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Research Article

Thermodynamic Analysis of Energy Transfer in Acidogenic Cultures

A global thermodynamic analysis, normally used for pure cultures, has been performed for steady-state data sets from acidogenic mixed cultures. This analysis is a combination of two different thermodynamic approaches, based on tabulated standard Gibbs energy of formation, global stoichiometry and medium compositions. It takes into account the energy transfer efficiency, ε, together with the Gibbs free energy dissipation, ΔG_o, analysis of the different data. The objective is to describe these systems thermodynamically without any heat measurement. The results show that ε is influenced by environmental conditions, where increasing hydraulic retention time increases its value all cases. The pH effect on ε is related to metabolic shifts and osmoregulation. Within the environmental conditions analyzed, ε ranges from 0.23 for a hydraulic retention time of 20 h and pH 4, to 0.42 for a hydraulic retention time of 8 h and a pH ranging from 7–8.5. The estimated values of ΔG_o are comparable to standard Gibbs energy of dissipation reported in the literature. For the data sets analyzed, ΔG_o ranges from –1210 kJ/mol$_x$, corresponding to a stirring velocity of 300 rpm, pH 6 and a hydraulic retention time of 6 h, to –20744 kJ/mol$_x$ for pH 4 and a hydraulic retention time of 20 h. For average conclusions, the combined approach based on standard Gibbs energy of formation and global stoichiometry, used in this thermodynamic analysis, allows for the estimation of Gibbs energy dissipation values from the extracellular medium compositions in acidogenic mixed cultures. Such estimated values are comparable to the standard Gibbs energy dissipation values reported in the literature. It is demonstrated that ε is affected by the environmental conditions, i.e., stirring velocity, hydraulic retention time and pH. However, a relationship that relates this parameter to environmental conditions was not found and will be the focus of further research.

Keywords: Anaerobic digestion, Biothermodynamics, Energy transfer efficiency, Gibbs energy dissipation

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1 Introduction

Anaerobic digestion processes are important for the treatment of urban and/or industrial organic wastes. Moreover, given the increasing interest in the effective use of natural resources nowadays, anaerobic digestion processes have become an important source for the recovery or production of bioenergy and/or chemical building blocks [1–3]. The bioenergy can be recovered as methane or hydrogen and among the chemical products, acetate, propionate, butyrate, formate, lactate and ethanol are produced. This products range gives a flexible character to these processes, and it is possible to maximize the productivity of any of the products. Anaerobic digestion (for waste treatment) is a mixed-culture fermentation, where the factors that determine product formation distribution under different environmental conditions are still unclear despite extensive research efforts. In order to obtain more insight into these processes, a global analysis of the energy transfer from the substrates to the products in acidogenic cultures (producing hydrogen and a mixture of chemicals) was performed based on thermodynamics, in order to allow one to study the optimization of the formation of products.

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As in any biotechnological process involving microbial cultures, biomass yield is one of the key parameters, since it determines the final biomass concentration reached after substrate consumption. Most of the mathematical models of biological systems use this parameter, which allows one to quantify substrate utilization, product formation and biomass generation [4], since it is important that these aspects are optimized in order to obtain reasonable productivities [5]. Prediction of biomass yield allows the optimization of biological processes using estimation methods, and this prediction requires a fundamental understanding of the phenomena controlling bacterial systems [4, 6]. Thermodynamics plays an important role in chemistry, chemical engineering and in chemical process development. The use of thermodynamic methods for the prediction of the true yield and stoichiometry of bacterial reactions has been widely applied in biotechnology [7]. However, these findings are sometimes very far from experimental results where many complicating factors including experimental errors, maintenance energy estimates, and simplifying assumptions, are present [6].

Microbial growth occurs objective and is a highly irreversible phenomenon. Therefore, it must be coupled with the production of entropy [6, 8]. Entropy may be exchanged with the environment due to heat transfer to or from the cell, and by exchanging products of higher entropy than substrates [5, 6]. The relationship between the driving force for microbial growth, ΔG_o (or dissipation energy), and the biomass yield, $Y_{x/s}$, is best understood by splitting the macrochemical reaction into catabolic and anabolic parts. The formation of biomass clearly produces matter with high Gibbs energy due to its low entropy content, and therefore, increases the Gibbs energy in the bioreactor. Thus, anabolic reactions alone are subjected to a driving force in the opposite sense of growth. In order to pull the anabolic reactions against this driving uphill force, they are coupled to catabolism, which is characterized by a large negative Gibbs energy change. The net driving force, ΔG_o, remaining for the whole process clearly depends on the stoichiometric load, anabolism has placed on catabolism, i.e., in the biomass yield [5]. Thus, the Gibbs energy lost due to the generation of entropy in the irreversible growth process is represented as a decrease in the energy available for synthesis [4]. In other words, this decrease would be treated as an efficiency of energy transfer, ε, from the substrates to the biomass. It has been widely hypothesized that energy transfer efficiency is not a constant, but instead, is controlled by environmental conditions. If this is true, the thermodynamic method for bacterial yield prediction will remain limited to cases when optimal conditions ensure predictable, high energy capture efficiencies. The next great challenge for bacterial yield prediction with thermodynamics is to understand how environmental conditions affect the energy transfer efficiency [5, 6].

The objective of this study is to describe acidogenic systems thermodynamically, using a combined approach resulting from the models of von Stockar et al. [8] and McCarty [9]. This approach is based on standard Gibbs energy of formation and global stoichiometry, and the only experimental measurements needed involve extracellular medium compositions.

2 Materials and Methods

2.1 Materials

Two different acidogenic (anaerobic) chemostat mixed culture reactor data sets were analyzed. The first are from the authors' laboratory (Aceves-Lara et al. [10]) and the second set are taken from Temudo et al. [11]. In the work of Aceves-Lara et al. [10], the aim was to determine the influences of pH, HRT, stirring velocity and their interactions on the hydrogen production by an acidogenic mixed culture at a constant temperature of 37 °C. The pH values were 5.5 and 6, HRT were 6 and 14 h, and the stirring velocity 150 and 300 rpm. Each operating condition was maintained until a steady state was reached.

The inoculum used was a sludge taken from an anaerobic methane digester fixed-bed reactor of wine distillery wastewater. A complex cultivation mixture including molasses resulting from sugar beet production was used as a carbon and energy source and diluted to a concentration of 9.4 g/L glucose equivalent by adding a nutritional medium rich in minerals.

The concentrations of acetic acid, propionic acid, butyric and iso-butyric acids, glucose, and biomass were measured in the liquid phase. The compositions of H_2 and CO_2 in the gas phase were also measured. All the measurements were performed after a steady state has been reached.

The experimental data taken from Aceves-Lara et al. [10] for use in this analysis are presented in Tab. 1.

In the work of Temudo et al. [11], in order to evaluate the impact of the pH on the product distribution during glucose fermentation by an undefined mixed culture, nine experiments were performed at pH values ranging from 4 to 8.5 and at a constant temperature of 30 °C. The experiments at pH 4, 4.75, 5 and 5.5 were performed at an HRT of 20 h, due to the difficulties in reaching a steady state at these pH values, while the experiments at pH 5.5, 6.25, 7, 7.75 and 8.5 were performed at an HRT of 8 h.

The inoculum consisted of a mixture of two sludges obtained from a distillery wastewater treatment plant and a sludge solution from a potato starch processing acidification tank. The reactor was freshly inoculated with 20 g of each inoculum before each experiment, and operated in batch mode until biomass growth was observed. A well-defined cultivation medium was used and it contained glucose as a carbon and energy source at a final concentration of 4.0 g/L and a solution of minerals as described in Temudo et al. [11].

The concentrations of glucose, acetic acid, propionic acid, butyric and iso-butyric acids, lactic acid, succinic acid, formic acid, glycerol, ethanol and biomass were determined in the liquid phase. The H_2 and CO_2 gas compositions were also measured. The data sets from Temudo et al. [11] used for the thermodynamic analysis are presented in Tab. 2.

Table 1. Experimental data set from Aceves-Lara et al. [10] utilized for the present thermodynamic analysis.

	Operational conditions								
	150 rpm				300 rpm				
	pH								
	5.5		6		5.5		6		
	HRT [h]								
	14	6	14	6	14	6	14	6	
Biomass	0.23	0.25	0.25	0.26	0.24	0.26	0.14	0.30	Y [mol/mol$_g$]
Acetic acid	1.06	0.68	1.36	1.00	1.10	0.61	0.67	1.18	
Propionic acid	0.06	0.03	0.08	0.07	0.10	0.13	0.03	0.06	
Butyric acid	0.41	0.53	0.32	0.40	0.35	0.42	0.39	0.44	
Glucose	2.1	2.1	2.1	2.1	2.1	2.1	2.1	2.1	Concentration [mM]
Acetic acid	55.2	35.5	70.8	52.3	57.5	31.8	34.7	61.7	
Propionic acid	3.4	1.5	4.2	3.6	5.3	6.6	1.5	3.0	
Butyric acid	21.4	27.8	16.6	20.7	18.4	22.0	20.3	23.1	
H^+	$3.2{\cdot}10^{-3}$	$3.2{\cdot}10^{-3}$	$1.0{\cdot}10^{-3}$	$1.0{\cdot}10^{-3}$	$3.2{\cdot}10^{-3}$	$3.2{\cdot}10^{-3}$	$1.0{\cdot}10^{-3}$	$1.0{\cdot}10^{-3}$	
CO_2	0.94	0.55	0.91	0.60	0.76	0.47	0.65	0.50	[atm]
H_2	0.06	0.45	0.01	0.40	0.24	0.53	0.35	0.50	

Table 2. Experimental data set from Temudo et al. [11] utilized for the present thermodynamic analysis.

	Operational conditions									
	HRT = 20 h				HRT = 8 h					
	pH									
	4	4.75	5	5.5	5.5	6.25	7	7.75	8.5	
Biomass	0.08	0.12	0.19	0.14	0.24	0.15	0.14	0.20	0.13	Y [mol/mol$_g$]
Acetic acid	0.42	0.45	0.36	0.36	0.40	0.62	0.70	0.56	0.68	
Propionic acid	0	0.05	0	0.04	0.03	0.02	0	0.04	0.03	
Butyric acid	0.58	0.42	0.55	0.66	0.52	0.04	0	0.18	0.03	
Formic acid	0	0.01	0.01	0.01	0.01	0.15	0.99	0.99	1.40	
Ethanol	0.02	0.09	0.14	0.05	0.03	0.64	0.69	0.55	0.58	
Lactic acid	0.06	0.16	0.05	0.02	0.01	0.01	0.01	0.10	0.06	
Succinic acid	0	0	0	0	0.01	0.08	0.11	0.10	0.06	
glycerol	0.18	0.12	0.06	0.14	0.09	0.14	0.06	0.05	0.17	
CO_2	1.28	1.40	1.66	1.50	1.19	1.22	0.55	0.62	0.56	
H_2	1.40	1.51	1.65	1.70	1.30	1.15	0.50	0.32	0.02	
Glucose	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	Concentration [mM]
Acetic acid	9.1	9.8	7.8	7.8	8.7	13.5	15.2	12.2	14.8	
Propionic acid	0	1.1	0	0.9	0.7	0.4	0	0.9	0.7	
Butyic acid	12.6	9.1	11.9	14.3	11.3	0.9	0	3.9	0.7	
Formic acid	0	0.2	0.2	0.2	0.2	3.3	21.5	21.5	30.4	
Ethanol	0.4	2.0	3.0	1.1	0.7	13.9	15.0	11.9	12.6	
Lactic acid	1.2	3.4	1.1	0.4	0.2	0.2	0.2	2.2	1.3	
Succinic acid	0	0	0	0	0.2	1.7	2.4	2.2	1.3	
Glycerol	3.9	2.6	1.3	3.0	2.0	3.0	1.3	1.1	3.7	
H^+	$1.0{\cdot}10^{-1}$	$1.8{\cdot}10^{-2}$	$1.0{\cdot}10^{-2}$	$3.2{\cdot}10^{-3}$	$3.2{\cdot}10^{-3}$	$5.6{\cdot}10^{-4}$	$1.0{\cdot}10^{-4}$	$1.8{\cdot}10^{-5}$	$3.2{\cdot}10^{-6}$	
CO_2	0.48	0.48	0.50	0.47	0.48	0.51	0.52	0.66	0.97	[atm]
H_2	0.52	0.52	0.50	0.53	0.52	0.49	0.48	0.34	0.03	

2.2 Methods

In order to thermodynamically analyze both experimental data sets, three different parts are differentiated, i.e., inputs, mathematical models and outputs, as depicted in Fig. 1. The inputs correspond to the experimental data concentrations, C_i, electron donor acceptor half-reaction stoichiometry, v_{ij}, and standard Gibbs energy of formation, $\Delta G_{f_i}°$. These inputs are combined into mathematical models to calculate the global yield coefficients, $Y_{i/s}$, standard Gibbs energy of reaction, $\Delta G_j°$, Gibbs energy of reaction at the actual compound concentrations, ΔG_j' and Gibbs energy of global catabolic, ΔG_e, and biosynthesis, ΔG_s, reactions. Two mathematical models are used in order to achieve this, i.e., von Stockar et al. [8, 12] and McCarty [9], and they are briefly explained in Sect. 2.2.2. Finally, the outputs are the energy transfer efficiency, ε, and the Gibbs energy of dissipation, ΔG_o.

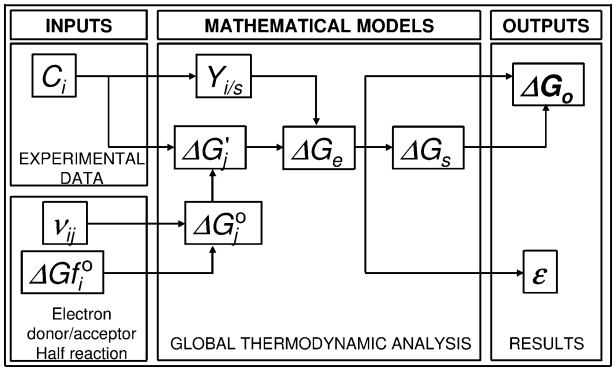

Figure 1. Simplified thermodynamic analysis diagram.

A further explanation of the construction of electron donor/acceptor half reactions and its stoichiometry is given in Sect. 2.2.1, together with the reactions utilized for both data sets. The calculation of $\Delta G_j°$, ΔG_j', ΔG_e, ΔG_s, ε and ΔG_o is also explained in Sect. 2.2.2.

2.2.1 Global Stoichiometry

The microbial heterotrophic metabolism can be simplified and represented as a global reaction that is the result of the combination of two processes, i.e., catabolism and anabolism. In catabolism, a substrate (an electron donor substrate) is converted into a product and metabolic energy is generated. In anabolism, the numerous biomass components are constructed from nutrients, i.e., carbon and nitrogen sources. Clearly, the electron and energy balances are inter-related [4, 13]. Uncoupling between the catabolism and anabolism can be established by the consumption of energy for non-growth related maintenance processes [13]. The catabolic and anabolic fluxes would be coupled by a stoichiometric relationship only in the case of neglect of energy consumption for maintenance purposes.

2.2.1.1 Catabolism

Catabolism, as outlined in Fig. 2, can be divided into pairs of electron donor and acceptor reactions. Half reactions for an electron donor and an electron acceptor can be combined to produce a global energy reaction with its associated Gibbs energy, ΔG_e. Half reactions for electron donor and cell synthesis can be combined to produce the synthesis reaction, from which the Gibbs energy for synthesis, ΔG_s, is derived. A global reaction for cell growth is obtained by combining the energy reaction and synthesis reaction (anabolism) in proper proportion. This proportion depends upon the energy transfer efficiency, ε, as explained by McCarty [9], and is represented by the experimental yield coefficients, $Y_{i/s}$.

2.2.1.2 Electron Donor/Acceptor Half Reactions

In order to balance the electron donor/acceptor equations, the electron balance, i.e., the reduction degree balance, γ, carbon balance and nitrogen balance are performed in this order. A reference oxidation state for each element of interest is arbitrarily defined to introduce the reduction degree of a compound, γ. In the present case, CO_2 is considered as a metabolically dependent product for carbon, CO_2 NH_3 and H_2O are considered as references. With this set of reference compounds and with the unit of the oxidation state defined as $\gamma_H = 1$, one

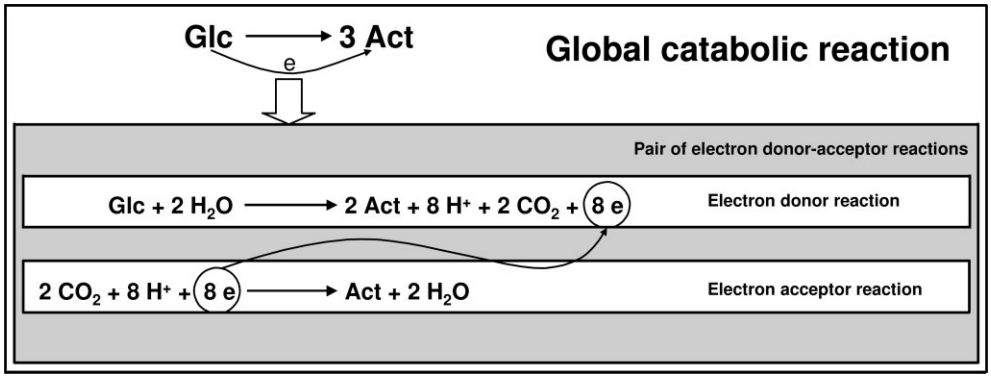

Figure 2. Simplified scheme for the global reaction.

obtains the following oxidation states for the three listed elements [14], i.e., $\gamma_C = 4$, $\gamma_N = -3$, and $\gamma_O = -2$.

Next, the degree of reduction of any compound taking place in this analysis can be calculated as the sum of the total reduction degree of each element in the compound as shown in Eq. (1):

$$\gamma_i = Cc_i\gamma_C + Nc_i\,\gamma_N + Oc_i\,\gamma_O + Hc_i\,\gamma_H \quad (1)$$

where Cc_i, Nc_i, Oc_i and Hc_i are the carbon, nitrogen, oxygen and hydrogen composition of compound *i*, respectively. Following this, for each electron donor-acceptor *j* half reaction, the electron balance is expressed as in Eq. (2):

$$\sum_i v_{ij}\gamma_i = -e_j \quad (2)$$

For each pair of electron donor-acceptor reactions, the electron balance must follow the relationship presented in Eq. (3):

$$e_{EA} = -e_d \quad (3)$$

The carbon and nitrogen balances follow Eqs. (4) and (5):

– Carbon balance:

$$\sum_i v_{ij}Cc_i = 0 \quad (4)$$

– Nitrogen balance:

$$\sum_i v_{ij}Nc_i = 0 \quad (5)$$

The electron donor-acceptor half reactions for both data sets used in this study are summarized in Tabs. 3 and 4. In the work of Aceves-Lara et al. [10] the electron acceptor compounds were acetic acid, propionic acid, butyric acid and hydrogen, while in the research of Temudo et al. [11], the electron acceptors were acetic acid, propionic acid, butyric acid, formic acid, ethanol, lactic acid, succinic acid, glycerol and hydrogen.

2.2.1.3 Biosynthesis Reaction

By utilizing a biomass composition of $C_5H_7O_2N$ [9], the biomass synthesis reaction was constructed as described by von Stockar et al. [8], using glucose as a carbon source and ammonia as a nitrogen source. This reaction is shown in Tab. 5, and was applied for the analysis of both sets of data.

2.2.2 Thermodynamic Analysis

2.2.2.1 Basis

The standard Gibbs energy changes, $\Delta G°$, associated with the partial reactions of energy metabolism have been calculated from the free energy of formation data, $\Delta G_{fi}°$, provided elsewhere [4, 9, 15] and the relationship with the stoichiometric coefficients, v_{ij}, as is shown in Eq. (6):

Table 3. Electron donor-acceptor half reactions from Aceves-Lara et al. [10].

		Aceves-Lara et al. [10]		
Electron donor	Acetic acid	Glc + $2H_2O$	→	2 Act + $8H^+$ + $2CO_2$ + 8e
	Propionic acid	Glc + $2H_2O$	→	Prn + $10H^+$ + $3CO_2$ + 10e
	Butyric acid	Glc	→	Btr + $4H^+$ + $2CO_2$ + 4e
	Hydrogen	Glc + $6H_2O$	→	$6H_2$ + $12H^+$ + $6CO_2$ + 12e
Electron acceptor	Acetic acid	$2CO_2$ + $8H^+$ + 8e	→	Act + $2H_2O$
	Propionic acid	$10H^+$ + $2.14CO_2$ + 10e	→	0.71Prn + $2.86H_2O$
	Butyric acid	$4H^+$ + $0.8CO_2$ + 4e	→	0.2Btr + 1.2 H_2O
	Hydrogen	$12H^+$ + 12e	→	$6H_2$

$$\Delta G_j^o = \sum_i^n v_{ij} \cdot \Delta Gf_i^o \quad (6)$$

$\Delta G°$ is the increment of free energy for the reaction under standard conditions, i.e., 298.15 K (25 °C) and a pressure of 1 atm. In aqueous solution, the standard condition of all solutes is 1 M and that of water is the pure liquid. $\Delta G_{fi}°$ refers to the standard free energy of formation of the substrates and the products from the elements [15].

However, under real conditions, the concentrations of substrates and products are different than 1 M and 1 atm. This is considered in $\Delta G'$, which is calculated using Eq. (7):

$$\Delta G_j' = \Delta G_j^o + R \cdot T^o \sum_i^n v_{ij}\, ln(C_i) \quad (7)$$

where C_i are the actual concentrations (in M for the aqueous phase, and in atm for the gas phase) of all compounds participating in the reaction, *R* is the universal gas constant and *T*° the standard temperature (298.15 K) [15]. The estimated $\Delta G'$ for both data sets are shown in Tabs. 6 and 7. All the Gibbs energy values are in kJ per mol of glucose consumed (kJ/mol_g).

2.2.2.2 Relationship between Thermodynamics and Biomass Yield

In anaerobic digestion, as in any living system, following the concepts of von Stockar et al. [8] and McCarty [9], the energy balance can be obtained, as shown in Eq. (8):

$$\Delta G_o = \frac{1}{Yx_{/s}}\left(\Delta G_e \frac{f_e}{f_s} + \Delta G_s\right) \quad (8)$$

given that the driving force for the growth, the Gibbs energy dissipation, ΔG_o [kJ/mol_x] must be equal to the energy re-

Table 4. Electron donor-acceptor half reactions from Temudo et al. [11].

	Temudo et al. [11]			
Electron donor	Acetic acid	$Glc + 2H_2O$	→	$2Act + 8H^+ + 2CO_2 + 8e$
	Propionic acid	$Glc + 2H_2O$	→	$Prn + 10H^+ + 3CO_2 + 10e$
	Butyric acid	Glc	→	$Btr + 4H^+ + 2CO_2 + 4e$
	Formic acid	$Glc + 6H_2O$	→	$Frm + 22H^+ 5CO_2 + 22e$
	Ethanol	$Glc + 3H_2O$	→	$EOH + 12H^+ + 4CO_2 + 12e$
	Lactic acid	$Glc + 3H_2O$	→	$Lct + 12H^+ + 3CO_2 + 12e$
	Succinic acid	$Glc + 2H_2O$	→	$Scn + 10H^+ 2CO_2 + 10e$
	Glycerol	$Glc + 3H_2O$	→	$GOH + 10H^+ + 3CO_2 + 10e$
	Hydrogen	$Glc + 6H_2O$	→	$12H^+ + 6H_2 + 6CO_2 + 12e$
Electron acceptor	Acetic acid	$8H^+ + 2CO_2 + 8e$	→	$Act + 2H_2O$
	Propionic acid	$10H^+ + 2.14CO_2 + 10e$	→	$0.71Prn + 2.85H_2O$
	Butyric acid	$4H^+ + 0.8CO_2 + 4e$	→	$0.2Btr + 1.2H_2O$
	Formic acid	$22H^+ + 11CO_2 + 22e$	→	$11Frm$
	Ethanol	$12H^+ + 2CO_2 + 12e$	→	$EOH + 3H_2O$
	Lactic acid	$12H^+ + 3CO_2 + 12e$	→	$Lct + 3H_2O$
	Succinic acid	$10H^+ + 2.86CO_2 + 10e$	→	$0.71Scn + 2.86H_2O$
	Glycerol	$10H^+ + 2.14CO_2 + 10e$	→	$0.71GOH + 2.14H_2O$
	Hydrogen	$12H^+ + 12e$	→	$6H_2$

Table 5. Biomass synthesis reaction from glucose.

Biomass synthesis	$Glc + 1.2NH_3$	→	$1.2X + 3.6H_2O$

leased from the energy source consumed, ΔG_e [kJ/mol$_g$] and the energy of the biomass synthesis, ΔG_s [kJ/mol$_g$]. However, by following the model presented by McCarty [9], one can reformulate Eq. (8) as shown in Eq. (9):

$$\varepsilon f_e \Delta G_e = -f_s \Delta G_s \tag{9}$$

This equation shows that the energy of biomass synthesis is equal to the energy released from the energy source multiplied by energy transfer efficiency, i.e., efficiency of energy captured by the organisms, ε, [6, 9]. In the scientific literature, this parameter is indeed considered (or fitted) as a constant [5, 6, 9]. However, as it will be demonstrated later, the analysis of experimental data obtained in the authors' laboratory [10] and in Temudo et al. [11] shows that ε should be better considered as a function of certain environmental variables, e.g., stirring velocity, HRT or pH.

2.2.2.3 *Equation for the Calculation of ε*

Global ΔG_e, as proposed by McCarty [9], is determined from the Gibbs energy change of the half electron donor, ΔG_d, and acceptor, ΔG_a, reactions, as in Eq. (10):

$$\Delta G_e = \Delta G_d + \Delta G_a \tag{10}$$

ΔG_s consists of two energy terms, i.e., one for the conversion of the electron donor to an intermediate compound, ΔG_{ic}, and another one for the conversion of the intermediate to cells, ΔG_{pc}, as in Eq. (11):

$$\Delta G_s = \frac{\Delta G_{ic}}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon} \tag{11}$$

Energy may be required to convert the cell carbon source to the intermediate compound, $\Delta G_{ic} > 0$, in which case $n = 1$, or it may be obtained from the conversion itself when $\Delta G_{ic} < 0$, in which case $n = -1$. The intermediate compound was taken to be acetyl-CoA with a half-reaction reduction potential, ΔG_{in}, of 30.9 kJ/eeq [9], as in Eq. (12):

$$\Delta G_{ic} = \Delta G_{in} + \Delta G_d \tag{12}$$

ΔG_{pc} is estimated from reported values of ATP in moles required for cell synthesis, and with an assumed cell relative composition of $C_5H_7O_2N$, and is set equal to 18.8 kJ/eeq when ammonia is the nitrogen source for cell synthesis [9]. It is then possible to calculate ε, i.e., if $\Delta G_{ic} < 0$ then $n = -1$, and

$$\varepsilon = \left(\frac{\Delta G_{pc}}{\Delta G_e \left[1 - \frac{\gamma_d}{\gamma_x \cdot Y_{x/s}} \right] - \Delta G_{ic}} \right)^{0.5} \tag{13}$$

However, if $\Delta G_{ic} > 1$, then $n = 1$, and

$$\varepsilon = \left(\frac{\Delta G_{pc} + \Delta G_{ic}}{\Delta G_e \left[1 - \frac{\gamma_d}{\gamma_x \cdot Y_{x/s}} \right]} \right)^{0.5} \tag{14}$$

Table 6. Estimated values of $\Delta G°$ and $\Delta G'$ from the data set of Aceves-Lara et al. [10].

			rpm = 150				rpm = 300			
			pH							
		Standard conditions	5.5	5.5	6	6	5.5	5.5	6	6
			HRT							
			14	6	14	6	14	6	14	6
			[kJ/mol$_g$]							
		$\Delta G°$	$\Delta G'$							
Electron donor	Acetic acid	−135.96	−386.47	−391.31	−408.22	−411.79	−387.32	−392.63	−413.43	−411.88
	Propionic acid	−152.58	−465.77	−471.79	−494.01	−497.45	−466.24	−469.25	−499.08	−499.32
	Butyric acid	−224.13	−344.23	−346.23	−356.44	−357.96	−345.66	−347.59	−357.60	−358.59
	Hydrogen	−25.87	−430.03	−408.04	−491.41	−442.74	−412.58	−407.94	−443.54	−442.13
Electron acceptor	Acetic acid	−55.05	189.22	190.78	212.83	214.14	190.37	191.29	212.72	215.45
	Propionic acid	−90.51	213.67	215.06	242.76	244.73	215.58	218.54	242.71	245.33
	Butyric acid	−39.65	84.13	85.33	95.49	96.42	84.48	85.52	96.26	96.84
	Hydrogen	0.00	334.86	364.83	342.46	397.33	355.48	367.27	395.34	400.64

Table 7. Estimated value of $\Delta G°$ and $\Delta G'$ from the data set of Temudo et al. [11].

			HRT = 20 h				HRT = 8 h				
		Standard conditions	pH								
			4.0	4.75	5.0	5.5	5.5	6.25	7.0	7.75	8.5
			[kJ/mol$_g$]								
		$\Delta G°$	$\Delta G'$								
Electron donor	Acetic acid	−135.96	−326.72	−360.58	−372.90	−396.07	−395.45	−427.15	−460.71	−494.92	−526.31
	Propionic acid	−152.58	−367.54	−427.21	−424.26	−470.77	−471.34	−514.60	−538.09	−596.65	−637.34
	Butyric acid	−224.13	−311.12	−329.00	−333.84	−345.13	−345.63	−368.74	−368.31	−398.03	−417.71
	Formic acid	17.45	−475.14	−590.14	−621.01	−684.64	−684.40	−770.94	−860.22	−951.54	−1040.14
	Ethanol	−130.43	−412.08	−459.65	−475.26	−512.73	−513.80	−556.85	−607.86	−657.51	−704.97
	Lactic acid	−72.13	−349.35	−398.17	−417.80	−454.82	−456.40	−507.21	−558.45	−602.40	−652.20
	Succinic acid	−143.52	−356.65	−399.42	−413.49	−442.36	−463.17	−500.46	−542.39	−584.29	−626.47
	Glycerol	−42.84	−271.55	−315.31	−330.98	−357.93	−358.88	−400.04	−444.82	−486.36	−523.30
	Hydrogen	−25.87	−301.64	−353.00	−370.10	−404.41	−404.38	−455.73	−507.12	−560.06	−639.81
Electron acceptor	Acetic acid	−55.05	119.62	154.00	164.66	187.82	187.99	222.95	257.41	289.96	322.80
	Propionic acid	−90.51	141.72	172.41	198.54	214.96	214.35	256.04	312.46	341.57	381.84
	Butyric acid	−39.65	50.97	67.92	73.68	85.31	85.16	100.86	121.45	135.36	150.84
	Formic acid	476.51	998.94	862.93	893.19	957.81	957.29	1123.28	1268.43	1356.33	1449.56
	Ethanol	−104.57	153.88	208.94	226.95	258.98	257.62	316.20	367.67	417.34	466.95
	Lactic acid	−46.27	216.60	270.41	284.41	316.89	315.02	365.84	417.08	472.44	519.71
	Succinic acid	−84.04	149.50	192.26	206.23	235.25	220.18	266.14	309.39	350.40	389.60
	Glycerol	−12.13	210.29	252.34	265.16	295.56	294.67	337.87	379.09	420.35	463.30
	Hydrogen	0.00	264.31	315.58	332.11	367.30	367.04	417.32	468.41	514.79	532.10

2.2.2.4 *Equation for Gibbs Energy Dissipation*

The Gibbs energy dissipation, ΔG_o, is calculated as in Eq. (8), with ΔG_e, ΔG_s and $Y_{x/s}$ are estimated from experimental data. For a detailed explanation of this methodology, readers can refer to the Appendix, where the procedure for the estimation of ΔG_e, ΔG_a, ΔG_d, ΔG_s, ε and ΔG_o is described and illustrated.

3 Results and Discussion

3.1 Influence of Environmental Conditions on ΔG_e and ΔG_s

The estimated values of ΔG_s and ΔG_e from the data sets of Aceves-Lara et al. [10] and Temudo et al. [11] are presented in Figs. 3 and 4, respectively. It is seen from both data sets that the environmental conditions have more influence on

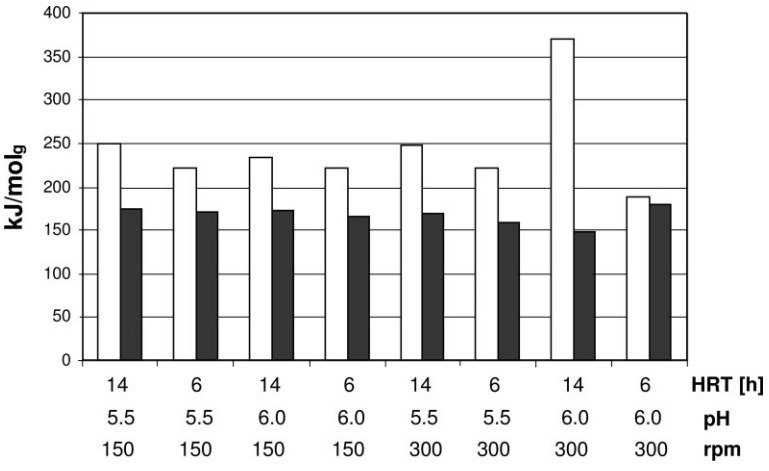

Figure 3. Estimated values of ΔG_s (white bars) and $-\Delta G_e$ (black bars) in kJ per mol of glucose [kJ/mol$_g$], under the environmental conditions studied by Aceves-Lara et al. [10].

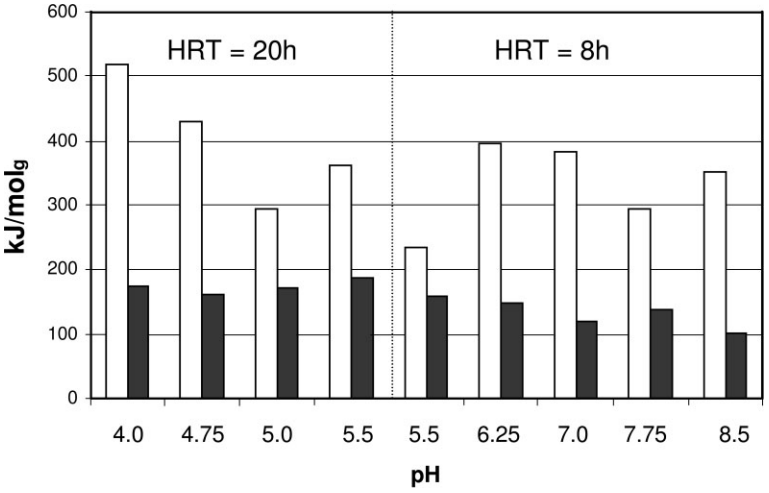

Figure 4. Estimated values of ΔG_s (white bars) and $-\Delta G_e$ (black bars) in kJ per mol of glucose [kJ/mol$_g$], under the environmental conditions studied by Temudo et al. [11].

ΔG_s than on ΔG_e. For Aceves et al. [10], ΔG_s ranges from 189 kJ/mol$_g$ at an HRT of 6 h, pH 6 and 300 rpm, to 370 kJ/mol$_g$ at an HRT of 14 h, pH 6 and 300 rpm, corresponding to an increase of 95 % from the lowest to the highest ΔG_s value. In the case of ΔG_e, the increase from the lowest to the higher values is only 21 %, corresponding to –149 kJ/mol$_g$ at an HRT of 14 h, pH 6 and 300 rpm, and –181 kJ/mol$_g$ at an HRT of 6 h, pH 6 and 300 rpm.

For the data of Temudo et al. [11] shown in Fig. 4, the increase on ΔG_s reaches 122 % between 234 kJ/mol$_g$ at an HRT of 8 h and pH 5.5, and 519 kJ/mol$_g$ at an HRT of 20 h and pH 4. ΔG_e increases by 85 % from –102 kJ/mol$_g$ at an HRT of 8 h and pH 8.5, and –189 kJ/mol$_g$ at an HRT of 20 h and pH 5.5.

3.2 Influence of Environmental Conditions on ε

The effect of environmental conditions on ε for the data set of Aceves-Lara et al. [10] is shown in Fig. 5. The increase of stirring velocity (in rpm), at pH 5.5 and an HRT of 14 h, is seen to lead to an increase of ε by 2.9 % from 150 to 300 rpm. A more pronounced increase, i.e., 11.4 %, is found at pH 5.5 and an HRT of 6 h for the same increase in rpm. In contrast, at pH 6, ε decreases on increasing the stirring velocity, i.e., at an HRT of 14 h, it decreases by 11.1 %, and at an HRT of 6 h, it decreases by 8.1 %. The reason for this influence is not clear, but it seems to be related to a combination between pH and stirring velocity effects.

The pH influence on ε is not clear either. At a stirring velocity of 150 rpm, the increase of pH from 5.5 to 6 results in an increase of ε by 2.9 % and 5.71 % for HRT values of 14 h and 6 h, respectively. But the same pH increase at a stirring velocity of 300 rpm results in a decrease of ε by 11.1 % and 12.8 % for HRT values of 14 h and 6 h, respectively.

In contrast to the stirring velocity and pH effects on ε, the influence of the HRT is more straightforward, and is clearly related to the maintenance energy consumption. An increase of HRT leads to a decrease in ε. The range of increment of ε is 0.16–7.3 % when the HRT decreases from 14 h to 6 h.

The effect of HRT and pH on ε from the data of Temudo et al. [11] is presented in Fig. 6. At an HRT of 20 h, ε is seen to have a maximum value of 0.33 at pH 5.0. For an HRT value of 8 h, ε reaches a maximum value of 0.42 in the pH range 7.0–8.5. The minimum value of ε (0.23) for pH 4.0 can be explained by a pH inhibition, and the decrease at pH 5.5 (HRT 20 h) is due to a change in metabolism shifting the product distribution from butyric acid, acetic acid and hydrogen (H_2) into acetic acid, ethanol and formic acid [11].

The results show that ε is not a constant and that it varies at the different environmental conditions studied. The influences of HRT and maintenance requirements in chemostat cultures are well known. Maintenance has been defined as "the energy consumed for functions other than the production of new cell material" [16]. This energy is related to the quantity of energy that is not transferred to the biomass production by ε, i.e., the uncoupling between catabolism and anabolism. As shown by Pirt [13], the effect of a maintenance requirement for the growth-limiting substrate increasing the value of HRT, i.e. the age of culture in a chemostat, will decrease the growth yield, and therefore, the value of ε. This phenomenon is well known and shown in Tabs. 1 and 2. Furthermore, the results demonstrate that the effect of pH on ε is related to shifts in metabolic pathways and osmoregulation as stated by van Bodegom [16], but since metabolic pathways

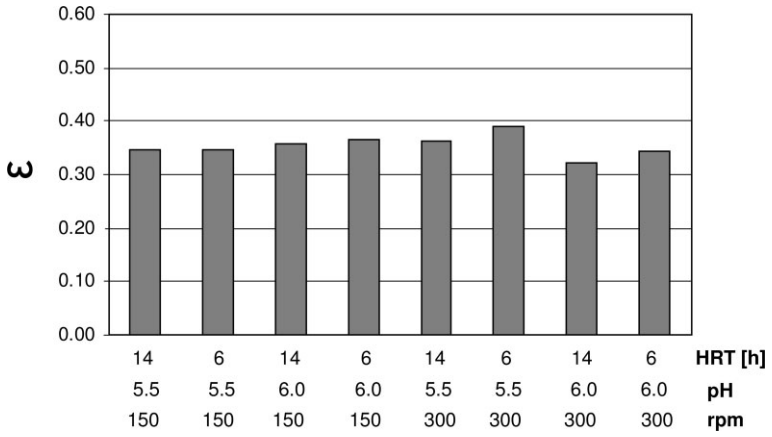

Figure 5. Estimated values of ε under the environmental conditions studied by Aceves-Lara et al. [10].

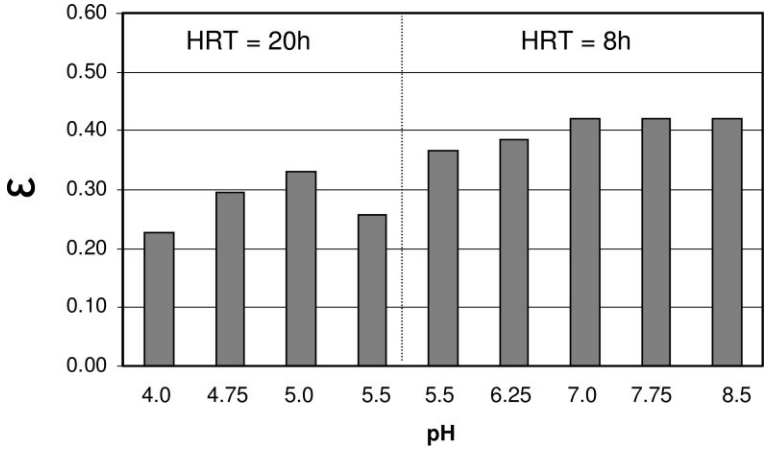

Figure 6. Estimated values of ε under the environmental conditions studied by Temudo et al. [11].

and transmembrane transport reactions are not taken into account in this analysis, it is difficult to make a decision concerning the influence of pH on ε. The effect of stirring velocity will be investigated in future experiments.

3.3 ΔG_o as a Function of $Y_{x/s}$

The glucose fraction for biomass synthesis, f_s, and the glucose fraction consumed for reaction energy, f_e, are linear functions of biomass yield, $Y_{x/s}$, as proposed by McCarty [9], Eqs. (15) and (16):

$$f_s = Y_{x/s}\frac{\gamma_x}{\gamma_d} \tag{15}$$

$$f_e = 1 - Y_{x/s}\frac{\gamma_x}{\gamma_d} \tag{16}$$

The slope of Eq. (15) corresponds to the ratio between the degrees of reduction of biomass, γ_x, and electron donor, γ_d, which are constants.

The estimated values of f_e and f_s as a function of experimental acidogenic biomass yield, $Y_{x/s}$, are shown in Fig. 7. The results agree with Eqs. (15) and (16), even though both data sets do not correspond to pure cultures. In contrast, they are from acidogenic mixed cultures with unknown microbial populations. The value of the slope from Fig. 7, i.e., 0.83, corresponds to the slope obtained with Eq. (15).

The values of ΔG_o estimated from both experimental data sets, and shown in Fig. 8, are comparable to the standard Gibbs energy dissipation for fermentative methanogenesis on acetate by *Methanosarcina barkeri* reported by von Stockar et al. [8]. Due to the fact the values of ΔG_o in the literature are given in kJ/c-mol$_x$, these values were multiplied by 5, since this is the carbon composition of 1 mol of biomass, as used in this analysis ($C_5H_7O_2N$). The standard Gibbs energy dissipation values from von Stockar range from –3000 to –5000 kJ/mol$_x$.

By using Eqs. (8), (15) and (16), one can analyze ΔG_o as a function of $Y_{x/s}$. The line in Fig. 8 corresponds to Eq. (8) using constant values of ΔG_e, i.e., –160 kJ/mol$_g$ and ΔG_s, i.e., 305 kJ/mol$_g$, which are the average of the estimated values shown in Figs. 3 and 4. The results show that decreasing the value of $Y_{x/s}$ results in larger growth driving force, ΔG_o. It is comparable to results shown in von Stockar et al. [8]

3.4 Relationship between ε and η

A new parameter is defined by von Stockar et al. [8], i.e., the growth efficiency, η. By combining the

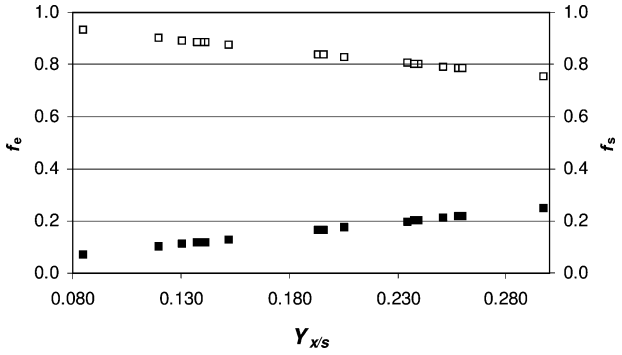

Figure 7. Estimated values of f_e (□) and f_s (■) versus experimental values of $Y_{x/s}$ from Aceves-Lara et al. [10] and Temudo et al. [11].

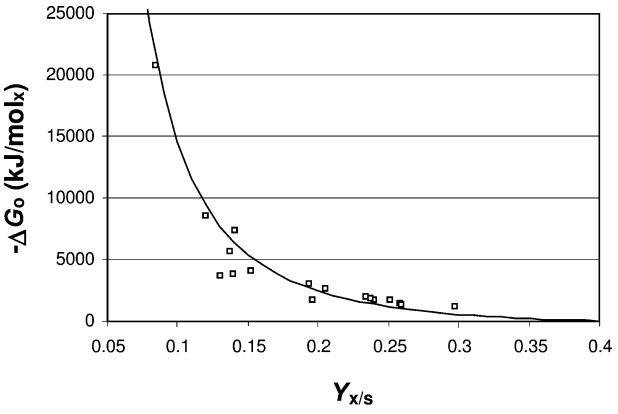

Figure 8. Gibbs energy dissipation [kJ/mol$_x$] as a function of $Y_{x/s}$. Squares (□) represent $-\Delta G_o$ estimated from experimental values of $Y_{x/s}$ used in this work. The line represents Eq. (8), where constant values for ΔG_e (–160 kJ/mol$_g$) and ΔG_s (305 kJ/mol$_g$), and the relationship between f_e, f_s and $Y_{x/s}$ derived from Fig. 7, are used.

approaches of von Stockar et al. [8] and McCarty [9], η can be redefined as in Eq. (17):

$$\eta = \frac{\Delta G_s}{\Delta G_e}\frac{f_s}{f_e}Y_{x/s} \tag{17}$$

Combining Eqs. (9) and (17) leads to a relationship between η, ε and $Y_{x/s}$, according to Eq. (18):

$$\eta = \varepsilon Y_{x/s} \tag{18}$$

From the estimation of η for each environmental condition analyzed, and by using the estimated values of ΔG_e, ΔG_s and $Y_{x/s}$, the results presented in Fig. 9, show a linear relationship between η and $Y_{x/s}$ where the slope, corresponding to ε, has a value of 0.38.

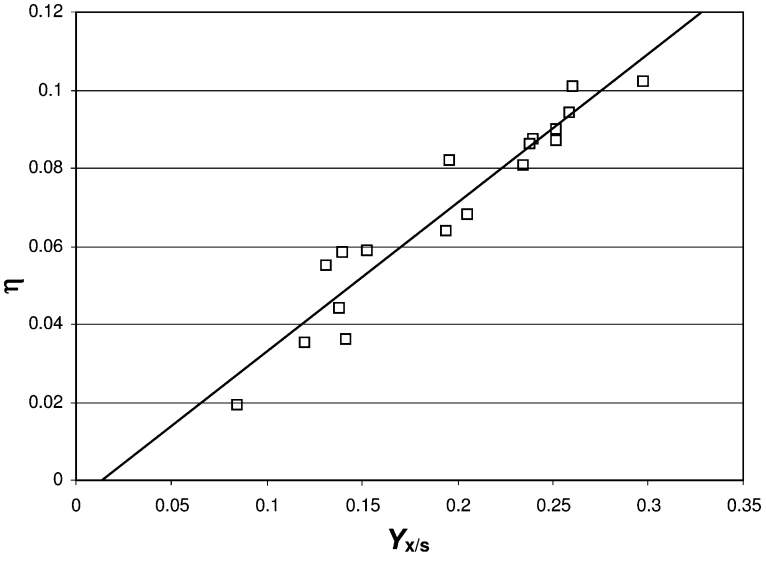

Figure 9. Values of η estimated using Eq. (15) as function of the estimated values of ΔG_e, ΔG_s, f_e, f_s and experimental values of $Y_{x/s}$.

4 Conclusions

The present thermodynamic analysis, which is a combination of two different approaches (from von Stockar et al. [8] and McCarty [9]), based on standard Gibbs energy of formation and global stoichiometry, allows the estimation of Gibbs energies dissipation, ΔG_o, in acidogenic mixed cultures from extracellular medium compositions, and does not require any heat measurement. The estimated values of ΔG_o in this study are comparable to standard Gibbs energy dissipation values reported in the literature. It was demonstrated that the energy transfer efficiency ε is affected by the environmental conditions, i.e., stirring velocity, hydraulic retention time and pH. The effect of hydraulic retention time on ε is related to the maintenance requirements in the cultures. The effect of pH on ε is related to shifts in the metabolic pathways and/or osmoregulation. The effect of stirring velocity is harder to analyze. However, a relationship that relates ε to the environmental conditions was not found and this will be the focus of further experimental studies. In contrast, the relationship found between the growth efficiency, η, and biomass yield, $Y_{x/s}$, predicts a constant value of ε, i.e., 0.38, for these acidogenic systems. Further research on acidogenic cultures will be based on thermodynamics together with detailed metabolic pathways and transmembrane transport models, in order to describe the influences of pH on ε, and to predict both biomass and products yields.

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Appendix

Case Study Example of the Thermodynamic Analysis Employed in this Study

In the following example, the methodology used for the estimation of ΔG_e, ΔG_a, ΔG_d, ΔG_s, ε and ΔG_o, is explained. The data used are from Aceves-Lara et al. [10] at the environmental conditions of 150 rpm, pH 5.5, and an HRT of 14 h. The substrate is glucose, and the products are biomass, acetic acid, propionic acid, butyric acid, CO_2 and H_2.

The $\Delta G°$ values (first column of ΔG values in Tab. 6) are estimated by Eq. (6) using the stoichiometry from Tab. 3 and the values of $\Delta G_f°$ from Thauer et al. [15]. Then, the $\Delta G'$ values (second column of the ΔG values in Tab. 6) are calculated

by Eq. (7), using the reactor's output concentrations (M) of glucose, acetic acid, propionic acid, butyric acid and protons, as well as CO_2 and H_2 partial pressures (atm).

The values of $Y_{i/s}$ (mol/mol$_g$) are calculated using the input and output concentrations of the substrate and products. From the stoichiometry in Tab. 3 and the $Y_{i/s}$ values, a new half-reaction stoichiometry is constructed. This new stoichiometry, presented in Tab. 8, represents the global growth stoichiometric reaction under the specific environmental conditions. The values of the global ΔG_e, ΔG_a, and ΔG_d, related to Eq. (10), are also presented in Tab. 8. The value of ε is then estimated by using Eq. (13) or Eq. (14). In this case, ε, has a value of 0.35. Following this, ΔG_s (248.95 kJ/mol$_g$) is estimated using Eq. (9), where the f_e and f_s values for glucose are represented by the rows K and L in Tab. 8, respectively. Finally, the value of ΔG_o (–2010.31 kJ/mol$_x$) is estimated by using Eq. (8).

Symbols used

ΔG_a	[kJ/mol]	Gibbs energy change of the electron acceptor half reaction
ΔG_d	[kJ/mol]	Gibbs energy change of the electron donor half reaction
ΔG_e	[kJ/mol]	Gibbs energy of catabolic reaction
$\Delta G_{f_i}°$	[kJ/mol]	Standard Gibbs energy of formation of compound i
ΔG_{ic}	[kJ/eeq]	Gibbs energy of the conversion of the electron donor to acetyl-CoA
ΔG_{in}	[kJ/eeq]	Gibbs energy of acetyl-CoA reduction
$\Delta G_j'$	[kJ/mol]	Gibbs energy of reaction j at the actual compound concentrations
$\Delta G_j°$	[kJ/mol]	Standard Gibbs energy of reaction
ΔG_o	[kJ/mol]$_x$	Gibbs energy of overall growth reaction per mol of dry biomass grown
ΔG_{pc}	[kJ/eeq]	Gibbs energy of the conversion of acetyl-CoA to biomass
ΔG_s	[kJ/mol]	Gibbs energy of anabolic reaction
C_{Ci}	[mol C/mol]	carbon content of compound i
C_i	[mol/L]	concentration of compound i
e	[–]	electron
e_{EA}	[–]	electrons from an electron acceptor reaction
e_d	[–]	electrons from an electron donor reaction
e_j	[–]	electrons participating in reaction j
f_e	[mol glucose/mol total glucose]	fraction of glucose used for energy reactions
f_s	[mol glucose/mol total glucose]	fraction of glucose used converted for biomass synthesis
H_{Ci}	[mol H/mol]	hydrogen content of the i compound
N_{Ci}	[mol N/mol]	nitrogen content of the i compound
O_{Ci}	[mol O/mol]	oxygen content of the i compound
X	[–]	biomass
$Y_{i/s}$	[mol biomass/mol substrate]	yield of i compound from electron donor substrate
$Y_{x/s}$	[mol biomass/mol substrate]	yield of biomass from electron donor substrate

Greek symbols

γ	[eeq/mol]	degree of reduction
γ_d	[eeq/mol]	degree of reduction of electron donor
γ_x	[eeq/mol]	degree of reduction of biomass
ε	[–]	energy transfer efficiency
η	[–]	growth efficiency
ν	[–]	stoichiometric coefficient

Abbreviations and Chemical Formulae

Act	acetic acid
Btr	butyric acid
EOH	ethanol

Table 8. New global stoichiometric coefficients for the experimental data from Aceves-Lara et al. [10] under the environmental conditions of 150 rpm, pH 5.5, and HRT 14 h.

				[mol/mol$_g$]									
				Glucose	Biomass	Acetic acid	Propionic acid	Butyic acid	CO_2	H_2O	H_2	Electrons	$\Delta G'$ [kJ/mol$_g$]
Electron donor		A	acetate	−0.35	0.00	0.71	0.00	0.00	0.71	−0.71	0.00	2.82	−136.45
		B	propionate	−0.04	0.00	0.00	0.04	0.00	0.11	−0.08	0.00	0.38	−17.62
		C	butyrate	−0.34	0.00	0.00	0.00	0.34	0.68	0.00	0.00	1.37	−117.67
		D	Hydrogen	−0.07	0.00	0.00	0.00	0.00	0.43	−0.43	0.43	0.86	−30.91
Electron aceptor		E	acetate	0.00	0.00	0.35	0.00	0.00	−0.71	0.71	0.00	−2.82	66.81
		F	propionate	0.00	0.00	0.00	0.03	0.00	−0.08	0.11	0.00	−0.38	8.08
		G	butyrate	0.00	0.00	0.00	0.00	0.07	−0.27	0.41	0.00	−1.37	28.76
		H	Hydrogen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.43	−0.86	24.07
Global electron donor	A+B+C+D	I		−0.80	0.00	0.71	0.04	0.34	1.93	−1.21	0.43	5.43	−302.65 (ΔG_d)
Global electon acceptor	E+F+G+H	J		0.00	0.00	0.35	0.03	0.07	−1.06	1.22	0.43	−5.43	127.72 (ΔG_a)
Global energy	I+J	K		−0.80	0.00	1.06	0.06	0.41	0.87	0.01	0.86	5.43	−174.93 (ΔG_e)
Synthesis		L		−0.20	0.23	0.00	0.00	0.00	0.00	0.70	0.00	0.00	
Global stoichiometry	K+L			−1.00	0.23	1.06	0.06	0.41	0.87	0.71	0.86	5.43	

Frm	formic acid
Glc	glucose
GOH	glycerol
Lct	lactic acid
Prn	propionic acid
Scn	succinic acid

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