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Towards large scale fermentative production of succinic acid

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Fermentative production of succinic acid (SA) from renewable carbohydrate feed-stocks can have the economic and sustainability potential to replace petroleum-based production in the future, not only for existing markets, but also new larger volume markets. To accomplish this, extensive efforts have been undertaken in the field of strain construction and metabolic engineering to optimize SA production in the last decade. However, relatively little effort has been put into fermentation process development. The choice for a specific host organism determines to a large extent the process configuration, which in turn influences the environmental impact of the overall process. In the last five years, considerable progress has been achieved towards commercialization of fermentative production of SA. Several companies have demonstrated their confidence about the economic feasibility of fermentative SA production by transferring their processes from pilot to production scale.

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Introduction

Since the US Department of Energy (DOE) and the BREW project published their reports on the most promising sugar-derived bio-products, of which SA is one, extensive research has been carried out on the fermentative production of this molecule [1,2]. SA is seen as a future important building block for the production of the biodegradable plastic polybutylene succinate (PBS), polyester polyols, plasticizers and polyurethanes (as replacer of the petrochemical-derived adipic acid) and, dependent on its final cost price realization, the bulk chemical 1,4-butanediol (1,4-BDO) [3–5]. These applications could potentially lead to a market in the order of one to several megatons [6•,7,8]. The current market for SA is in the order of 30–50 kton/year based pre-dominantly on petrochemical-based production via maleic-anhydride [9,10]. To bridge the gap in manufacturing, several companies and consortia have begun the development of industrial production of bio-based SA (Table 1, partly derived from [11••]).

SA producing microorganisms

Natural producers vs engineered strains

Microbial production of SA can be achieved using natural producers, such as bacteria isolated from rumen, like *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*, *Mannheimia succiniciproducens* and *Bacillus fragilis* and certain fungi such as *Fusarium*, *Aspergillus* and *Penicillium* species [12••]. Genome sequencing and efforts to develop genetic tools have led to the application of metabolic engineering to increase the performance of rumen bacteria [13,14]. A disadvantage of using rumen bacteria as SA producers is that they contain several auxotrophies because vitamins and amino acids are readily available in their natural habitat. Accordingly, complex media are required to support growth of these organisms [12••].

An alternative strategy is to use commonly applied and genetically well accessible industrial micro-organisms which do not naturally produce significant amounts of SA, such as *Escherichia coli*, *Corynebacterium glutamicum* or *Saccharomyces cerevisiae* and to apply metabolic engineering to convert them into high producers [15].

In some cases, less common microorganisms such as the strictly aerobic yeast *Yarrowia lipolytica* [16] have been demonstrated.

Surprisingly, the majority of the recent research in this area has been carried out with *E. coli*, an organism which brings some disadvantages for industrial SA fermentation. The most important are the susceptibility of *E. coli* to bacteriophage infections [26] and the need for a near-neutral cultivation pH which requires the addition of alkali during fermentation and a more complicated down-stream processing design (see the section on process options below).

Current industrial strains

Several companies and consortia have started or are about to start large-scale fermentative production of SA, using different producing strains. DSM/Roquette

Table 1

Overview of industrial parties active in the field of succinic acid production, their presumed technologies and challenges

Producer	Capacity (kton/year)	Start-up	Location	Presumed technology	Challenges
Myriant	14	Q2, 2013	Lake Providence, Louisiana, USA	Ammonia precipitation	- Co-product to sell - Effect of ammonia in fermentation - Recovery of succinic acid in di-ammonium sulfate stream
BioAmber, Mitsui	30	Q4, 2014	Sarnia, Ontario, Canada	- Electrodialysis - Low pH fermentation	- Electricity costs and performance of EDBM - Effect of sodium in fermentation - Fermentation performance (low pH)
Succinity (BASF/ Corbion-Purac)	10	2013	Barcelona, Spain	Mg-based process	- Dependency on two recycles in process - Costs and performance of $MgCl_2$ cracking - Recovery of Succinic acid present in $MgCl_2$-stream
Reverdia (DSM/Roquette)	10	Q4, 2012	Cassano Spinola, Italy	Low pH fermentation	-Effect low pH on fermentation performance

(Joint Venture Reverdia) have constructed a high SA producing *S. cerevisiae* strain [17], Bioamber/Mitsui has initially chosen *E. coli* as producing organism, but more recently appears to have switched to the yeast *Candida krusei*. They have licensed exclusively a high producing strain developed by Cargill [18,19]. This in contrast to Myriant, which seems to have retained *E. coli* as its production host [20]. BASF/Corbion-Purac (Joint Venture Succinity) isolated a new member of the family Pasteurellaceae from bovine rumen and named it *Basfia succiniproducens* [21]. This natural producer, which has a high yield of 0.75 mol of SA per mol of glucose, was further optimized through metabolic flux analysis and subsequent metabolic engineering [13•].

Feed-stocks and fermentation strategies

Feed-stocks

Commonly used feed-stocks for large scale fermentative production of organic acids are refined sugars (sucrose, glucose and fructose), starch and beet or cane molasses.

In parallel, efforts are currently being undertaken to apply second generation feed-stocks, that is, non-edible plant biomass such as waste streams from agriculture, forestry and paper milling [22]. An advantage is that these feed-stocks have the potential to be generally cheaper. A disadvantage is that the sugars present in such plant material are difficult to access and thus pretreatment and hydrolysis is required to release the sugars from the cellulose, hemicellulose and lignin present in the plant biomass. As a consequence, a variety of impurities and sugar degradation products is formed, such as furfural, 5-hydroxymethylfurfural, acetate, formate and soluble lignin products which can act as inhibitors [23•]. Limiting the impact of these components [24] requires substantial efforts [23,25] and thus additional costs in the downstream processing section; especially if high purity, polymer grade SA is to be produced.

One way to overcome the inhibitory impact of these impurities and sugar degradation products is by increasing the furfural tolerance of producing strains. This was attempted for an ethanol producing strain of *E. coli* and four genetic properties were identified, and subsequently altered, resulting in increased furfural tolerance [23•].

Fermentation strategies

Many different fermentation strategies for the production of succinic acid have been described in recent literature. In addition to the common batch and fed-batch [27] approaches, more exotic systems such as fed-batch with cell recycling, single- and two-stage continuous cultures [27–29], and continuous cultures with integrated membranes for cell recycling [30] have been proposed. An extensive overview of the recent literature on these approaches can be found in [12••]. It should be appreciated, however, that SA is a future bulk chemical and has to be produced on a large scale, typically in fermentation vessels of 100 m^3 and larger. Therefore the applicability of such approaches for industrial fermentative production of SA can be questioned, as the required complex fermentation systems are expensive in terms of both investment and operation, more difficult to maintain and present higher risks on malfunction and contamination. Also the large scale application of membranes, e.g. for cell recycle is not trivial [31].

Carbon dioxide as additional substrate for SA production

To achieve the highest possible yield of SA on the supplied substrate it is essential that the flux towards SA is preferentially via the reductive part of the tricarboxylic acid (TCA) cycle, resulting in the net fixation of carbon dioxide [12••]. This carbon dioxide needs to be produced in another part of metabolism (e.g. substrate catabolism, biomass formation) or externally supplied to the fermentation (as gas or carbonate salt). In addition, rather high concentrations (partial pressures) of carbon dioxide are needed to drive sufficient flux through the