Bioresource Technology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

A review on the bioenergetics of anaerobic microbial metabolism close to the thermodynamic limits and its implications for digestion applications

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ARTICLE INFO

Keywords:
Anaerobic digestion (AD)
Syntrophy
Thermodynamics
Kinetics
Electron transfer

ABSTRACT

The exploration of the energetics of anaerobic digestion systems can reveal how microorganisms cooperate efficiently for cell growth and methane production, especially under low-substrate conditions. The establishment of a thermodynamically interdependent partnership, called anaerobic syntrophy, allows unfavorable reactions to proceed. Interspecies electron transfer and the concentrations of electron carriers are crucial for maintaining this mutualistic activity. This critical review summarizes the functional microorganisms and syntroph partners, particularly in the metabolic pathways and energy conservation of syntrophs. The kinetics and thermodynamics of propionate degradation to methane, reversibility of the acetate oxidation process, and estimation of microbial growth are summarized. The various routes of interspecies electron transfer, reverse electron transfer, and Poly-β-hydroxyalkanoate formation in the syntrophic community are also reviewed. Finally, promising and critical directions of future research are proposed. Fundamental insight in the activities and interactions involved in AD systems could serve as a guidance for engineered systems optimization and upgrade.

1. Introduction

Anaerobic digestion (AD) is a biological process in which microorganisms mineralize organic materials in the absence of molecular oxygen. This process is widely used in industrial and municipal wastewater treatment for biogas recovery. Recently, the global challenges in energy and environmental arena necessitate a facelift of AD, with respect to maximizing energy production and enhancing treatment efficiency. Insight in the microbial mutualism that underpins AD and functions close to the thermodynamic limits, is the key to elucidate the black box and upgrade this process to a new renaissance (Tan et al., 2016).

AD for methane production is less exergonic than aerobic degradation or alternative forms of anaerobic respiration; for example, the conversion of hexose to methane and carbon dioxide releases only 15% of the energy generated by aerobic degradation (Schink, 1997). This small amount of energy generation in AD forces the microorganisms into a very close and efficient cooperation. Syntrophy is a particularly mutualistic partnership in AD, defined as a thermodynamically interdependent life style where neither partner can operate without the other (Morris et al., 2013). Interspecies electron transfer and the concentrations of electron carriers in the system are crucial for maintaining this cooperative metabolic activity. It has been postulated that a bacterium needs a minimum of about $-20\,kJ\,mol^{-1}$ (one third of the energy for the synthesis of an ATP molecule) to exploit the free energy change of a reaction, which is the smallest quantum of metabolically convertible energy for an ion transported across the cytoplasmic membrane and the amount for a living cell cooperating in syntrophic fermentation (Schink, 1997). However, it was reported that this minimum energy is considerably lower with the evidences from anaerobic mixed culture chemostat studies (i.e. $-8.0 \pm 3.1\,kJ\,mol^{-1}$ of Gibbs free energy available at growth equilibrium (ΔG_E) for propionate conversion to acetate plus hydrogen) (McCarty and Bae, 2011).

The AD process begins with the microbial hydrolysis of proteins, fats, carbohydrates, and some other biodegradable polymers, releasing amino acids, fatty acids, and sugars. Hydrolytic bacteria are phylogenetically diverse but mostly fall into two phyla, *Bacteroidetes* and *Firmicutes* (Venkiteshwaran et al., 2015). Acidogenic bacteria then

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http://dx.doi.org/10.1016/j.biortech.2017.09.103
Received 7 July 2017; Received in revised form 9 September 2017; Accepted 15 September 2017

convert amino acids, fatty acids, and sugars into ammonia, short-chain fatty acids (SCFAs), carbon dioxide (CO_2), hydrogen (H_2), and alcohols. Most species of acidogenic bacteria belong to the phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Chloroflexi*, and *Actinobacteria*. The genera under these phyla have been commonly identified as *Clostridium* and *Bacillus*, *Bacteroides* and *Proteiniphilum*, *Desulfovibrio* and *Geobacter*, *Chloroflexus*, and *Mycobacterium* (Cai et al., 2016). While acetate, formate, H_2/CO_2, and methyl compounds can be directly utilized by methanogens, other compounds resulting from acidogenesis such as butyrate, propionate, lactate, and ethanol are further biodegraded by a group of syntrophic acetogens into acetate, formate, and H_2/CO_2. Syntrophic acetogens generally include *Syntrophobacter*, *Pelotomaculum*, *Smithella*, *Syntrophus*, *Syntrophomonas*, and *Syntrophothermus*. The first three genera are typically involved in propionate degradation, whereas the others are commonly responsible for the oxidation of butyrate and other fatty acids (Cai et al., 2016; Venkiteshwaran et al., 2015).

Syntrophic acetogenesis is thermodynamically unfavorable under standard conditions. The syntrophic partnership with methanogens, which maintain a low H_2 partial pressure (p_{H2}) and low formate and acetate concentrations, allows this process to occur (Stams and Plugge, 2009). For example, the p_{H2} is crucial in the control of a syntrophic partnership between *Syntrophobacter* bacteria and hydrogenotrophic methanogens. p_{H2} measured at steady-state conditions were in the range of 1–20 $\times$ 10^{-5} atm, under which propionate consumption is thermodynamically favorable (McCarty and Smith, 1986). In addition, *Smithella* bacteria in syntrophic partnership with H_2-consuming methanogens have a larger H_2 window than the classical syntrophic acetogens due to a different propionate degradation pathway (Dolfing, 2013). Syntrophic electron flow during methanogenesis can also be achieved by interspecies formate transfer. In microbial flocs, more than 90% of syntrophic ethanol conversion to methane by *Desulfovibrio vulgaris* and *Methanobacterium formicicum* was mediated via interspecies formate transfer (Thiele and Zeikus, 1988). There are four main pathways for methane (CH_4) production: (i) acetoclastic methanogens utilize acetate to directly produce CH_4 and CO_2; (ii) hydrogenotrophic methanogens use H_2 or formate to reduce CO_2 to CH_4; (iii) methylotrophic methanogens metabolize methyl compounds to produce a small amount of CH_4; (iv) syntrophic partnerships of acetate-oxidizing bacteria and hydrogenotrophic methanogens convert acetate to CH_4 via the intermediates H_2 and CO_2. In anaerobic wastewater treatment, around 70% of the CH_4 is produced from acetate and the remainder mostly comes from H_2 and CO_2 (Venkiteshwaran et al., 2015). In terms of thermodynamics, the overall energy generated via acetoclastic methanogenesis (Pathway i) is the same as the energy generated via acetate oxidation and hydrogenotrophic methanogenesis based on anaerobic syntrophy (Pathway iv). The difference is that in acetoclastic methanogenesis all energy goes to one type of microorganisms. In syntrophic acetate oxidation, the energy is shared by two different species. Based on energetics alone, acetoclastic methanogens should outcompete syntrophic acetate-oxidizing bacteria. Hydrogenotrophic methanogens are crucial for the electron flow in the AD process because of their ability to scavenge H_2/formate at low levels and promote syntrophic acetogenesis. The most abundant genus of methanogens found in two anaerobic digesters was *Methanosarcina* (Cai et al., 2016), which are facultative acetoclastic methanogens that can also utilize H_2/CO_2 and C-1 compounds for methane production (Liu and Whitman, 2008). *Methanosaeta* are obligate acetoclastic methanogens that are known to use only acetate or acetate plus electrons obtained via direct interspecies electron transport (DIET) (Venkiteshwaran et al., 2015). DIET raises the intriguing possibility that the organism gets additional energy with electrons transferred via DIET (Shrestha et al., 2013). *Methanoculleus*, *Methanospirillum*, *Methanoregula*, *Methanosphaerula*, *Methanobacterium*, *Methanobrevibacter*, and *Methanothermobacter* are the most commonly observed hydrogenotrophic methanogens in anaerobic digesters (Cai et al., 2016). The H_2 can also be thought of as protons (H^+) associated with electrons, and syntrophic bacteria share electrons with methanogens in the form of H_2 could also be possible in a particular form of interspecies electron transfer separately with H^+. DIET proceeds via electrically conductive pili or c-type cytochromes from anaerobic syntrophs to methanogens resulting in methane production from ethanol, as observed for associations between *Geobacter* as electron producer and *Methanosaeta* or *Methanosarcina* as electron consumer (Rotaru et al., 2014a). The DIET will be discussed in more detail later in the review.

The focus of this review is on the literature that touches on anaerobic syntrophy as thermodynamically-limiting step in the AD process, with special attention to the functional microorganisms, syntrophic partners, microbial growth kinetics, and metabolic pathways involved in syntrophic processes, and to the molecular bioenergetics of syntrophic metabolism.

2. Thermodynamic and kinetic perspectives of propionate degradation

2.1. Thermodynamic perspective

Oxidation of propionate is energetically unfavorable because of the standard Gibbs free energy change, ΔG^0, of this reaction is positive. Propionate can be oxidized only if a syntrophic association occurs between propionate-oxidizing bacteria and H_2-consuming methanogens, such that the overall reaction is thermodynamically feasible (McInerney et al., 2009). The accumulation of propionate, an important intermediate, causes acidification of anaerobic digestion systems and deterioration of digestion performance (Smith and McCarty, 1989). Its degradation into acetate and H_2/CO_2 (and then to CH_4) accounts for approximately 6–35% of the total methanogenesis (Smith and McCarty, 1989). Therefore, the degradation of propionate is crucial, and as propionate degraders, syntrophic propionate-oxidizing bacteria (SPOB) play an imperative role in the metabolic network.

Thermodynamic laws can act as a vital tool to provide the theoretical basis for analyzing experimental results and providing important information regarding bacterial growth and metabolism. Thermodynamics also play an important role in understanding the pathway reversibility. The possible pathway reversibility of specific anaerobic catabolic reactions opens a new paradigm in the development of biofuels and chemicals with high energy density (Leng et al., 2017). As anaerobic bioprocesses occur in an energy-scarce environment in which concentrations of substrates remain at a relatively low level, the metabolic pathways take place very close to thermodynamic equilibrium with minimum energy dissipation. Therefore, a slight change in substrate/product concentrations or environmental conditions can alter the direction of the pathway.

Using thermodynamic principles, the formation mechanisms of the intermediate compounds can also be analyzed (Smith and McCarty, 1989). Later, efforts relating the thermodynamics with the process kinetics were made to characterize the operation of anaerobic digestion systems (McCarty and Bae, 2011). In addition, the correlation between microbial yield and Gibbs free energy changes of microbial conversions is a well-known application of thermodynamic principles. Thermodynamics also performs an essential function in kinetic models. Reactions can occur if the end products contain less free energy than the reactants, which means that the net Gibbs free energy ($\Delta G'$) is negative. This understanding can help in the investigation of the product concentrations that cause inhibition to a bio-reaction operating close to its thermodynamic equilibrium. Upon reaching the dynamic equilibrium, the reaction will cease before all of the substrate is converted. McCarty and Bae (2011) proposed a model that couples anaerobic process kinetics with biological growth equilibrium thermodynamics. Subsequently, González-Cabaleiro et al. (2013) linked thermodynamics and kinetics to assess pathway reversibility in anaerobic bioprocesses. In this section, the thermodynamics related to propionate degradation in anaerobic digestion processes will be discussed.