Glucose contents in anaerobic ethanol stillage digestion manipulate thermodynamic driving force in between hydrogenophilic and acetoclastic methanogens

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ABSTRACT

In disciplines related energy conversion, thermodynamics is the cornerstone for interpretation of process performance. It can provide the basis for the estimation of maximum convertible energy and spontaneity in novel, sustainable, anaerobic digestion processes. Here electrochemical thermodynamics is applied to the interpretation of glucose decomposition in anaerobic digestion of ethanol distillation residues (stillage) containing residual ethanol and ammonia. Under saturated conditions for biochemical mediators such as NAD$^+$/NADH the microbial oxidations and reductions between hydrolytic fermentative bacteria, syntrophic acetogens and methanogens will always be close to or at the electrochemical equilibrium state through the mediator. The product distribution arising from the up-take of glucose is found to be sensitive to the electrochemical potential which is a measure of the thermodynamic driving force, ΔG and related to both ΔH and ΔS via the standard relationships. Glucose decomposition is strongly favorable and only weakly dependent on electrochemical potential in the range investigated. However the system showed overall $\Delta H < 0$, indicating the potential for significant exotherm but ΔS was also found to be less than zero, indicating that the forward reaction was not spontaneous. This work also finds that as the mole fraction of glucose, x, is increased up to $x = 0.001$, the thermodynamic driving force for hydrogenophilic methanogenesis is dramatically decreased whilst that for acetoclastic methanogenesis is increased, and the thermodynamic "inhibition" of beta oxidation of volatile fatty acids is reduced. This may be interpreted as the diversion of the metabolic pathway from the conventional route 'glucose–acetate–CH$_4$/CO$_2$' towards glucose–butrate–proprionate–acetate–CH$_4$/CO$_2$.

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1. Introduction

Ethanol, whether for human consumption or for industrial purposes, is traditionally produced through yeast fermentation, of mashed starchy foods such as grain (wheat, rye, rice, maize), and potatoes, and molasses into "wine" or "beer", which is distilled to yield product ethanol. The bottom sludge from the distillation process has, historically, been treated as strong organic waste which has approximately 2400–50,000 mg l^{-1} BOD$_5$ and range of pH (3.5–5.4). In particular, it may contain complex mixture comprising pieces of pomace, fruit skins, seeds, pulp and "lees" solids coagulated with bentonite (>4%), ethanol (4–12% w/w) and variety of volatile acids including acetate and surfactants [1,2]. Ethanol (>11% w/w) induces cell lysis in most bacterial species including Escherichia coli and significantly impacts on the cell-membranes of hydrolytic bacteria preventing the production of extracellular enzymes [3]. This effect can be utilised for prevention of bacterial infection in humans, but it creates difficulties in the biological treatment of distillery sludge. Secondly according to the concentration

of coagulation agent 'bentonite', the concentrations of calcium and magnesium ions may potentially increase pH value in biological stabilization of the stillage. Thirdly, volatile matter including surfactants in stillage have a potential to cause significant foaming in aerobic biological treatment processes [4].

Anaerobic digestion has been used for many decades to treat the strong organic wastes with simultaneously production of renewable energy such as biogas. The digestion has a complex process through interaction amongst microbial species, which are broadly divided into three groups: (i) hydrolytic fermentative bacteria (ii) syntrophic acetogenic bacteria, and (iii) acetoclastic and hydrogenophilic methanogens. Hydrolytic fermentative bacteria attach to a complex organic particle, where they produce extra-cellular enzymes to hydrolyse the solid matter and then they use the soluble products (i.e. glucose, long chain fatty acids, amino acids and other organic acids) converting into fermentation products (i.e. ethanol, lactic acid, short chain fatty acids or benzoate) for maintenance of their cells [5,6]. Syntrophic acetogenic bacteria convert the fermentation products into acetate and hydrogen as protons and electrons and molecular H_2 in the process. However in the absence of coupling the acetogenic conversions of the fermentation products are not generally thermodynamically feasible biological reactions ($\Delta G \gg 0$), only becoming feasible at very low H_2 partial pressures [7]. Thus, the metabolic efficiency and the growth yield of the acetogenic bacteria which produce hydrogen, depend strongly on the rate of the H_2 removal by a consuming species [8,9]. This tells us that a number of microorganisms must coexist in anaerobic digesters, and are metabolically coupled, sharing their metabolites to produce methane. Even if a single monomer substrate such as 'ethanol' or 'glucose' is utilised, their concerted activity is necessary for the complete bioconversion of the organic material to methane.

Conventionally the complex interactions between species in anaerobic digestion have been understood in terms of "inter species hydrogen transport" and modelled in terms of molecular hydrogen [8–13]. Low observed hydrogen partial pressures have been interpreted as the consequences of product inhibition in the syntrophic acetogens. This approach suffers from a number of significant problems. The basic premise is to explain the observations entirely through a kinetic argument whilst largely ignoring the underlying thermodynamics. The solubility of molecular hydrogen in the digesting medium (henry's law coefficient 8.58×10^{-4} mol atm^{-1} l^{-1}) is low as is the hydrogen diffusion coefficient (4.50×10^{-9} m^2 s^{-1}). Together these militate against the high rate of interspecies transport required to support observed rates of methanogenesis. More recently the interactions have been understood in terms of biochemical mediators such as NAD$^+$/NADH; NADP$^+$/NADPH; HNQ$^+$/HNQH. Greater concentrations of NAD$^+$ (0.8–1.6 mM/g-biomass [14]) can be achieved in the digesting medium than molecular hydrogen. However the diffusivities of NAD$^+$ and NADH are low due to their relatively high molecular weight. The close proximity of cells in flocs and films tends to mitigate this effect by offering particularly short diffusion pathways. Reguera et al. [15] observed "nano-wires" between the individual cells of a microbial consortium. Such wires may offer routes for electron and proton transport between cells through complex ionic and/or hydrogen bonded structures. Hydrogen bonding of water has been used in the past to explain the anomalously high proton diffusion coefficient observed in electrolytes [16]. This suggests that interspecies hydrogen transport can occur independently of the conventional biochemical carriers. Thus, the success of the digestion process might be attributed to the efficiency of the proton and electron transfer processes.

In systems comprising high concentrations of low diffusivity mediators (NAD$^+$/NADH) or relatively low concentrations of highly mobile equivalents (H$^+$/e$^-$) the inter-species transport will be at the equilibrium state whilst there is a quasi-steady state microbial growth. On this basis this work establishes an electrochemical equilibrium potential network model for the catabolic reactions involved in the anaerobic digestion of ethanol stillage liquors which assumes that both H$^+$/e$^-$ and NAD$^+$/NADH pairs are inter cellular mediators between fermentative bacteria, syntrophic acetogenic bacteria, acetoclastic and hydrogenophilic methanogens and anaerobic oxidising bacteria. The resulting model is used to investigate the magnitude of the thermodynamic driving force (i.e. ΔG, ΔH and ΔS) for the stillage digestion process.

2. Model development

The electrochemical equilibrium is modelled and simulated in an isothermal and isobaric (298.15 K and 1 atm) batch digester. The anaerobic digester is assumed to contain a 'fully acclimatised' hydrolytic fermentative bacteria, syntrophic acetogenic bacteria and methanogenic consortium with anaerobic ammonium oxidising bacteria. A two-phase (solution and gas) digester is assumed.

In this case, the authors propose the slow addition of glucose in anaerobic ethanol stillage digestion for an intensive production of biogas and a stabilisation of ethanol stillage. The initial substrate considered is glucose ($C_6H_{12}O_6$) in constant concentrations (0.0, 0.01, 0.05, 0.1 and 0.25 mol l^{-1}) of ethanol (C_2H_6O) with constantly 0.1 mol l^{-1} of ammonia (NH$_3$), which are in range approximately 0.01–0.25 mol l^{-1} as brewery sludge [17,18]. 0.001 mol of NADH is considered as a biochemical mediator to tradeoff hydrogen molecule between micro-organisms. The authors thus investigated a sensitive change in equilibrium state in the variety of initial concentration of glucose at conserved system mass (1 kg). As the initial mole fraction of glucose ($x = C_6H_{12}O_6/(H_2O + NH_3 + C_2H_6O + C_6H_{12}O_6 + TNADH)$) is increased, the initial moles of solvent water (H$_2$O) is decreased to conserve the system mass of 1 kg. The authors investigated in the whole range of initial glucose mole fraction ($0 < x < 1$) in the conserved mass (1 kg of two phased reactor), where the low range of initial glucose mole fraction ($x < 0.01$) have practically been operated [18–20]. Furthermore, the hypothetical high range of initial glucose mole fraction ($x > 0.01$) of solution phase was also included.

The electrochemical framework comprises two parts. The first includes equilibrium expressions that relate the relative activities and or fugacities of all chemical species in the two phases to each other. The second consists of the equations of state that quantify the relationships between activity in the liquid phase and fugacity in the gas phase to the observable concentrations and partial pressures via the respective activity and fugacity coefficients. The completed model consists of a set of highly non-linear simultaneous equations which was solved using an algorithm based on the Newton–Raphson technique [21,7]. The following sections describe the network of microbial electrochemical equilibria including phase transition and ionisation of solutes.

2.1. Overall stoichiometric model

Stoichiometric expressions were developed in the conventional manner to quantify the possible relationships between the initial materials and the various products and intermediates. Table 1 shows the stoichiometric relationship for ethanol–glucose digestion. Fermentative bacteria degraded glucose to butyrate and acetate, which are continuously degraded to lower chain fatty acid into acetate and hydrogen by syntrophic acetogenic bacteria, and then methanogenic bacteria convert hydrogen and the residuals to methane. These stoichiometric relationships are categorised by three equilibrium relationships which are electrochemical reactions, phase transitions and electrolytes reaction.