Review

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Thermodynamics-based design of microbial cell factories for anaerobic product formation

Hugo F. Cueto-Rojas, A.J.A. van Maris, S. Aljoscha Wahl, and J.J. Heijnen

Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

The field of metabolic engineering has delivered new microbial cell factories and processes for the production of different compounds including biofuels, (di)carboxylic acids, alcohols, and amino acids. Most of these processes are aerobic, with few exceptions (e.g., alcoholic fermentation), and attention is focused on assembling a high-flux product pathway with a production limit usually set by the oxygen transfer rate. By contrast, anaerobic product synthesis offers significant benefits compared to aerobic systems: higher yields, less heat generation, reduced biomass production, and lower mechanical energy input, which can significantly reduce production costs. Using simple thermodynamic calculations, we demonstrate that many products can theoretically be produced under anaerobic conditions using several conventional and non-conventional substrates.

Anaerobic production of bio-based chemicals

In the past years the use of renewable sources for production of chemicals has gained significant notoriety [1–3], particularly since the US Department of Energy (DOE) published a list of chemicals that are relevant for the transition to a bio-based economy (Table 1) [4,5]. Design of cost-effective processes demands maximization of product titer, production rate, and yield, and minimization of waste streams and both fixed and variable costs [6]. These imperatives encourage the development of anaerobic processes as opposed to aerobic cultivations. Most notably, energy requirements for mixing, aeration, and heat removal are significantly reduced using anaerobic methods, and the production rate, titer, and yield are increased.

This review highlights a general approach to assessing the feasibility of anaerobic substrate-to-product conversions based on basic thermodynamic calculations. The proposed thermodynamic analysis requires minimum input data, and allows a quick and simple feasibility check of the anaerobic process taking into account different substrates and full-scale process conditions. Special attention is given to the DOE list of key compounds and some relevant amino acids (Table 1).

Choosing the right substrate

The choice of a suitable substrate for production of bio-based chemicals under anaerobic conditions is a crucial first step because it will determine important features of the process, especially if the substrate is not glucose, which is widely used. Depending on the type of biorefinery, different feedstocks may be used as fermentation substrates [7].

First-generation substrates

Carbohydrates and lipids can be obtained from crops such as sugar cane, palm trees, corn, and others. These feedstocks are advantageous to the fermentation process because high substrate concentrations are possible. This facilitates a minimal water requirement, allowing the achievement of higher product concentrations. The disadvantage of these feedstocks, which is widely discussed, is competition for land used for food production [8,9].

Second-generation substrates

These substrates are usually rich in C_6 and C_5 sugars, and lignocellulosic molecules [10]. The advantage of these substrates is that they reduce competition with food crops. Their main disadvantage is that they require expensive pretreatment processes to obtain fermentable C_6 and C_5

Glossary

γ Value: the degree of reduction of a molecule, describes the electron content of a chemical compound based on the biological frame of reference where water, CO_2, protons, NH_4^+, and SO_4^{-2} have a $\gamma = 0$.

$\Delta_e G^{0'}{}_P$ or $\Delta_e G^{0'}{}_S$: Gibbs free energy content per electron of a product or substrate, respectively (kJ/mol e^-); derived from half-redox reaction using the biological frame of reference; where H_2O, CO_2, H^+, NH_4^+, and SO_4^{-2} have a $\Delta_f G = 0$ kJ/mol under standard conditions.

$\Delta_f G^{0'}$ and $\Delta_f H^{0'}$: standard Gibbs free energy and enthalpy of formation (kJ/mol), that relate to a frame of reference where all elements have $\Delta_f G = 0$ kJ/mol and $\Delta_f H = 0$ kJ/mol under standard conditions: pH = 7, T = 298 K, partial pressure of 1 bar for all gaseous compounds, and concentrations of 1 mol/L for all dissolved compounds.

$\Delta_{bb} G^{0'}$: Gibbs free energy of formation under the biological frame of reference, where H_2O, CO_2, H^+, NH_4^+, and SO_4^{-2} have a $\Delta_f G = 0$ kJ/mol under standard conditions (kJ/mol).

$\Delta_r G^{0'}$, $\Delta_r H^{0'}$, $\Delta_r S^{0'}$: Gibbs free energy, enthalpy, and entropy of the product reaction (kJ/$mol_{Product}$). Calculated from the reaction stoichiometry and $\Delta_f G^{0'}$, $\Delta_f H^{0'}$, and $\Delta S^{0'}$ values of all compounds involved in the product reaction under standard conditions.

Corresponding authors: Wahl, S.A. (S.A.Wahl@tudelft.nl); Heijnen, J.J. (J.J.Heijnen@tudelft.nl).

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Table 1. Key compounds that can be produced from biomass [4,5,61]

Compound	Chemical formula (γ)	$\Delta_e G^{0'}$ (kJ/mol)	Potential uses of derivatives
Carbon monoxide	CO (2)	49.7188 ± 0.4330	Fuels, synthesis precursors, others
3-Hydroxypropionic acid	$C_3H_6O_3$ (12)	32.5994 ± 0.2165	Fibers, absorbent polymers
Propionic acid	$C_3H_6O_2$ (14)	27.8648 ± 0.2585	Building block
3-Hydroxybutyrolactone	$C_4H_6O_3$ (16)	37.2073 ± 0.2706	Pharmaceuticals, solvents and fibers
Fumaric acid	$C_4H_4O_4$ (12)	35.4060 ± 0.3805	Solvents, fibers and water-soluble polymers
Succinic acid	$C_4H_6O_4$ (14)	29.9962 ± 0.5533	Solvents, fibers and water-soluble polymers
Arabitol	$C_5H_{12}O_5$ (22)	39.9152 ± 0.6223	Sweeteners, new polymers, antifreeze fluids
Furfural	$C_5H_4O_2$ (20)	41.1388 ± 0.4138	Building block
Itaconic acid	$C_5H_6O_4$ (18)	34.8205 ± 0.2992	Solvents, copolymers
Levulinic acid	$C_5H_8O_3$ (22)	30.9370 ± 0.2756	Fuels, solvents, catalysts, polymers
Xylitol	$C_5H_{12}O_5$ (22)	39.8023 ± 0.3036	Sweeteners, new polymers, antifreeze fluids
Xylonic acid	$C_5H_{10}O_6$ (18)	41.6261 ± 0.1964	Building block
2,5-Furan dicarboxylic acid (FDCA)	$C_6H_4O_5$ (18)	29.7816 ± 0.3368	PET analogs, new polyesters, polyamides, and nylons
Glucaric acid	$C_6H_9O_8$ (18)	42.8509 ± 0.2640	Solvents and nylons
Gluconic acid	$C_6H_{12}O_7$ (22)	40.4469 ± 0.2114	Building block
Levoglucosan	$C_6H_{10}O_5$ (24)	37.8972 ± 0.2526	Building block
Lysine	$C_6H_{14}N_2O_2$ (28)	31.1489 ± 0.3209	Feed and food, building block
Alanine[a]	$C_3H_7NO_2$ (12)	32.0038 ± 0.3775	Feed and food
Valine[a]	$C_5H_{11}NO_2$ (24)	29.7322 ± 0.3493	Feed and food

[a]Included as interesting additional products; these are analyzed in more detail in the examples found in the text boxes.

sugars diluted in aqueous solutions, which leads to low product titers and high downstream processing costs. In addition, pretreatment processing produces a wide variety of molecules including toxic compounds such as furfural [11,12].

Third-generation (water-free) substrates

Other potential (non-conventional) substrates are biogas, synthesis gas (H_2/CO) obtained from biomass [11], ethanol, methanol, or glycerol waste from biodiesel and ethanol production. Ethanol might be an attractive non-conventional substrate because it is cheap and can be obtained from second-generation substrates. The advantage of these substrates is that they do not contain water, and this increases product titers and decreases downstream processing costs. By contrast, these substrates will impose important challenges, for instance gaseous substrates require efficient gas-to-liquid mass transfer [13].

Thermodynamic analysis of anaerobic product reactions under standard conditions

For each substrate and product, $\Delta_f G^{0'}$ [14–16] and $\Delta_f H^{0'}$ [15,17] (see Glossary) can be obtained from databases [16,18] (http://webbook.nist.gov) or from calculations using methods reported in literature [19,20], for example group contribution [19]. For a complete list with the standard thermodynamic properties of relevant substrates and products see Table S1 in the supplementary material online.

Given that water, CO_2, protons, N-source (NH_4^+ in most cases) and sulfate (SO_4^{2-}) are found universally in anaerobic substrate-to-product reactions, it is useful to employ a different frame of reference; this frame of reference will be called the anaerobic biological reference, indicated by the subscript *fb* [15]. $\Delta_{fb} G^{0'}$ for each organic compound (substrate or product) is calculated by setting up a redox half-reaction as shown in Equation 1 using 1 mole of compound and the reference components and elements discussed previously:

$$-1 \cdot Compound + \upsilon_{CO_{2(g)}} \cdot CO_{2(g)} + \upsilon_{H_2O_{(l)}} \cdot H_2O_{(l)} + \upsilon_{H^+_{(aq)}} \cdot H^+_{(aq)} + \upsilon_{NH^+_{4(aq)}} \cdot NH^+_{4(aq)} + \upsilon_{SO^{-2}_{4(aq)}} \cdot SO^{-2}_{4(aq)} + \gamma \cdot electron^{-1} = 0 \quad [1]$$

In Equation 1 the six unknown stoichiometric coefficients are calculated by setting up the five elements (C, H, O, N, S) and charge balances. Note that the stoichiometric coefficient of electrons is, by definition, equal to γ [21]. $\Delta_{fb} G^{0'}$ is now defined as the opposite of the Gibbs free energy of the redox half-reaction (1), calculated from the standard Gibbs free energy of formation of each component as shown in Equation 2:

$$\Delta_{fb} G^{0'}_{compound} = \Delta_f G^{0'}_{compound} - (\upsilon_{CO_{2(g)}} \cdot \Delta_f G^{0'}_{CO_{2(g)}} + \upsilon_{H_2O_{(l)}} \cdot \Delta_f G^{0'}_{H_2O_{(l)}} + \upsilon_{H^+_{(aq)}} \cdot \Delta_f G^{0'}_{H^+_{(aq)}} + \upsilon_{NH^+_{4(aq)}} \cdot \Delta_f G^{0'}_{NH^+_{4(aq)}} + \upsilon_{SO^{-2}_{4(aq)}} \cdot \Delta_f G^{0'}_{SO^{-2}_{4(aq)}} + \gamma \cdot \Delta_f G^{0'}_{electron^-}) \quad [2]$$

Because of this definition, the values of $\Delta_{fb} G^{0'}$ for CO_2, H_2O, H^+, NH_4^+, SO_4^{2-} and electrons become zero. Under anaerobic conditions, no external electron acceptors are present, and consequently all electrons from the substrate will be found in the product. Because of this link, as shown in Equation 3, it is useful to define $\Delta_e G^{0'}{}_{Compound}$ [22]:

$$\Delta_e G^{0'}_{compound} = \frac{\Delta_{fb} G^{0'}_{compound}}{\gamma_{compound}} \quad [3]$$

Note that $\Delta_e G^{0'}$ is related to the redox potential using Faraday's constant; γ and $\Delta_e G^{0'}$ values for the DOE list of products were calculated (Table 1).