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Biotreatment of Simulated Tannery Wastewater Containing Reactive Black 5, Aniline and CrVI Using Biochar Packed Bioreactor

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Complete List of Authors:	Mahmood, Shahid; PMAS-Arid Agriculture University, Rrawalpindi, Department of Environmental Sciences Khalid, Azeem; PMAS-Arid Agriculture University, Rrawalpindi, Department of Environmental Sciences Mahmood, Tariq; PMAS-Arid Agriculture University, Rrawalpindi, Department of Environmental Sciences Arshad, Muhammad; University of Agriculture, Faisalabad, Institute of Soil and Environmental Sciences Loyola-Licea, Juan Carlos; Institute of Saltillo, Blvd. V. Carranza 2400, C.P. 25230, Saltillo, Coah., Mexico, Department of Metal Mechanics, Technological Crowley, David; University of California, Riverside, Department of Environmental Sciences
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10	**Authors:**	Shahid Mahmood[a], Azeem Khalid[a]*, Tariq Mahmood[a],
11		Muhammad Arshad[b], Juan Carlos Loyola-Licea[c] and
12		David E. Crowley[d]
13	**Affiliation:**	[a]Department of Environmental Sciences, PMAS Arid
14		Agriculture University, Rawalpindi 46300, Pakistan
15		[b]Institute of Soil and Environmental Sciences, University
16		of Agriculture, Faisalabad-Pakistan
17		[c]Instituto Tecnológico de Saltillo Blvd. V. Carranza 2400
18		Saltillo, Coah. Mexico. 25205
19		[d]Department of Environmental Sciences, University of
20		California, Riverside, CA 92521, USA
21	***Corresponding Author:**	Dr. Azeem Khalid
22		Department of Environmental Sciences, PMAS-Arid
23		Agriculture University, Rawalpindi-46300 Pakistan
24		Email: azeem@uaar.edu.pk, azeemuaf@yahoo.com
25		Tel. +92519292135

ABSTRACT

Azo dyes and hexavalent chromium (CrVI) are common pollutants in wastewater generated by the leather processing industry. This study was designed to develop a treatment strategy that can simultaneously remove azo dyes, their byproducts and CrVI using biochar packed bioreactor. Various feedstock materials were evaluated, after which, biochar produced from pyrolysis of corn cobs at 400 ºC was selected as a packing material for the reactor, based on its large surface area (1275 $m^2 g^{-1}$), microporosity (2–5 μm), and ability to support microbial biofilm formation. In the bioreactor experiments, simulated tannery wastewater containing 100 mg L^{-1} Reactive Black-5 azo dye and 10-100 mg L^{-1} aniline (byproduct of azo dye) was treated in the presence and absence of CrVI using bacterium *Pseudomonas putida* strain KI. The results showed complete biodegradation of Reactive Black 5 (100 mg L^{-1}) within 5 h. Strain KI could also reduce 100 mg L^{-1} dye and 10 mg L^{-1} CrVI simultaneously in 24 h in a 2 L continuous packed bed bioreactor. Complete biodegradation of aniline (byproduct of Reactive Black 5) in the bioreactor was obtained within 24 h in the absence of CrVI, whereas degradation was decreased to 84% in the presence of CrVI. LC-MS analysis confirmed the biodegradation of Reactive Black 5 and aniline. This study clearly illustrates the feasibility of using bacterial strains having multifaceted function for the biological treatment of tannery wastewater in bioreactor containing pyrolyzed carbon (biochar) as a support matrix for bacterial cells.

Keywords: Reactive black 5, Hexavalent Chromium, Bioreactor, Biochar, Anilin, Leather Industry

Introduction

Environmental contamination from wastewater effluent generated by the leather tanning industry is a serious concern in developing countries where leather products are produced.[1,2] Along with high levels of salts, tannery wastewater contains residual azo dyes and hexavalent chromium that are toxic, carcinogenic and mutagenic.[3,4,5,6] Currently, different combinations of chemical, physical, and biological methods are used to decolorize, degrade, and adsorb residual dyes, whereas hexavalent chromium (CrVI) is treated by reduction of CrVI to trivalent chromium (CrIII), which is much less soluble, followed by adsorption on to various materials that can be processed to recycle the metal.[7] Biological processes for treating azo dyes are attractive for their low costs, and generally involve the use of batch or continuous flow bioreactor systems in which specific bacteria are introduced. Ideally, strains selected for treatment systems should lead to complete mineralization to avoid the generation of toxic aromatic amines that are produced by reductive cleavage of azo bonds. [8,9] To this end, many bioreactor systems use a two stage, anaerobic/aerobic system to first decolorize the dyes under low oxygen conditions and then are switched to aerobic conditions to degrade aniline and other byproducts that are generated during decolorization.

To date, many bacteria and fungi have been evaluated for their ability to reduce azo dyes or to reduce hexavalent chromium. However, bioreactor systems that simultaneously treat both the chromium and azo dye components of tannery wastewater are still in the early stages of development.[2,10,11] In prior research, Mistry *et al*. [12] used an anaerobic packed bed bioreactor for the reduction of hexavalent chromium, in which a chromium resistant bacterium *Vogococcus fluvialis* st KKF was isolated from chromium contaminated soil for use in the bioreactor. About 92% reduction of hexavalent chromium was achieved in 6 h at 37°C. The results of this study show that

packed bed reactors can be used for treating CrVI in wastewater and suggests that bioreactors might be designed for full treatment of both the dye and chromium components of tannery wastewater. More recently, bacteria have also been isolated that can simultaneously reduce CrVI and azo dyes.[2] These strains hold promise for improving treatment technologies for tannery wastewater.

One of the main considerations in the development of bioreactor treatment processes is the requirement for a short residence time, which is determined by the population density and activity of the degrader population, and the degradation rate that can be achieved under the specific conditions that are imposed in the reactor system. Successful treatment of wastewater requires stable maintenance of microbial cells in the bioreactor system so that they do not wash out with the effluent. Activated carbon has long been used for wastewater treatment and provides both a support matrix for biofilm development as well as a large surface area for adsorption of substances that pass through the reactor.[13,14] As an alternative to activated carbon, pyrolyzed biomass, also known as biochar, is a low cost option that is being investigated for wastewater treatment. Prior studies have shown that biochar can be used for adsorption of CrVI [15] and that it is also an effective adsorbent for azo dyes and metal complex dyes containing chromium.[16] In the research reported here, we examine the use of biochar from different feedstocks as a packing material for a continuous flow, packed bed bioreactor using an azo dye degrading , *Pseudomonas putida* strain K1, for simultaneous treatment of azo dyes and CrVI,

Material and Methods

Chemicals, bacterial strains and culture medium

Analytical grade chemicals were purchased from Sigma Aldrich, Merck Chemicals or Fisher Scientific. Diphenyl carbazide reagent was used as a color developing agent for the spectrophotometric determination of CrVI. *Pseudomonas putida* strain KI, which is capable of simultaneously reducing azo dyes and CrVI in liquid media was used in the bioreactor.[2] Bacteria were cultured in mineral salts medium containing the azo dye Reactive Black as a carbon source, [17] and amended with $K_2Cr_2O_7$ at concentrations determined previous individual experiments.

Evaluation of biochar as a packing material for bioreactor systems

Biochar was prepared at different temperatures from different feedstocks including avocado seeds (400 ^{o}C and 500 ^{o}C), nutshells (400 ^{o}C) and corncobs (400 ^{o}C). Each material was characterized for surface area, porosity and biofilm formation and evaluated in experiments to determine potential chromium adsorption and flow characteristics for use in the bioreactor.

Specific surface area was determined by the ethylene glycol monoethyl ether (EGME) method.[18] Each type of biochar was ground into fine particles with a mortar and pestle, passed through a 4 mm sieve and dried with P_2O_5. Approximately 3 ml of EGME was added to 1 g biochar. Thereafter, the biochar was placed in a desiccator, and air was removed using a vacuum at 635 mm Hg. The sample was weighed to determine the mass of biochar and EGME mixture daily until they reached equilibrium. Specific surface area was calculated using the formula below.

$$\text{Specific surface area } (m^2\ g^{-1}) = \frac{\text{Weight of the EGME retained by sample (g)}}{0.000286 \times P_2O_5 \text{ treated dried weight of the soil}}$$

A scanning electron microscope (Hitachi TM-1000) was used to examine the pore diameters of the biochar materials. The samples were cut into thin sections and were examined at 20X and 1000X magnifications. During the treatment process, biofilm formation and cell penetration into the pores of the biochar were also examined. The samples were cut into thin sections for the best cross-sectional view and to examine colonization of the bacterial cells in the biochar. Images were taken at 20X and 1000X magnification. Further, the bacterial population in inoculated biochar was estimated by using the dilution plate technique. Biochar (1 g) was suspended in 100 ml of phosphate buffer solution (pH 7.2), followed by stirring at 150 rpm for 15 min. Serial dilutions were prepared and plated on agar media. The colonies thus formed were counted as colony forming units (CFU) per gram of biochar.

Adsorption and desorption of the dye on the material packed in the column was also examined for the different biochar materials. For this purpose, Reactive Black 5 (100 mg L^{-1} dye) was mixed with 5 g biochar in 250 ml conical flasks and stirred at 150 rpm at room temperature. Samples were taken at 2 h intervals for 12 h and again after 24 h. The color removal of the supernatant was determined spectrophotometrically at 597 nm. For the desorption isotherm, 100 ml of NaCl solution (2 g L^{-1}) was added to each flask containing dye adsorbed on to biochar.[19] The mixture was shaken at 150 rpm for 72 h on an orbital shaker to equilibrate. The supernatant was collected and centrifuged for 5 min at 8000 rpm. The desorbed dye concentrations were measured using a spectrophotometer at 597 nm.

Biotreatment of Reactive Black 5, aniline and hexavalent chromium in packed bed bioreactors.

Biotransformation of Reactive Black 5 dye, aniline (intermediate) and CrVI was examined in packed bed bioreactors using *Pseudomonas putida* strain KI. The bioreactors were built with ~ 6.4 cm diameter acrylic pipes, polypropylene lids with 40 cm height. The reactor column can hold an empty bed volume of 1.3 L in single column. While shape of the reactor column was cylindrical. Flow rate was maintained using multichannel peristaltic pump (WATSON MARLOW: Model B051917). Transparent tubes from Watson Marlow with bore size 1.6 mm were used to connect the different reactor pipes. Based on preliminary studies using different types of biochar, a biochar produced by slow (1.5 hr) pyrolysis of corncobs at 400 ºC was selected to be used for packing columns of the continuous flow reactor to examine simultaneous reduction of Reactive Black 5 and CrVI. After filling with the packing materials (300 g), now each column had a capacity of approximately one liter void volume for liquid.

Cells of *Pseudomonas putida* strain KI were harvested from liquid culture after growth to midlog phase (OD= 1.0±0.01 at 578 nm) and were collected by centrifugation for inoculation of the bioreactor. The inoculum was applied once at the start of experiment and allowed to stand for 24 hours for attachment of the bacteria and biofilm development. All experiments were then carried out using the same bioreactors. In the continuous-flow reactor, the dye solution was pumped through the column at a rate of 250 ml h^{-1}. The reactor was continuously run for 150 days. Yeast extract (4 g L^{-1}) was applied as co-substrate during each column run. Aliquots from each column were collected every 2 h for periods up to 24 hr after introduction of dye or dye and chromium mixtures on the column. Percent decolorization of the dye was determined

by measuring the absorbance at 597 nm with a spectrophotometer. The treatment efficiency in bioreactors was compared based on the time required for decolorization of the dye, disappearance of aniline, and rate of chromium reduction.

Different concentrations (2, 4, 6, 8 and 10 mg L^{-1}) of CrVI individually as well as with 100 mg L^{-1} of the azo dye, Reactive Black 5 was used. The flow rate was 250 ml h^{-1}. In these experiments, eluent passing through the column was collected every 4 h. Diphenyl carbazide reagent was used as a color developing agent to determine the residual concentration of CrVI by spectrophotometry,[20] while dye decolorization was measured by spectrophotometry at 597 nm.

Aniline, an aromatic amine is produced as a metabolic product of azo dye degradation, by reductive cleavage of the azo bond in the dye molecule. Biodegradation of aniline was studied alone and in the presence of 4 mg L^{-1} CrVI in continuous and sequential packed bed bioreactors respectively. Three different levels (10, 50 and 100 mg L^{-1}) of aniline were used. The flow rate was maintained as 125 ml h^{-1}. In the two-stage bioreactor system, the first column was continuously sparged with nitrogen gas (@ 1 atm) to create anaerobic conditions for the reduction of CrVI, while air (@ 1 atm) was sparged into the remaining four columns to create aerobic conditions for the oxidation of aniline. Aniline degradation was determined by taking a 1.5 ml aliquot, followed by centrifugation at 8000 x *g* for 10 min. Aniline residues were determined at 254 nm by HPLC (Agilent Technologies 1200 series) with a C18 reverse phase column (Supeclo, 5 µm particle size, 15 cm x 4.6 mm). Acetonitrile and water (40:60, *v/v*) were used as a mobile phase at a flow rate of 0.8 ml min^{-1}. The injection volume was 20 µL.

Analysis of metabolic products of Reactive Black 5 and aniline by LC-MS

Biodegradation products of Reactive Black 5 and aniline were further examined by LC-MS (Agilent, Model 6210) using C18 reversed phase column (5 µm particle size, 15 cmm x 4.6mm). Acetonitrile and water (50:50, *v/v*) at 0.8 ml min^{-1} flow rate was used as the mobile phase. The injection volume was 10 µL. Mass spectra were obtained using an ion trap mass spectrometer at 254 nm, fixed with an electron spray interface operating in negative ionization mode at a capillary temperature of 275 ^{o}C. Data analysis was performed with the Mass Hunter software (Agilent Version B02).

Data are presented as percentage reduction of dye and CrVI, and were calculated using Microsoft Excel$^{(R)}$ spreadsheets 2010. Standard errors were calculated and are shown in the line graphs depicting disappearance rates for azo dyes and hexavalent chromium.

Results

Characterization of biochar as a bacterial support matrix

Specific surface areas of the biochar materials used here are summarized in Table 1. Biochar produced by pyrolysis of corncobs at 400 ^{o}C had the highest surface area (1275 $m^2 g^{-1}$), which was 2.1 times larger than that observed for biochar made from nutshells. The specific surface areas of biochars produced from avocado seeds were dependent on the pyrolysis temperature, increasing nearly 4-fold from 124 $m^2 g^{-1}$ for char produced at 400 ºC to 442 $m^2 g^{-1}$ for char produced at 500 ºC. Similarly, the highest number of bacterial cells (5 x 10^7) was associated with biochar produced from corn cobs pyrolyzed at 400 ºC, followed by avocado seed biochar (1.6 x 10^7) (Table 1). Of the four types of biochar, nutshells supported the lowest number of bacterial cells.

SEM analysis showed that the pores formed in biochar varied greatly in size (Fig. 1). Corncob biochar had a large number of micropores ranging in size from 2–5

213 μm, in a symmetrical arrangement, whereas biochar produced from avocado seeds (500
214 ºC) had larger pores (8–20 μm). In the case of nutshell biochar, large pore spaces with
215 flat surfaces were observed. Biochar produced from the avocado seeds was similar to
216 that produced from nutshells. While the cell densities associated with char produced
217 from nutshells and avocado seeds were lower, the hardness properties of these materials
218 may contribute to maintenance of good flow characteristics and resistance to collapse
219 of the column matrix over time. SEM images taken at 100X showed the formation of a
220 biofilm on the surface of corncob biochar. Similarly, high bacterial cell density was
221 found inside the particles when thin cross sections of biochar were analyzed by electron
222 microscope (Fig. 2).

223 Experiments examining the adsorption and desorption of the dye on the
224 different types of biochar revealed that the corncob biochar (400 ºC) adsorbed 8% of
225 the applied dye (100 mg L^{-1}) in 12 h (Fig. 3). A similar trend was observed in case of
226 biochar prepared from avocado seeds at 500 ºC (data not shown). Lower dye adsorption
227 (1.5%) was observed in case of biochar made from nutshells. Adsorption of Reactive
228 Black 5 was initially rapid and continuous during the first 6 hours of exposure, and then
229 gradually decreased with the increasing incubation time (12 h). Approximately 87% of
230 the dye adsorbed on biochar was desorbed in 2 hours, while the rest was desorbed
231 within 12 h (Fig. 3).

232 Overall, the corn cob biochar was found to be the most suitable for use in
233 packed bed bioreactor due to its large specific surface area, porosity, and support for
234 the growth of bacterial cells and the biofilm formation. Relatively small differences
235 were observed in the rate of decolorization of the dye in columns with biochar (avocado
236 and pistachio nutshells) packing material and decolorization ranged from 70 to 74%

after 4 h (data not shown). However, maximum decolorization of dye (95%) after 10 h was observed in columns packed with biochar prepared from corncobs.

Simultaneous Treatment of Reactive Black 5 and Hexavalent Chromium in Continuous Packed Bed Bioreactor

Based on the efficacy of treatment, the continuous packed bed bioreactor was used for the simultaneous treatment of dye and hexavalent chromium. Using strain KI, complete decolorization (100%) of Reactive Black 5 (100 mg L^{-1}) and 100% reduction of CrVI (2 mg L^{-1}) was observed in 20 h in the continuous packed-bed bioreactor. At a higher concentration using 4 mg L^{-1} of CrVI, complete reduction was obtained within 24 h (Fig. 4). At still higher concentration using 6 mg CrVI L^{-1}, the dye decolorization in 24 h was 90% while at the same time 82% of the CrVI was reduced. This trend in which dye reduction rates were affected by increasing concentrations of Cr was also observed in columns with 10 mg L^{-1} CrVI, in which only 45% decolorization was achieved within 24 h while simultaneous reduction of 66 % of CrVI. In general, the rate of dye decolorization was satisfactory when the CrVI concentration ranged from 2 to 8 mg L^{-1} with respect to the reduction of CrVI, but at 10 mg L^{-1} the rate of Cr reduction exceeded the rate of dye decolorization. The steady state was achieved at 2 and 4 mg L^{-1} of CrVI concentrations for both reactive black 5 and CrVI after 24h retention time. Individual reduction of hexavalent chromium by *Pseudomonas putida* strain KI in a continuous packed bed reactor run with minimal media at varying (2-10 mg L^{-1}) concentrations of CrVI was also studied. It was observed that up to 4 mg L^{-1} concentration, CrVI was completely reduced (100 %) in 12 h. While at 6 mg L^{-1} concentration 90% reduction of the CrVI was observed. Individually least reduction (55%) of the CrVI was occurred at 10 mg L^{-1} concentration (Fig 5).

Simultaneous Treatment of Aniline and Hexavalent Chromium in Continuous Packed-Bed Bioreactor

Degradation of aniline individually and in combination with CrVI was examined in a continuous packed-bed bioreactor. HPLC analysis showed 92% degradation of aniline (10 mg L^{-1}) after 32 h retention time, while complete (100 %) degradation took 40 h (Fig. 6). At 50 mg L^{-1} aniline degradation increased gradually up to 32 h, after which it leveled off, resulting in 75% degradation after 48 h. At 100 mg L^{-1} aniline, a lag of phase of 8 h was observed, and its biodegradation rate then increased gradually, leading to 37% degradation after 48 h. The experiment was reached to steady state after 40 h retention time at 10 mg L^{-1} aniline concentration, while at 50 mg L^{-1} and 100 mg L^{-1} aniline concentration the steady state was reached after 56 and 64 h respectively.

In simultaneous treatment, the degradation of aniline (10 mg L^{-1}) was 89% after 64 h at 4 mg CrVI L^{-1} (Fig. 7). This was followed by 72 % and 33% degradation at 50 and 100 mg CrVI L^{-1} in the same retention time. After 56 h there was no change in the degradation of aniline at 100 mg CrVI L^{-1}, while in case of 50 mg CrVI L^{-1} aniline, the steady sate was reached in 64 h. Likewise, the rate of CrVI reduction was decreased with increasing concentrations of aniline. At 10 mg L^{-1} aniline, 93% of CrVI was reduced in 8 hours, with 100% reduction occurring at 16 hours retention time. At an aniline concentration of 50 mg L^{-1}, complete reduction of CrVI required 32 h. At the highest concentration of 100 mg L^{-1}, the reduction of CrVI was 75% after 48 h and there was no significant change occurred even up to 72 h.

Biodegradation Products of Reactive Black 5 and Aniline

LC-MS analysis was performed to identify the biodegradation products of Reactive Black 5 and aniline. In the case of Reactive Black 5, the molecular weights of the

287 degradation products ranged from 111-293 kDa with retention times between 2.5 and
288 4.5 min (Fig. 8a). The major peak was resolved at 3.7 min with 165 kDa molecular
289 weight, and was identified as benzaldehyde 3, 4 di-methoxy. Molecular weights of
290 metabolic products from aniline degradation obtained were between 119 kDa and 290
291 kDa (Fig. 8b). In this case, a major peak was detected at a retention time of 3.5 min
292 with a molecular weight of 119 kDa, which corresponded with the molecular mass of
293 benzaldehyde 4-methyl.
294

295 ## Discussion

296 This study clearly illustrated the potential use of *Pseudomonas* sp. for simultaneous
297 treatment of tannery wastewater containing CrVI, azo dyes and their toxic metabolites
298 in packed bed bioreactors. Performance of the continuous-flow, packed-bed bioreactor
299 was generally superior to the batch bioreactor. This is in agreement with prior research
300 showing that bioreactors facilitate the treatment of azo dyes by enabling a high reaction
301 rate for a long period of time with stable maintenance of cell biomass on the column
302 packing material.[21] In the present study, the bioreactors were run under clean, but
303 nonsterile conditions for 150 days with a single application of the bacterial inoculum
304 immobilized on biochar. Corncob biochar was selected as the best matrix for the
305 immobilization of the bacterial cells in the continuous flow bioreactor and facilitated
306 the adsorption of a large number of bacterial cells and biofilm formation both within
307 and on the surface of the biochar. Furthermore, studies of the adsorption and desorption
308 also indicate better retention of Reactive Black 5 dye on to corncob biochar as
309 compared to the other tested biochars. On the other hand, Reactive Black 5 dye was
310 also readily desorbed, indicating that the biochar does not permanently hold the dyes.

311 The results further demonstrated that even relatively high concentrations (100
312 mg L^{-1}) of Reactive Black 5 and CrVI (10 mg L^{-1}) can be treated in a continuous
313 packed-bed bioreactor using a two-stage system, in which the first stage is maintained
314 under low oxygen conditions, and the second stage is used to aerobically degrade the
315 metabolites of the azo dye. In the first stage, aromatic compounds are initially formed
316 as a result of the anaerobic decomposition of the azo bond (-N = N-) by azoreductase
317 enzyme, which are then metabolized under aerobic conditions.[22] While during
318 experimentation for simultaneous reduction of CrVI and azo dyes, hexavalent
319 chromium reduce first and followed by the dye decolorization.[2] Higher concentrations
320 of CrVI showed more inhibitory effect on the reduction (decolorization) of azo dye
321 than the reduction of CrVI itself.[2] The continuous flow system eliminates possible
322 problems that may be caused by accumulation of toxic degradation products that could
323 otherwise be toxic and eventually inhibit survival and activity of the degrader
324 bacterium. In addition, continuous types of bioreactors have the ability to process large
325 volumes of industrial effluents,[10] and can easily be scaled up for use by commercial
326 industry that generate wastewater contaminated with synthetic dyes and CrVI
327 Aniline is an intermediate of Reactive Black 5.[23] Here, degradation of aniline,
328 alone and in combination with CrVI also was monitored in the continuous-flow,
329 packed-bed bioreactor under aerobic conditions. Strain KI showed great potential to
330 degrade aniline, although the presence of high concentrations of CrVI in the reactor
331 reduced the rate of biodegradation. LC-MS analysis of Reactive Black 5 azo dye and its
332 products confirmed the degradation of dye by *Pseudomonas putida* strain KI in the
333 continuous packed bed bioreactor. The results clearly showed the presence of low
334 molecular weight products of Reactive Black 5 after the treatment process. One of the
335 degradation products of Reactive Black 5 was identified as benzaldehyde 3,4 di-

methoxy having a molecular weight of 165 kDa. Other unidentified products were also lower in molecular weight than the molecular weight (991.8 kDa) of the parent (Reactive Black 5) compound. The by-products of degradation of the aniline had a molecular weight of 119-290 kDa, with a product identified as benzaldehyde 4-methyl with a molecular weight of 119 kDa.

Conclusions

The present study demonstrated the potential of using *Pseudomonas putida* strain KI for accelerated decolorization of azo dyes and CrVI in packed bed bioreactors. The continuous-flow, packed-bed bioreactor could remove the dye, dye-originated amine (aniline) and CrVI more efficiently than the batch type bioreactor. Biochar produced from corncobs and other low cost agricultural waste can all be used as packing materials for the bioreactor, and provided an effective matrix that supported formation of a biofilm of the degrader bacteria that remained active for at least 5 months after a one-time inoculation.

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440 Table 1 Specific surface area of different types of biochar and cell density of bacterial

441 strain KI on different types of biochar

Biochar/plastic	Biochar characteristics	
	Colony forming units (CFU g^{-1})	Specific surface area ± S.E.* (m^2 g^{-1})
Avocado 400 ºC	1.6×10^7	124 ± 3.7
Avocado 500 ºC	1.5×10^7	442 ± 4.2
Nutshell 400 ºC	6.6×10^6	612 ± 4.1
Corn cob 400 ºC	5.1×10^7	1275 ± 5.7
PET caps	5.5×11^5	-

442 *Standard error

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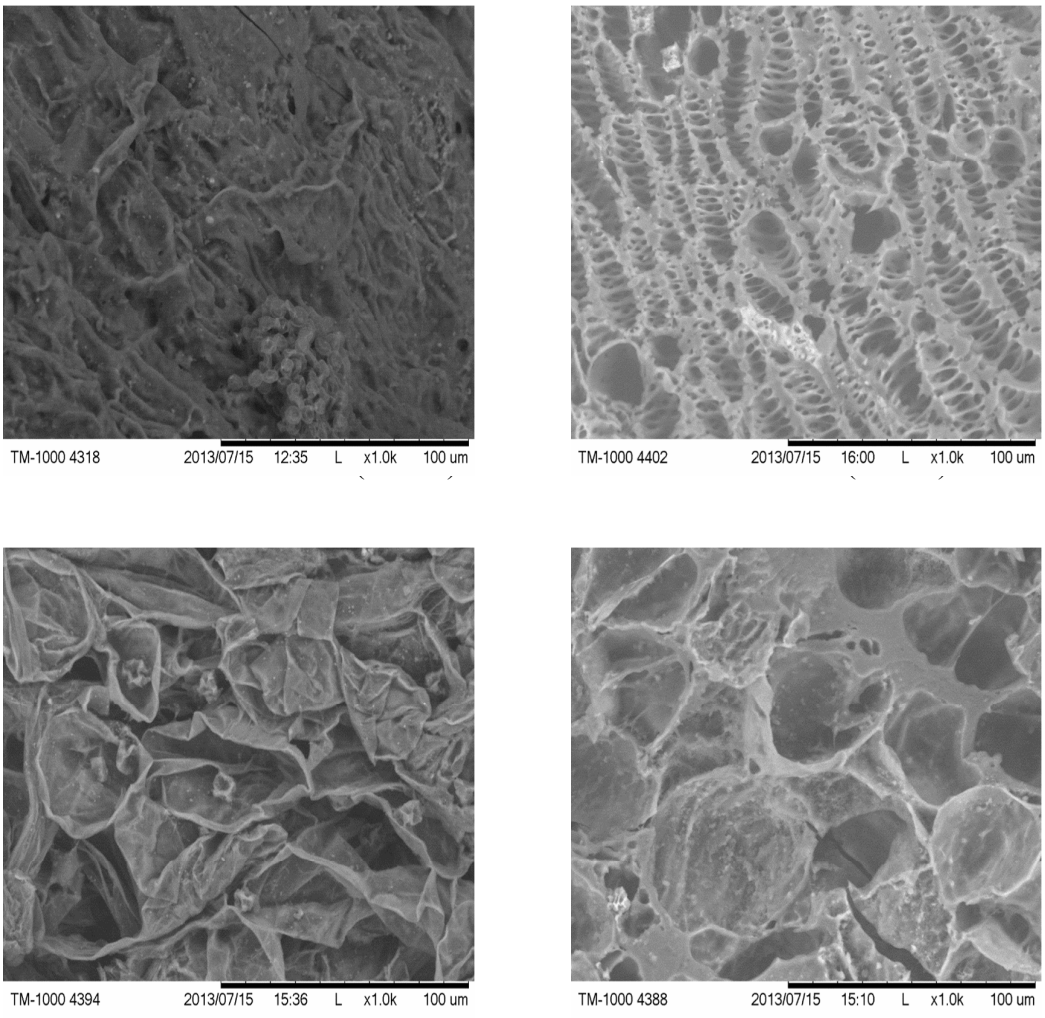
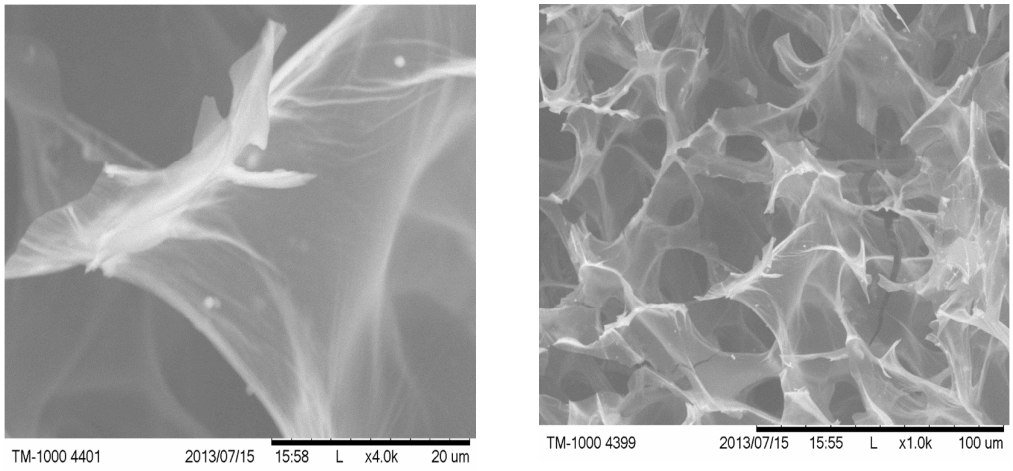


Nutshells (400 ºC) Avocado seed (500 ºC)

Fig. 1 Scanning electron microscope images showing the pore spaces associated with biochar produced from avocado seeds, corncob and pistachio nutshells.

(a)



(b)

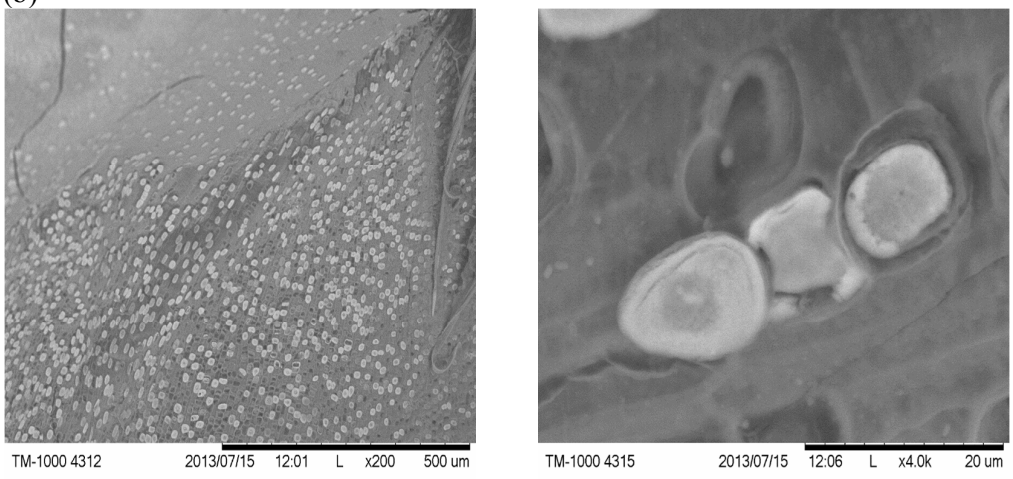


Fig. 2 a) Biofilm formation on the biochar surface, b) Penetration of the bacteria cells inside the biochar

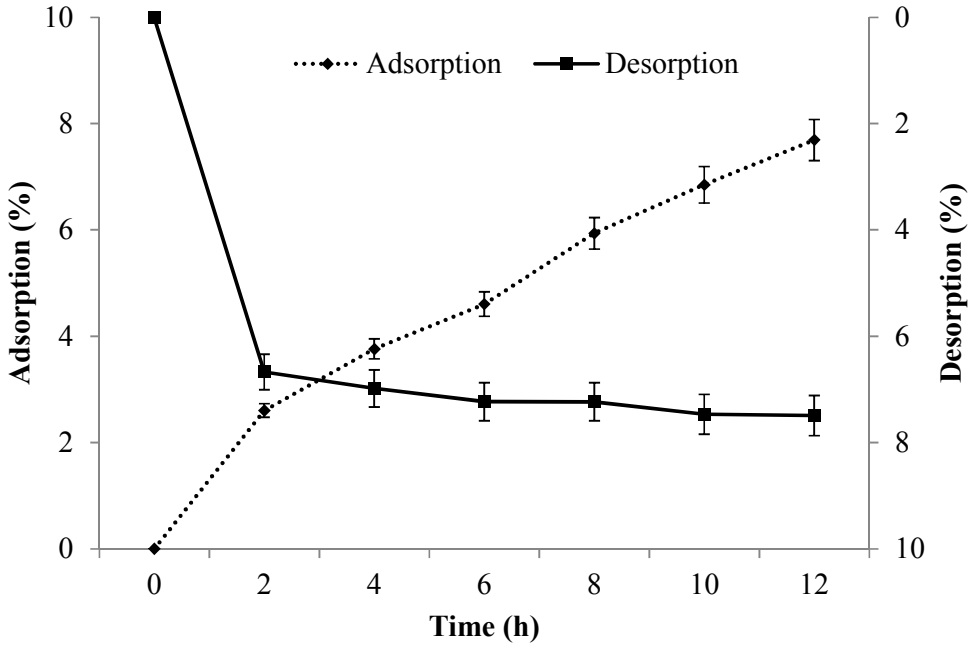


Fig. 3 Percent adsorption and desorption of Reactive Black 5 on biochar produced by pyrolysis of corncobs at 400 ºC.

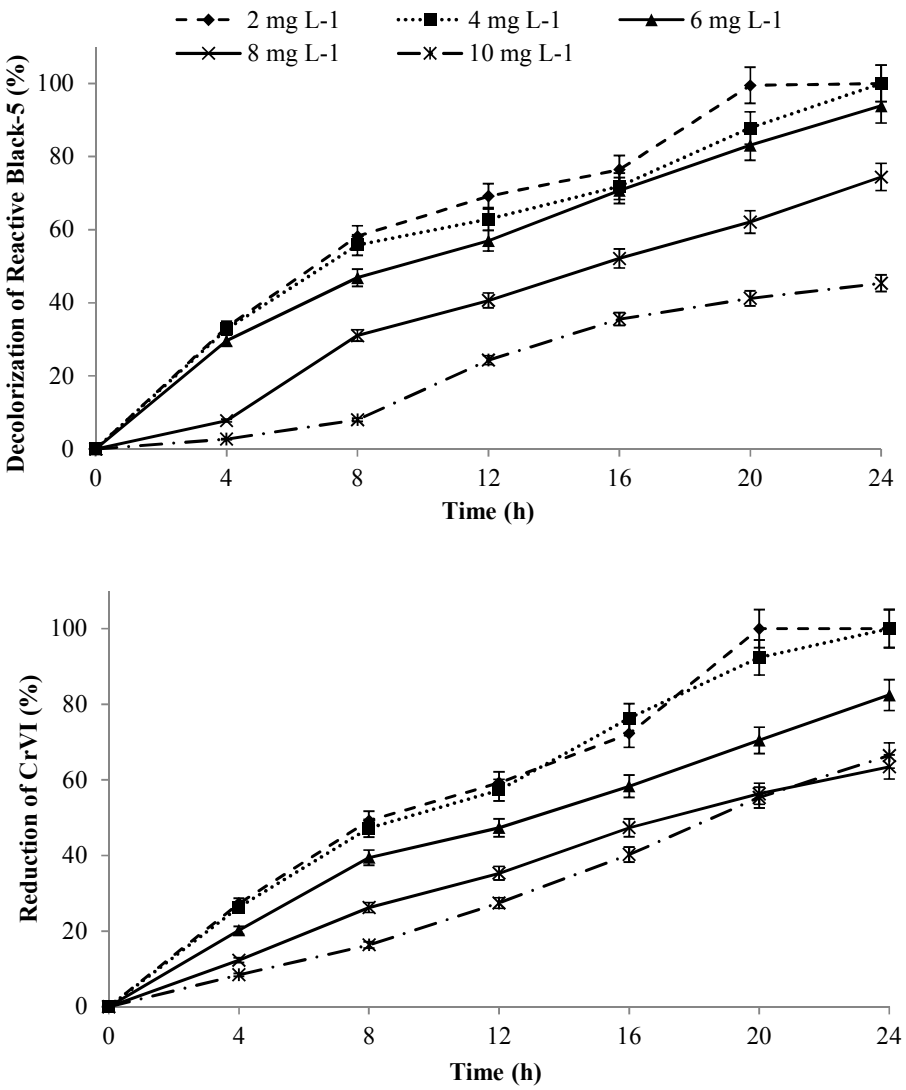


Fig. 4 (A) Decolorization of Reactive Black-5 azo dye and (B) Reduction of hexavalent chromium by *Pseudomonas putida* strain KI in in a continuous packed bed reactor run with minimal media containing 100 mg L^{-1} RB as a sole carbon source at varying (2-10 mg L^{-1}) concentrations of CrVI.

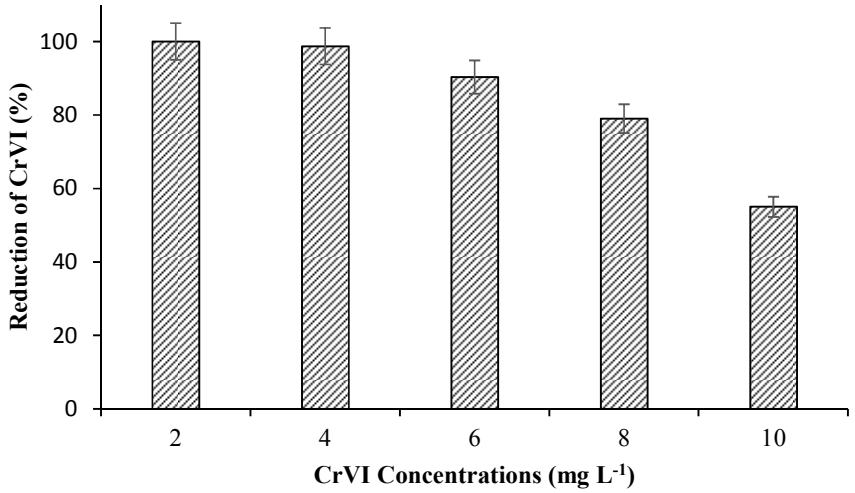


Fig. 5 Individual reduction of hexavalent chromium by *Pseudomonas putida* strain KI in a continuous packed bed reactor run with minimal media at varying (2-10 mg L^{-1}) concentrations of CrVI

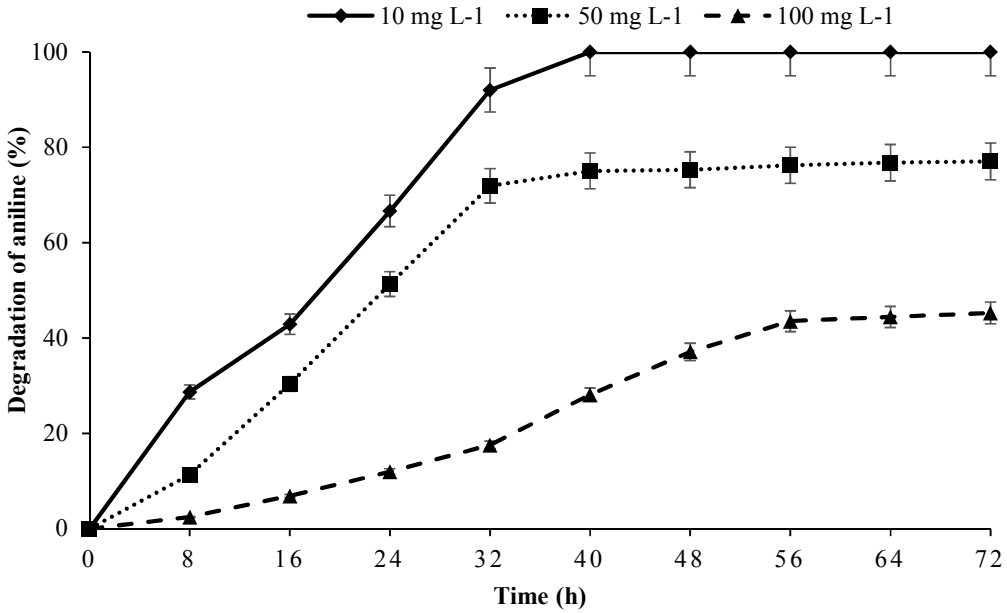


Fig. 6 Effect of hexavalent chromium concentration on aniline degradation by *P. putida* strain KI in a continuous-flow, packed-bed reactor using biochar produced from corn cob feedstock as a support for bacterial growth.

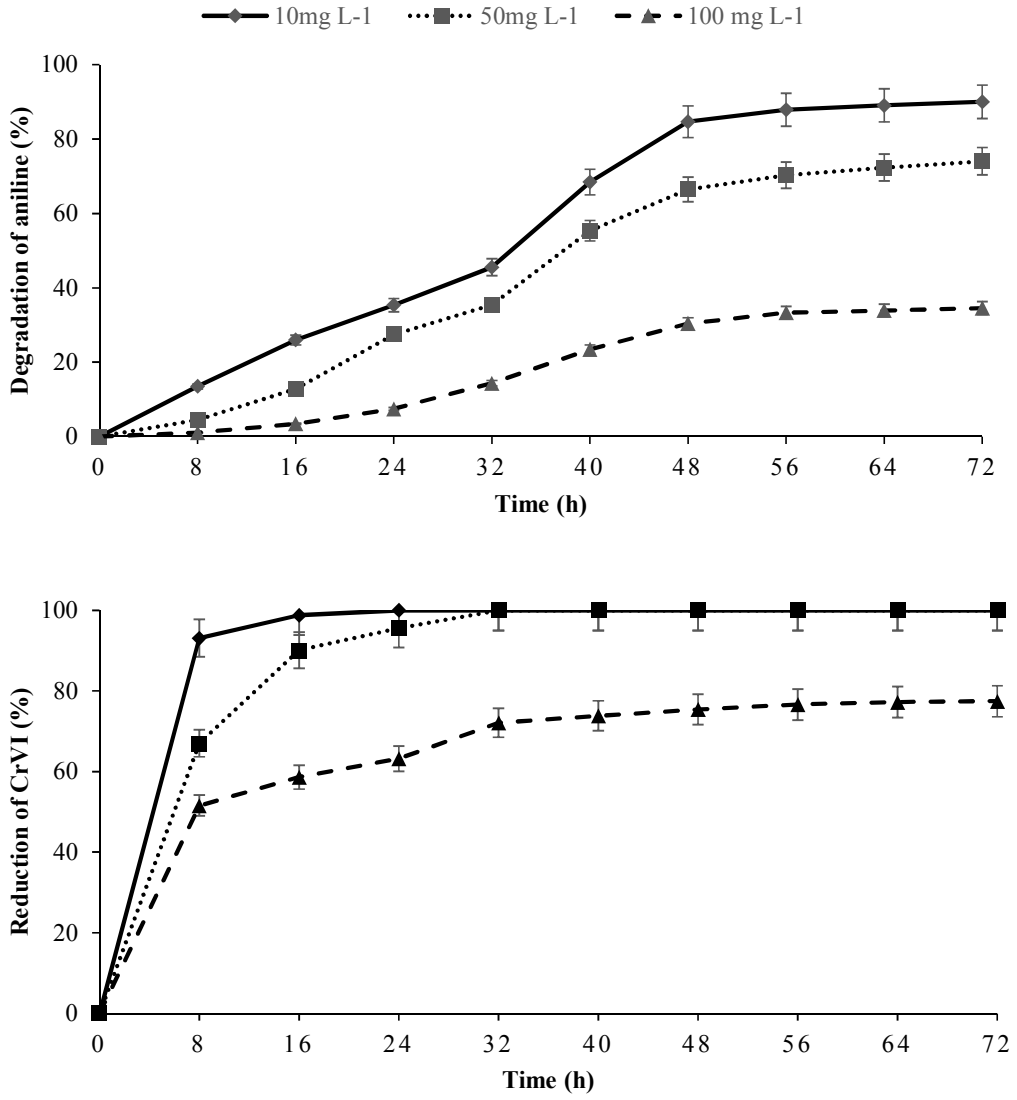


Fig. 7 Effect of aniline concentrations (10, 50 and 100 mg L^{-1}) on aniline degradation and CrVI (4 mg L^{-1}) reduction by *P. putida* strain KI in a continuous packed bed reactor using biochar produced from corn cob feedstock as a support for bacterial growth.

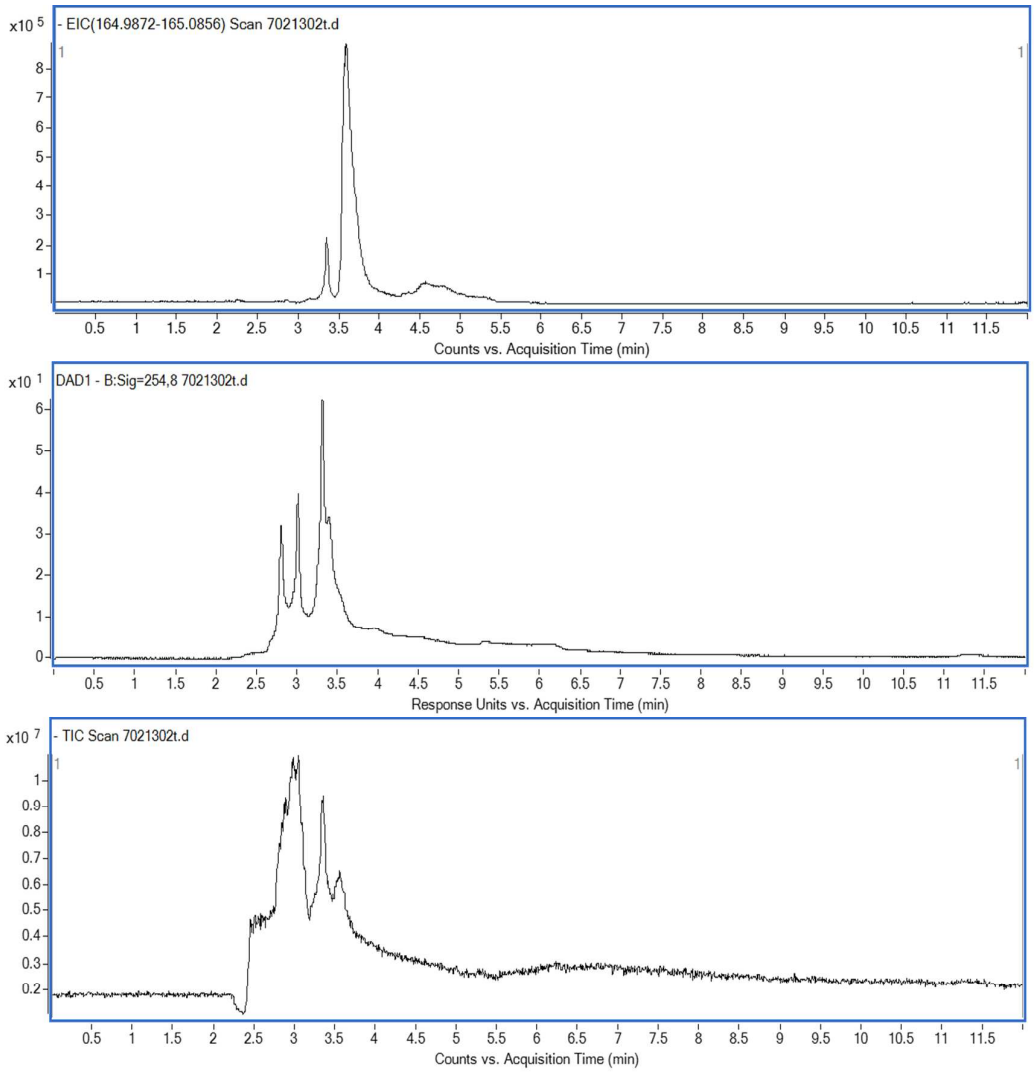


Fig. 8a LC-MS spectra indicating the degradation products of Reactive Black 5 after treatment with strain KI in continuous-flow, packed-bed bioreactor.

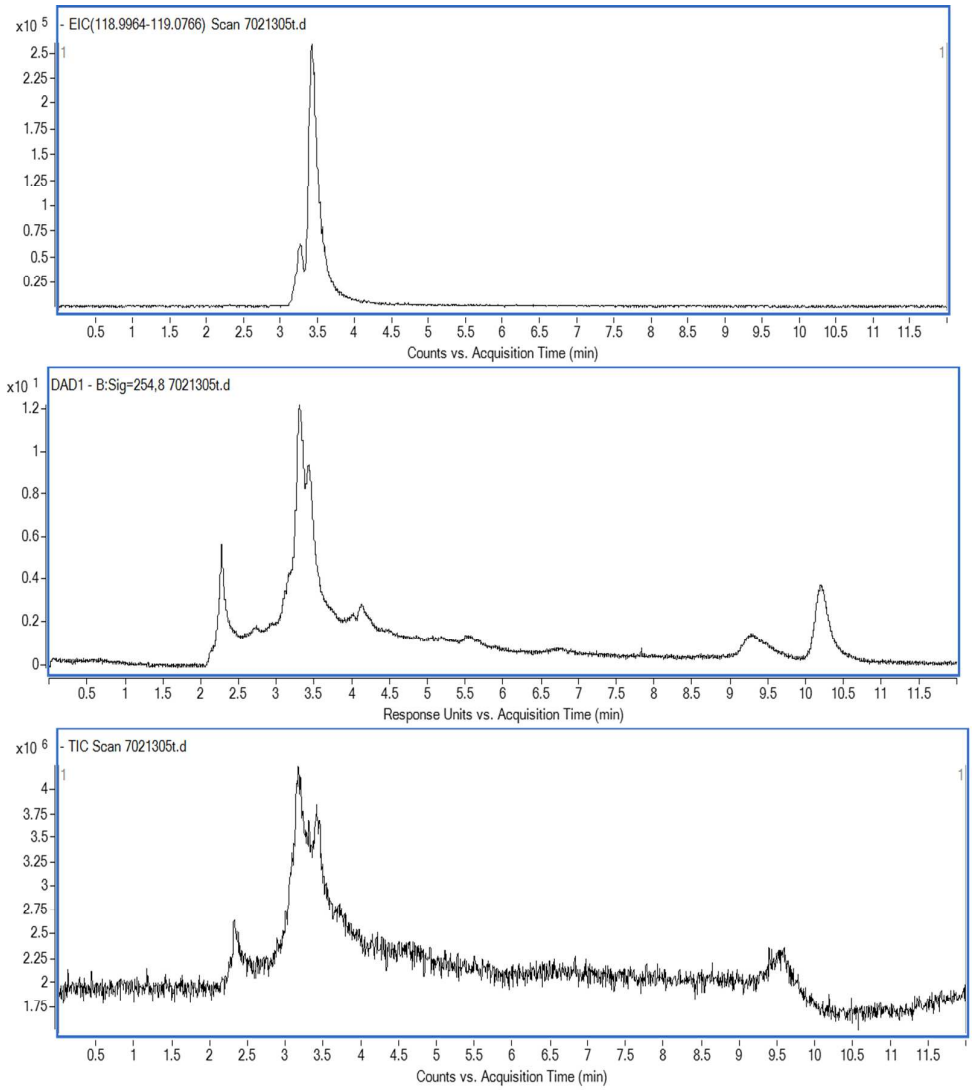


Fig. 8b LC-MS spectra indicating the degradation products of aniline after treatment with *Pseudomonas* strain KI in continuous-flow, packed-bed bioreactor.