Chemicals from CO_2: Hydrogenotrophic production of acetate with *Acetobacterium woodii*

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Great interest has emerged in biological CO_2-fixing processes in the context of current climate change discussions. One example for such a process is the hydrogenotrophic production of acetic acid by anaerobic microorganisms. Acetogenic microorganisms can make use of synthesis gas (carbon monoxide, carbon dioxide and hydrogen) to produce acetic acid.

The formation of acetate by *Acetobacterium woodii* was studied systematically in batch-operated stirred-tank reactors at different hydrogen and carbon dioxide partial pressures in the gas phase. 48 parallel stirred-tank reactors on a millilitre-scale as well as stirred-tank reactors on a litre-scale were applied for the detailed reaction engineering analysis of hydrogenotrophic acetate formation by the strictly anaerobic *Acetobacterium woodii*:

$$4\ H_2 + 2\ CO_2 \quad \rightarrow \quad 1\ CH_3COOH + 2\ H_2O$$

Waste gas analysis and mass balances confirmed, that acetate was produced by *Acetobacterium woodii* with 90 % of the theoretical yield in the stirred-tank reactors.

The cell-specific acetate formation rate of *Acetobacterium woodii* increased with increasing hydrogen partial pressure and increasing power input due to the low solubility of hydrogen gas in water. A maximum cell-specific acetate productivity of 21 $g_{acetate} \cdot g_{cell\ dry\ weight}^{-1} \cdot d^{-1}$ was measured at a pH_2 of 1700 mbar in the gas-phase and at a power input of 5.5 $W \cdot L^{-1}$. Controlling gas-liquid mass transfer resulted in final acetate concentrations of 50 $g \cdot L^{-1}$ after a process time of 3 days, if the pH of the batch-process was controlled at pH 7.0 (average cell density of 1.2 $g_{cell\ dry\ weight} \cdot L^{-1}$). This is an increase by a factor of 5 compared to the best data so far reported in literature for hydrogenotrophic acetate production with *Acetobacterium woodii.*

References:

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Heterogeneity in liquid cultures of *Aspergillus*: boon or bane?

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Filamentous fungi such as *Aspergillus niger* are widely used in biotechnology for the production of various proteins, enzymes, food ingredients and pharmaceuticals. During recent years, *A. niger* became an industrial model fungus, due to its well annotated genome sequence and newly established gene transfer systems allowing efficient and targeted genetic and metabolic engineering approaches.

Filamentous growth is characterised by polarised extension of cells, whereby a mycelial network is formed which is heterogeneous in terms of morphology, cell age and product formation. Filamentous fungi grow either as pellets or as freely dispersed mycelium during submerged growth. Both macromorphologies depend among other things on hyphal branching frequencies – pellets are formed when hyphae branch with a high frequency, dispersed mycelia are a result of low branching frequencies. Whereas the formation of pellets is less desirable because of the high proportion of biomass in a pellet that does not contribute to product formation, long, unbranched hyphae are sensitive to shear forces in a bioreactor. Lysis of hyphae and the subsequent release of intracellular proteases have thus a negative effect on protein production. Hence, from an applied point of view, the preferred fungal macromorphology would consist of dispersed mycelia with short filaments derived from an optimum branching frequency.

As part of our effort to understand the connection between the processes of polarized growth and protein secretion in the industrially important fungus *A. niger*, we used several genome-wide expression profiling studies to predict and identify the morphogenetic machinery of *A. niger*. Our data suggests the participation as well as interconnectedness of several regulatory and metabolic pathways in these processes including phospholipid signaling, sphingolipid signaling, TORC2 signaling, calcium signaling and CWI signaling.

Intriguingly, mycelial heterogeneity, however, can also offer leads to new products. This becomes most compelling, when *A. niger* is cultivated in retentostats at very low growth rates approaching almost zero. In such cultivations, asexual development becomes initiated which is accompanied by high expression of genes encoding potentially new products including secondary metabolites and difficult to express small cysteine-rich proteins. We thus established two new promoter systems which should be active under zero growth conditions to explore the potential of retentostat cultivation as a new production system.

Miniplanttechnik in der Bioprozessentwicklung

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Der Miniplantmaßstab beschreibt eine Größe von Anlagen und Apparaten, die im Bereich eines Durchsatzes einiger Kilogramm pro Stunde arbeiten. Im Gegensatz zu Laborapparaten bieten Miniplantapparate speziell in der thermischen Verfahrenstechnik die Möglichkeit eines direkten Scale-ups in den Pilot oder Produktionsmaßstab und gleichzeitig die Möglichkeit des kontinuierlichen Betriebes. So lassen sich ganze Produktionsprozesse in Miniplants darstellen und beispielsweise auch Recyclesströme untersuchen. Die wichtigen Daten für die Prozessentwicklung können im Technikumsmaßstab nur unter erheblich größerem Kosten- und Zeitaufwand gewonnen werden. Aus diesem Grund haben sich Miniplant in der chemischen Technik gut etabliert. In der Bioverfahrensentwicklung hingegen besteht hier ein Nachholbedarf.

Im Rahmen eines Forschungsprojektes zur bioverfahrenstechnischen Herstellung von 1,3-Propandiol wurde am Institut für Bioprozess- und Biosystemtechnik an der TU Hamburg-Harburg eine Miniplant für einen möglichen Prozess entwickelt und genutzt. Diese Anlage setzt Rohglycerin aus der Biodieselherstellung fermentativ in einem 12L Fermenter anaerob zu 1,3-Propandiol um. Anschließend wird die Fermentationsbrühe durch Ultrafiltration von der Biomasse getrennt und die klare Fermentationsbrühe destillativ eingeengt. Abschließend erfolgt dann eine zweischrittige Rektifikation in einer d=50mm Rektifikationskolonne im Vakuum um 1,3-Propandiol mit einer Reinheit von 99% zu erhalten.

Neben der erfolgreichen absatzweisen Durchführung und Optimierung des Prozesses war es möglich Fermentation, Biomasseabtrennung und Destillation kontinuierlich zu verknüpfen und damit verbunden einen Zell- und Wasserrecycle zu etablieren. Der Einfluss von Fermentation auf den DSP wurde durch den Einsatz unterschiedlicher Titrationslaugen in der Fermentation deutlich, der ein verändertes Trennverhalten von Nebenprodukten im DSP zur Folge hatte. Weiterhin zeigten die apparativen Herausforderungen die speziellen Ansprüche an Apparate für Bioprozesse hinsichtlich von Schaumbildung, Salzausfällungen und Membranfouling.

Optimizing protein secretion in *Corynebacterium glutamicum:* A combined question of biological and bioprocess parameter heterogeneity

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Within bioprocess development many biological and bioengineering parameters influence the expression and secretion of a protein of interest. Promoter strength and induction strategy, fused signal peptides, temperature, different substrates and feeding profiles, as well as the protein and expression host itself, to mention only some of them. This leads to a high number of possible parameter combinations to be tested for each new target protein de-novo. To manage the high cultivation effort, miniaturized but scalable parallel fermentation systems with increased throughput became an important field of research during the last decade.

In our work we have implemented a micro titer plate (MTP) based cultivation system (BioLector) within a liquid handling robotic setup to facilitate the analysis of a broad range of parameter combinations. Upstream processes like media preparation and preculture handling are carried out automatically in a sterile environment. Furthermore, the integration of MTP cultivation in a liquid handling platform enables automated media preparation as well as the transfer of liquid in and out of each well also during the running cultivation. Those sampling or dosing events (e.g. IPTG) are triggered independently for each well taking online data from the cultivation (e.g. biomass, pO_2).

This setup enables acquiring reliable performance data for protein expression successfully demonstrated in several applications. Among them are a secretory signal peptide library, IPTG dosage time and concentration, media composition and glucose feeding rate showing distinct dependencies between biological and bioprocess parameters. The complex task of media optimization is supported with evolutive software algorithms and automated media preparation by the robot, going along with a dramatically increased throughput. The optimized process from MTP correlated very good with scale up results from 1 L and 20 L bioreactor cultivations, strongly supporting this automated cultivation platform for its relevance for routine application.