Karlsruher Institut für Technologie

Institute of Process Engineering in Life Sciences
Section II: Technical Biology

Microbial utilization of process waters derived from thermochemical biomass conversion processes

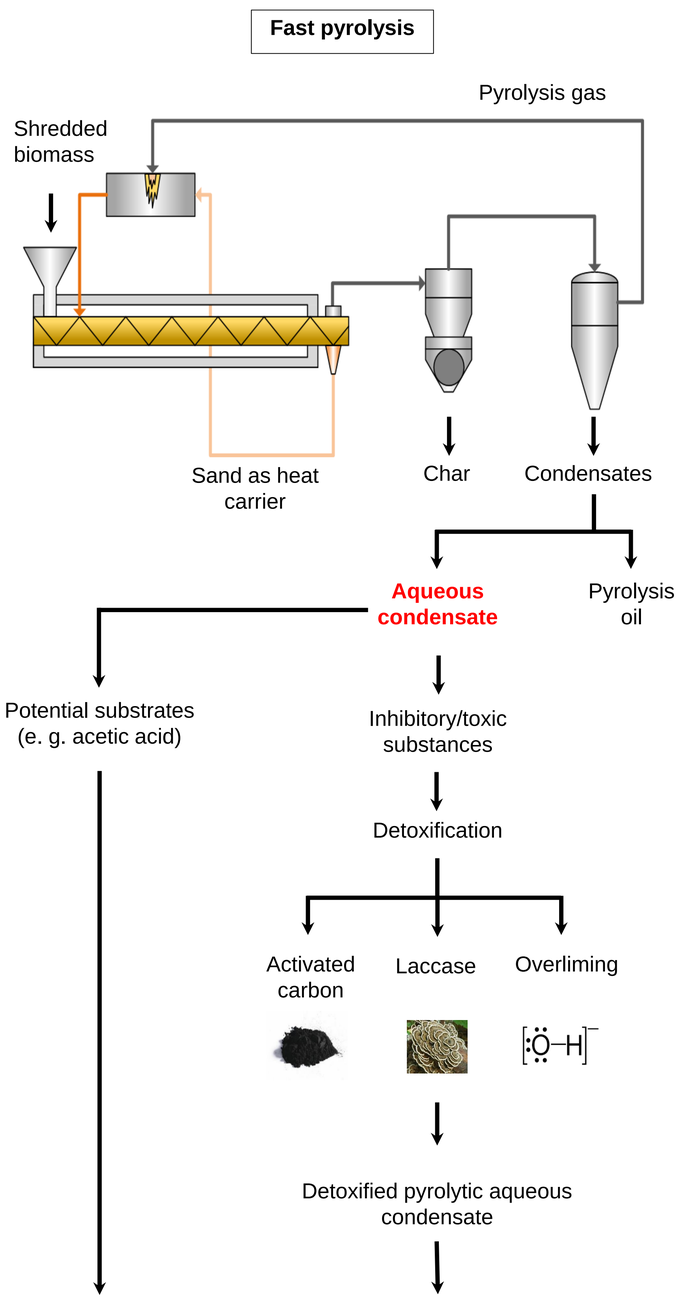
Christin Kubisch, Katrin Ochsenreither

Karlsruhe Institute of Technology (KIT); Institute of Process Engineering in Life Sciences; Section II: Technical Biology; Fritz-Haber Weg 4; 76131 Karlsruhe, Germany; e-mail: christin.kubisch@kit.edu

Introduction

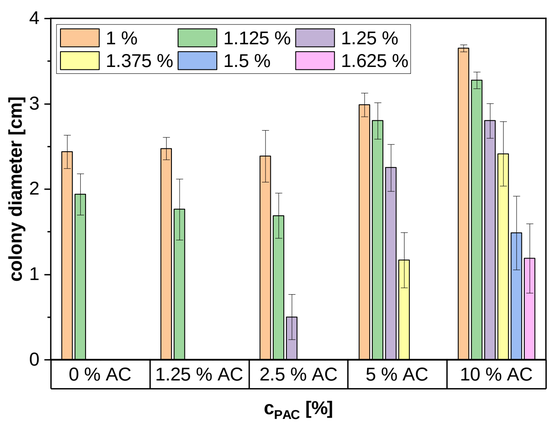
The pyrolytic aqueous condensate (PAC) is formed as a side stream during the fast pyrolysis of lignocellulosic biomass. Due to its high content of organic carbons such as acetic acid and acetol it can potentially serve as an inexpensive renewable substrate for microbial fermentation processes.
A promising candidate for such a process is the L-malic acid production with *Aspergillus oryzae* as the fungus is characterized by a high tolerance against pyrolysis products [1] and shows the ability to use acetic acid as substrate for L-malate production [2].
However, to enable a L-malate formation by *A. oryzae* on PAC as sole carbon source the fungal tolerance needs to be further increased. This work provides an overview about different pretreatment methods and evaluates their impact on the PAC tolerance of *A. oryzae*.

Methods

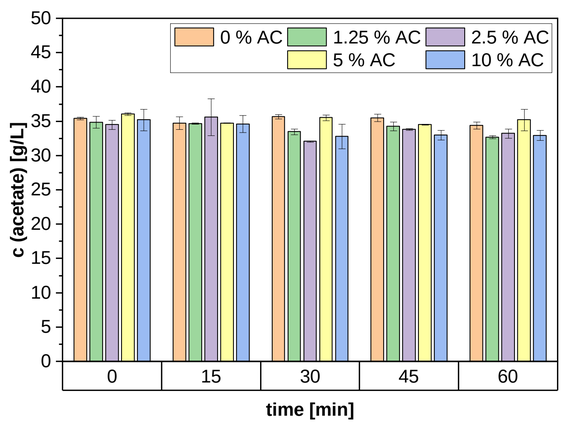


Results

Activated carbon



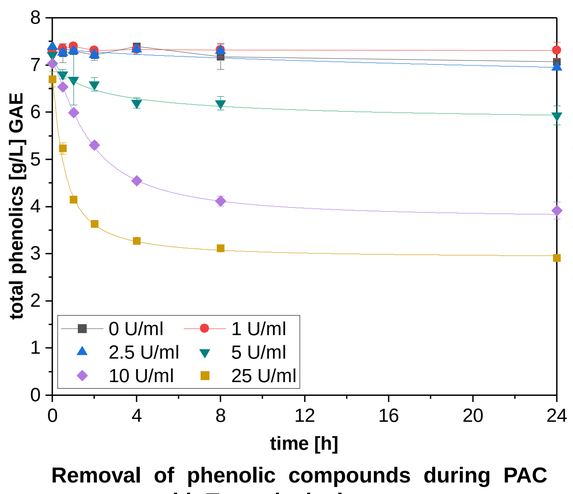
Impact of different activated carbon (AC) loads on the PAC tolerance of *A. oryzae*
Colony diameters on agarplates with glucose after 5 d incubation at 30 °C



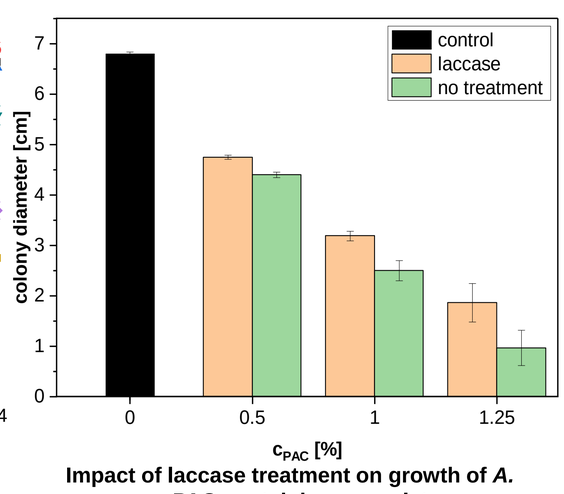
Acetic acid content in PAC treated with different activated carbon loads

- Carbon load dependent increase in PAC tolerance
- Maximum improvement of 44 % for 10 % carbon
- No loss of acetic acid as potential substrate

Laccase from *Trametes versicolor*



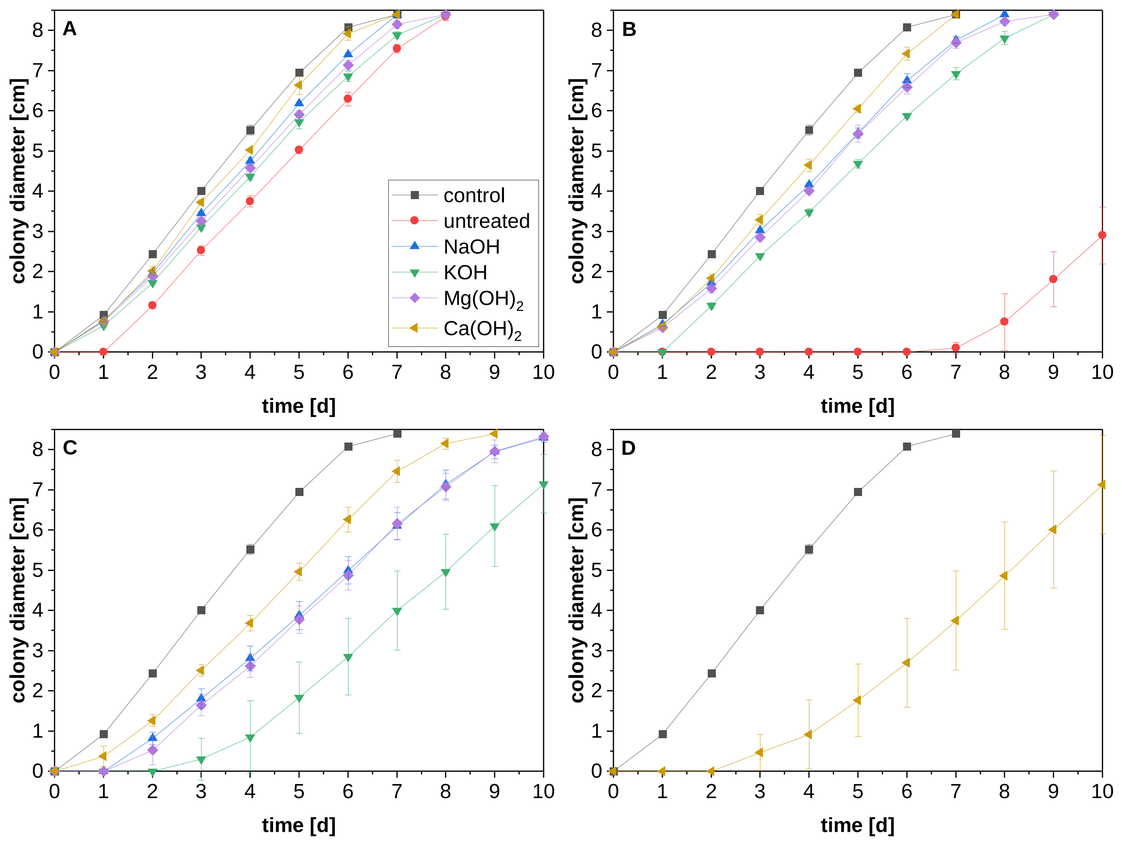
Removal of phenolic compounds during PAC treatment with *T. versicolor* laccase
Total phenolics measured as gallic acid equivalents (GAE) using Folin-Ciocalteu method



Impact of laccase treatment on growth of *A. oryzae* on PAC containing agar plates
Enzymatic treatment of PAC was performed with 25 U/ml of *T. versicolor* laccase for 24 h at 30 °C & 180 rpm

- Removal of phenolics for laccase concentrations >5 U/ml
- Laccase treatment leads to increased colony diameters
- No increase in *A. oryzae* overall PAC tolerance level

Overliming with different hydroxids



Growth of *A. oryzae* on agar plates containing PAC treated by overliming using different hydroxids
Colony diameters after 5 days incubation at 30°C and PAC contents of: A: 0.5 % ; B: 1 %; C: 2 %; D: 4 %

- Comparable growth for all conditions on 0.5 % PAC
- No more growth on ≥ 2 % of untreated PAC
- Maximum increase in fungal tolerance achieved by $Ca(OH)_2$ overliming

Conclusions

The results indicate that a physical detoxification using ≥ 2.5 % activated carbon as well as overliming treatments are suitable approaches to improve the growth of *A. oryzae* on PAC. The highest tolerance of 4% was reached by $Ca(OH)_2$ overliming corresponding to an fourfold increase compared to the untreated control. An enzymatic treatment with *T. versicolor* laccase had no positive effect on the fungal growth on PAC even though a reduction in phenol concentration was detected. In addition, this method is relatively expensive making it unsuitable for the detoxification of the pyrolytic side stream. In contrast to that a promising approach for the further increase of the fungal PAC tolerance could be the combination of several pretreatment methods and should therefore be investigated in the future.

References

[1] Dörsam et al. (2016). Frontiers in microbiology. doi: 10.3389/fmicb.2016.02059
[2] Kövilein et al. (2021). Biotechnology for biofuels. doi: 10.1002/jctb.6269

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