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Review

Biological strategies for enhanced hydrolysis of lignocellulosic biomass during anaerobic digestion: Current status and future perspectives

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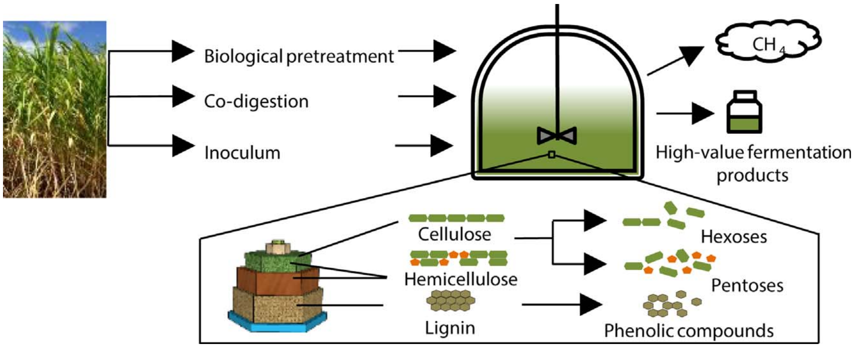
GRAPHICAL ABSTRACT



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ABSTRACT

Lignocellulosic biomass is the most abundant renewable bioresource on earth. In lignocellulosic biomass, the cellulose and hemicellulose are bound with lignin and other molecules to form a complex structure not easily accessible to microbial degradation. Anaerobic digestion (AD) of lignocellulosic biomass with a focus on improving hydrolysis, the rate limiting step in AD of lignocellulosic feedstocks, has received considerable attention. This review highlights challenges with AD of lignocellulosic biomass, factors contributing to its recalcitrance, and natural microbial ecosystems, such as the gastrointestinal tracts of herbivorous animals, capable of performing hydrolysis efficiently. Biological strategies that have been evaluated to enhance hydrolysis of lignocellulosic biomass include biological pretreatment, co-digestion, and inoculum selection. Strategies to further improve these approaches along with future research directions are outlined with a focus on linking studies of microbial communities involved in hydrolysis of lignocellulosics to process engineering.

1. Introduction

Anaerobic digestion (AD) of abundant lignocellulosic biomass remains a promising option to produce renewable energy. AD has been widely adopted to convert agri-residues, animal manure, and municipal and industrial wastes into biogas, while simultaneously treating or stabilizing these waste streams. Other merits of AD include nutrient recovery and mitigation of the release of greenhouse gases. Lignocellulosic materials, which include agri- and forestry residues, energy crops, and municipal paper and food waste, are attractive feedstocks for AD (Sawatdeenarunat et al., 2015). Over 17,240 operational AD plants across Europe collectively produced 63.3 TWh of

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electricity from biogas, which is equivalent to an annual electricity consumption of 14.6 million European households in 2014 (European Biogas Association, 2015). AD plants in the U.S. are mainly employed in stabilization of residuals generated at municipal wastewater treatment plants, as well as in food waste and animal manure digestion. The American Biogas Council estimates that 2,000 AD plants are operational in the U.S. in 2015 (American Biogas Council, 2015). Regulatory changes, such as SB-1383, which requires 40 percent reduction in dairy and livestock methane emissions by 2030 in California (California, 2016), have the potential to greatly expand the application of AD. A large number of the AD plants in Europe, particularly those in Germany, currently use food crops (e.g., maize, sugar beet etc.) as feedstocks. However, the European Union's biofuel policy does not provide incentive for their further use due to negative impacts on land use and food production (food/feed versus fuel debate) (Pols, 2015). Thus, agri-residues, organic waste streams, manure, and other lignocellulosic feedstocks, which do not compete with food/feed production, serve as more sustainable substrates for AD. Furthermore, developing countries that rely heavily on imported fossil fuels to meet energy demands, while generating considerable amounts of untapped renewable feedstocks, such as crop residues and energy crops, can benefit by using these lignocellulosic feedstocks for biogas production (Surendra et al., 2014). For example, Thailand has been focusing its energy policies on the use of Napier grass, a high yielding energy crop, for bioenergy production via AD to curtail its dependency on fossil fuels imports (Waramit and Chaugool, 2014).

Lignocellulosic biomass sources can be distinguished from other AD feedstocks by their abundance, low price, relatively consistent composition, and relatively high yield (Wu and He, 2013). However, due to the presence of poorly biodegradable components such as lignin, hemicellulose and cellulose, which are intertwined to form highly recalcitrant lignocellulosic structures, the methane yield of such biomass sources often does not exceed 60% of the theoretical value (Frigon and Guiot, 2010). Specifically, the methane yields of lignocellulosic feedstocks range from 0.17 to 0.39 Nm^3kg^{-1} volatile solids (VS) depending on their composition and associated hydrolysis rates (Frigon and Guiot, 2010). Moreover, recent advances in the AD field towards producing other high value fermentation products, such as volatile fatty acids (VFAs) (Surendra et al., 2015), medium chain fatty acids through chain elongation (Angenent et al., 2016), and hydrogen (Ren et al., 2016), depends heavily on improving the hydrolysis rate. The conversion of lignocellulosic biomass to methane is catalyzed by a diverse consortium of anaerobic microbes, including cellulolytic/hemicellulolytic microbes, non-cellulolytic fermentative or acidogenic microbes, syntrophic hydrogen-producing bacteria, and methanogenic archaea. To design an efficient and stable digester using lignocellulosic substrates, and to overcome the slow hydrolysis rate, it is important to identify key microbial players as well as the metabolic pathways involved during hydrolysis. This will help us better understand how operational and environmental parameters that affect these microbial populations can be optimized to maintain a favorable environment for their growth and activity, and thus to improve the efficiency and stability of AD operation.

The lack of efficient methods to overcome the refractory properties of lignocellulosic feedstocks is one of the main bottlenecks for their widespread utilization in AD. Specifically, it is well established that the conversion of lignocellulosic materials into desirable products is limited by hydrolysis, the first step of AD (Noike et al., 1985). So far, a variety of strategies, such as physical and chemical pretreatment of feedstocks (Gianico et al., 2013; Li et al., 2014), co-digestion (Fonoll, 2015; Lin et al., 2014; Zhao et al., 2014; Zheng et al., 2015), and use of an inoculum rich in cellulolytic/hemicellulolytic microorganisms (Gu et al., 2014; Shrestha, 2015) have been examined to address this problem. Another method, which remains relatively unexplored, is the simulation of natural ecosystems that contain microbial populations adapted to degrading lignocellulosic biomass (Bayané and Guiot, 2011; Godon et al., 2013).

Research on AD of lignocellulosic biomass has accelerated greatly during the last decade and a number of reviews have been published recently, focusing on process microbiology in general (Tsavkelova and Netrusov, 2012), the challenges during the digestion process (Sawatdeenarunat et al., 2015), and different hydrolytic bacteria involved (Azman et al., 2015). None of these reviews have linked these different approaches to improve lignocellulosic biomass degradation in AD systems. The current review encapsulates both process engineering and microbiology perspectives, and explores how they can be integrated to improve the AD of lignocellulosic biomass. The review first presents the structural composition of lignocellulosic biomass reflecting its recalcitrant nature. Next, the literature on relevant anaerobic microorganisms, with a particular focus on bacteria capable of degrading lignocellulosic biomass in AD systems and natural ecosystems (gastro-intestinal tract of ruminant animals and termites) is summarized. Furthermore, various strategies are discussed to optimize the digestion process and highlight areas where improvements can be made with a focus on advancing the understanding on how studies of microbial community structure and function can be used to help design and operate efficient AD systems.

2. Structure and composition of lignocellulosic biomass

Lignocellulose forms the principal building block of plant cell wall, and consists primarily of cellulose (most abundant), hemicellulose, and lignin (Martínez et al., 2005; Sawatdeenarunat et al., 2015). In addition, non-structural carbohydrates, such as glucose, fructose, and sucrose, as well as proteins, lipids, and pectin are present in varying amounts (McDonald et al., 1991). The specific composition of lignocellulosic biomass depends on the plant species, its growth stage, and the environment (Surendra and Khanal, 2015).

Hemicellulose serves to link lignin and cellulose fibers. Hemicellulose restricts access to cellulose cores by coating them, and its removal reduces the amount of cellulases required to convert cellulose into glucose (Himmel et al., 2007). Hemicellulose is relatively easy to hydrolyze compared to cellulose, which exhibits more crystallinity and a higher degree of polymerization, and hemicellulose is broken down and metabolized before other structural components (Surendra and Khanal, 2015). Lignin acts as the glue that binds the different components together providing structural support to the plant and contributing resistance against microbial or enzymatic degradation (Martínez et al., 2005; Sawatdeenarunat et al., 2015; Zeng et al., 2014). Besides being a physical barrier that limits access to hemicellulose and cellulose, other negative effects of lignin in AD include non-specific adsorption of hydrolytic enzymes to "sticky" lignin, interference with and non-productive binding of cellulolytic enzymes to lignin-carbohydrates complexes, and toxicity of lignin derivatives to microorganisms (Agbor et al., 2011).

To degrade lignocellulosic materials, cellulose and hemicellulose must be hydrolyzed into their respective building blocks. The ether and C–C linkages present in lignin are not susceptible to hydrolytic attack, which makes it highly resistant to breakdown (Bugg et al., 2011). Cleavage of linkages between lignin units, within aromatic rings of lignin monomers, and of bonds between lignin and hemicellulose (benzylether, benzylester, phenylglyoside, and acetal type) can all release lignin from the lignocellulosic matrix (Zeng et al., 2014). The lignin content determines the extent of the achievable degradation of