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PERSPECTIVES

Developing "precise-acting" strategies for improving anaerobic methanogenesis of organic waste: Insights from the electron transfer system of syntrophic partners

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Abstract Methanogenesis is the last step in anaerobic digestion, which is usually a rate-limiting step in the biological treatment of organic waste. The low methanogenesis efficiency (low methane production rate, low methane yield, low methane content) substantially limits the development of anaerobic digestion technology. Traditional pretreatment methods and bio-stimulation strategies have impacts on the entire anaerobic system and cannot directly enhance methanogenesis in a targeted manner, which was defined as "broad-acting" strategies in this perspective. Further, we discussed our opinion of methanogenesis process with insights from the electron transfer system of syntrophic partners and provided potential targeted enhancing strategy for high-efficiency electron transfer system. These "precise-acting" strategies are expected to achieve an efficient methanogenesis process and enhance the bio-energy recovery of organic waste.

Keywords Methanogenesis, Anaerobic digestion, Enhancing strategy, Electron transfer, Organic waste

1 Introduction

As the by-product of the world's economic development and population increase, considerable organic waste (OW) generated every day and the overall volume is increasing rapidly (Li et al., 2021a). Under the consideration for harmless treatment, resources recovery, and carbon emissions reduction, the application of anaerobic digestion (AD) has been viewed as an important and significant process in OW treatment, which requires low operation cost, stabilizes OW and produces biomass energy (i.e., biogas) (Pace et al., 2018). Methanogenesis is the last step in AD, after which the biological treatment of OW is finished and biogas is generated. Typically, there are three aspects to evaluate methanogenesis process in practice, that is, methane production rate, methane yield, and methane content in biogas. However, methanogenesis in the biological treatment is usually a rate-limiting step and the conversion degree from complex organic compounds to biogas also remains low. What's more, CO_2 content in biogas can be up to 50% and much of it must be removed before utilization to meet the standards similar to natural gas (85-96% CH_4) (Liu et al., 2021). To sum up, the low methanogenesis efficiency (low methane production rate, low methane yield, low methane content) substantially limits the development of AD technology.

2 Conventional enhancing strategies have been challenged

Methanogenesis pathways can be mainly classified into three categories according to their substrates for methanogens, namely aceticlastic, hydrogenotrophic, and methylotrophic (Xu et al., 2021). By contrast, organic wastes usually have multiple organic compositions and different chemical structures, which cannot be converted to methane directly and efficiently. Therefore, during anaerobic methanogenesis, multiple metabolism pathways are involved in the degradation of complex OWs and in which methanogenesis is often not robust. To address the low efficiency of methanogenesis process, studies have focused on the property optimization of OW and bio-stimulation of methanogens. On the one hand, considering

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the limited substrate scope for methanogens to use, multiple pretreatment methods (biological, physical, and chemical approaches and integrated protocols) have been adopted to enhance the solubilization and conversion of complex OWs. On the other hand, strategies for improving key enzymes (e.g., coenzyme M and F_{420}) activities and alleviating inhibition (e.g., ammonia nitrogen, amines) effects have been developed to enhance the metabolic ability of methanogens (Yan et al., 2020). However, traditional pretreatment methods (acid-base, ultrasonication, thermal hydrolysis, etc.) can only enhance the solubilization and hydrolysis of organic wastes to an uncertain extent with no access to provide proper substrate for methanogenesis precisely. Common bio-stimulation strategies (trace elements regulation, exogenous material addition, etc.) also fail to stimulate the methanogenic metabolism of methanogens precisely. What's worse, the concentration of metabolites such as ammonia nitrogen could be significantly increased after pretreatment, which would aggravate feedback inhibition on methanogens activity. Under certain circumstances, new refractory substances would generate by the physical and/or chemical reactions during pretreatment process, which would further decrease the organics conversion rate and methane yield. For example, furfural, phenol, and other products could be formed after thermal hydrolysis pretreatment via the Maillard reaction (Wilson and Novak, 2009).

In conclusion, these conventional strategies for improving methanogenesis result in consecutive concerns and dilemmas. Taking one step further, we define these conventional strategies as "broad-acting" strategies, which have an impact on the entire anaerobic system and cannot directly enhance methanogenesis in a targeted manner. In an attempt to overcome the bottlenecks of traditional methods, "precise-acting" strategies, that is, potential targeted enhancing methods for methanogenesis need to be further developed.

3 Electron transfer of syntrophic partners is important for methanogenesis

In fact, methanogenesis process represents a significant portion of carbon flow in anaerobic biological treatment of OW, which requires methanogens to build syntrophic association with functional bacteria for sequential organics conversion. The fundamental principle for this syntrophy is to achieve redox balance, that is, the redox state and energy conservation of methanogens and their partners highly rely on the property and availability of initial electron donor and terminal electron acceptor. For example, when growing solely in pure culture, *Ruminococcus albus* converts the electron donor glucose to ethanol with 2 ATP generated. However, when the syntrophic relationship is built between *R. albus* and hydrogen-consuming methanogens, glucose is converted to acetate with 4 ATP generated (Stams and Plugge, 2009). Thus, large amount of energy is conserved by such syntrophic partners via possible thermodynamic reactions.

Generally, there are five basic elements in the electron transfer system for syntrophic methanogenesis: 1) electron donor, 2) syntrophic bacteria, 3) electron transfer carrier, 4) methanogens, and 5) electron acceptor. In most cases, H_2/formate is the electron carrier during the interspecies electron transfer (IET) process between syntrophic bacteria and methanogens. Hydrogen formation and consumption are simple redox reactions, which nevertheless require multiple enzymes with complex active centers to catalyze reversible conversion of H_2 into electrons and protons. Actually, the production, distribution, and consumption of the electron carriers occupy a significant proportion of energy requirements during syntrophic methanogenesis. Luckily, the discovery of direct interspecies electron transfer (DIET) provides an efficient and energy conserving electron transfer process between syntrophic bacteria and methanogens (Lovley, 2017), which maintains a biological film touch or electrical connection via cytochromes, e-pili or abiotic conductive materials with no need for H_2/formate serving as electron carriers (Li et al., 2021b). Previous studies have proved that the methanogenic efficiency of simple substrates is largely improved via DIET pathway, which further indicates that electron transfer system has a significant influence on the metabolic driven force of syntrophic partners. Thus, we here provide the hypothesis that a high-efficiency electron transfer system drives a high-efficiency syntrophic methanogenesis process, that is, the targeted enhancing strategy for high-efficiency electron transfer system leads to the "precise-acting" effect on syntrophic methanogenesis of OW.

4 Potential targeted strategies for high-efficiency electron transfer system deserve more investigation

Based on these backgrounds, developing the high-efficiency electron transfer system between syntrophic bacteria and methanogens is of great importance, encompassing optimized electron donor (for improving methane yield), direct electron transfer process (for improving methane production rate), and proper electron acceptor (for improving methane content). First of all, targeted fermentation process is necessary for OW to become optimized electron donor. The fermentation process of organic waste may be managed by the design of specific fermentation microorganisms or microbiota. For example, the utilization of substrates, as well as the intracellular electron generation, can be broadened and strengthened by synthetic biology strategies (Li et al., 2018), genetic technologies, in particular the genomic editing tools, can play an important role (Bai et al., 2021). Also, anodic electro-fermentation (AEF) is another promising biotechnology which can

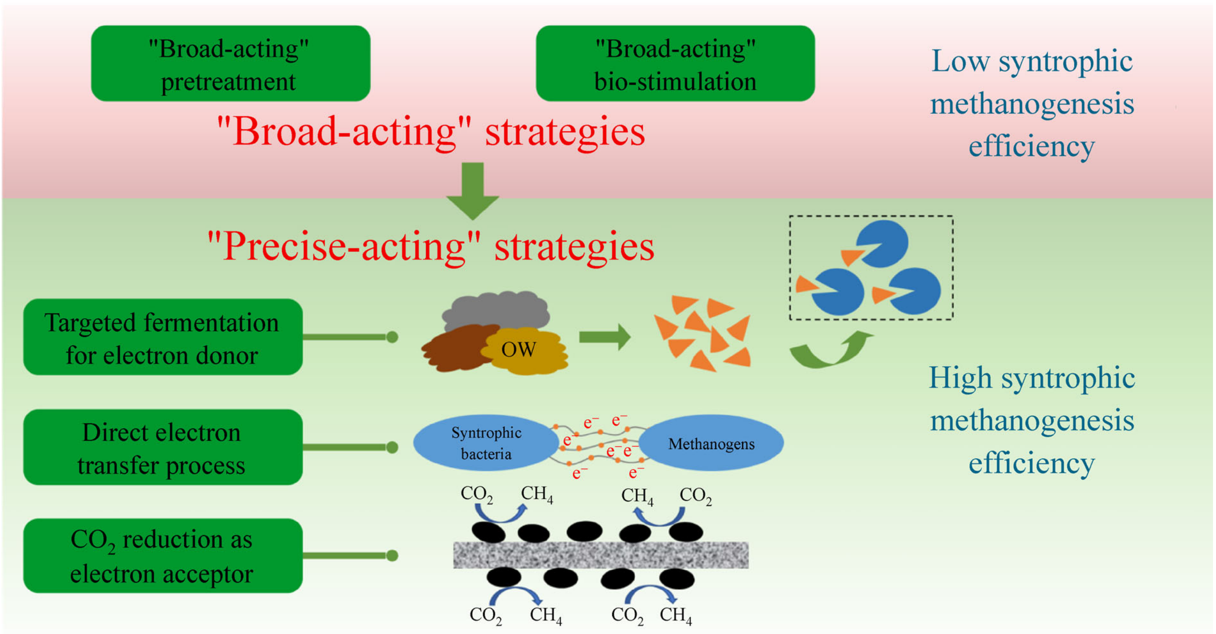

Fig. 1 Diagrammatic sketch of the evolution from "broad-acting" strategies to "precise-acting" strategies with different syntrophic methanogenesis efficiency (OW: organic waste).

achieve electrochemical control of the redox metabolism and energy conservation, steering the carbon flow of OW toward a product of interest (Vassilev et al., 2021). Secondly, recyclable or fixed conductive materials and biosynthetic e-pilis can be further explored for their stable application in direct electron transfer process between syntrophic partners. Also, microbial 3D printing technology can be further developed to prepare functional living materials with controllable and desirable microbial syntrophy structure (González et al., 2020). Lastly, the application of bio-electrochemical system (BES) in anaerobic methanogenesis needs more investigation and the bio-electrochemical CO_2 reduction by methanogens can lead an economic and promising biogas upgrading process (Dykstra et al., 2020). By the effective combination of these sections, a high-efficiency electron transfer system can be constructed with the successful production, distribution, and consumption of electrons (Fig. 1). Therefore, the targeted enhancing goal of methanogenesis improvement of OW can be achieved.

5 Conclusions

Based on the discussion above, we analysis the present limitations in anaerobic methanogenesis of OW, summarize the traditional enhancing strategies and argue their bottlenecks, further gain insights from the electron transfer system of syntrophic partners and draw a specific conclusion that developing targeted strategies to construct a high-efficiency electron transfer system is of great significance for improving syntrophic methanogenesis efficiency. More profound investigations of "precise-acting strategies" based on targeted fermentation for electron donor, direct electron transfer process, and CO_2 reduction as electron acceptor are still needed. With these efforts, the resource utilization of OW can be maximized by a promising efficient methanogenic process.

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References

Bai X, Lin T, Liang N, Li B Z, Song H, Yuan Y J (2021). Engineering synthetic microbial consortium for efficient conversion of lactate from glucose and xylose to generate electricity. Biochemical Engineering Journal, 172: 108052

Dykstra C M, Cheng C, Pavlostathis S G (2020). Comparison of carbon dioxide with anaerobic digester biogas as a methanogenic biocathode feedstock. Environmental Science & Technology, 54(14): 8949–8957

González L M, Mukhitov N, Voigt C A (2020). Resilient living materials built by printing bacterial spores. Nature Chemical Biology, 16(2): 126–133

Li F, Wang L, Liu C, Wu D, Song H (2018). Engineering exoelectrogens by synthetic biology strategies. Current Opinion in Electrochemistry, 10: 37–45

Li L, Cai C, Chen Y, Liu H, Liu R, Yang D, Dong B, Dai X (2021a). Secondary acidogenic fermentation of waste activated sludge via voltage supplementation: Insights from sludge structure and enzymes activity. Science of the Total Environment, 797: 149161

Li L, Xu Y, Dai X, Dai L (2021b). Principles and advancements in improving anaerobic digestion of organic waste via direct interspecies electron transfer. Renewable & Sustainable Energy Reviews, 148: 111367

Liu C, Xiao J, Li H, Chen Q, Sun D, Cheng X, Li P, Dang Y, Smith J A, Holmes D E (2021). High efficiency in-situ biogas upgrading in a bioelectrochemical system with low energy input. Water Research, 197: 117055

Lovley D R (2017). Happy together: microbial communities that hook up to swap electrons. The ISME Journal, 11(2): 327–336

Pace S A, Yazdani R, Kendall A, Simmons C W, Vandergheynst J S (2018). Impact of organic waste composition on life cycle energy production, global warming and Water use for treatment by anaerobic digestion followed by composting. Resources, Conservation and Recycling, 137: 126–135

Stams A J, Plugge C M (2009). Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nature Reviews. Microbiology, 7(8): 568–577

Vassilev I, Averesch N J H, Ledezma P, Kokko M (2021). Anodic electro-fermentation: Empowering anaerobic production processes via anodic respiration. Biotechnology Advances, 48: 107728

Wilson C A, Novak J T (2009). Hydrolysis of macromolecular components of primary and secondary wastewater sludge by thermal hydrolytic pretreatment. Water Research, 43(18): 4489–4498

Xu Y, Gong H, Dai X (2021). High-solid anaerobic digestion of sewage sludge: Achievements and perspectives. Frontiers of Environmental Science & Engineering, 15(4): 71

Yan W, Qian T, Soh Y N A, Zhou Y (2020). Micro-level evaluation of organic compounds transformation in anaerobic digestion under feast and famine conditions assisted by iron-based materials: Revealing the true mechanism of AD enhancement. Environment International, 135: 105362