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Forum

Lignocellulolytic microbiomes for augmenting lignocellulose degradation in anaerobic digestion

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Bioaugmenting lignocellulose digestion with potent lignocellulolytic microbiomes (LMs) facilitates efficient biomethanation. Assessing the metabolic roles of microbial communities of the LMs and their complex interactions with the indigenous anaerobic digester microbiome is pivotal in implementing bioaugmentation. Multiple meta-omics are the frontline approaches to investigating gene functions, metabolic roles, and the ecological niches of LMs.

LMs in bioaugmentation of anaerobic digestion

Restricted hydrolysis of recalcitrant lignocellulosic biomass in anaerobic digesters may fail to generate sufficient intermediate products that act as substrates for subsequent metabolic processes during anaerobic digestion (AD). Although conventional AD microbiomes have lignocellulose-degrading microbial communities they still lack the adequate lignocellulolytic capacity to keep up with the rate of metabolic processes in AD [1]. Bioaugmentation, or microbial reinforcement, of AD with engineered LMs, that is, the addition of a specific microbial species or consortium that has potent lignocellulolytic activities, is a promising approach to enhance the digestion of lignocellulosic biomass towards higher biomethane yields [2]. However, the application of LMs in bioaugmenting lignocellulose degradation in AD has still not reached its full potential due to the selection of incompetent consortia, microbiome instability, washout of lignocellulolytic communities during lignocellulose digestion, and the complex interaction of those communities with the indigenous AD microbiome. Therefore, a deep understanding of lignocellulolytic communities' functions, robustness, ability to withstand invasion and/or competition from indigenous species, and how long they can thrive throughout prolonged and continuous AD operation is key to ensure long-term efficacy of AD bioaugmentation [3]. The combined application of multiple meta-omic techniques will allow elucidation of the exact dynamics and functions of LMs and their complex interaction with the indigenous AD microbiome after their addition to digesters that utilize lignocellulose.

The functional stability of LMs underlies the success of AD bioaugmentation

To exert a potent bioaugmentation effect, microbial communities which are actively involved in degrading lignocellulose – and which are shared across the LMs used for AD bioaugmentation – should be (i) resistant to environmental changes to a certain degree, (ii) functionally redundant, and (iii) functionally resilient [4]. The source of lignocellulolytic microbes is critical in designing the engineered microbiomes for the bioaugmentation of AD. Enhanced lignocellulose digestion has been achieved by using an acclimatized microbiome comprising core communities of potent hydrolytic, acidogenic, syntrophic, and methanogenic members [2]. Rumen fluid and cattle manure are vital sources for enriching LMs because of the presence of a cellulosome (large multienzyme complexes comprising cellulase, hemicellulase, and other related enzymes) containing microbes such as *Ruminococcus* sp., *Butyrivibrio* sp., *Clostridium thermocellum*, *Clostridium cellulolyticum*, *Clostridium cellulovorans, Neocallimastix, Piromyces*, and *Orpinomyces* [2]. The synergistic action of anaerobic rumen fungi (ARF) – including *Anaeromyces*, *Neocallimastix*, *Orpinomyces*, and *Piromyces* – at various ratios seems to be a better alternative to overcome the difficulties arising during lignocellulose biodegradation, showing improved digestion of lignocellulose with up to 33% higher methane yield (Table 1) [1]. ARF and cellulolytic rumen-fluid bacteria – including *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus* – were found to be more effective due to faster cellulose degradation in less retention time and improved degradation of lignocellulosic biomass with enhanced methane production during AD [5].

A rational goal of an LM is to enable engineers to directly add or maintain a specific microbiome function to enhance methane productivity in AD over a longer period and range of operational conditions. Successful integration of a few key members of lignocellulolytic communities – such as species from the families *Ruminococcaceae*, *Clostridiaceae*, and *Bacteroidaceae* – undertaking lignocellulose degradation in bioaugmented AD has been confirmed [2]. Nevertheless, a challenge with bioaugmentation of AD is maintaining the functional stability of engineered microbiomes which may not be competitive enough to survive invasion by indigenous species and washout in a harsh, dynamic, and heterogeneous AD ecosystem. The disappearance of some key members of the LM – including *Ruminococcaceae*, *Bacteroides, Fibrobacter,* and *Acetivibrio* – may occur quickly following bioaugmentation during AD of lignocellulosic biomass [6]. Additionally, ARF also do not really