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Starch hydrolysis in autogenerative high pressure digestion: Gelatinisation and saccharification as rate limiting steps

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ABSTRACT

Autogenerative high pressure digestion (AHPD) provides an integrated biogas upgrading technology, capable of producing biogas with a CH_4 content exceeding 95% at pressures up to 90 bar. Hydrolysis is generally regarded as the rate-limiting step in the anaerobic digestion of complex organic matter, governing the volatile fatty acid (VFA) production rate for subsequent conversion to methane. Starch hydrolysis rates in AHPD systems were studied and the potential risk for VFA accumulation was assessed. Under the anticipated practical moderate pressure conditions at 30 °C, experimental CH_4-content of the biogas improved from 49 to 73 ± 2% at atmospheric and elevated pressure, respectively. Furthermore, no significant effect of pressure on the hydrolysis was found. Like under atmospheric pressure, gelatinisation was the rate-limiting step for particulate starch (0.05 d^{-1}) and saccharification for gelatinised starch (0.1 d^{-1}). Because no effect was observed on starch, an effect on the hydrolysis rate of more complex organic matter like (ligno-)cellulose is also not anticipated.

1. Introduction

Biogas production from organic waste is regarded as an important potential source of renewable energy, suitable for partial substitution of fossil fuels. However, the relatively low quality and sometimes variable composition of the biogas, may pose a significant bottleneck for further dissemination of anaerobic digestion technology. Besides the main component CH_4 biogas generally contains CO_2, H_2O and depending on the substrate also low levels of H_2S and NH_3. These compounds lower the calorific value of the produced gas. Moreover, biogas

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impurities cause corrosion of piping and equipment in the transport and combustion of biogas [1]. Conventional ex situ upgrading of biogas to high quality methane adds to the operation and investment costs and thereby often hampers the beneficial use of biogas. At gas flows <100 m^3/h in situ CH_4 enrichment was calculated to reduce total upgrading costs by a factor three [2]. The Autogenerative High Pressure Digestion (AHPD) technology uses microbial-generated pressure to control the distribution of gaseous compounds such as CO_2, H_2S and NH_3 over gas and liquid phase as predicted by Henry's law [3]. In addition, the water vapour content of the pressurised gas is obviously lower than biogas released at atmospheric pressures. Using acetate as the substrate autogenerated biogas pressures between 5 and 90 bar were attained containing >95% methane [3]. Owing to increased CO_2 solubility at increased operational pressures any possible volatile fatty acid (VFA) accumulation will result in a decreasing reactor pH and deterioration of the digestion process. This is particularly relevant when easily degradable substrates such as monomeric sugars are digested [4,5]. Most anaerobic digestion processes for wastes and slurries are fed with complex particulate organic matter consisting of (ligno-) cellulose, lipids and proteins. The aim of this work is to define the rate-limiting steps under anticipated AHPD conditions and thereby assess the risk of VFA accumulation for more complex substrates.

The first-order empirical hydrolysis model [6] shows that hydrolysis of complex particulate organic matter is the rate limiting step at atmospheric pressure and ambient temperature [7,8] and therefore reduces the risk of VFA accumulation. Carbohydrate hydrolysis typically only results in the formation of monomeric sugars [9], for which the subsequent conversion in AHPD reactors could result in a pH drop [4]. Within the group of carbohydrates, first-order empirical hydrolysis constants observed for starch generally are significantly higher than for (ligno-)cellulose [8]. Therefore it is postulated that if fatty acid accumulation can be prevented with starch as a model compound, it should certainly be possible to prevent fatty acid accumulation with (ligno-) cellulose.

Multiple authors demonstrated that the particle surface area available for enzyme binding is generally determining the overall hydrolysis rate [7,10,11]. Gelatinisation, i.e. the conversion of crystalline starch into amorphous starch by inclusion of water molecules in the crystal lattice, is the mechanism that enlarges the physical contact area and subsequently increases the hydrolysis rates of starch and cellulose [7,8,12–14]. Amorphous starch is then dissolved in a much faster enzymatic step, in which dextrin and oligosaccharides are formed, i.e. the liquefaction. In the last step of the hydrolysis, saccharification, dissolved oligosaccharides are then converted into monosaccharides by an also relatively fast enzymatic reaction [12].

Pressure treated starches were found more susceptible to α-amylase degradation than non-pressure treated starches and therefore pressure can theoretically be used, besides temperature, to increase the enzyme binding capacity of the starch [15]. Nevertheless, pressures between 1000 and 6000 bar at 29 °C with a 15 min exposure time, were required to significantly enhance the degree of gelatinisation of particulate starch [16]. These pressures are however way above the envisaged operational pressure of an AHPD system. Likewise, structural changes in enzymes resulting from compression or decompression of intermolecular cavities are theoretically feasible [17,18], but in order to increase the α-amylase activity of *Bacillus licheniformis* significantly a pressure of over 100 bar was required [19,20]. Nevertheless, Francisco and Sivik [21] found that chemical effects of dissolved CO_2 improved the degree of gelatinisation by 10–15% after 20 min exposure by using a relatively low pressure of 80 bar pCO_2 at 46 °C. A pCO_2 of 1–10 bar can already significantly decrease pH depending on the buffering capacity [4]. So, this may influence the gelatinisation, enzyme-substrate binding, and enzyme denaturation by strong interdependent relations between ionic strength, salt effect, pH, temperature and substrate characteristics, according to the theory described by Morild [17]. Consequently, despite the fact that operational pressure and temperatures in AHPD systems can be characterised as still moderate, some impact of dissolved CO_2 concentrations up to 150 mmol L^{-1} (at 5 bar), combined with a hydraulic retention time in the order of days, instead of minutes, can be anticipated.

This work describes two series of duplicate experiments. The first series of experiments aims to clarify the influence of physical pressures up to 20 bar on enzyme-based hydrolysis rates of gelatinised starch using a non-adapted mixed anaerobic culture. The second experimental series aims to determine whether particulate starch hydrolysis remains the rate-limiting step under AHPD conditions (up to 20 bar and 30 °C), with little risk of VFA accumulation during the pressurised anaerobic digestion process.

2. Materials & methods

2.1. Experimental setups

The 1st set of experiments was performed in three 0.6 L AHPD reactors as described in more detail elsewhere [5]. For the 2nd set of experiments, an 8 L AHPD reactor setup (Parr Instruments, model 910908, Moline, USA) was used [4].

All setups were controlled at 30 °C by a water bath (Julabo MP, Seelbach, Germany). Despite the fact that mesophilic processes generally proceed at maximum velocity at 35–37 °C in practice, the operational temperature of 30 °C ensures that in case of temperature upsets the chance on denaturation of enzymes is minimised and the long term stability of the experiment is maximised. Total pressure (Ashcroft A-series 1000 PSI, Stratford, USA), temperature (PT100) and pH were measured online and data was logged with Compact field point modules (cFP1804 and cFP-CB1) and stored with Labview 7.1 (National Instruments Corporation, Austin, USA) on the PC. The 0.6 L setups contained Prosense high pressure pH probes (Prosense serial nr. 34002/002, Oosterhout, The Netherlands) and the 8 L setup contained Bűchi high pressure probes (Bűchi Labortechnick AG, Flawil, Switzerland). Two six bladed impellers with a 9.8 cm diameter attached to a central stirrer shaft (Parr Instruments, type A709HC, Moline, USA) were used to stir the reactors continuously at 150 rpm for the 8 L reactor. Two 4 bladed impellers with a 3.5 cm diameter were used to stir the 0.6 L reactor at 60 rpm (Parr Instruments, type A837HC,