at different pH of the medium

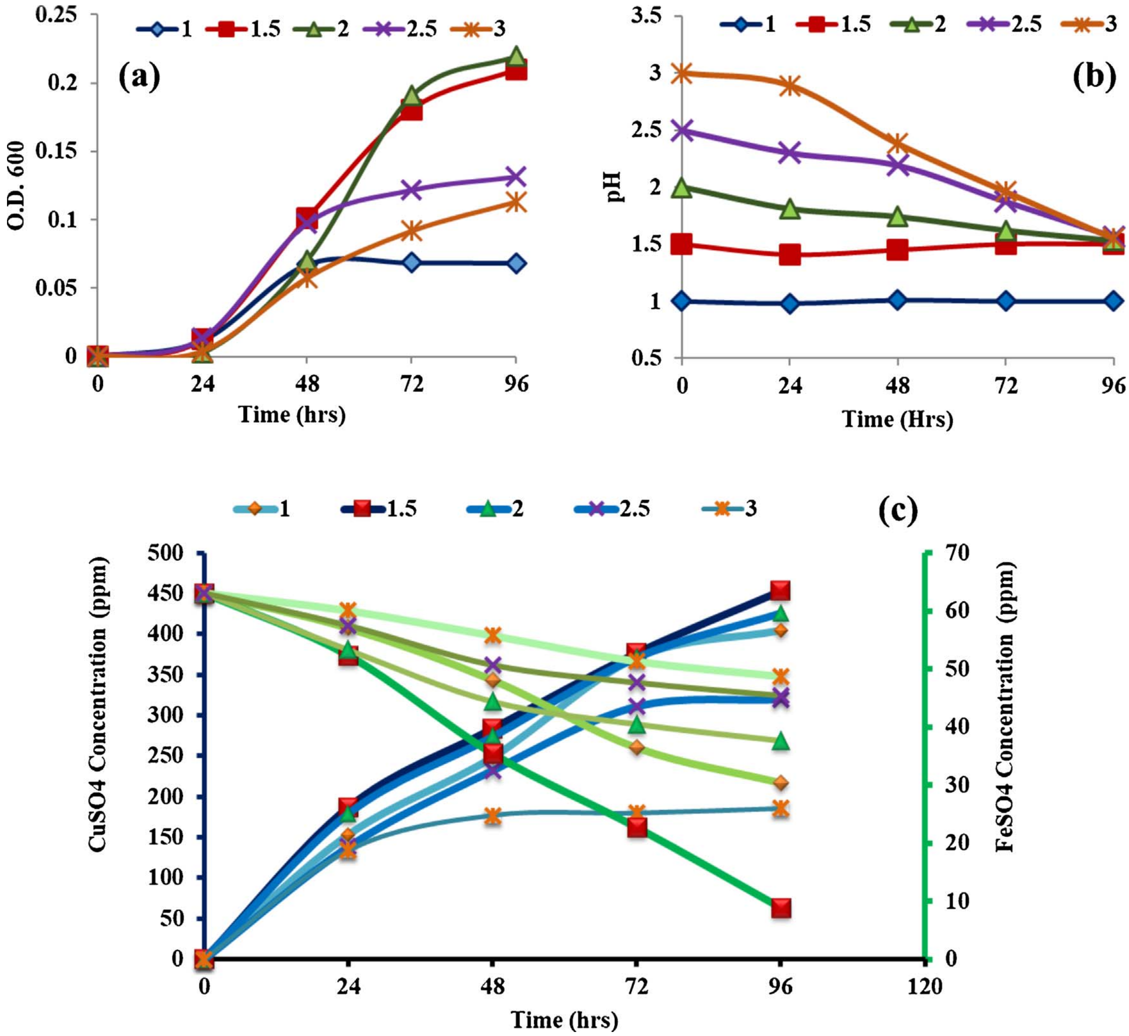

Fig. 2. (a) Effect of pH on Growth of USCT-R010 (b) Change in pH with Time (c) Effect of pH on bioleaching of Cu (Lines in Blue shade: Copper Dissolution; Green shade: $FeSO_4$ utilization).

during bioleaching process. Maximum growth of the microorganism was observed at pH = 1.5–2, whereas slower growth kinetics was observed at pH above or below the given range (pH = 1.5–2). The pH of the system was also monitored with time and the obtained results are shown in Fig. 2(b). It can be seen in Fig. 2(b) that pH of the media decreases to pH = 1.5 for all the medium with starting pH > 1.5 within 96 h while no changes were observed in the medium pH when starting pH was less than 1.5. Results were found to be in concordance with literature where the pH = 1.5 was considered the optimum pH for the solubilization of metal using *A. ferrooxidans* [19].

The concentration of copper and $FeSO_4$ was regularly measured in order to conclude the system pH with optimum copper leaching efficiency and results are shown in Fig. 2(c). It can be inferred from Fig. 2(c) that maximum copper leaching (450 mg/L) as well as maximum $FeSO_4$ utilization (86%) was observed at pH = 1.5. With the increase in solution pH from pH = 1.5 to pH = 3, significant decrease in bioleaching efficiency was observed as shown in Fig. 2(c). It could be attributed to the adsorption of the metal complexes on epoxy waste and precipitation of the dissolved metals rather than dissolution. Leaching at a low pH allows epoxy fractions of the waste to overcome metal complex adsorption which further checks the metal hydroxyl precipitation and passivation.

3.2.2. Effect of varying concentration of ferrous ions

Bioleaching of metals from the WPCB may involve indirect leaching mechanism by the biogenic H_2SO_4 reagent. The *A. ferrooxidans* species forms a biolayer around the $FeSO_4$ ions and produce sulphuric acid which in turn reacts with elemental copper present in the PCB to produce $CuSO_4$. Therefore, $FeSO_4$ was considered as an important growth factor for *A. ferrooxidans* species in present study. Bas et al. [20] suggested that Fe^{2+} is the only energy-yielding substrate for bacteria to support its growth. In this respect, the external addition or increasing the concentration of Fe^{2+} is highly recommended to significantly enhance the dissolution rate and extent of copper removal. ICP-OES analysis suggested nearly 8.97 mg/100 g Fe content in WPCB which may be utilized for the growth of microorganism responsible for bioleaching process. However, in view of the low iron content of WPCB, additional $FeSO_4$ solution was added into the medium in order to provide enough substrate concentration for growth of microorganisms. Substrate concentration was varied for a wide range from 63.1 mg/L to 177.4 mg/L and the effect of increasing Fe^{2+} ion concentration on growth of *USCT-R010* is shown here in Fig. 3(a). Batch experiments were conducted for the medium having 1.0 g/100 ml pulp density at optimized pH = 1.5. Fig. 3(a) indicates growth curve of the isolated strain at different concentrations of $FeSO_4$ in the medium during bioleaching process. Maximum growth of the microorganism was observed with medium having an initial $FeSO_4$ concentration in the range 91.71–148.85 mg/L, whereas slower growth kinetics was observed at concentration beyond the given range (91.71–148.85 mg/L) due to precipitation of the dissolved $FeSO_4$ at reaction temperature At high

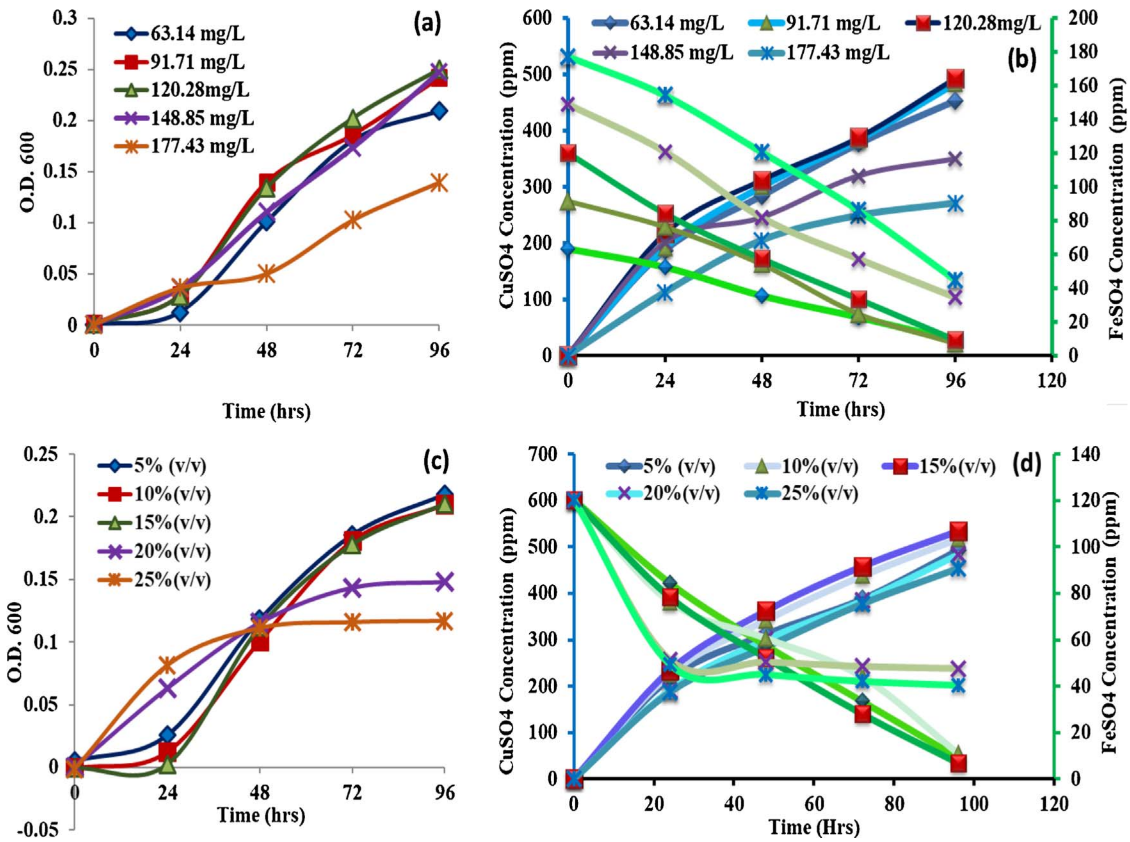

Fig. 3. (a) Effect of $FeSO_4$ concentration on growth of *USCT-R010* (b) Effect of $FeSO_4$ concentration on bioleaching of Cu (c) Effect of inoculum size on growth of USCT-R010 (d) Effect of inoculum size on bioleaching of copper (Lines in Blue shade: Copper Dissolution; Green shade: $FeSO_4$ utilization).

levels of Fe^{2+}, the bioleaching of copper gets limited by the strength of inoculum (size of active population of bacteria) relative to available Fe^{2+}, the availability/transfer of O_2 and CO_2, and other factors [21]. Also almost no growth was supported below the minimum $FeSO_4$ concentration (63.14 mg/L) required for medium preparation.

The enhanced copper leaching and $FeSO_4$ utilization is directly dependent on the efficient growth of *USCT-R010*. Fig. 3(b) demonstrate the effect of $FeSO_4$ concentration on bioleaching of Cu from WPCB. It can be observed from Fig. 3(b) that the dissolution of copper increased with increasing concentration of $FeSO_4$. The observations could be related with the oxidation of Fe^{2+} available in the medium by bacteria and generation of Fe^{3+} which reacts with the copper present in WPCB sample to produce $CuSO_4$. Maximum copper leaching (493.66 mg/L) along with 92.3% $FeSO_4$ utilization was observed with medium having initial $FeSO_4$ concentration of 120.28 mg/L. Therefore, 120.28 mg/L of $FeSO_4$ concentration in medium was considered optimum substrate concentration for efficient leaching of Cu in present study.

3.2.3. Effect of varying inoculum size

Effect of inoculum size was investigated on the growth of *USCT-R010* (Fig. 3(c)) and on the dissolution of copper from WPCB (Fig. 3(d)) by varying inoculum size for a wide range from 5%(v/v) to 25%(v/v).

No any significant difference in growth kinetics was observed while varying inoculum size from 5%–15% (v/v) whereas, a larger inoculum size resulted into rapid utilization of the substrate and therefore, a steady state was reached within 48 h as shown in Fig. 3(c). Inoculum size beyond 15% (v/v) was not found suitable for the optimum growth of microorganism in present study. Inoculum size significantly affects the rate and efficiency of bioleaching [22,23] as the number of active bacteria increases with an increase in the size of inoculum. A substantive increase particularly in the initial rate of bioleaching of copper with increasing the concentration of inoculum from 5%(v/v) to 25%(v/v) was observed as shown in Fig. 3(d), however increase in inoculum size causes deficiency of substrate to the active bacteria. The enhanced dissolution of copper at 15%(v/v) inoculum size compared to 5%(v/v) inoculum size can be attributed to the higher conversion of Fe^{2+} to Fe^{3+} at larger bacterial population, although it is controlled by transfer of O_2 and CO_2 in the bioleaching medium [20]. An optimum copper leaching with sufficient $FeSO_4$ utilization was observed with 15%(v/v) inoculum size therefore, further experiments were carried out at 15%(v/v) inoculum size.

3.2.4. Effect of particle size and pulp density

Particle size of WPCB and its concentration in the solution (pulp density) play an important role in the bioleaching of metals. The WPCB was crushed and then sieved to form homogenous powder of desired particle size 2810 µm, 1680 µm, 420 µm and 250 µm. Effect of particle size on growth of *USCT-R010* and on copper dissolution was investigated and results are shown in Fig. 4(a) and (b) respectively. It can be depicted from Fig. 4(a) that growth of microorganisms was significantly hindered with the decrease in particle size. Small particles increase the apparent viscosity of the slurry and decrease oxygen transfer rates. Also, presence of fine particles may damage the structure of the cells, resulting in their inability to oxidize pyrite. It can be inferred from Fig. 4(a) that growth of *USCT-R010* was hindered at particle size below 420 µm due to formation of particle layer on the surface of the medium and may cause lack of sufficient oxygen for bacterial growth. Maximum copper leaching as well as maximum $FeSO_4$ utilization was observed with particle size of 420 µm.

Pulp density was also varied from 0.5 g/100 ml to 2.5 g/100 ml in the solution to investigate the effect of WPCB concentration on bioleaching efficiency. Significant growth of microorganism was observed for the WPCB loading of 0.5–1 g/100 ml. On the other hand, growth inhibition was noted with the increase in WPCB loading beyond 1.0 g/

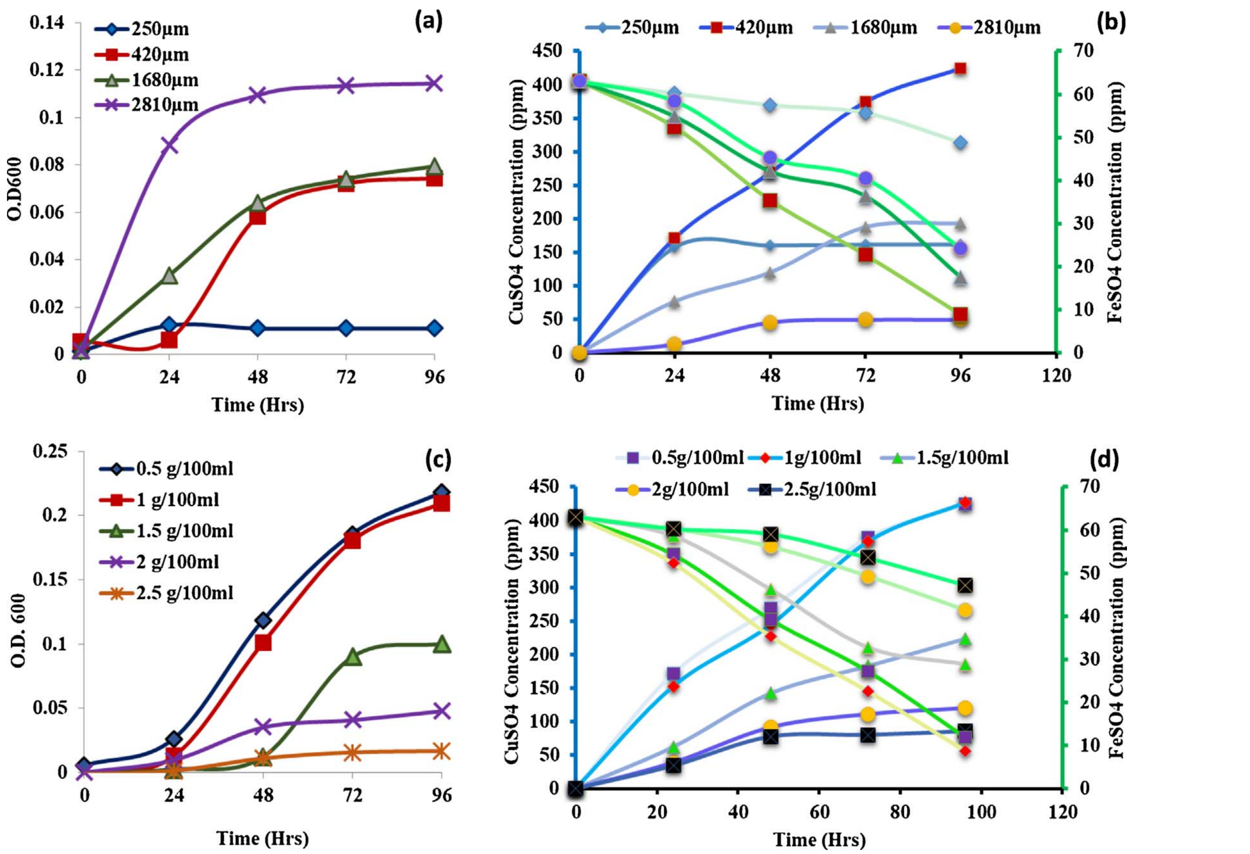

Fig. 4. (a) Effect of particle size on growth of USCT-R010 (b) Effect of particle size on bioleaching of copper. (c) Effect of pulp density on growth of the USCT-R010 (d) Effect of pulp density on bioleaching of copper (Lines in Blue shade: Copper Dissolution; Green shade: $FeSO_4$ utilization).

100 ml. Negligible growth was obtained at pulp density 2.5 g/100 ml as shown in Fig. 4(c). Optimum growth was observed with a WPCB loading of 0.5–1 g/100 ml but non-significant growth was observed when the WPCB loading was increased to 2–2.5 g/100 ml. Bioleaching activity of *USCT-R010* was also observed in the similar manner as growth of the strain at different pulp densities. Maximum copper leaching as well as maximum $FeSO_4$ utilization was observed with 1 g/100 ml loading of WPCB. It can be clearly inferred from Fig. 4(d) that leaching activity of microorganisms is inhibited at high pulp densities which can be attributed to mechanical damage, insufficient adaptation of cells, metabolic stress and nutrient limitation [24]. The inhibition is accompanied by an increase in ferrous ion concentration pointing out at a decreased biooxidation rate as shown in Fig. 4(d).

3.3. Biosorption-desorption cycle

Nearly 85% copper was leached out in leach liquor when bioleaching experiments were conducted under optimized process parameters obtained in Section 3.2. for 15 day time duration. This leach liquor may contain various metals salts along with $CuSO_4$, therefore, a purification step was included in present study to obtain pure copper from leach liquor. The leach liquor was subjected to biosorption process in order to analyze sorption behavior of metal-sorbent system using *A. oryzae* and Baker's yeast. Biosorption experiments were conducted for removal of copper from leach liquor and synthetic solution of same copper concentration under optimized reaction conditions adopted from our other piece of work (submitted for publication). Nearly 88.6% and 91.2% Cu^{2+} was sorbed from leach liquor and synthetic solution respectively using *A. oryzae* under optimum process conditions i.e. contact time: 300 min, solution pH: 2, temp: 30 °C, biosorbents dosage: 1 g, rotation speed: 120 rpm. Similarly, 70.9% of Cu^{2+} was sorbed from leach liquor as compared to 83.6% from synthetic medium using Baker's yeast at optimum reaction conditions i.e. solution pH: 5, contact time: 60 min, temp: 30 °C, biosorbents dosage: 1 g, rotation speed: 120 rpm. Difference in sorption efficiency of biomass for leach liquor and synthetic solution could be attributed to presence of various interfering metal ions and impurities in the leaching solution extracted from WPCB. Desorption studies were conducted using 0.1N HCl as an eluant to desorb metal ions from Baker's Yeast and *A. oryzae* and results are shown in Fig. A.4 (Appendix A in Supplementery information). 91.5% and 85.9% copper was desorbed from dead biomass of Baker's yeast and *A. oryzae* respectively when biosorption experiments were conducted for leach liquor. Slightly higher desorption efficiency was obtained in case of synthetic solution (93.5% for Baker's yeast and 95.6% for A. oryzae).

3.4. Recovery and reusability of copper

Electrowinning experiments were conducted to recover copper metal from eluate ($CuCl_2$) of desorption step. Batch experiments were conducted by varying electrolysis time for a wide range from 0 to 180 min at 15 min time interval and electric current supply from 0–3A in order to maximize the amount of recovered copper [25]. Maximum 94.7% and 92.7% Cu was deposited on graphite electrodes from synthetic solution and leach liquor respectively at electric supply of 2A and 150 min electrolysis time. Optimization results are shown in Fig. A.5 (Appendix A in Supplementery information). ICP-OES analysis was conducted to ensure the purity level of copper which suggested recovery of 95.4% pure copper from WPCB.

The antibacterial activity of recovered pure copper was studied against the commonly available gram-negative *E. coli* bacteria via well diffusion method. After incubation for 24 h, a clear zone of inhibition was observed around the wells inoculated with copper as shown in Fig. 5. High concentration of copper resulted into a larger diameter of

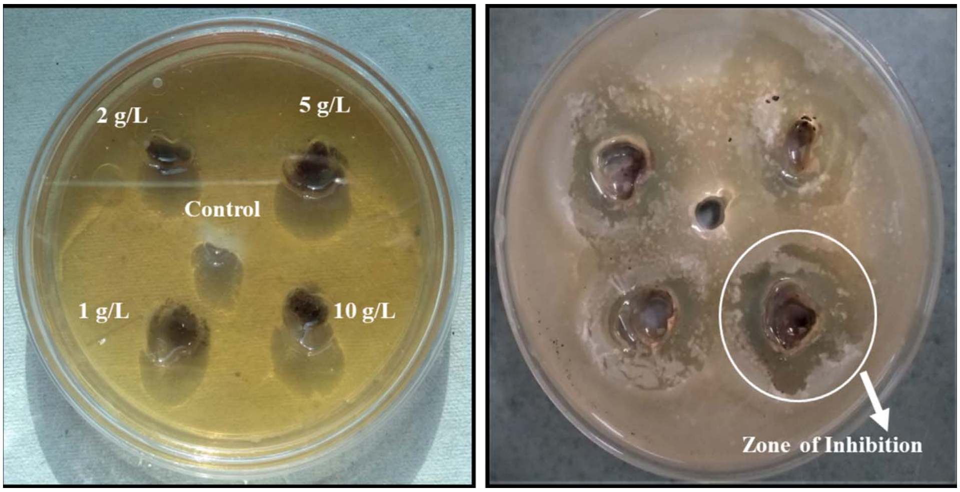

Fig. 5. Antibacterial activity of recovered copper against *E.coli.* a.) Well plates inoculated with *E. coli* before incubation b.) Well plates inoculated with *E. coli* after 24 h of incubation.

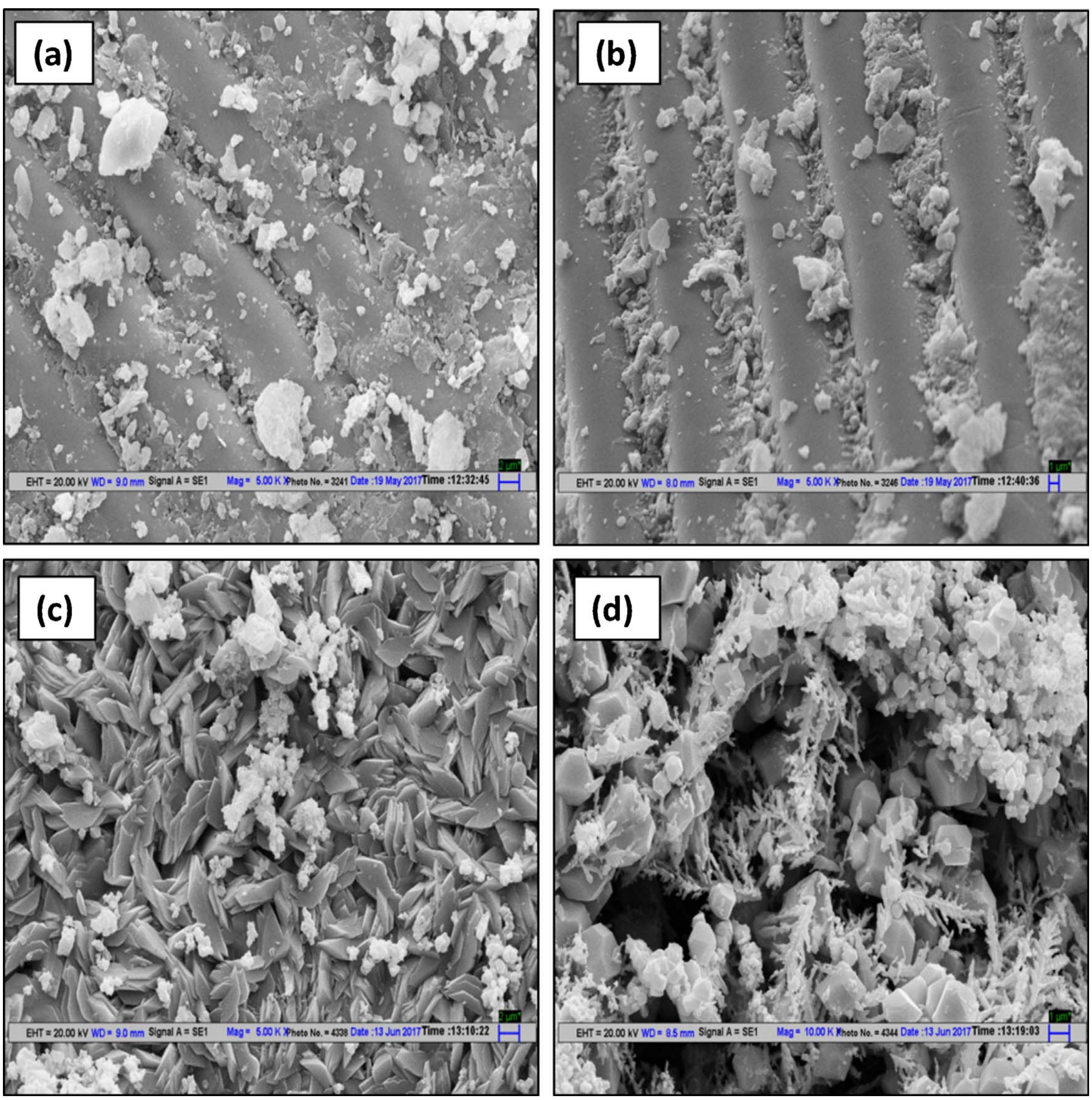

Fig. 6. SEM images of (a) raw WPCB (b) pre-treated WPCB (c) Jarosite (d) Recvoered Copper.

zone whereas inhibition zone was completely absent around the well inoculated with distilled water. Maximum diameter of the zone of inhibition was seen to be 13.5 mm at 10 g/L concentration of copper as shown in Fig. 5. The observations affirmed the significant antibacterial activity of copper against *E. coli*. Results were found to be in concordance with literature [26].

3.5. Characterization studies

SEM analysis was used to study the changes in surface morphology of WPCB during bioleaching process. Fig. 6(a) demonstrates the SEM images of WPCB, pretreated WPCB, jarosite (residue obtained after bioleaching) and recovered copper respectively. Presence of dust particles and polymer can be clearly seen in the SEM image of the raw WPCB as shown in Fig. 6(a). Agglomeration of particles, lump formation, and non-uniform distribution of metals were also observed. WPCB was pretreated with ethanol to remove some of its non-metallic components namely epoxy resins, glass fibers and brominated flame retardants and consequently to reduce the toxic effect of these materials on the activity of micro-organisms. It is clearly evident in Fig. 6(b) that all the impurities, polymeric coating and dust particle were removed after ethanol wash and a clearer image with just the presence of metal compounds was obtained. Fig. 6(c) illustrates individual euhedral crystals ranging in size from ~1.0 to 7.4 μm which represent the formation of Jarosite in the bioleaching residue [27]. Literature indicates the formation of Jarosite (a mixture of various monovalent cations, moderate to high ferric ion and sulfate concentrations) formation in residue obtained after bioleaching experiments at solutions of pH = 1.5–2 [28]. Trace amount of other impurities were also observed in the SEM image of Jarosite. SEM image of recovered copper is show in Fig. 6(d). A clear picture of copper dendrites was obtained which affirms the recovery of pure copper from WPCB using the proposed novel two stage biorecovery process.

Fig. A.6 (Appendix A in Supplementery information) demonstrates the XRD patterns for raw WPCB, pretreated WPCB, jarosite and recovered copper. Presence of Cu^{2+} ions in WPCB was affirmed by having peaks at 2θ of 19.0°(2 1 2), 29.2°(1 1 1), 38.4°(2 2 0), 43.7°(1 1 1), 50.8°(2 0 0) and 74.4°(2 0 0) in the XRD spectrum of WPCB. Several small peaks at 2θ angle of 31.04°–36.8° were seen which may represent epoxy resins and other impurities present on WPCB surface. These peaks were completely lost when WPCB was washed with ethanol as shown in XRD pattern of pretreated sample whereas strong peaks for Cu^{2+} were observed at 18.3°, 43.0°, 50.1°, 73.8°. XRD analysis of the bioleaching residue was consistent with the SEM observation and contains dominant phase being jarosite. The best fit to database patterns was for a hydronium substituted jarosite (K, H_3O^+) $Fe_3(SO_4)_2(OH)_6$). Similar observations were reported in literature where sample, collected from sediment cores, contains Jarosite as primary phase, however some impurities of quartz and illite was also reported in XRD spectra [27]. No significant peak for copper or any other impurities was observed in the bioleaching residue which ensure efficient extraction of copper from WPCB in leach liquor. A clear observation about the purity level of copper is reflected in the XRD spectra of recovered copper where peaks with very high intensity were obtained for copper. Major peaks at 2θ angle of 38.5° and 43.3° representing the cubic face centered plane of Cu^{2+} metal ion, was observed in XRD spectra of recovered copper. Several other peaks for copper metal ions were obtained and validated the extraction of copper with 95.4% purity.

4. Conclusion

A novel hybrid two stage biorecovery approach followed by electrochemical treatment was employed to recover copper from WPCB with high purity level. The isolated strain obtained from the e-waste dumping site showed significant potential to leach copper (> 85%) from WPCB under optimized reaction condition. The eco-friendly, low-cost biosorbents (A.oryzae and Baker's yeast) demonstrated more than 86% biosorptive potential to recover copper from mixture of various metals present in leach liquor. Significant antibacterial activity of recovered copper (95.4% PURE) against *E. coil* corroborates the reusability of the extracted metal from WPCB. Proposed novel combination of biohydrometallurgical method and electrotreatment can be reckoned as an environmentally benign approach to recover and reutilize heavy metals present in waste material. This work offers an incentive to the industrial practice for waste minimization, recycling of extracted metals, and the noncorrosive, ecofriendly approach for metal extraction from the fastest growing waste stream.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jece.2018.01.030.

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