SEQUESTRATION OF MULTIDRUG-RESISTANT CLINICAL BACTERIAL ISOLATES BY PRECISION ENGINEERED WASTE LIGNOCELLULOSIC BIOCHARS

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1. Keywords

Pyrolysis, Biochars, Lignocellulose, Multidrug resistance, Filtration

2. Highlights

- Biochars from walnut shells sequester up to 94% of *Pseudomonas aeruginosa* RP73
- Biochars from walnut shells sequester up to 85% of *Staphylococcus aureus* EMRSA15
- Alkaline pretreatment and peak pyrolysis temperature affect adsorption
- This work is the first to show adsorption of MDR human clinical pathogen isolates.

3. Purpose

Bacterial drug-resistant infections (DRIs) directly kill ~1.3M people per annum and are associated with ~5M deaths per annum (as of 2019 estimates).[1] These infections arise because of antimicrobial resistance (AMR), a natural phenomenon by which micro-organisms gain the ability to survive in the presence of previously lethal or inhibitory concentrations of antimicrobial compounds. When an organism is resistant to more than one compound, it is known as multidrug-resistant (MDR).

While it is clear that driving down DRIs will require innovation and massive sustained investment in the anti-infective drugs space, this alone is not a solution. The time between a drug entering the marketplace and resistance to that drug being detected has shrunk since the introduction of penicillin, the first antibiotic, to the point of resistance detection sometimes preceding market authorisation. As such, creative non-pharmaceutical interventions are required in the fight against AMR and DRIs.

One such solution is the inline filtration of hospital effluent to sequester MDR bacteria. Hospitals act as large sources of environmental contamination with MDR organisms, as these slough off sick patients into sinks and shower drains. There are currently no mitigation measures in the place in the United Kingdom to prevent the release of MDR organisms into waterways via effluent. Wastewater treatment plants do not sequester MDR organisms—acting instead as hotspots for horizontal gene transfer of AMR genes.[2] Upstream mediation measures are therefore an attractive solution to curbing the spread of MDR organisms. Repurposing waste lignocellulosic biomass into biochar for this filtration application exemplifies valorisation.

The objective was therefore to determine what biochar production conditions have the greatest effect on the adsorption or sequestration of two clinical bacterial species with different physicochemical characteristics. A further objective of this work was to establish relationships between the production conditions and the biochar characteristics which affect the adsorption of bacterial isolates.

4. Materials and methods

A bespoke pyrolysis reactor was built, as described in Barr *et al* (2021).[3] The walnut shells used as a waste lignocellulosic biomass feedstock were milled and packed into quartz tubes for pyrolysis; these quartz tubes were then also used as filtration cells, through which bacterial cultures were flowed.

Walnut shells were either pre-treated with alkaline solution (200mM NaOH) or left untreated. beds were convectively heated by a 3L/min stream of resistively preheated argon at a rate of 6°C/min to peak temperatures of 250 °C, 350 °C, and 450 °C. Beds were held at peak temperature for 30 min before cooling to 70 °C under the same gas flowrate

Clinical bacterial isolates (*Pseudomonas aeruginosa* RP73 and Staphylococcus aureus EMRSA15) were obtained from an existing culture collection. These were selected for their complementary and differing physicochemical characteristics—the former being Gram-negative, and the latter Gram-positive. Cultures were grown in rich media, centrifuged into a pellet, and resuspended in saline (0.9% NaCl) to an appropriate optical density known to correspond to a certain number of colony-forming units (CFUs). Suspensions were flowed through filtration cells centrifugally, and aliquots of the outputs plated on rich media agar plates and incubated at 37 °C overnight before being subjected to colony counting. Technical duplicates were performed for each condition, and comparisons were made between maximum pyrolysis temperatures, organism filtered, and pre-treatment (or lack thereof).

5. Results and discussion

There is already an established body of work looking at sequestration of micro-organisms by char-based filtration methods. However, studies published to date suffer from two main pitfalls: 1) the bacterial strains tested are always "lab" strains which are irrelevant to the study of pathogenic bacteria and their dynamics; 2) the sorbents tested are always poorly characterised—usually commercially produced and obtained with little to no information on the feedstock or production conditions.

As regards point 1, a very common test sorbate in studies published to date is *Escherichia coli* K-12.[4] This is a laboratory strain with 'genetic lesions arising from years of laboratory growth and treatment with mutagens', which has lost all pathogenicity along with 20% of its genes.[5] This work presents the first ever studies on clinical isolates: *P. aeruginosa* RP73 is sequestered much better than *S. aureus* EMRSA-15 across all temperatures, with up to 94% mean removal of the former and 85% of the latter.

As regards points 2, almost no studies thoroughly characterised the sorbents used to sequester bacteria. In few cases, some details are available in the original text, but few published study specify heating rates, maximum temperatures, or reactor design. This work shows that these conditions are key: temperature directly affects sequestration capacity, with higher temperatures associated with higher removal. In addition, alkaline pre-treatment of walnut shells improves sequestration of EMRSA15 but not RP73.

6. Conclusions and perspectives

In this first-in-kind study, we show that alkaline pre-treatment and peak pyrolysis temperatures drastically affect bacterial adsorption capabilities, on a per-organism basis. These results provide a platform to inform selection of biochar production conditions for MDR pathogen sequestration.

7. References

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