Journal of Biotechnology 228 (2016) 82–94

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Comparative reaction engineering analysis of different acetogenic bacteria for gas fermentation

Anna Groher, Dirk Weuster-Botz*

Institute of Biochemical Engineering, Technische Universität München, Boltzmannstr. 15, D 85748 Garching, Germany

ARTICLE INFO

Article history:
Received 23 February 2016
Received in revised form 8 April 2016
Accepted 19 April 2016
Available online 20 April 2016

Keywords:
Acetobacterium
Acetogen
Autotrophic
Clostridium
Gas fermentation
Sporomusa

ABSTRACT

The production of chemicals by syngas fermentation is a promising alternative to heterotrophic fermentation processes. The autotrophic process performances of the so far not well studied acetogens *Acetobacterium fimetarium*, *Acetobacterium wieringae*, *Blautia hydrogenotrophica*, *Clostridium magnum*, *Eubacterium aggregans*, *Sporomusa acidovorans*, *Sporomusa ovata* and *Terrisporobacter mayombei* were characterized. *Acetobacterium woodii* was used as reference strain. Standardized batch experiments with continuous supply of the gaseous substrates CO_2 and H_2 were performed in fully controlled stirred-tank bioreactors. *A. wieringae* and *S. ovata* showed by far the highest growth rates and maximum biomass concentrations among the acetogens under study. Aside from the reference strain *A. woodii*, highest volumetric (17.96 g L^{-1} d^{-1}) as well as cell specific acetate formation rates (21.03 g g^{-1} d^{-1}) were observed with *S. ovata* resulting in a final acetate concentration of 32.2 g L^{-1}. Accumulation of formate with up to 4.8 g L^{-1} was observed with all acetogens. Ethanol was produced autotrophically with up to 0.42 g L^{-1} by four of the acetogenic bacteria under study (*A. wieringae*, *C. magnum*, *S. acidovorans* and *S. ovata*) and also by *A. woodii*. Butyrate was formed with up to 0.14 g L^{-1} by three of the acetogenic bacteria under study (*C. magnum*, *B. hydrogenotrophica* and *E. aggregans*). Due to its superior process performances *S. ovata* may be a promising host for redirecting carbon fluxes by applying metabolic engineering and tools of synthetic biology to produce non-natural chemicals from syngas.

1. Introduction

Reducing CO_2-emissions is a major demand for industry. Trying to make use of renewable carbon sources instead of fossil gas and oil may be a solution. A putative promising alternative to liquid fuels derived from fossil resources were the first generation biofuels, that are produced from renewable resources but this strategy has two major drawbacks: On the one hand, first generation biofuels are in competition with the supply of food. The increasing demand of crops and land for the production of biofuels is one of the reasons for the rising food prices. Furthermore first generation biofuels do not contribute to the solution of the climate crisis but aggravate it due to the burning of native vegetation or the microbial decomposition of organic carbon stored in plant biomass and soil (Fargione et al., 2008). These disadvantages are the origin of the second generation biofuels. In this strategy ligno-cellulosic or any further carbon containing residual biomass is used instead of food crops. For this purpose, there are three major approaches: The disintegration of lignocellulose to simple, fermentable sugars. Secondly, the gasification of poorly degradable biomass or carbon containing residues of any kind to synthesis gas (CO_2, CO and H_2) and the subsequent use of the Fischer-Tropsch process to convert CO and H_2 to liquid hydrocarbons. And last, gas fermentation (Daniell et al., 2012). Here, synthesis gas serves as substrate for the production of chemicals and biofuels by microorganisms (Henstra et al., 2007). Even though the first two approaches are currently the focus of research, gas fermentation has some overall beneficial aspects like contribution to the reduction of the atmospheric greenhouse effect (Schiel-Bengelsdorf and Dürre, 2012) and high feedstock flexibility and additionally some advantages over chemical catalysts that are used in the Fischer-Tropsch process: high substrate specifity, tolerance to tar and trace contaminants as well as low operation temperatures (Munasinghe and Khanal, 2010). Acetogens, carboxytrophs and methanogens are able to utilize hydrogen and carbon dioxide or carbon monoxide as sole carbon and energy source (Liew et al., 2013). The reductive acetyl-CoA pathway, also known as Wood-Ljungdahl pathway enables the autotrophic metabolism of acetogens and the production of C1-, C2- and C4-chemicals like formate, acetate, ethanol, butyrate, butanol and 2,3-butanediol (Drake et al., 2008). To current knowledge, acetogenic microorganisms

* Corresponding author.
E-mail address: D.Weuster-Botz@lrz.tum.de (D. Weuster-Botz).

http://dx.doi.org/10.1016/j.jbiotec.2016.04.032
0168-1656/

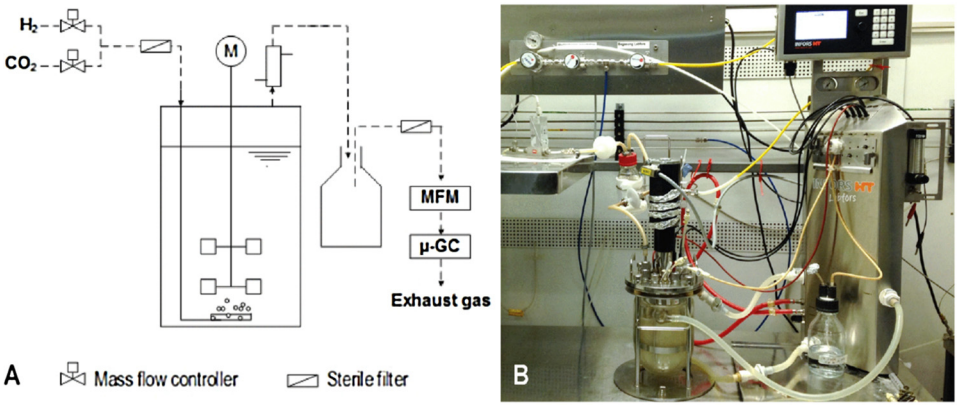

Fig. 1. Scheme (A) and photo (B) of the fully controlled bioreactor system with a continuous gas supply (H_2 and CO_2) and an exhaust gas analysis.

comprise 23 different genera with over 100 species that can be found ubiquitously in nature (Drake et al., 2008). But despite this plurality only few microorganisms have been studied in detail to date. The best characterized and studied strains are of the genera *Clostridium* (Daniell et al., 2012; Köpke et al., 2010) and *Acetobacterium* (Bertsch and Müller, 2015). While in most cases *Clostridia* depict a manifold product spectrum, *Acetobacterium woodii* exhibit extremely high growth and acetate formation rates as well as acetate concentrations (Kantzow et al., 2015).

Interest in gas fermentation has increased significantly in the last few years and three companies (INEOS Bio, Coskata Inc., LanzaTech) had already announced the approach of commercialization in 2012. But despite intensive research and development so far only INEOS Bio started a commercial facility in the middle of 2013 to produce ethanol from synthesis gas which is generated by the gasification of vegetative and wood waste. Low product yield and selectivity as well as low biomass densities and inefficient utilization of gas substrates are some of the large challenges that prevent a further commercialization (Liew et al., 2013). Since genome sequences have been published for several acetogenic bacteria (Bruant et al., 2010; Hemme et al., 2010; Köpke et al., 2010; Pierce et al., 2008; Poehlein et al., 2012, 2013; Roh et al., 2011) metabolic engineering is a promising approach to overcome these problems and to enlarge the product portfolio but the lack of genetic tools and the associated difficulty of introducing foreign DNA into acetogens impede this strategy. The application of new bioreactor designs depict an additional solution strategy by providing a high gas-liquid mass transfer efficiency (Munasinghe and Khanal, 2010). The isolation of new acetogenic bacteria as well as the further characterization of the huge number of acetogens that are so far only characterized to a very small extent by anaerobic flask experiments present also promising tools to cope with the mentioned challenges of commercialization (Munasinghe and Khanal, 2010). The autotrophic process engineering characterization of so far not studied acetogenic bacteria will probably reveal strains that exhibit improved growth rates and product yields with efficient syngas utilization.

The present study focuses on the reaction engineering analysis of the autotrophic process performances and the production capabilities of 8 different acetogenic bacteria that have not been studied in stirred-tank reactors so far. Batch cultivations with continuous gas supply are performed in stirred-tank bioreactors at fully controlled reaction conditions with CO_2 and H_2 as sole carbon and energy sources. Furthermore, reaction engineering characterization of all bacterial strains is performed at identical process conditions (medium, power input) to enable an unbiased and reliable comparison of the acetogenic microorganisms under study. To evaluate the autotrophic process performances on the basis of one of the most well studied acetogenic bacteria, *A. woodii* was cultivated as reference strain at identical process conditions.

2. Material and methods

2.1. Microorganisms

Acetobacterium fimetarium (DSM 8238), *Acetobacterium wieringae* (DSM 1911), *A. woodii* (DSM 1030), *Blautia hydrogenotrophica* (DSM 10507), *Clostridium magnum* (DSM 2767), *Eubacterium aggregans* (DSM 12183), *Sporomusa acidovorans* (DSM 3132), *Sporomusa ovata* (DSM 2662) and *Terrisporobacter mayombei* (DSM 6539) were provided by the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany).

2.2. Phylogenetic tree

The phylogenetic tree was generated with the program MEGA 6.06. ClustalW algorithm was used for the multiple sequence alignment. Maximum-likelihood algorithm was used for the construction of the tree. Underlying data are 16S rRNA gene sequences that were deposited at GeneBank under the accession numbers that are given in the supplementary material.

2.3. Batch cultivation in stirred-tank bioreactors

A general medium for acetogens (GA-medium) was used for the autotrophic cultivation of all acetogenic bacteria under study. The GA-medium contained: NH_4Cl 1 g L^{-1}; K_2HPO_4 0.35 g L^{-1}; KH_2PO_4 0,23 g L^{-1}; $MgSO_4 \times 7H_2O$ 0.5 g L^{-1}; $FeSO4 \times 7H_2O$ 4 mg L^{-1}; NaCl 2.25 g L^{-1}; Yeast extract 2 g L^{-1}; $CaCl_2 \times 2H_2O$ 0.15 g L^{-1}; Cys-HCl 0.4 g L^{-1}; $Na_2SeO_3 \times 5H_2O$ 3 μg L^{-1}; $Na_2WO_4 \times 2H_2O$ 4 μg L^{-1}; $FeCl_2 \times 4H_2O$ 3 mg L^{-1}; $ZnCl_2$ 0.14 mg L^{-1}; $MnCl_2 \times 4H_2O$ 0.2 mg L^{-1}; H_3BO_3 12 μg L^{-1}; $CoCl_2 \times 6H_2O$ 0.38 mg L^{-1}; $CuCl_2 \times 2H_2O$ 4 μg L^{-1}; $NiCl_2 \times 6H_2O$ 48 μg L^{-1}; $Na_2MoO_4 \times 2H_2O$ 72 μg L^{-1}; Biotin 40 μg L^{-1}; Folic acid 40 μg L^{-1}; Pyridoxine-HCl 0.2 mg L^{-1}; Thiamine- $HCl \times 2H_2O$ 0.1 mg L^{-1}; Riboflavin 0.1 mg L^{-1}; Nicotinic acid 0.1 mg L^{-1}; D-Ca-Pantothenate 0.1 mg L^{-1}; Vitamin B_{12} 2 μg L^{-1}; *p*-Aminobenzoic acid 0.1 mg L^{-1}; Lipoic acid 0.1 mg L^{-1}.

Pre-cultures were grown heterotrophically in GA- medium with additional 2 g L^{-1} casitone, whereas the following carbon sources were used: Fructose for *A. fimetarium*, *A. wieringae*, *A, woodii*, *B. hydrogenotrophica*, *E. aggregans*, *S. acidovorans* and *T. mayombei*; glucose for *C. magnum*; betaine for *S. ovata*. Pre-cultures were started with the inoculation of 1–5 mL of either a frozen cell stock (*A. fimetarium*, *A. wieringae*, *A. woodii*, *B. hydrogenotrophica*, *E. aggre-*