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Old obstacles and new horizons for microbial chemical production

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Microorganisms appear as ideal catalysts for chemical conversions. Diverse metabolic routes seem to open doors to the whole range of chemistry. Indeed, a vast amount of scientific papers suggesting new microbial cell factories for old and new products is published every year. However, only very few of them reached industrial relevance. Chemical balances and some metabolic tricks allow natural microorganisms the efficient production of some chemicals, but not others. So first of all it is important to choose metabolically feasible products of value for synthetic chemistry. Here we see a clear task for the chemical and biotechnology industries to communicate for defining the right target molecules. Finally, despite our limited current knowledge, synthetic biology points to a future independent from natural strain backgrounds.

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Introduction

Commodity organic chemicals pervade our daily life. They are produced on a very large scale to satisfy global markets. Before the petrochemical era, microbial processes contributed significantly to chemical synthetic processes. However, with the development of the petro industry after 1950, microorganisms lost their valuable position. Petrochemistry was and is more economical, so petroleum is still the principal raw material today. The huge variety of the obtained useful products have enabled to better tailor the quality of life. However, the lack of sustainability of products based on petroleum gets more and more into the center of our attention. Simply aimed to alternative productions first and to reduce the dependency from petroleum later, the concept of carbohydrate and renewable biomass use to produce chemicals has been proposed.

Microorganisms appear as ideal catalysts, given their efficient ability to transform molecules from organic to inorganic and from complex to simple state, and *vice versa*. Strikingly, only few carbon sources are recalcitrant for microorganisms, opening up a scenario of many possible transformations, which can match our need for alternative sources.

Among the first industrialized microbial commodity productions from sugar was citric acid. Since 1919 this commodity is industrially produced by the filamentous fungus *Aspergillus niger* [1,2]. Since then, but particularly in recent years, a vast amount of publications and patents related to the production of commodities from microbial cell factories appeared [3–5]. However, only very few products have really been commercialized up to now.

Few microbial products are on the market: where are the obstacles?

The constraints for creating a viable business with the production of commodities with a selling price lower than 2 Euro/kg starting from renewable resources are tight. In order to establish an economically viable industrial process the classical parameters to fulfill are titer, yield and productivity [6]. Titer (product concentration in the fermentation broth) is important to keep the purification costs as low as possible. Yield (product per substrate ratio) is important to gain sufficient revenue from the invested substrate costs. Productivity (product per bioreactor volume and time) relates to the size of the bioreactors and therefore to the investment costs. Traditionally, to achieve useful values of these parameters the focus for strain development was strictly on the metabolic pathway responsible for the production [7]. Rational engineering was focused on overexpressing genes for enzymes involved in the pathway and knocking out genes responsible for competing reactions (to abolish by-product formation). However, in most cases this is not sufficient to come even close to industrial relevance. Many projects arrive at a proof-of-principle stage, showing that a substance can be produced microbially at all. The final jump over the gap to industrial reality is rarely successful.

Table 1

Main metabolic features constituting successful microbial production processes

Metabolic feature	Significance
Thermodynamic feasibility	Thermodynamics is ultimately decisive if a chemical reaction or metabolic pathway is possible. Single reaction steps can be driven uphill by coupling them to energy release, but inevitably this comes at a cost
Stoichiometric balance	The theoretical stoichiometric yield — especially of carbon atoms — defines the potential economical feasibility of a production process
Redox balance	If substrate and product have a different degree of reduction re-equilibration of the electron balance leads either to by-product formation or energy overflow
Energy balance	If production requires metabolic energy, part of the substrate is lost for its supply. Oxidized products lead to energy overflow which may be dissipated as metabolic heat, requiring high cooling capacity during fermentation
Flux coupling	Coupling the product flux to the central carbon flux by a shared reaction may create a strong driving force towards product formation
Irreversible reactions	Including an irreversible step, like decarboxylation, creates a metabolic driving force pulling flux towards the product

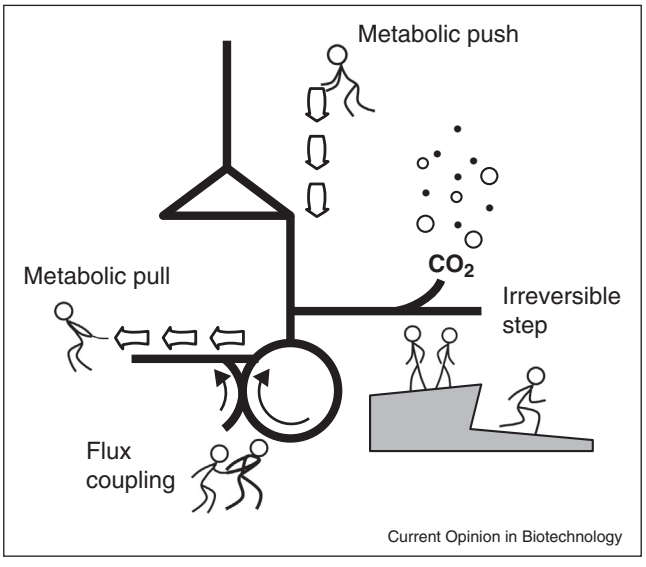
The question arises, where the true obstacles are, preventing the microbial production of many compounds. To reveal the answer, it appears worthwhile to have a deeper look at processes which have successfully been implemented at industrial level. Strikingly, a significant fraction of current bioprocesses for large scale microbial chemical production rely on selected wild type organisms. Only very few examples employ rationally engineered cell factories.

Prominent examples of successful primary metabolite production are citric acid produced by *A. niger* [8], ethanol produced by *Saccharomyces cerevisiae* [9] or glutamic acid produced by *Corynebacterium glutamicum* [10]. Pathway engineering allowed to establish a chimeric synthesis route to 1,3-propanediol (1,3-PDO) in *Escherichia coli* [11], while different other bacteria (e.g. *Lactobacillus* sp.) naturally convert glycerol to 1,3-PDO [12,13]. Lactic acid is a microbially produced bulk chemical [14,15], and succinic acid made by engineered microorganisms entered the market recently [16,17]. Furthermore, the first industrial plant producing butanediol from renewable feedstocks with a recombinant microbial cell factory has recently been announced [18].

One common feature of efficient metabolic pathways (apart from thermodynamic feasibility) is their metabolic balance [19,20]: stoichiometry, redox and energy household need to be balanced. Furthermore, flux coupling and irreversible reactions add to the efficiency of the pathway when the balances are fulfilled (Table 1). Figure 1 illustrates the concepts of metabolic push or pull, flux coupling, and flux irreversibility to enhance engineered metabolic pathways.

An ideal full stoichiometric balance could result in a total conversion of substrate into a product. Practically, most metabolic pathways lead to by-products (at least some biomass is formed), which inevitably reduce the product yield. Investigating the examples above, ethanol production from glucose comes with an intrinsic loss of $^1/_3$ of the carbon into CO_2, while citric acid or lactic acid are stoichiometrically balanced at the carbon level. However stoichiometric and redox balances are interconnected, so that metabolic redox balance may lead to a significant decrease in yield, when the product is more reduced than the substrate. Again the paradigm is ethanol, being more reduced than sugars. To close the redox balance, $^1/_3$ of the substrate carbon is oxidized to CO_2 so that the overall metabolic pathway is extremely efficient — at the expense of yield losses. Contrary, lactic acid production from glucose is fully balanced both on the levels of stoichiometry and degree of reduction, so that this metabolic pathway can run very efficiently with only minor losses in yield [21].

Figure 1



General concepts for the enhancement of engineered metabolic pathways. Metabolic push utilizes enhancement of the entry reaction as a driving force, while metabolic pull drives a pathway by depleting intermediate products towards the end of the pathway. Flux coupling enforces an engineered reaction by coupling it metabolically to a strong essential pathway. Including an irreversible step into an engineered pathway creates a strong driving force towards the product.