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Towards biobased industry: acetate as a promising feedstock to enhance the potential of microbial cell factories

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One sentence summary: Acetate is discussed as a promising feedstock to improve microbial cell factories for production of value-added chemicals and other compounds towards biobased industry.

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ABSTRACT

A broad range of different chemical and pharmaceutical compounds have been produced in microbial cell factories. To compete with traditional crude oil based production processes, the use of complex alternative raw materials such as lignocellulosic biomass, waste streams and utilization of CO_2 in gas fermentations has been suggested. All of these streams contain acetate, a cheap and potentially interesting carbon source for microbial production processes. Acetate (co-)utilization remains challenging, which is the reason for extensive research on the use of acetate for the production of value-added compounds. For industrial implementation of microbial conversion processes using acetate as a feedstock gaining a deeper insight into acetate metabolism of microorganisms is essential. Systems level analyses and manipulation of potential host organisms should be applied to achieve full utilization of this prospective substrate.

Keywords: acetate metabolism; metabolic engineering; microbial cell factories; acetate utilization; co-utilization of glucose and acetate; *Escherichia coli*

INTRODUCTION

Extensive research efforts are underway to develop microbial cell factories for the production of proteins and chemicals of interest (Ohta *et al.* 1991; Atsumi *et al.* 2010; Nielsen *et al.* 2010; Lee *et al.* 2012; Eggenreich *et al.* 2016). With the advent of recombinant DNA technologies, the speed of development of production platforms has been further increased. That way, biobased production of a wide variety of different compounds using an array of different host organisms has been achieved. As the focus for most of these studies was proof-of-principle, they rely on expensive pure carbon substrates and complex media components. However, the biobased production of chemicals for everyday products as well as pharmaceutical products requires economically sensible production routes. In order to achieve competitiveness with crude oil based production, cheap substrates are necessary (Lim *et al.* 2018), which include but are not limited to hydrolysates from lignocellulosic biomass, industrial and landfill waste streams and streams containing acetate derived from industrially available CO_2 (Crank *et al.* 2005). These streams represent, either alone or in combination, mixed feed systems. These systems contain a number of different carbon sources including hexoses, pentoses and organic acids such as acetate (Ko *et al.* 2015; Jönsson and Martín 2016). Acetate represents an

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Table 1. Examples of potential acetate sources with approximate acetate concentrations of individual streams.

Source	Process	Acetate concentrations	Reference
	Potential sources of acetate		
Lignocellulosic biomass	Hydrolysis, typical process	1 - > 10 g/l	(Mills, Sandoval and Gill 2009)
Waste	Anaerobic digestion, typical process	<15 g/l	(Braun 2013)
Industrial CO_2 and H_2	Batch gas fermentation with *A. woodii* (30°C, 1200 rpm, 0.5 vvm. 1 bar, H_2:CO_2:N_2 = 40:17:43)	59.2 g/l	(Kantzow, Mayer and Weuster-Botz 2015)
	Continuous gas fermentation with *A. woodii* (30°C, 1200 rpm, 1 bar, 0.5 vvm. H_2:CO_2:N_2 = 60:25:15)	18–23 g/l	(Kantzow, Mayer and Weuster-Botz 2015)
Syngas/CO	Continuous gas fermentation with *C. authoethanogenum* (37°C, 510–1000 rpm, 0.06–0.15 vvm, varying CO, H_2, CO_2)	4–8 g/l (4–12 g/l ethanol)	(Valgepea *et al.* 2018)
CO_2 and electricity	Microbial electrosynthesis (–590 mV vs SHE, 100 % CO_2)	10.5 g/l	(Marshall *et al.* 2013)

interesting carbon substrate as it usually inhibits microbial production processes. Here, it is argued that the use of acetate as carbon substrate could significantly increase the performance of microbial cell factories and allow for more efficient and economic production of a wide range of products.

ACETATE—POTENTIAL SOURCES

To achieve economic production and to compete with fossil fuels, it is necessary to use low-cost feedstocks (Lim *et al.* 2018). Feed-stocks such as pure sugars from corn, sugar beet, etc., are often criticized, since they compete with food industry and occupy arable land (Sitepu *et al.* 2014). Hence, raw materials which do not compete with the food and feed industry will likely play a key role in future applications of microbial cell factories.

In this respect, plant biomass seems to be the only available feedstock for the foreseeable future that is able to both satisfy this requirement and is available in sufficient quantities. However, the use of biomass in most cases requires pretreatment and the use of lignocellulosic hydrolysates in microbial fermentations is often inhibited by an array of different compounds including organic acids such as acetate. To achieve full conversion of available carbon sources, acetate would need to be utilized in hydrolysate-based processes as well.

When acetate should serve as an additional carbon source for fermentation processes, several potential resources can be imagined.

Plant biomass

Extraction of carbohydrates from pretreated plant biomass provides streams containing a mixture of hexoses and pentoses (Ko *et al.* 2015; Jönsson and Martín 2016). Ideally, high concentrations of fermentable sugars such as glucose are obtained. However, inhibitory compounds such as organic acids (e.g., acetate) that are released during hydrolysis hamper microbial conversion (Jönsson and Martín 2016). Full conversion of all available carbon sources is crucial for successful application of such streams for microbial production processes (Duque, Cardona and Moncada 2015).

Food waste

Food waste can be imagined for utilization in microbial fermentations, because it contains both sugars and proteins, making it an attractive alternative feedstock (Matsakas *et al.* 2014). Additionally, reducing the large quantities of waste production by today's society would help improve the economical footprint. However, due to fast partial anaerobic digestion it usually also contains organic acids such as acetate as a result of partial anaerobic digestion. Valorization of these streams is further complicated by their heterogeneity as well as seasonal and process-related variations in composition and pH (Pfaltzgraff *et al.* 2013).

Gas fermentation

In addition to biomass-derived materials, gaseous substrates such as CO, CO_2 and H_2 that are available in large quantities from industrial sources represent an interesting alternative carbon source (Schiel-Bengelsdorf and Dürre 2012). A very interesting group of carbon-fixing organisms that have recently gained more attention are acetogenic bacteria, which can be used for production of different chemicals such as acetate (Schiel-Bengelsdorf and Dürre 2012), ethanol (Valgepea *et al.* 2018), 2,3-butanediol (Köpke *et al.* 2011), and butanol (Liou *et al.* 2005). In a subsequent microbial conversion process, acetate could be upgraded by production of high value-added compounds. This principle has already been used in an integrated two-phase process, where acetate produced from syngas is continuously converted into C_{16}-C_{18} triacylglycerides (Hu *et al.* 2016).

Each of these potential streams has distinct characteristics which need to be considered for microbial conversion, where fast substrate co-conversion into products at high yields is required for economic and sustainable processes. With this background in mind it is important to select, manipulate and improve suitable organisms for acetate (co-)utilization. Additionally, it might not be possible to directly use the described acetate streams, as further processing such as purification or detoxification might be of need.

Table 2. Selected examples of microbial cell factories used for production of value-added compounds by (co-)utilization of acetate.

Strain	Source	Product	Reference
	Microbial cell factories using acetate as sole carbon source		
E. coli BL21 pET-22b + (MNEI)	Acetate	180 mg/l monellin (protein)	(Leone *et al.* 2015)
E. coli W Δ*iclR* pCDF(*cad*), pET(*acs*), pCOGA5(*gltA*, *aceA*)	Acetate	3.6 g/l itaconic acid	(Noh *et al.* 2018)
E. coli MG1655 Δ*sdhAB* Δ*maeB* Δ*iclR* *gltA*	Acetate	7.3 g/l succinate	(Li *et al.* 2016)
E. coli BL21 Δ*fadE* pYX26(*tesA*) pYX30(*acs*)	Acetate	1 g/l fatty acids	(Xiao *et al.* 2013)
R. toruloides AS 2.1389	Acetate	2.1 g/l fatty acids	(Huang *et al.* 2016)
Y. lipolytica Po1g pMT065 (*acc*, *dga*)	Acetate from syngas fermentation	18 g/l C_{16}-C_{18} triacylglycerids	(Hu *et al.* 2016)
Mixed culture of glycogen accumulating organisms	Acetate	41 % of dry cell weight polyhydroxyalkanoates	(Dai *et al.* 2007)
	Microbial cell factories for acetate co-utilization		
E. coli BW25113/F′ Δ*adhE* Δ*frd* Δ*ldhA* Δ*pta* Δ*pflB* Δ*fnr* pAL953(*ackA-pta*) pAL603(*alsS-ilvCD*, *kivd-adhA*) pAL991(*atf1*)	Glucose and acetate	19.7 g/l isobutyl acetate	(Tashiro, Desai and Atsumi 2015)
S. cerevisiae SR7(D452-2 Δ*ald6* *xyl1*, *xyl2*, *xks1*) *adhE*	Glucose, xylose and acetate	42 g/l ethanol	(Wei *et al.* 2013)
E. coli BL21 Δ*fadE* pYX26(*tesA*) pYX30(*acs*)	waste streams from dilute acid hydrolysis of lignocellulosic biomass	0.43 g/l fatty acids	(Xiao *et al.* 2013)
E. coli BL21 Δ*fadE* pYX26(*tesA*) pYX30(*acs*)	effluent from anaerobic-digested sewage sludge	0.17 g/l fatty acids	(Xiao *et al.* 2013)

(CO-)UTILIZATION OF ACETATE AS CARBON SOURCE

Co-utilization of acetate and other carbon sources is of great interest for microbial production processes as the use of acetate in addition to carbohydrates could potentially assist in improving target product yields or to decrease the formation of byproducts (Wu *et al.* 2016). The current state of the art for production of chemicals with microbial systems is the use of cultivation media containing glucose as the sole source of carbon, rather than complex raw materials (Baez, Cho and Liao 2011; Xu *et al.* 2014). Even co-utilization of glucose and acetate in a defined background is still challenging, because of the toxicity and inhibitory effects of acetate on many microorganisms (Salmond, Kroll and Booth 1984; Roe *et al.* 1998; Russell and Diez-Gonzalez 1998) as well as the underlying regulatory networks of metabolism favoring glucose utilization (Stülke and Hillen 1999; Schmidt and Schaechter 2012; Enjalbert *et al.* 2017). As shown in Table 2, metabolic engineering has been shown to be helpful in addressing these problems by improving natural acetate utilization capacities in well-studied organisms such as *Escherichia coli* (Castaño-Cerezo *et al.* 2015; Noh *et al.* 2018; Novak *et al.* 2018). In addition to relying on model organisms, another potential strategy could be the implementation of extraordinary mechanisms found in efficient natural utilizers of acetate in model organisms such as *E. coli* and *Saccharomyces cerevisiae*. One such example would be the acetate uptake and metabolism of *Azotobacter vinelandii* in which glucose uptake is inhibited in the presence of acetate, thus resulting in a reversed diauxic growth pattern (George, Costenbader and Melton 1985).

Escherichia coli using glucose as the carbon source has been used for the production of a wide range of chemicals as well as proteins (Ohta *et al.* 1991; Atsumi *et al.* 2010; Nielsen *et al.* 2010; Ferrer-Miralles *et al.* 2015). The most important pathways of acetate utilization are the low affinity and reversible phosphate acetyltransferase (Pta) and acetate kinase (AckA) node as well as the high affinity acetyl-CoA synthetase (Acs) (Wolfe 2005; Enjalbert *et al.* 2017). The manipulation of these pathways was shown to have a positive effect on acetate (co-)utilization (Novak *et al.* 2018). Furthermore, regulation of acetate metabolism to explain phenomena such as overflow metabolism has been extensively studied (Valgepea *et al.* 2010; Enjalbert *et al.* 2017).

Recently, acetate as carbon source has gained more interest (Table 1). Tashiro, Desai and Atsumi (2015) could demonstrate potential benefits of co-utilization of glucose and acetate. It was shown that product yield of isobutyl-acetate can be increased by simultaneous use of glucose and acetate as in this case carbon loss by decarboxylation of pyruvate to acetyl-CoA can be circumvented, resulting in an increased product yield. Apart from this example, co-utilization of acetate for production of value-added products with *E. coli* is still in its early stages and the focus of attention has mainly been on improving co-utilization of glucose and acetate and understanding underlying mechanisms (Lin *et al.* 2006; Castaño-Cerezo *et al.* 2015; Ding *et al.* 2015).

Saccharomyces cerevisiae is another well-studied and well-used organism in biotechnology. Ethanol production using baker's yeast is among the best studied examples of microbial chemical production. Recent studies addressed the well-known phenomenon of glycerol formation during ethanol fermentation by providing acetate as alternative to regenerate NADH, thus increasing ethanol yield by directing more carbon towards ethanol, both from glucose and acetate (Henningsen *et al*. 2015; Papapetridis *et al*. 2016).

CONCLUSION AND OUTLOOK

Its low cost makes acetate a promising feed-stock for biotechnological processes with different applications. Already today, it plays an important role as an intermediate product in anaerobic digestion and can be indirectly used for the production of heat from biogas. To provide alternative and potentially more valuable chemicals as final product of acetate (co-)utilization, deeper insights will have to be gained. Shedding light on mechanisms of acetate uptake and metabolism through systems biology tools, would allow for more efficient manipulation of microbial cell factories. Finally, the use of acetate as a carbon source could contribute towards (co-)utilization of complex raw materials, which can make microbial production processes more competitive and thus accelerate industrial implementation. All in all, acetate is worth to be considered as a potential substrate for the improvement of microbial cell factories.

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