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Waste biorefineries — integrating anaerobic digestion and microalgae cultivation for bioenergy production

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Commercialization of microalgal cultivation has been well realized in recent decades with the use of effective strains that can yield the target products, but it is still challenged by the high costs arising from mass production, harvesting, and further processing. Recently, more interest has been directed towards the utilization of waste resources, such as sludge digestate, to enhance the economic feasibility and sustainability of microalgae production. Anaerobic digestion for waste disposal and phototrophic microalgal cultivation are well-characterized technologies in both fields. However, integration of anaerobic digestion and microalgal cultivation to achieve substantial economic and environmental benefits is extremely limited, and thus deserves more attention and research effort. In particular, combining these two makes possible an ideal 'waste biorefinery' model, as the C/N/P content in the anaerobic digestate can be used to produce microalgal biomass that serves as feedstock for biofuels, while biogas upgrading can simultaneously be performed by phototrophic CO_2 fixation during microalgal growth. This review is thus aimed at elucidating recent advances as well as challenges and future directions with regard to waste biorefineries associated with the integration of anaerobic waste treatment and microalgal cultivation for bioenergy production.

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Introduction

Anaerobic digestion (AD) is one of the most widely applied biological processes for the conversion of organic biomass to bioenergy (e.g. H_2 and CH_4). Dark fermentation (DF) with anaerobic bacteria (e.g. *Clostridium* spp.) is the major process for the conversion of biomass to hydrogen but during the DF process, most of the carbon matters remain in the liquid phase in the form of volatile fatty acids, alcohols and acetone. During methanogenesis process, COD reduction is more efficient, whereas most H_2 generated from acidogenesis phase is consumed but nitrogen and phosphorus contents still remain to certain extent. In addition, the biogas generated from anaerobic digestion still contains a significant amount of CO_2, which decreases the efficiency of power generation with the biogas and causes global warming when emitting to the atmosphere. Thus, these interrelated anaerobic processes generate: first, an effluent with high chemical oxygen demand (COD) contributed by the high concentrations of volatile fatty acids (VFAs), total nitrogen (TN) and total phosphorus (TP) [1]; and second, low biogas (CH_4 or H_2) yield and purity of due to the incomplete conversion of organic carbon. The biogas from AD contains approximately 20–60% CO_2 and 0.005–2% H_2S, and thus does not meet fuel gas specifications unless a proper purification process is employed [2]. Therefore, developing a low-cost strategy to treat fermentation effluents and upgrade biogas quality to meet fuel specifications is essential. In this review, microalgal cultivation is presented as a valorization method for the utilization of fermentation effluents, including both liquid and gases components. Integration of AD with microalgal cultivation has the dual benefits of reducing the carbon footprint of AD and managing the high production costs associated with conventional microalgal cultivation.

Mechanism and major metabolites of anaerobic digestion and dark fermentation

Fermentation of complex organic materials by anaerobic bacteria results in the decomposition of the carbon in the biomass to either CO_2 or CH_4. AD is a multi-step process, with four different phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis, and the initial organic compounds are decomposed to methane and VFAs, with concomitant release of gaseous products [1]. DF is the acidogenesis phase of AD, where VFAs, hydrogen and CO_2 are generated as the main products [3••]. These two processes are the major anaerobic pathways for treating

waste and generating hydrogen and methane. While AD can be used for treating various biomass compounds, including lignocellulosic biomass, DF is usually carried out with simpler substrates or with pretreated complex substrates. A mixed microbial consortium, like activated sludge, is generally used for AD, and in some cases for DF as well. The microbial community present in activated sludge is diverse, including both bacteria and archaea. The maintenance of the pH at acidic over neutral conditions determines the outgrowth of hydrogen-producing over methane-producing bacteria, and hence DF is carried out under acidic conditions [4]. DF can also be performed with pure cultures, comprising species from the genera *Clostridium*, *Enterobacter*, *Thermotoga*, *Bacillus*, and some genetically engineered *Escherichia coli* [1]. The major VFAs released during these fermentations are acetate and butyrate from the acidogenesis phase of AD, while the subsequent phases of AD generate propionate, formate, lactate, ethanol, valerate, isovalerate, acetone, acetoin and butanol [3••]. The AD effluent also contains other uncharacterized organic materials that contribute to the overall COD, but the influence of these compounds on AD performance is unclear [3••]. The final products of methanogenesis phase are mainly CH_4 and CO_2 with a significant reduction of the COD level in the liquid phase, whereas TN and TP contents could not be completely removed.

Growth of microalgae on gaseous and liquid fermentation effluents

Microalgae own the advantages of high growth rate, superior environmental adaptability, high nutrient-removal ability, no competition with food or arable land, year-round cultivation, higher lipid productivities and photosynthetic efficiencies compared with other terrestrial plants or microorganisms [5,6], which are regarded as a potential solution for the valorization of AD waste. Biogas is the gaseous counterpart of the AD products, and the liquid effluent or slurry is rich in organic matter, as shown by the high COD levels, while DF effluents are rich in VFAs. The uncharacterized organic matter represented by COD (including VFAs) can be effectively reduced by the growth of microalgae via heterotrophic or mixotrophic growth, accompanied by the removal of nitrogen and phosphorus, greatly reducing the nutritional levels of effluents [7]. Under mixotrophic conditions, microalgae are capable of simultaneously converting the waste biogas (i.e. CO_2) and AD effluent (i.e. COD, TN, TP and VFAs) into oil-rich biomass for biodiesel production [8,9]. The CO_2 released during dark hydrogen fermentation has been successfully used for microalgal cultivation in both photoautotrophic and mixotrophic cultivation [9,10]. Since the tolerance levels of microalgae for various metabolites are different, it is of importance to identify the optimal loading level of the fermentation effluent or biogas to achieve better growth performance for the microalgae used. Some microalgal strains, such as *Chlorella sorokiniana*, are able to grow on raw DF effluent [11,12]. In contrast, the biogas and slurry from an AD process should be sterilized prior to use for the cultivation of other microalgal strains (e.g. *Chlorella* sp. and *Scenedesmus* sp.) [13]. Biogas is a mixture of CH_4 and CO_2 with a relatively lesser amount of H_2S, and microalgae are used to capture the CO_2 from biogas, upgrading the CH_4 content. Biogas upgrading by microalgae and concomitant COD removal from the AD effluent are summarized in Table 1. The CO_2 content in biogas ranges from 20 to 60% in general, and microalgae tolerant to high CO_2 concentrations are preferred. Random mutagenesis has been used to increase the CO_2 tolerance levels in *Chlorella* sp. [3••]. The tolerance of microalgae to CH_4 is also strain dependent. A mutant *Chlorella* sp. MM2 can tolerate up to 80% of CH_4 [14], while a native strain of *Nannochloropsis gaditana* can tolerate 100% CH_4 without any significant changes in growth and biomass production [15]. The H_2S concentrations in the biogas can affect microalgal growth since dissolution of H_2S in the culture would lower the pH. Hence, H_2S-tolerant strains should be used. A *Scenedesmus* sp. that is highly tolerant to up to 3000 ppm of H_2S has been reported [16]. In some cases, the H_2S content needs to be reduced before introducing the biogas into the microalgae culture [13], whereas some microalgae species are able to carry out biogas upgrading with direct use of raw biogas rich in H_2S [16,17].

Microalgae can transport and metabolize VFAs efficiently, albeit in a hierarchical manner. Reports on the assimilation of various VFAs by microalgae are summarized in Table 2. Notably, microalgae can rapidly convert the acetate in effluents into acetyl-CoA (precursor for lipid synthesis) [18], suggesting that the VFAs produced from AD could be removed by microalgae through their metabolic functions [3••]. Acetate is also the central metabolite favoring lipid synthesis in microalgae, and the addition of acetate during nitrogen limitation promotes the expression of Acetyl-CoA carboxylase gene (ACS), committing the cells to lipid accumulation [18,19]. Butyrate is also converted to acetyl CoA via Crotonyl CoA in the glyoxysome, while it has been proposed that the energy expense for the transport of butyrate is compensated by the energy produced by the butyrate metabolism, with no net energy gain [20]. Butyrate is inhibitory as the sole carbon source above 0.1 g/L [9], but can be consumed by microalgae in a diauxic pattern when present with other VFAs, preferably with a higher acetate to butyrate ratio [21•] or at higher substrate to microorganism ratio [9,10]. The other major VFA, propionate, can be utilized by microalgae by converting it to either acetyl CoA or succinyl CoA, but it cannot also support growth as a sole carbon source [22]. Valerate and isovalerate have also been reported to be preferentially used for heterotrophic growth of *Chlorella protothecoides* [23••]. Lactate at a concentration above 0.5 g/L could inhibit the growth of *Chlorella vulgaris*