# Integrated uses of alkali in lignin-first pretreatment and arrested anaerobic digestion for volatile fatty acids from yard waste

Jiachen Sun [a], Le Zhang [b, c], Kai-Chee Loh [a, b, c*]

[a] Department of Chemical and Biomolecular Engineering, National University of Singapore, 117585, Singapore
[b] NUS Environmental Research Institute, National University of Singapore, 138602, Singapore
[c] Energy and Environmental Sustainability Solutions for Megacities (E2S2) Phase II, Campus for Research Excellence and Technological Enterprise (CREATE), 138602, Singapore
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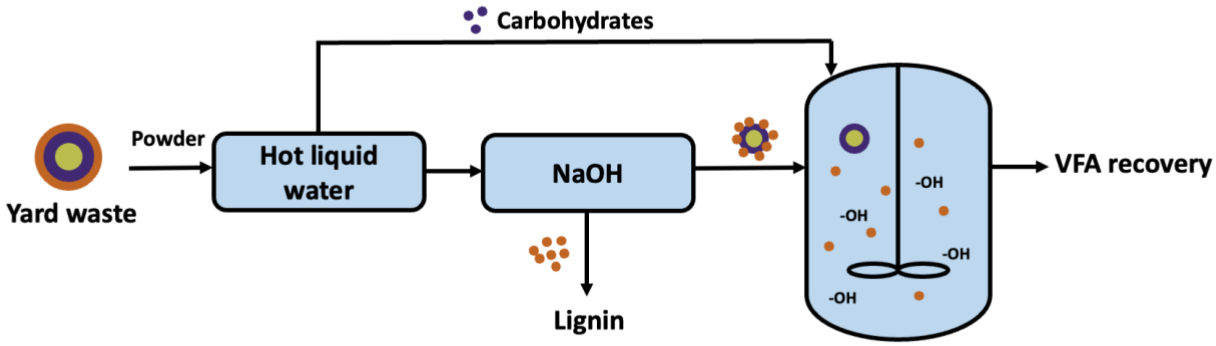
Presenter: Jiachen Sun Email: jiachen.sun@u.nus.edu
*Corresponding author: Kai-Chee Loh E-mail: chelohkc@nus.edu.sg

Lignocellulosic waste is the most voluminous waste worldwide. Singapore produces about 330 thousand tons of yard waste (YW) annually, a quarter of which is disposed of via incineration. Similarly, in other regions like the United States, 3.54 million tons of yard trimmings are generated annually and 30% of them are landfilled. This underutilized lignocellulosic waste is an ideal feedstock for sustainable biofuels and biochemicals towards net-zero targets.

Anaerobic digestion (AD) is a well-developed technology for converting lignocellulose to energy (biomethane, $150/ton). AD of YW comprises four steps i.e., hydrolysis, acidogenesis, acetogenesis, and methanogenesis, each of which can form individual intermediate compounds. Interrupting AD prior to methanogenesis accumulates volatile fatty acid (VFA, C2-C5, $600 - $3000/ton), a precursor for value-added biofuels and biochemicals, such as aviation fuels, bioplastics, etc (Calt, 2015). The conventional route of lignocellulose digestion pursues easily accessible carbohydrates by pretreatment and rapid-accumulated VFA in digesters. This valorization pathway introduces undigested lignin into anaerobic digesters, which impedes lignin recovery and makes biofuels less competitive (Ragauskas *et al.*, 2014). To address this, lignin is recommended to recover during pretreatment, theoretically producing 30% higher lignin-based biofuels (Ragauskas *et al.*, 2014). In the lignin-first route, the reaction conditions in pretreatment and bioconversion used to be contradictory, due to the heterogeneity of cellulose/hemicellulose and lignin (Abu-Omar *et al.*, 2021). For example, alkali-catalytic pretreatments are widely used to fragment lignin from lignocellulose, albeit most microbial consortium producing short-chain fatty acids are neutrophilic or slightly acidophilic (Shahab *et al.*, 2020; Abu-Omar *et al.*, 2021). Thus, post-pretreatments are required to compensate for the pH difference between the pretreatments and AD process, thereby offsetting the biofuels profit.

Anaerobic digester harbors diverse microorganisms. Recently the acidogens that can work at alkaline conditions (pH of 8-10) were identified in AD when treating wastewater sludge and food waste (Wang *et al.*, 2019; Owusu-Agyeman *et al.*, 2022). The occurrence of these alkaliphilic acidogens broadens the pH range of AD to the alkaline, which is promising to narrow the gap between alkali-catalytic lignin-first pretreatment and arrested AD. We anticipated that biodiverse anaerobic digesters could also incubate alkaliphilic hydrolytic bacteria to degrade cellulose. Moreover, pretreatment and alkaline AD broth can minimize the solid lignin inside the digesters, thereby alleviating the aromatic inhibition and strengthening cellulose exposure, finally maximizing the VFA yield.

Herein, we combined hot liquid water and NaOH in lignin-first pretreatment to fractionize lignocellulose and recover lignin (Scheme 1). Expressly, hot liquid water pretreatment preserves 25% sugars (wt of YW) in case of loss to the lignin stream, followed by lignin recovery of 21% (wt of YW) through NaOH-treated lignocellulose fractionization. Then, the carbohydrate fractions were digested for VFA with different initial pH (8~12). Alkaline digestion broth was found effective to solubilize lignin residue from cellulose surface (Fig. 1a). The highest VFA production was generated with initial pH of 10, contributed by arresting methanogenesis and bacterial viability recovery (Fig. 1b). Lastly, the reactors were scaled up to the working volume of 6 L and produced VFA yield of 0.45 g/gYW (Fig. 1c). This study considers lignin recovery during bioconversion of biomass and contributes to understand alkaline AD of lignocellulose.




Scheme 1 Integrated lignin-first pretreatment and alkaline arrested AD for VFA

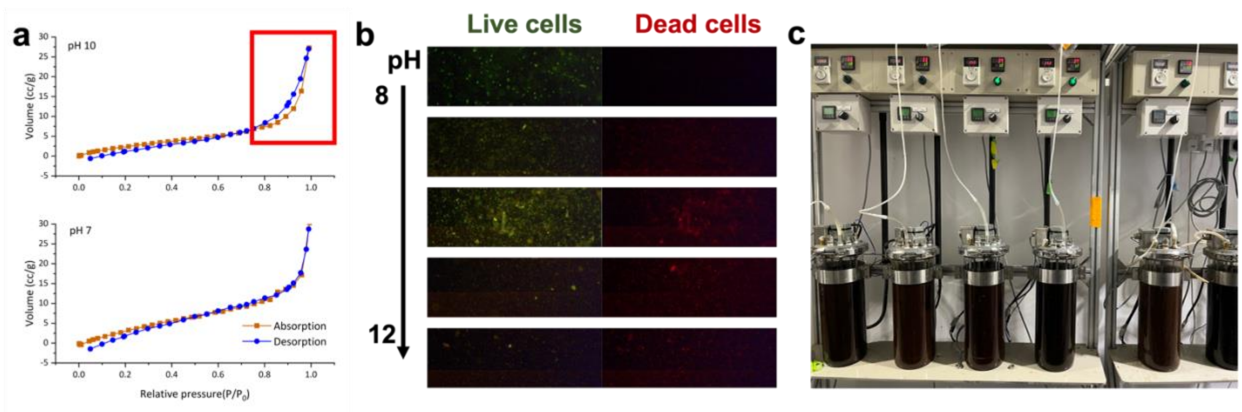



Figure 1 a) nitrogen absorption/desorption characterized enhanced YW exposure in alkaline solutions, b) live and dead cells inside digesters characterized by fluorescence microscope and c) anaerobic digesters for VFA

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