

Crystal ball – 2011

In this feature, leading researchers in the field of microbial biotechnology speculate on the technical and conceptual developments that will drive innovative research and open new vistas over the next few years.

Bioproducts from undefined mixed cultures: electron pushing

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A thermodynamic state analysis can inform the bioprocess engineer if a biological redox reaction is energetically feasible. This understanding is especially pertinent in complex systems, such as undefined mixed cultures that convert organic wastes, as a result of the many possible pathways that could operate in sequence or in parallel (Angenent *et al.*, 2004; Kleerebezem and van Loosdrecht, 2007; Agler *et al.*, 2011). In theory, the bioprocessing of organic substrates in an open microbial community-based process will result in the production of the end-product with the lowest free energy content per electron. This state can be considered as the thermodynamic equilibrium state and guarantees a maximum amount of free energy to be harvested by the microbial community catalysing the process (Hanselmann, 1991). In the presence of a strong electron acceptor, such as oxygen, the end-product of organic carbon conversion is carbon dioxide (0 kJ e-mol⁻¹, reference state) via complete mineralization. In the absence of an electron acceptor or external energy source, the end-product is methane (–23.0 kJ e-mol⁻¹). Combined with the *in situ* product separation of a gaseous end-product, these thermodynamic properties provide a firm basis for the anaerobic digestion process. Full-scale anaerobic digesters are, therefore, widely used to treat a wide range of solid substrates and wastewaters and the generated biogas is used for heat and electric power production – of note are the ~30 million domestic digesters in China. Still, despite these intrinsic advantages, methane is not the most valuable end-product because as a gas it has a lower energy density and is harder to store/transport than a liquid fuel. For this reason, bioprocess engineers are now looking for ways to produce more valuable liquid fuels or chemical building blocks from organic residues.

To produce a product that is more valuable than methane under anaerobic conditions with undefined mixed cultures, methanogenesis should be prevented. If acetoclastic methanogens are inhibited by, for example, lowering the pH from ~7 to ~5.8, acetate (–26.9 kJ e-mol⁻¹) accumulates because hydrogenotrophic methanogens still maintain low hydrogen partial pressures, allowing for anaerobic oxidation of intermediately formed short-chain carboxylates and ethanol. A further decrease in pH will inhibit hydrogenotrophic methanogens as well, and will shift the fermentation end-product spectrum to a mixture of carboxylates (e.g. propionate: –27.0 kJ e-mol⁻¹; and butyrate: –27.1 kJ e-mol⁻¹) and ethanol (–30.5 kJ e-mol⁻¹). At longer cell residence times, n-butyrate is often found at high relative ratios within the fermentation product mixture. At shorter residence times and with readily degradable substrates, lactate (–31.6 kJ e-mol⁻¹) dominates in some cases.

The environmental conditions in the reactor are such that these fermentation end-products, such as n-butyrate and lactate, cannot be further oxidized anaerobically. The production of short-chain carboxylates at relatively low concentrations (< 50 g l⁻¹), however, is not very attractive because it requires major efforts to recover them from the fermentation broth. The future quest in development of bioprocesses for fuel or building blocks should, therefore, aim at products that require a limited effort for product recovery. To date, *in situ* product separation has been accomplished by generating intracellular storage polymers (polyhydroxyalkanoates) after aerobic conversion of carboxylates, but new anaerobic production pathways are currently under investigation as well. These novel production routes reduce short-chain carboxylates to alcohols or medium/long chain fatty acids that can be separated from the fermentation broth by precipitation, distillation, or by concentration in an organic solvent. We believe that the reduction of carboxylates (also called biohydrogenation or chain elongation) by pushing electrons into the undefined mixed culture offers a prosperous route.

In situ upgrading of fermentation end-products by pushing with external reducing power (electrons) is envisioned to produce:

- (i) a single main end-product rather than a mixture; and
- (ii) a compound that can be separated from the fermentation broth.

Recently, a ground-breaking study by Steinbusch and colleagues (2010) demonstrated the production of

n-caproate [i.e. hexanoate ($-27.2 \text{ kJ e-mol}^{-1}$)] with an undefined mixed culture under anaerobic conditions after complete inhibition of methanogenesis with an antibiotic compound. The microbial communities catalysed a biological two-carbon chain-elongation reaction with n-butyrate to form n-caproate, using electrons from externally supplied ethanol that was oxidized to acetate. Importantly, this C6 carboxylate is relatively easy to separate from water because of its maximum solubility of $\sim 10 \text{ g l}^{-1}$ at 30°C . To generate a valuable fuel, the extracted n-caproate with an energy density of -144.8 kJ C^{-1} , should be further upgraded to, for example, n-hexanol with an energy density similar to ethanol and n-butanol (-182.7 and -171.4 kJ C^{-1}); or even better alkanes (-178.6 kJ C^{-1}), by additional external reactions.

Future research should focus on different methods to accomplish electron pushing: electrons can be supplied in many forms other than ethanol, such as by adding synthesis gas (syngas: mainly hydrogen and carbon monoxide); or directly at the cathode in bioelectrochemical systems. Particularly interesting of the latter system is that energy levels at which electrons are introduced can be manipulated by the power supply, or that hydrogen gas can be generated within the bioprocess at the cathode. Recently, researchers have demonstrated that organic molecules can be produced from carbon dioxide in a biocathodic compartment, whereas the electrons were generated from organic waste oxidation in the bioanodic compartment (Clauwaert *et al.*, 2008).

Thermodynamic estimates can help to predict which chemicals may accumulate when an external electron donor is supplied. Molecular hydrogen is a stronger electron donor ($-40 \text{ kJ e-mol}^{-1}$) than most (but not all) organic compounds, and therefore the net driving force in the system still aims for production of organic carbon in its most reduced form (methane). Upon selective inhibition of methanogenesis, as suggested above, there is a driving force for the production of other strongly reduced organic molecules. We also want to point out that, most likely, the production of strongly reduced compounds will be stimulated by carbon limited operational conditions. In addition, the end-product composition will depend strongly on the microbial community structure in the system and the extent to which the microbes are capable of harvesting the small amounts of energy available for the electron pushing reactions. Classical tools can be used to drive the process in a required product direction, such as bioaugmentation, adaptive evolution or selecting specific process conditions. Finally, ecology theory should be applied to synthesize communities that are stable to perturbations. After accomplishing these tasks successfully, we predict that undefined mixed cultures (carboxylate platform) will be integrated within a biorefinery concept with other platforms, such as sugar platform (ethanol), syngas

platform (hydrogen and carbon monoxide) or renewable electricity platform (electrons) to generate bioproducts that can be upgraded further to bulk chemicals or liquid fuels.

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Looking through the crystal ball: where will our next generation drugs come from?

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Starting from recombinant DNA and production of industrial quantities of human insulin by gene cloning in *Escherichia coli* to the present day synthetic biology through chemical synthesis of a complete genome and introducing it in specific *Mycoplasma* strains or the role of gut microflora in obesity and other modern day illnesses, microbial biotechnology has come a long way. A classical example of the role of soil microorganisms in alleviating diseases is the production of antibiotics giving rise to a huge anti-infective industry. With the decline in the search for new antibiotics, and with the emergence of new infectious agents, or even aggressive forms of cancer, it is imperative that we seek help from bacteria, particularly the difficult to treat pathogenic bacteria causing chronic infections and having long-term residence in the human body, for fighting the life-threatening diseases.

Take for example the case of cancers, HIV/AIDS, malaria or tuberculosis, for which, in spite of years of research, no good drugs or vaccines exist. This is because these disease agents are very smart and quickly change the target(s) for the drugs or the epitope(s) for vaccines. The current technology used by the pharmaceutical industry is to rationally design inhibitors as drugs