# Sewage sludge derived biochar accelerates toluene removal by *Pseudomonas plecoglossicida*

T.T. Shen[1,2], R.A. de Toledo[1], Y.Y. Tang[2], H. Shim[1]

[1]Department of Civil and Environmental Engineering, Faculty of Science and Technology, University of Macau, Macau SAR, P.R. China
[2]School of Environmental Science and Engineering, Southern University of Science and Technology, Shenzhen 518055, P.R. China

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Presenting author email: hjshim@umac.mo

The increasing production of sewage sludge from wastewater treatment plants has become a huge environmental problem worldwide. In China, from 2007 to 2013, the total sludge production had an average annual growth rate of 13% with 6.25 million tons of dry solids produced in 2013 (Zhang *et al.* 2018a). Some common sludge treatment and disposal technologies include landfilling, incineration, and composting. However, the presence of heavy metals, persistent organic pollutants, microorganisms, and micropollutants limits the options in treatment and disposal processes considerably (Shao *et al.* 2015). One potential and effective technology for sludge management is its conversion into biochar through pyrolysis, which is considered a "win-win" strategy. The produced biochar is an alternative adsorbent due to its relatively low cost and strong adsorption affinity to different types of environmental contaminants (Srinivasan *et al.* 2015). In addition, biochar is known to increase the microbial abundance and change the microbial community composition when added to soil due to its sorption capacity and stability against biodegradation (Kappler *et al.* 2014). Moreover, a new but significantly important functionality of biochar has been revealed recently. It has been demonstrated that biochar can participate in redox reactions of biogeochemical relevance due to its electron-chemical properties (redox properties and electron transferring capacities) (Klüpfel *et al.* 2014). However, the impact of inorganic components on biochar electron-chemical properties is less understood. It is necessary to figure out the unique role of minerals in biochar, especially for sludge biochar that contains much higher inorganic constituents than plant-based biochar. The objective of this study is to investigate the influences of biochar addition on toluene removal by *Pseudomonas plecoglossicida*, aiming especially 1) to study whether toluene removal is accelerated after biochar addition, 2) to evaluate the contribution of biochar electron-chemical properties as well as the contribution of biochar adsorption affinity in the system, and 3) to reveal the role of biochar inorganic components, biochar structure, and biochar leachate during this process.

Sewage sludge was collected at local wastewater treatment plant, dried at 80°C, crushed, and sieved. The sludge biochars (BCs) were prepared under $N_2$ atmosphere at 300°C, 500°C, and 700°C ($BC_{300}$, $BC_{500}$, and $BC_{700}$) for 4 h at a heating rate of 5°C $min^{-1}$ in a tube furnace (OTF-1200X-II-100, Kejing). Microcosms were prepared in 160-mL serum bottles containing 45 mL mineral salts medium (pH 7), 5 mL inoculum, 250 mg/L toluene, and 50 mg biochar. The bottles were incubated for 3 days (150 rpm and 30°C) to estimate the effectiveness of biochar on toluene removal. The adsorption ability of biochar and the removal capacity of microorganisms were set as controls. To evaluate the biochar inorganic components and soluble constituents influence, deash $BC_{500}$ and leachate from $BC_{500}$ were applied. $BC_{500}$ was washed with acid (1 M HCl and 1 M HF) three times, then washed with water and dried at 80°C. The obtained sample was named as deash $BC_{500}$. $BC_{500}$ leachate was obtained by adding 50 mg $BC_{500}$ in 45 mL sterilized mineral salts medium, bubbling with $N_2$ for 10 min, sealed and shaken for 3 days, and then filtered to remove the solid materials. The concentration of toluene was checked daily using GC/FID (Thermo Scientific, USA). The one-way analysis of variance (ANOVA) at 95% confidence was used to calculate the statistical significances of different biochar temperatures on the toluene removal.

As shown in Figure 1(a), approximately 20% toluene were removed by biochar after 3 days of incubation, suggesting adsorption did not play a major role on the contaminant removal. On the other hand, around 40% toluene were biologically removed in the absence of biochar at the same period. For the systems with biochar and microorganisms, toluene removal sharply increased and it was almost completely removed within 3 days of incubation. Regardless of different temperatures used to produce biochar ($BC_{300}$, $BC_{500}$, and $BC_{700}$), toluene removal (99.80% ± 0.23, 98.66% ± 0.45, and 99.83% ± 0.17, for $BC_{300}$, $BC_{500}$, and $BC_{700}$, respectively) was not statistically significant ($P=0.1176$) for the microcosms with biochar and microorganisms. However, within 2 days of incubation, toluene removal (99.80% ± 0.07, 91.84% ± 0.88, and 99.74% ± 0.12, for $BC_{300}$, $BC_{500}$, and $BC_{700}$, respectively) was statistically significant ($P<0.05$). The obtained results were consistent, considering the biochar adsorption capacity of toluene, with $BC_{500}$ showing the poorest adsorption affinity [Figure 1(a)]. Moreover, this behavior could be further related to the biochar electron-chemical properties as they can be changed with biochar pyrolysis temperature (Klüpfel *et al.* 2014; Yu *et al.* 2015). To evaluate the effect of biochar inorganic constituents, deash $BC_{500}$ and leachate from $BC_{500}$ ($BC_{500}$-leaching) were tested [Figure 1(b)]. Compared with $BC_{500}$ (20.78% ± 0.67), the toluene removal efficiency by deash $BC_{500}$ adsorption was enhanced to 32.18% ± 0.44. In addition, toluene was almost completely removed within 2 days of incubation when deash $BC_{500}$ was added to the microcosm with microorganisms, suggesting the partial removal of minerals from biochar may improve its ability to accelerate toluene bioremoval as well as its adsorption capacity. The results are in accordance with Zhang *et al.*

(2018b) where ash played a negative role in neonicotinoids' sorption onto biochar. Toluene was also completely removed within 2 days of incubation in the microcosm containing leachate from $BC_{500}$ and microorganisms, which further indicates compounds leaching out from biochar could enhance toluene bioremoval to some extent. Since biochar has a complex composition, it is still not clear what kinds of organic compounds or microelements could contribute to enhance toluene bioremoval. According to Yang *et al.* (2015), biochar can form environmentally persistent free radicals on its surface and they may be released to improve the contaminant removal. Further experiments are underway to evaluate the contribution of biochar electron-chemical properties as well as the contribution/role of inorganic components, biochar structure, and biochar leachate to accelerate toluene bioremoval.

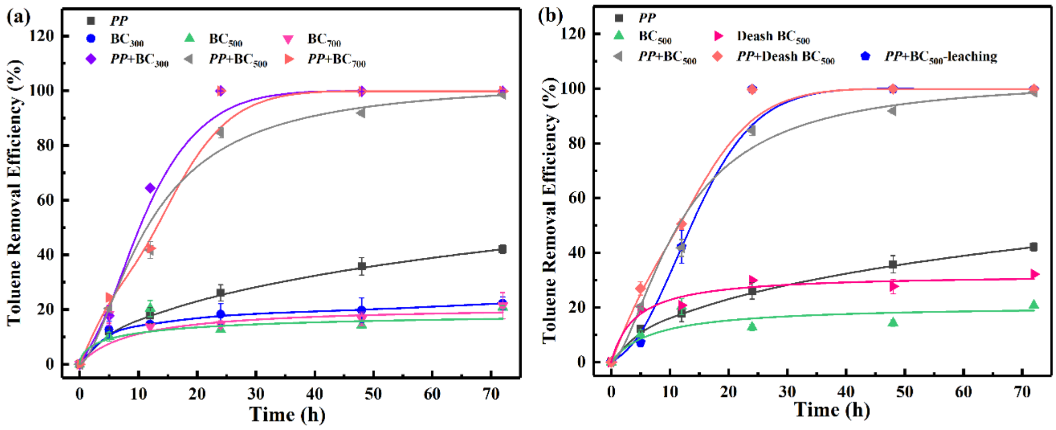


Figure 1. (a) Toluene removed by *Pseudomonas plecoglossicida* (*PP*) with biochar produced under different temperatures ($BC_{300}$, $BC_{500}$, and $BC_{700}$). (b) Toluene removed by *PP* with $BC_{500}$, deash $BC_{500}$, and leachate of $BC_{500}$ ($BC_{500}$-leaching).

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