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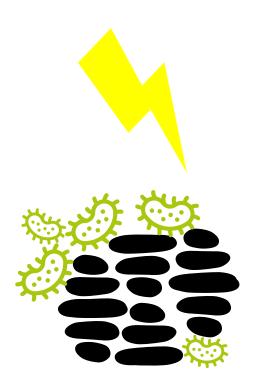
## Elucidating mechanisms of bacterial adsorption to biochar by 3D X-ray image analysis

## Aim:

The environment is a major reservoir for the spread of antimicrobial resistance (AMR). Resistant human pathogens enter the environment via wastewater, which can be untreated due to combined sewer overflows and other bypass events. To avoid resistance spreading in hotspots like combined sewers and in the environment more broadly, high-risk wastewater (such as that from hospitals) can be filtered using biochar, which adsorbs microorganisms.

Biochar is a carbon-rich material produced from inedible biomass. Its porous structure enables biochar to adsorb bacteria effectively, making it a valuable tool for wastewater filtration while aligning with sustainability goals. This is just one way biochar can interact with microorganisms to reduce pollution. It can both remove harmful microorganisms from, and add helpful microorganisms to, the environment. Biochar can not only filter pathogens from wastewater, but also selectively adsorb them from soil and waterways. It can also seed and promote growth of microorganisms that degrade chemical pollutants or outcompete pathogens.

This project used a 3D imaging technique called X-ray nanotomography to investigate the mechanisms of bacterial adsorption to biochar. High-resolution imaging enabled detailed characterisation of biochar morphology and its relationship to adsorption of resistant human pathogens. A significant outcome of the project was the development of an innovative image analysis pipeline. Using machine learning-based segmentation tools, the pipeline accurately distinguished bacterial clusters from biochar structures and mapped their spatial distribution. The distinct X-ray attenuation properties of bacteria enabled precise identification of bacterial adsorption patterns within biochar particles.



**Results:** The research revealed that specific features of local biochar structure play a critical role in promoting bacterial adsorption. Larger bacterial clusters were primarily found between (rather than inside) plant cell structures in biochar, where porosity was lower and tortuosity higher. This suggests either that larger clusters became lodged in smaller pores, or that they formed by agglomeration following adsorption of smaller clusters in these regions. In either case, results suggest that a mixture of pore sizes relative to the target adsorbate is preferable, ideally with larger openings towards biochar particle surfaces.

The findings provide a clear pathway for enhancing biochar's performance in wastewater filtration and other microbial adsorption applications. By optimising production parameters—such as pyrolysis temperature, feedstock selection, and pre- and post-treatment methods—manufacturers can design biochar with tailored pore structures that maximise bacterial adsorption. These targeted improvements can enhance the efficacy of biochar-based filtration and other adsorption systems, providing sustainable and cost-effective methods to prevent and remediate water and soil pollution.