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Improvement in treatment of soak liquor by combining electro-oxidation and biodegradation

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Abstract

Soak liquor generated from leather processing industry contains high organic load, making it a challenge to treat it efficiently. A combined process involving electro-oxidation and biodegradation by halophilic bacteria was applied to treat the wastewater effectively for discharge. Electrolysis was performed prior to biological treatment in an electrochemical reactor at a current density of 0.012 A/cm^2 for a period of 30 minutes. Titanium Substrate Insoluble Anode (TSIA) was used as an anode and titanium mesh used as cathode. Biological degradation was carried out with two isolated microbial strains at pH 6. The combination of electro-oxidation and biodegradation was recorded as a good technique in terms of COD (Chemical Oxygen Demand), BOD (Biological Oxygen Demand) and TKN (Total Kjeldahl Nitrogen) removal efficiency of 95%, 85%, and 88% respectively. The present study claims that the integrated process gives better performance on reduction of COD while comparing previous studies.

Keywords: Tannery; Soak liquor; Electro-oxidation; Biodegradation.

1 Introduction

Soak liquor, a primary effluent from the tannery industry has intense brown colour, hypersalinity, and stinky odour. Soak liquor is composed of 2-4% of sodium chloride by weight and contains traces of calcium chloride along with organic contaminants (flesh, skin, blood, humic substances and other suspended particles) such as nitrogen and phosphorous containing compounds [1, 2]. If this effluent is discharged into nearby lands without any prior treatment, it will pollute the lands and groundwater. The treatment method in use is solar evaporation but the evaporation rate is affected due to higher total dissolved solids (TDS), organic loads and suspended solids (SS)[3]. The other additional problem of this effluent treatment is the presence of humic substances. These compounds form complexes with proteins and other organic contaminants making it difficult for biological degradation[4]. The breaking down of these complexes is necessary for effective biological treatment of soak liquor. Some reports also indicated that bacteria found in the hypersaline environment have greater potential in degrading pollutants [3, 5]. The native concentration of chloride favours the pre-treatment in electro-oxidation. Szpyrkowicz *et al.*[6] Srinivasan *et al.*[7] and Kanagasabi *et al.*[8] did pre-treatment processes viz. electro-oxidation, chemical oxidation (ozone treatment) before biological treatment. The electrochemical pre-treatment was used to convert bio-recalcitrant organics to biodegradable compounds [9]. The secondary oxidants such as chlorine, hypochlorite liberated during the electro-oxidation process enhance the biodegradability, resulting in the removal of organic pollutants in the effluent. Basha *et al.*[2] stated that the rate of biological treatment can be enhanced by reducing the molecular size of the organic pollutant present in the effluent by electro-oxidation, where TSIA (Titanium substrate insoluble anode) was used as the anode. Sundarapandiyan *et al.*[10] performed electrochemical

oxidation of synthetic saline waste water by employing graphite electrodes and suggested that 0.012 A/cm^2 is the optimum current density for the treatment of saline wastewater with the COD reduction of 89.11% within 2 h where biological degradation was not investigated.

In recent years, a few studies (Table 1) have been carried out on combined process viz electro-oxidation and biological treatment of tannery soak liquor, where the COD reduction was in the range between 64% and 90% [(76%) [6], (64%) [7], (66.2%) [8], (90%) [11],] where the current density was 0.02 &0.04 A cm^{-2} [6], 0.05 A cm^{-2} [8], 0.024 A cm^{-2} [11] respectively in electro-oxidation. Some of the studies on biological degradation alone in soak liquor, the COD reduction was about 96% in 300 days[3], 74 to 88% in 45 days [4] and 80% in 72 h [12]. Senthilkumar *et al.* [12] used halophilic bacteria collected from marine and tannery saline wastewater for degradation of soak liquor and the COD removal efficiency was about 80% within 3 days whereas initial COD was about 2512 ppm in raw tannery saline wastewater.

In the present communication, the importance of electro-oxidation prior to biological treatment of soak liquor containing high COD was explored. Electrochemically generated secondary oxidants were removed by solar exposure. The combined process viz electro-oxidation and biological treatment using (electro-biodegradation) halophilic bacteria was investigated to improve the COD reduction. The initial COD of the soak liquor was about 7300 mg/L which is the highest when compared to previous works presented in Table 1. The soak liquor was electro-oxidized with low current density (0.012 Acm^{-2}) compared to previous works using triple oxide coated electrode (TSIA) to break the humic acid organic complex followed by biodegradation using *Bacillus cereus* and *Klebsiella oxytoca* with constant agitation (150 rpm). The removal of organic contaminants during the process was

investigated by monitoring COD, BOD, TKN, lipid, and protein. removal efficiency before and after electro-biodegradation.

2 Materials and methods

Soak liquor was collected from Leather processing division, CSIR-Central Leather Research Institute (CLRI), Chennai and transported in the icebox to the CSIR-Central Electrochemical Research Institute (CECRI) Karaikudi. The sample was filtered using Whatman No: 1 filter paper to avoid the contamination of suspended particles such as hair, sand etc., present in the soak liquor. The filtered particles were discarded carefully and the filtered soak liquor was further used in the study.

The physical and chemical properties of filtered soak liquor were characterized using standard methods [13]. COD and BOD were determined using the dichromate open reflux method and Winkler's method respectively by strictly following the American Public Health Association (APHA) procedures[13]. The interference of chloride during COD measurement was overcome by adding 10/L weight ratio of mercuric sulphate to chloride [3]. The protein [14] and lipid[15] were measured before and after electro-biodegradation using standard estimation procedures. Electro-bio degraded samples were centrifuged at 6000 rpm for 30 minutes and the supernatant used for protein and lipid estimation in order to avoid the interference of cell suspensions.

112 **2.1 Electro-oxidation of soak liquor**

113 **2.1.1 Electrodes**

114 IrO_2-RuO_2-TiO_2 film coated titanium expanded mesh prepared by thermal
115 decomposition method was used as anode. The respective metal chloride was dissolved in
116 isopropyl alcohol and brushed on pre-treated titanium substrate layer by layer until required
117 coating solution was brushed on the titanium [16, 17]. After brushing each layer, the substrate
118 was heated in a furnace at 450^{o}C in the presence of air. The coating consists of TiO_2: 76%;
119 RuO_2: 23%; IrO2: 1% and the coating was characterized by SEM and EDAX and presented
120 in supplementary Fig 1 and 2 and Table 1. Titanium expanded mesh was used as cathode.

121 A rectangular undivided cell of dimension 15×5 cm^2 was designed and fabricated
122 using polypropylene solid material of volume 350 ml with TSIA anode and titanium mesh
123 cathode. The anode and cathode were kept apart at an interelectrode distance of 1 cm. The
124 soak liquor was taken into the cell and electrolysed at the current density of 0.012 A/cm^2 for
125 30 minutes. The experiments were done at a galvanostatic condition using a DC power
126 supply (Aplab power supply model: Regulated DC power supply L3205). Anodic and
127 cathodic potentials were measured using a multimeter (Agilent U1232A) with the help of
128 saturated calomel electrode (SCE) as a reference electrode. The excess hypochlorite present
129 after electro-oxidation was decomposed by exposing to sunlight for 1 h at 1.0× 10^5 lux as
130 measured by Lux meter (Digital 128 instruments). The same electrode was used to treat
131 multiple batches (6 times) of soak liquor to find the fouling and the nature of coatings on the
132 electrode surface.

133

134

135 **2.2 Isolation and identification of bacterial strain**

136 The bacterial strains were isolated from the soak liquor. The isolated bacterial strains
137 were enriched in nutrient agar medium [8]. Pure cultures were obtained by picking
138 morphologically dissimilar and dominant isolates were stored at 4°C with periodic
139 subculturing was done. These strains were identified using 16s rRNA sequencing in Defence
140 Research and Development Organisation (DRDO), Gwalior.

141

142 **2.3 Electro-biodegradation of soak liquor**

143 The isolated bacterial strains were inoculated in hypochlorite free electro-oxidized
144 soak liquor (250 ml) supplemented with 1% of glucose as energy source [18]. The electro-
145 oxidized soak liquor was inoculated with 5% of 5-day old culture and agitated at 150 rpm at
146 30 °C. The growth was monitored by measuring the optical density at 600 nm using UV-
147 Spectrophotometer (Thermo scientific evolution 201). The growth conditions such as pH (4
148 to 9), temperature (25°C to 40°C), inoculum concentration (5% to 25%), the percentage of
149 carbon source (1% to 5%) and agitation (100 rpm to 250) were optimized by measuring the
150 biomass concentration at 600nm. The condition for biodegradation study was chosen based
151 on higher yielding biomass.

152

153 **2.4 Decolourization and degradation studies**

154 UV-Vis spectra were recorded between 190 and 1100 nm using UV-Vis spectrometer
155 (Thermo scientific evolution 201) and the spectrum was compared for soak liquor before and
156 after electro-oxidation and biological degradation. The colour removal efficiency and organic
157 load removal pattern were analysed. The variation in the functional group during the electro-

158 oxidation and biodegradation were determined using FT-IR (Bruker Optik GmbH, model no -
159 Tensor 27). The samples were mixed with KBr and the transmittance percentage was
160 observed between the wavenumber range of 400-4000 cm^{-1}.The variation in the percentage of
161 hydrogen, nitrogen and sulphur content of the soak liquor, before and after electro-oxidation
162 and electro-biodegradation were measured by CHNS (Carbon, Hydrogen, Nitrogen, Sulphur)
163 analysis (Elementar Cube Vario). The soak liquor before and after electro-oxidation and
164 biodegradation were lyophilized and used for high-pressure liquid chromatography (HPLC)
165 analysis. 20µL of the sample was injected into liquid chromatographic system (Prominence,
166 Shimadzu) with a photodiode array (PDA), The chromatographic separation was performed
167 on a C18 column (100mm × 4.6mm) using methanol/water (acidified 1%, v/v) as mobile
168 phase at a flow rate of 0.4 ml min^{-1} [19].
169
170 **2.5 Energy consumption**
171 Energy consumption in electro-oxidation of soak liquor was calculated on the basis of
172 COD reduction[20]

173 $$\text{Energy consumption} = \frac{tVA/SV/1\times10^{3}}{\Delta COD/1\times10^{6}}\ \text{KWh/Kg COD}$$

174
175
176 where t is the time of electrolysis in hours, V is the average cell voltage, A is current in
177 ampere, SV is sample volume in litres and ΔCOD is the difference in COD in time t in mg/L.
178
179
180

3 Results and Discussion

The physiochemical properties of soak liquor during various stages of the process are mentioned in Table 2. The soak liquor contained protein (13 g/L), lipid (89 g/L) and chloride (17.08 g/L) as major components where COD was 7300 mg/L.

3.1 Electro-oxidation of soak liquor

The electro-oxidation of soak liquor was done at the current density of 0.012 A/cm^2 for 30 minutes to reduce the cost of the process using TSIA electrode. The oxidation of active chlorine molecules reduces total energy required for the treatment [21]. The impact of pH on electro-oxidation of soak liquor was studied by varying pH from 2 to 12 with an increment of 2. The amount of hypochlorite produced during the course of electro-oxidation at pHs 2, 4, 6, 8, 10, 12 was 401.8 ppm, 446.4 ppm, 483.6 ppm, 111.6ppm, 74.4 ppm and 37.2 ppm respectively. The reduction in organic load was higher at pH 6 when compared to other pHs, where the COD removal efficiency was 41%. At acidic conditions, the OCl^- ions are unstable and forms HOCl as well as chlorine gas which are strong oxidant.

$$HOCl \leftrightarrow H^+ OCl^-$$

The reduction in the concentration of hypochlorite was noticed at high pHs (pH 8 - pH 12)[10]. It can be concluded that near neutral pH is favourable for electro-oxidation of soak liquor with a higher quantity of hypochlorite production [10].

In the electrochemical cell, chlorine formed at anode and hydroxides formed at cathode which reacts to form chlorine and hypochlorites respectively [10,19]. Both the hypochlorite and free chlorine are chemically reactive and oxidize the organic pollutants in the effluent to

carbon dioxide and water. The following reactions are taken place during electro-oxidation in the presence of sodium chloride.

At Anode

$$2Cl^- \longrightarrow Cl_2 + 2e^- \quad (1)$$

$$4OH^- \longrightarrow O_2+2H_2O+4e- \quad (2)$$

At cathode

$$2H_2O+2e^- \longrightarrow H_2+2OH^- \quad (3)$$

$$Cl_2+ H_2O \longrightarrow H^+ + Cl^-+ HOCl \quad (4)$$

The HOCl further dissociates into OCl^- and H^+

$$HOCl \longleftrightarrow H^+ + OCl^- \quad (5)$$

Hypochlorite ions act as main oxidizing agent in organic degradation.

The overall desired reaction of electrolysis is:

$$\text{Organic matter} + OCl^- \longrightarrow \text{Intermediates} + CO_2+Cl^- + H_2O \quad (6)$$ [12]

These oxidizing species can diffuse into the areas away from electrodes and continue to oxidize the pollutants [10]. The UV absorbance spectra for electro-oxidized soak liquor at various pH and commercial grade humic acid is presented in Fig. 2. It can be assumed that the presence of humic acid at pH 6 was due to efficient oxidation of organics present in soak liquor and release of humic acid bound to it. The free humic acid could not be noticed at pH 2 and 4 though there is no significant variation in hypochlorite formation. It can be assumed that the hypochlorite does not break the organics with humic acid significantly at low pH (2 to 4). This is because the organics can be removed from humic acid at pH 5.5 to 6.5 and also the humic acid complex is stable in the range 3 to 5 [22]. Hence, the optimum pH for electro-oxidation to break the humic acid present in the soak liquor was about 6.

In the present study, the anode potential was in the range of 1.32-1.7 V vs SCE. Salazar-Gastélum *et al.* [23] noticed the formation of hypochlorite at 1.7 V vs SCE. In the present study, the oxidation of the organic complex is due to indirect electro-oxidation at TSIA electrode [24]. The presence of high concentration of sodium chloride (17.08 g/L) in soak liquor makes it even more compatible for TSIA electrode with the current density of 0.012 A/cm^2 which is necessary for indirect oxidation [23]. Sundarapandiyan *et al.*[10] performed electro-oxidation of tannery saline wastewater for 120 minutes by employing graphite electrode, where the COD removal efficiency at a current density of 0.012 A/cm^2 was about only 89.11%. In the present study, the electro-oxidation time of soak liquor was reduced to 30 minutes employing TSIA electrodes thus, the energy consumption can be reduced which helped to overcome the shortcomings of the work done by the previous group[10]. The reduction of time for electro-oxidation will enhance the life of the electrode thereby the cost can be reduced. Besides, 6 cycles of electro-oxidation were done and there was no coating damage and fouling on the electrodes which was confirmed by SEM and EDAX (supplementary Fig. 1 and 2 and Table 1). Kanagasabi *et al.* [8] described that lower COD concentration results in efficient degradation by microbes, which supports the present study.

3.2 Electro-biodegradation of soak liquor

The solar treatment after electro-oxidation helped in the removal of the oxidants generated during the electrochemical process [28]. The complete removal of hypochlorite from electro-oxidized soak liquor was achieved by exposing it to 1.0×10^5 lux solar light for 1hour for complete removal of hypochlorite followed by biodegradation [25]. The biological process was enhanced by an increase in biomass concentration consecutively improves the

degradation efficiency [8]. Thus supplementing the medium with the simpler substrate as primary carbon source such as glucose increased the biodegradation efficiency [8, 26]. Thus, the organics present in the solution were co-metabolised along with primary carbon source. Two bacterial strains were isolated from the soak liquor and identified as *Bacillus cereus* and *Klebsiella oxytoca*. The phylogenetic tree was constructed included in supplementary Fig. 3. The electro-oxidized soak liquor was treated using the bacterial strains isolated from soak liquor as a mixed culture after removal of hypochlorite by solar exposure [27]. The bacterial growth conditions were optimized and presented in Table 3. The growth curve of the mixed culture and individual isolates are mentioned in Fig. 1. It was indicated in earlier reports, microbes in the hypersaline environment have greater potential in degrading pollutants [12, 28]. In the present study, the chloride concentration was about 17.08 g/L. Hence, these isolates can be claimed as halotolerant [29, 30].

3.3 Chemical characterisation

UV-Vis study was carried out for soak liquor before and after electro-oxidation and electro-biodegradation and presented in Fig. 3. Two major peaks were observed at 197 nm and 200 nm, with an additional hump in the range of 252 to 294 nm. The presence of all peaks and shoulder in the region of 250-270 nm denote humic acid [29]. After electro-oxidation of soak liquor, only one major peak was found at 210 nm which indicates the presence of hypochlorite. The peak at 210 nm corresponds to $\pi^{\cdots}\pi^*$ electronic transition of carboxylic and phenolic groups [1]. Upon treatment of electro-oxidized solution using microbes the intensity of the peak at 210 nm has further reduced significantly. The shifting of the peak

from 210 to 230 nm in the electro-biodegraded and biodegraded sample is due to secondary metabolites of bacteria.

The FT-IR spectrum of soak liquor before and after electro-oxidation and electro biodegradation along with commercial grade humic acid is given in Fig. 4. The FT-IR spectrum of soak liquor is similar to commercial grade humic acid, which also confirms the presence of humic acid. It can be noticed that there is a significant decrease in humic acid bound with primary amine R-NH_2 (3440 cm^{-1}) after electro-oxidation, which is oxidized by OCl^- and converted as CO-NHR (1648 cm^{-1}). Another interesting observation is an increase in the intensity of peaks in 3000 cm^{-1} to 3150 cm^{-1} region is due to break down of the humic acid organic complex. Tatzber *et al.* [31] reported that humic acids are always involved in the formation of complexes with sodium salts of phenols to form sodium carboxylate salts (1700 cm^{-1}). It reveals that humic acid complex is broken during electro-oxidation and in the biological treatment of soak liquor major peaks (OH, -NH_3^+, -NH_2^+, -CO-NH_2, -CO-NH, S-H, and P-H) could not be noticed. The humic acid exists in the form of sphere colloids, a rigid molecule [29], which was broken down into smaller molecules during electro-oxidation by active oxidizing species and further used for biodegradation. The electro-biodegradation of soak liquor helped to break the rigid molecules, which can be observed through the intensity of peaks. The implementation of biological treatment after electrochemical oxidation has helped to oxidize the organic contaminants completely (Fig. 4) from the soak liquor.

The CHNS analysis was carried out to measure the percentage of the respective elements present in the soak liquor before and after electro-oxidation and electro-biodegradation (supplementary Fig. 4). After electro-oxidation of soak liquor, no significant reduction of hydrogen was found, whereas the nitrogen and sulphur contents reduced by 46%

and 76% respectively. During electro-biodegradation of soak liquor, the removal efficiency of hydrogen, nitrogen and sulphur were 70%, 100%, and 84% respectively which indicates that electro-biodegradation increased removal efficiency of the elements [19]. On the other hand, in the stand-alone process of biological treatment, the removal efficiency of nitrogen and hydrogen was 50% and 60% respectively but the sulphur removal was similar to electro-biodegradation. These results support electro-biodegradation which is efficient in treating the soak liquor.

The pollution parameters COD, BOD and TKN were measured before and after electro-oxidation and electro biodegradation (Fig. 5a). After electro-oxidation, 60%, 36% and 64% of BOD, COD, and TKN reduced within 30minutes respectively. After electro-biodegradation, the above parameters further reduced to 85%, 95%, and 88% respectively which supports with FTIR analysis. In the present study, the increased COD removal efficiency (95%) is due to electro-biodegradation and application of mixed halophilic bacterial strains used in the study[12]. It can be claimed that the degradation efficiency is higher when compared with the previous studies [6,8].

The protein and lipid content of the soak liquor was measured before and after electro-oxidation and electro-biodegradation (Fig. 5b). After electro-oxidation 7.6% and 31% reduction in protein and lipid were found, whereas after electro-biodegradation the above values further increased by 57% and 91% respectively. The biological treatment of soak liquor has led to a poor reduction in protein and lipid, the reduction efficiency was 14% and 27%. The lesser reduction of protein (7.6%) during electro-oxidation is due to the fact that protein was not completely mineralized instead it is broken down into simpler molecules such as smaller peptides and amino acids [10]. The microbes were able to mineralize the amino acids

only after the molecular breakdown of humic acid organic complex[2]. This is the reason for the lesser reduction in organic load during biodegradation alone when compared to electro-biodegradation. It can be concluded that electro-biodegradation gives better efficiency in the treatment of soak liquor within 7 days.

The soak liquor was analysed by HPLC before and after electrochemical, biological treatment and electro-biodegradation (Fig. 6). The untreated soak liquor has three peaks at retention time around 2.85, 3.161 and 5.482 min. After electro-oxidation, the peak intensity got reduced and the peak was found with retention time around 2.898 and 3.080 min. A new peak was observed at 5.471 min which is due to the molecular breakdown of the humic acid organic complex. After biological treatment, of electrolysed soak liquor the chromatogram showed six important peaks at retention time around 1.507, 2.703, 3.056, 3.181, 3.348, and 5.482 where the original peaks in the effluent disappeared, the formation of new peaks can be explained as metabolites of bacteria [19]. During electro-biodegradation, the peak at 2.740, 3.041 observed in soak liquor decreased about 75.92% and 87.58% respectively. In biodegradation process, 29.67% and 22.71% reduction was observed respectively. These results also support that the electro-biodegradation promotes significant degradation of organics present in the soak liquor.

3.4. Mechanism proposed for electro-biodegradation of soak liquor

Considering the results from FT-IR, a possible mechanism can be proposed for the present study (Fig. 7). The humic acid bound with primary amine R-NH_2 (3440 cm^{-1}) is oxidized by OCl^- and converted as CO-NHR (1648 cm^{-1}). It can be assumed that small degraded molecules formed after electro-oxidation [5-cyano-2,3,3',4'-tetrahydroxy biphenyl,

2-(4-hydroxyphenyl)-3H indole-4,7-dione derivative, 10H-phenoxazine-1,4-dione derivative, and 1,6,7-trihydroxyphenanthro[2,3]benzofuran-9,10-dione derivative (supported with FT-IR results)] are consumed by bacteria. CHNS analysis also revealed that 70% of hydrogen, 100% of nitrogen and 84% of sulphur were consumed by bacteria in the integrated process. Besides sulphur, the remaining counterparts of amino acids can be utilized by the bacteria. It can be concluded that electro-oxidation alone cannot be a complete treatment technique for soak liquor. Further, it can be explained that the electro-oxidised hydrogen, nitrogen and sulphur molecules from amino acids can be easily consumed by bacteria.

4 Conclusions

The electro - oxidation process has the ability to convert the biologically recalcitrant complex humic acids into biodegradable compounds. The smaller molecules present after electro-oxidation were effectively treated biologically using mixed cultures collected from tannery effluent. In this study by applying a current density of 0.012 A/cm^2 for 30minutes followed by biological degradation for seven days with primary substrate enhanced the organic load reduction in the soak liquor. The release of humic acid during electro-oxidation helped the biodegradation process. For the first time, the combined process achieved higher COD reduction (95%) using halophilic bacteria at pH 6 for rich organic containing soak liquor. The higher cost efficiency of 0.03 dollars/cm^3 subsequently reducing the cost by 97% and 90 % when compared with works of Sundarapandiyan *et al.*[10] and Kanagasabi *et al.* [8]. The electro-biodegradation can be employed for effective treatment of soak liquor in the leather industry.

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387 **References**

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Fig. 1 Growth curve of mixed and individual isolates comprising *Bacillus cereus* and *Klebsiella oxytoca*

Fig. 2 UV-Vis spectra of soak liquor electro-oxidized at different pH (2-4) with soak liquor and humic acid.

Fig. 3 UV-Visible spectrum of humic acid, soak liquor before and after electro-oxidation, biodegradation and electro-biodegradation.

Fig. 4 FT-IR spectrum of soak liqour (A) Soak liquor, (B) After electro-oxidation, (C) After biological process, (D) After electro-biodegradation, (E) Humic acid.

Fig. 5 (A) Estimation of BOD, COD, TKN, (B) Estimation of protein and lipid in soak liquor before and after electro-oxidation and electro-biodegradation.

Fig. 6 HPLC analysis of soak liquor, after biodegradation, electro-oxidation and after electro-biodegradation.

Fig. 7 Possible mechanism of electro-biodegradation of soak liquor.

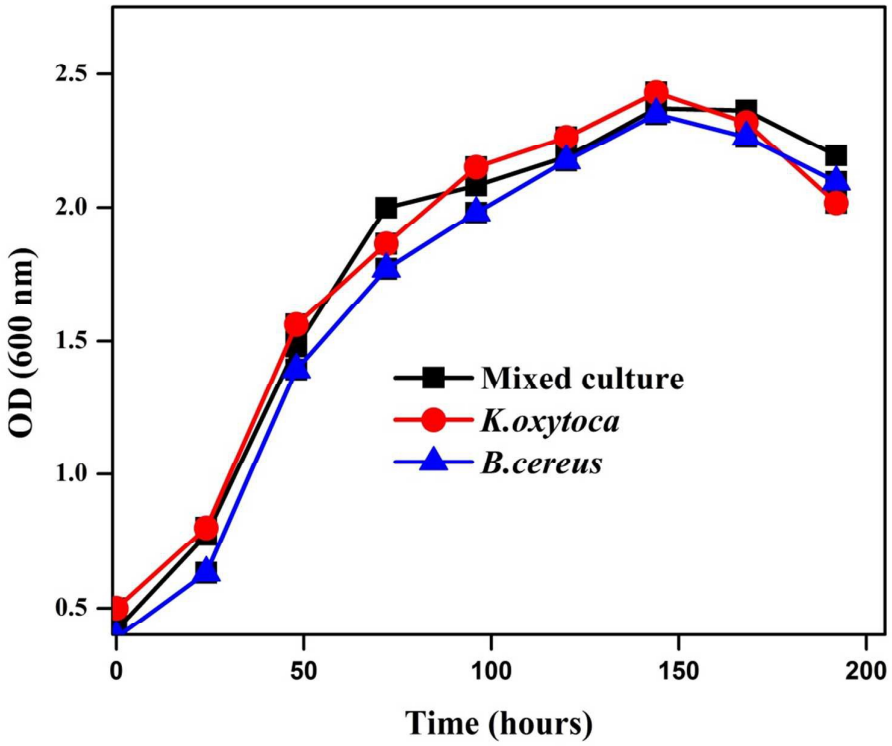


Fig. 1 Growth curve of mixed and individual isolates comprising *Bacillus cereus* and *Klebsiella oxytoca*

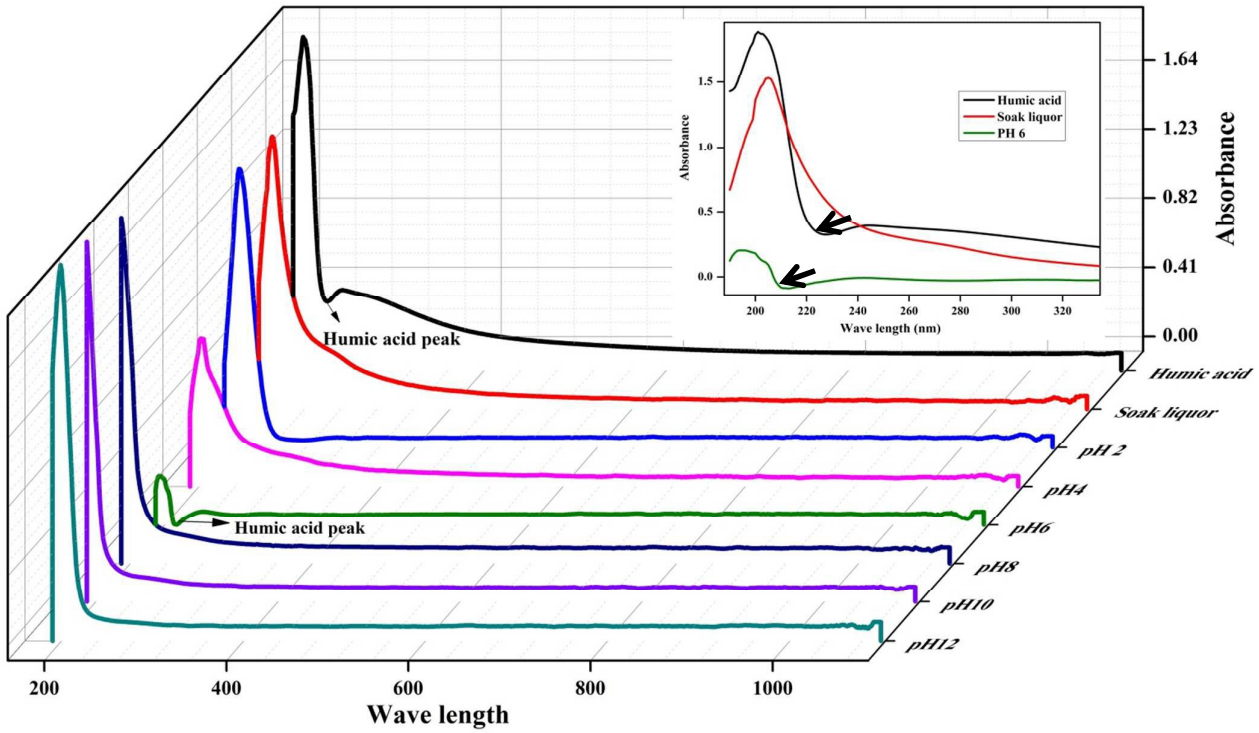


Fig. 2 UV-Vis spectra of soak liquor electro-oxidized at different pH (2-4) with soak liquor and humic acid.

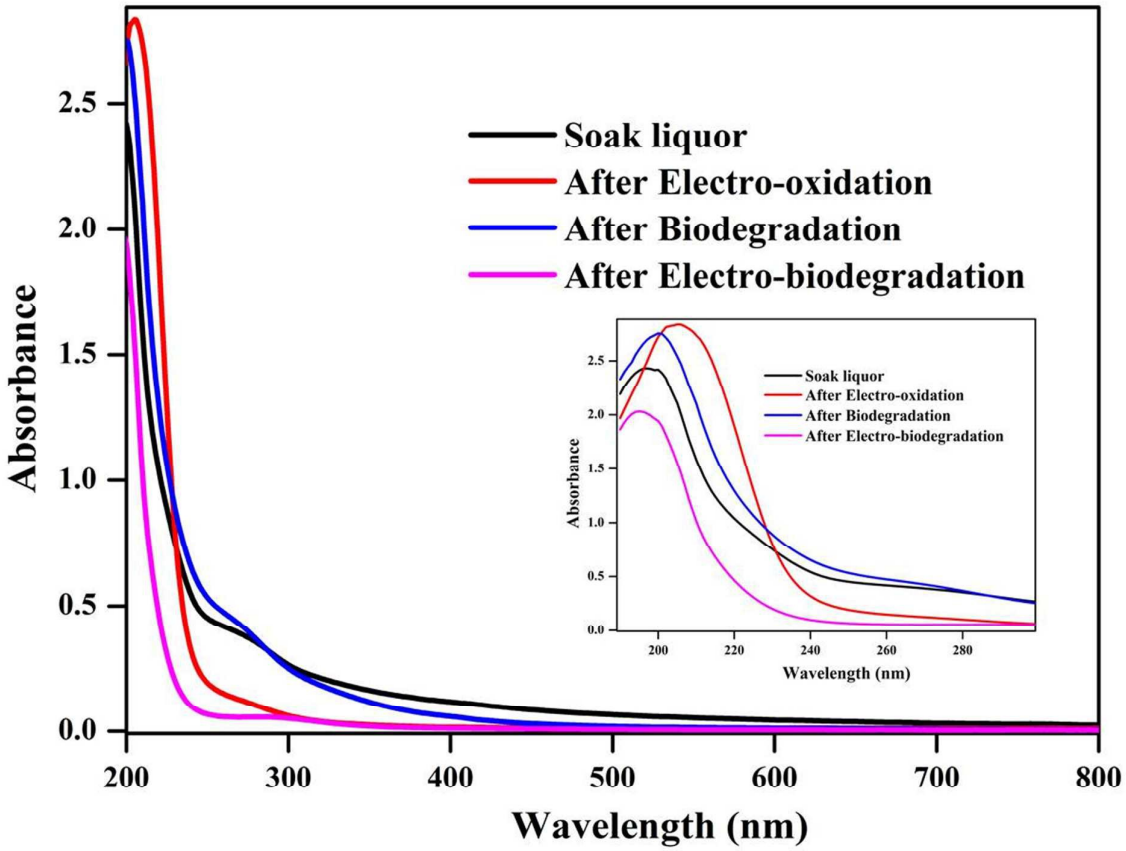


Fig. 3 UV-Visible spectrum of humic acid, soak liquor before and after electro-oxidation, biodegradation and electro-biodegradation.

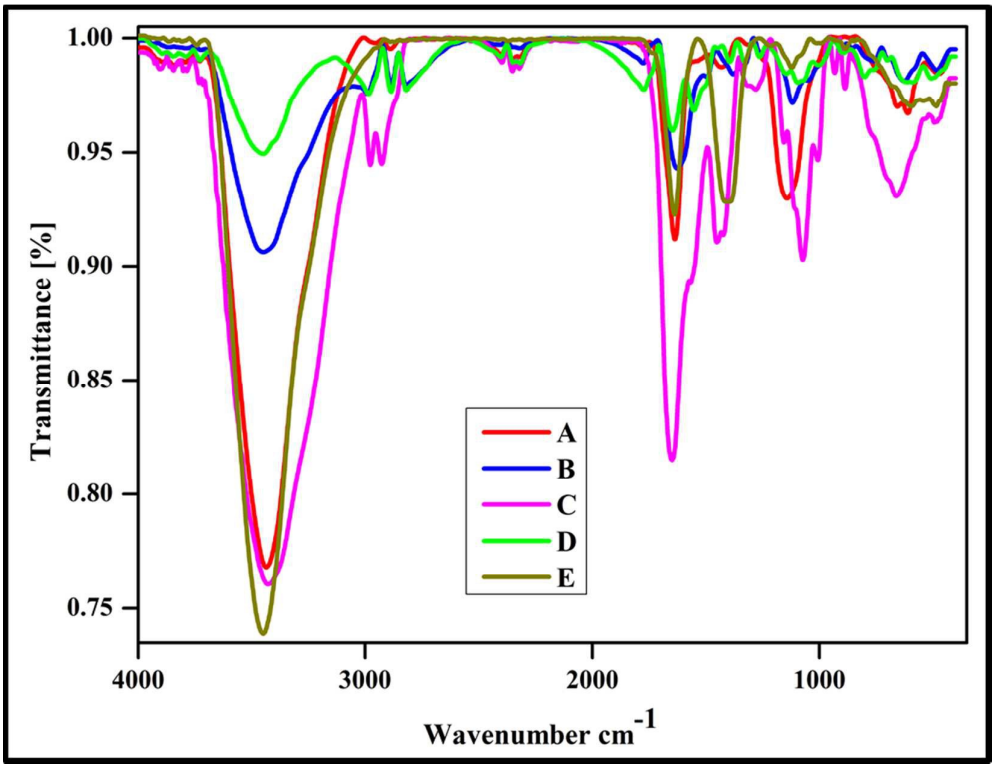


Fig. 4 FT-IR spectrum of soak liqour (A) Soak liquor, (B) After electro-oxidation, (C) After biological process, (D) After electro-biodegradation, (E) Humic acid.

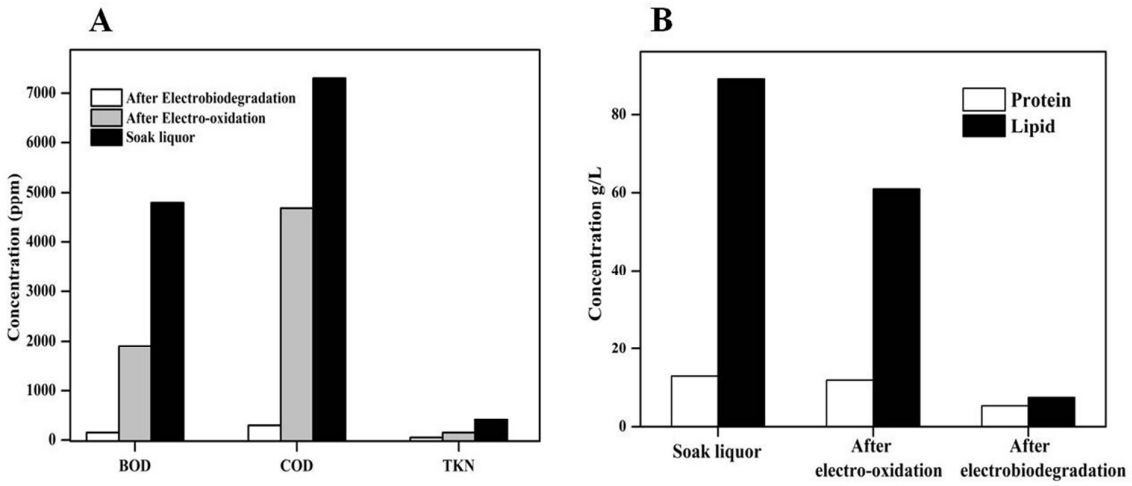


Fig. 5 (A) Estimation of BOD, COD, TKN (B) Estimation of protein and lipid in soak liquor before and after electro-oxidation and electro-biodegradation.

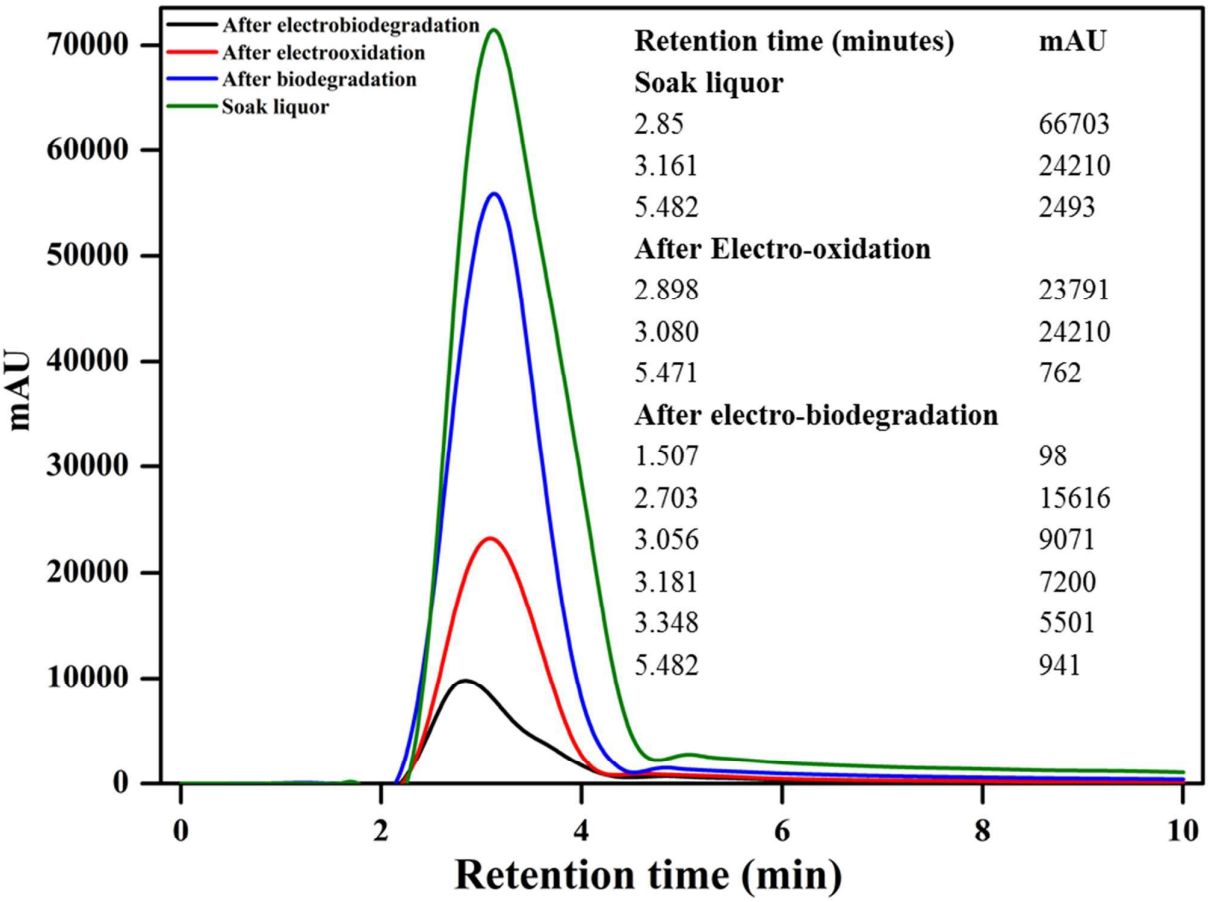


Fig. 6 HPLC analysis of soak liquor, after biodegradation, electro-oxidation and after electro-biodegradation.

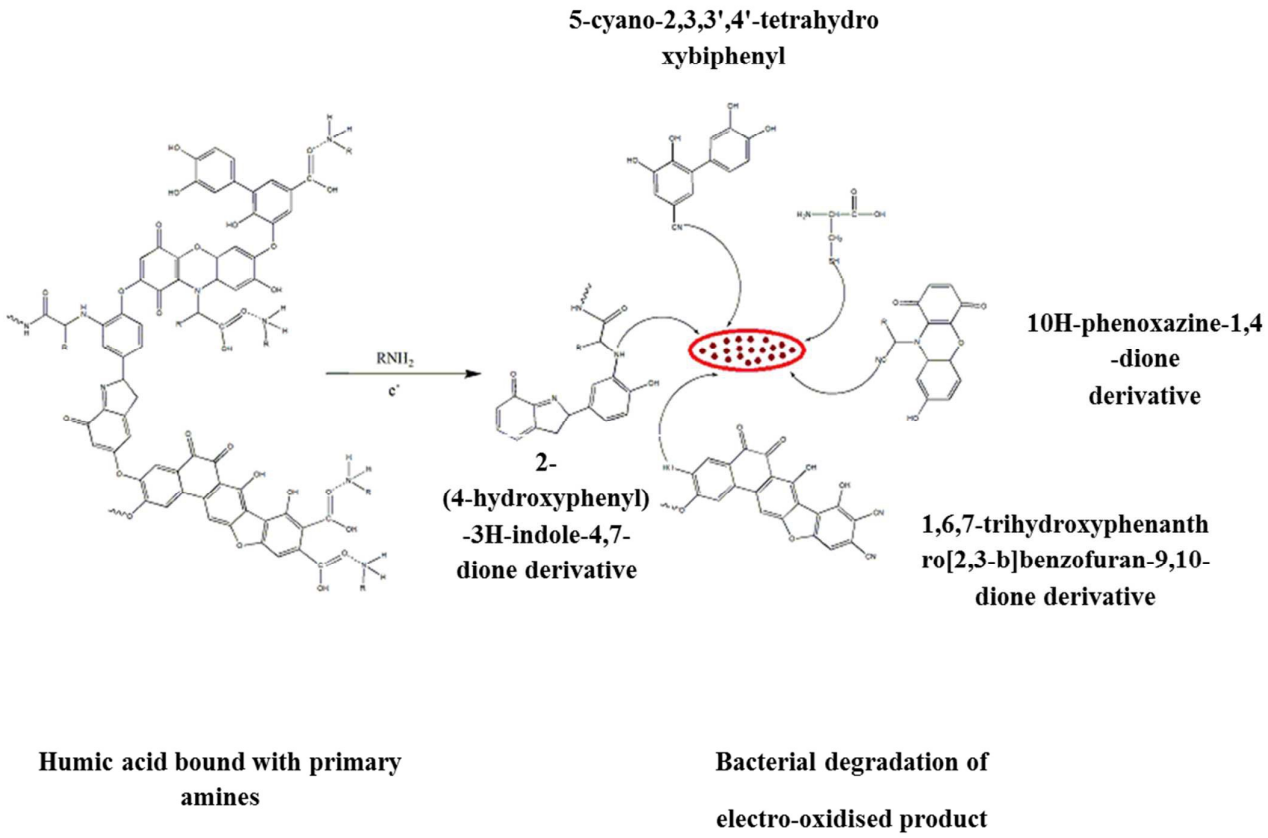


Fig. 7 Possible mechanism of electro-biodegradation of soak liquor

Table 1

Previous studies on degradation of tannery effluent.

Reference	Type of effluent	Initial COD(ppm)	COD removal efficiency (%)	Single/integrated process	Remarks
[2]	Soak liquor	5800	94.8%	electrochemical method	Current Density -0.058 A cm^{-2} Experiment: 7.05 h
[3]	Soak liquor	1500-4400	96%	biological method (combined anaerobic and aerobic process)	Duration of the experiment: 300 days
[4]	Tannery wastewater	4800±350	74-88%	biological method	Duration of the experiment:45 days
[6]	Raw wastewater	2386	76%	Integrated Process (electrochemical and anaerobic process)	Current Density – 0.02 and 0.04 A cm^{-2} Electrode selection Ti-Pt-Ir and Ti/PdO-Co_3O_4
[7]	Primary tannery effluent	890 – 1600	64%	combined (chemical and biological oxidation)	Duration of the experiment: 36 h
[8]	Chrome tannery	4600-6000	66.2%	combined (electrochemical and biological oxidation)	Current Density - 0.05 Acm^{-2} Duration of the experiment: EO - 90 mins and Biodegradation: 7 days
[10]	Soak liquor	3000-6000	89.11%	electro-oxidation	Current Density – 0.012 A cm^{-2} Duration of the experiment:2 h
[11]	Soak liquor	4466	90%	combined (electrochemical and photovoltaic stand-alone system)	Current Density:0.024 A cm^{-2} Duration of the experiment: 3 days
[12]	Saline wastewater	2512	80% in 8% salinity	biological method	Duration of the experiment: 48 to 72 h
[13]	Evaporated residue of soak liquor	5570±0.04	75%	electrochemical method	Current Density : 0.05 A cm^{-2} Duration of the experiment: 4 h
Present study	Tannery soak liquor	7300±0.10	96%	combined (electrochemical and biological)	Current Density: 0.012 A cm^{-2} Duration of the experiment: EO–30mins and Biodegradation–7 days

Table 2

Physiochemical properties of soak liquor

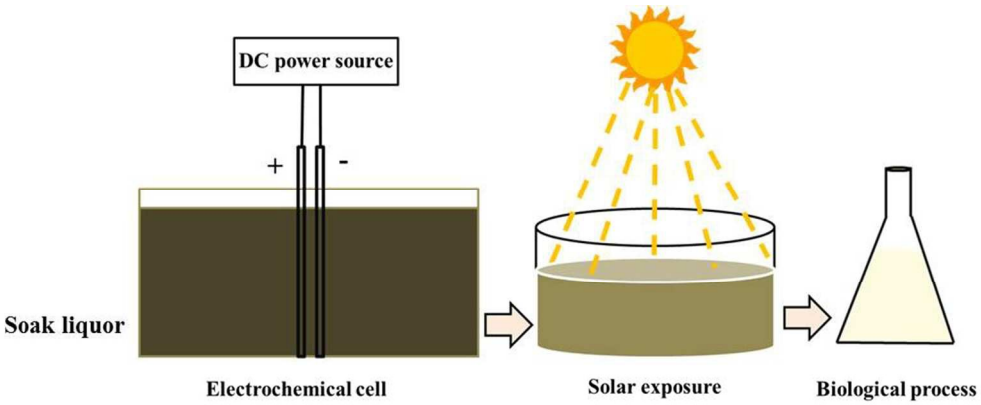
Parameters	Soak liquor	E.O.S	E.O-ST	HF-E.O-BT
pH	7.9±0.6	7.75±0.4	7.61±0.3	3.2±0.4
Colour	Intense brown	Pale yellow	Pale yellow	transparent
Odour	Foul smell	Bleach smell	Nil	Nil
Protein (g/L)	13±0.2	12±0.2	11.64±0.2	5.5±0.2
Lipid (g/L)	89±0.2	61±0.2	60.86±0.2	7.6±0.2
TKN (mg/L)	420	150	143	50
Chloride (g/L)	17.08	16.17	16.21	16.23
Hypochlorite (mg/L)	Nil	186	Nil	Nil
TDS (mg/L)	33.01	32.06	31.64	52.72
COD (mg/L)	7300±0.10	4326	4294	292
BOD (mg/L)	4800	1900	1850	138

E.O.S electro-oxidized soak liquor, E.O-ST electro-oxidized soak liquor after solar treatment, HF-E.O-BT Hypochlorite free soak liquor after biological treatment.

Table 3

Optimized parameters for the growth of mixed culture for efficient biodegradation

Parameters	Optimized conditions for efficient biodegradation
Time of electro oxidation	30 minutes
Primary carbon source	Glucose
Percentage of Glucose	1%
pH	6
Percentage of inoculum	5%
Temperature	28°C
Agitation	150rpm



Electro-biodegradation of soak liquor

174x96mm (150 x 150 DPI)