ENABLING COST EFFECTIVE BUTANOL PRODUCTION WITH DAB.bio's UNIQUE BIOREACTOR TECHNOLOGY

Thanos Pappas, Arjan Oudshoorn
Delft Advanced Biorenewables, Alexander Fleminglaan 1, 2613 AX Delft, NL

ABSTRACT

Biotechnology provides novel opportunities for sustainable production of existing and new chemicals, often focusing on strain development. However, state of the art fermentation processes continue to be characterized by low productivities and titers which translates into diluted product streams and costly separation in mostly batch-based production processes. Overcoming the large working volumes needed to provide the desired market volumes of low-cost bio manufactured products is the challenge of industrial biotechnology today.

Continuous removal of inhibiting compounds from the fermentation broth could significantly improve the viability and duration of fermentation processes leading to increased productivity, higher titers and concentrated product streams, thereby reducing the high capital and product costs caused by low volumetric productivity. This study demonstrates how DAB.bio's continuous organic overlay/extractive fermentation platform known as Fermentation Accelerated by Separation Technology (FAST) prolongs the process and robustly increases butanol production of an anaerobic Clostridium strain.

INTRODUCTION

Butanol is hydrophobic and its toxicity restricts the productivity of the fermentation making it suitable as a showcase example of our technology platform. *In-Situ Product Removal* (ISPR) of n-butanol via gas-stripping can be used to relieve fermentation toxicity, however, removal rates are limited by the temperature and low flow rates of fermentation compatible stripping gases. Higher inflow gas rates typically cause foaming & sludging, high gas hold-ups and excessive compressor costs.

Using hybrid ISPR, i.e., both gas stripping and a secondary extractant phase, is a powerful tool to further alleviate the toxic effect of butanol. Batch organic overlays are currently used in fermentations to remove toxic compounds from the broth. However, in these processes, equilibrium and saturation of the organic and gas phase is inevitable, which leads to an accumulation of toxic products in the broth, and a resulting decrease in the overall productivity and duration of the fermentation. A solution to this limitation is the in-situ implementation of a *continuous* organic phase overlay technology which is the core of our technology (FAST).

MATERIALS AND METHODS

Two ISPR butanol fermentations were carried out and compared in productivity, overall production capacity and duration: A lab scale batch overlay fermentation and a pilot scale continuous overlay FAST fermentation. Both processes are described in Table 1.

Scale effects were considered negligible between the systems as they are both mixed and anaerobic. Gas stripping is similar for both systems since temperature and aeration (vvm) are the same and gas phase is saturated with the volatile component in both systems.

Feed strategy:
Glucose was adjusted to maintain a concentration of 10 g/L, because at lower concentrations fermentation performance is significantly diminished. This feeding strategy was followed in both processes.

Solvent dosing strategy (FAST):
At the end of the batch phase, oleyl alcohol was continuously added at a rate of 2 kg h-1 to match the targeted butanol productivities. The solvent was separated and harvested continuously at the set dosing and recovery rate.

Solvent dosing strategy (Batch overlay):
At the start of the fed-batch phase, 0.5 L of solvent (Oleyl alcohol) was added.

FAST **Batch Overlay**

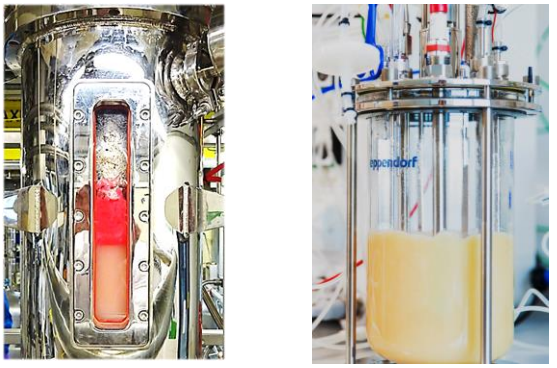

Figure 1: FAST shows a visible organic layer in red, batch overlay appears to be a homogonous mixture.

Table 1: Comparison of FAST and batch overlay fermentation processes to produce butanol from anaerobic Clostridium.

	FAST	Batch overlay
Strain	Clostridium	Clostridium
Mode	Batch followed from fed-batch	Batch followed from fed-batch
ISPR technique	Hybrid gas & continuous organic overlay	Hybrid gas & batch organic overlay
Reactor	FAST reactor	CSTR
Overall volume	100 L	3 L
Aqueous phase volume	70 L	1 L
Organic phase volume	(< 2 % overall working volume)	(33 % overall working volume)
Solvent	Oleyl alcohol	Oleyl alcohol
Temperature	32 °C	32 °C
pH	6.2 (batch phase) 5.3 (feed phase)	6.2 (batch phase) 5.3 (feed phase)
Airflow (N_2)	35 L·min^{-1} (0.5 vvm)	0.5 L·min^{-1} (0.5 vvm)
Glucose feeding rate	3 (g·L^{-1}h^{-1})	3 (g·L^{-1}h^{-1})

RESULTS AND DISCUSSION

Butanol production occurs in the aqueous phase and mass transfers from aqueous to organic and gas phase until aqueous/gas & aqueous/organic equilibrium is reached. In batch overlay fermentations (Figure 2a), butanol content in aqueous phase (purple line) steeply decreases when the extractant solvent is introduced (~ 24 h), then increases again (~ 31 h) indicating a saturation of the gas and organic phases leading to an accumulation of butanol in the aqueous phase.

The resulting product toxicity leads to stagnation of production and loss of activity. In the FAST case (Figure 2b), product equilibrium is reached between aqueous, gas, and organic phases and is maintained throughout the continuous feed phase. Because FAST continuously removes butanol-rich organic phase and replenishes the fermentation with fresh solvent, aqueous butanol content is kept below inhibition limits until the end of the process.

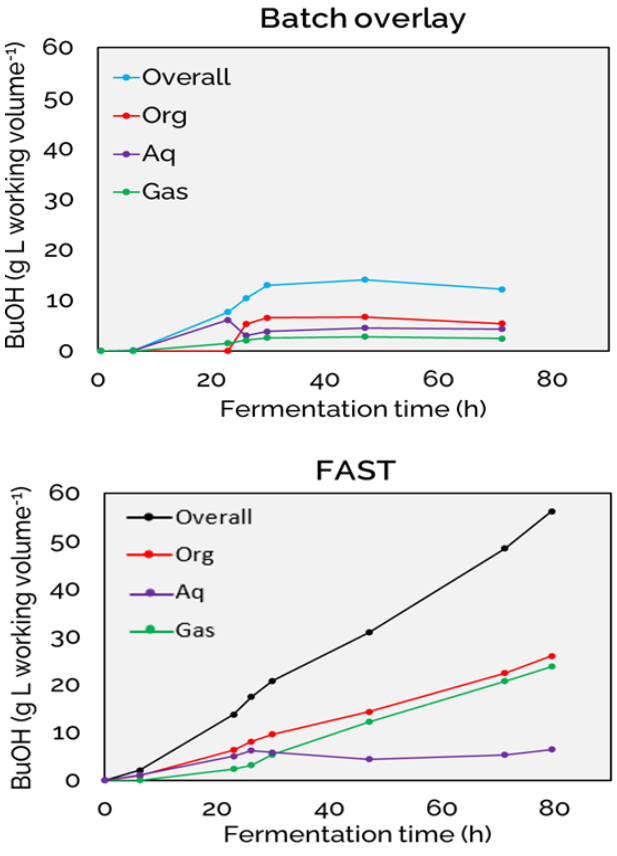

Figure 2: Cumulative production of butanol per phase for Batch overlay (**a**bove) and FAST (**b**elow).*

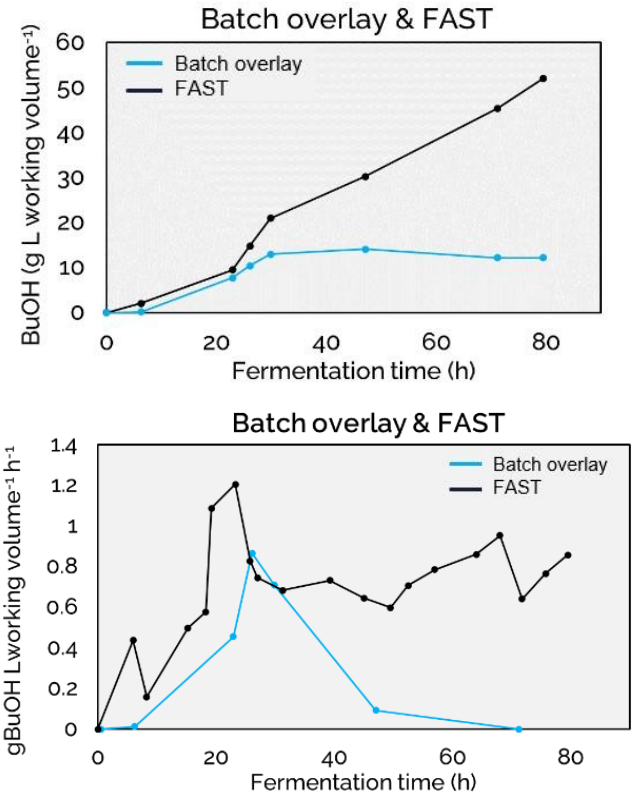

Figure 3: Butanol production per fermenter volume (above) and total butanol productivity (below) for batch overlay (blue) & FAST (black).*

Between the fermentation start and 25 hours after inoculation, both processes give similar production outlooks with comparable butanol removal rates from gas and organic phase. At approximately 30 h, production of butanol in the ***batch overlay*** stops and the overall amount stagnates, indicating that gas stripping removal capacity is reached, and the organic overlay is saturated. A resulting increase in the amount of butanol is seen in the aqueous phase of the base case.

Conversely, when ***FAST*** with continuously extracted organic overlay is used, *product titer is 3.4 times higher* than the batch overlay, and butanol production continues until the fermentation is manually ended at 80 h (Figure 3). Because FAST is not limited in product removal capacity, overall achievable productivity can be further optimized.

*All amounts are normalized and expressed per L of overall fermenter working volume.

The *average overall productivity increased 2.4-fold with **FAST*** (from 0.3 g.L.-1 h-1 to 0.71 g.L.-1h-1). The *duration of **FAST**'s productive phase was extended 2-fold*, when the fermentation was ended for operational reasons. The continued high productivity indicates the fermentation could be extended longer, further improving the economic benefits.

To better illustrate FAST benefits for production at scale, a short case study was carried out assuming a desired annual production capacity of 100 ktonne of butanol for the shown overall productivities. A conventional stripping organic extractive overlay fermentation would require an installed capacity of around 500 fermenters of 100 m3 working volume. When applying FAST, the number of fermenters would be reduced to 220. This would reduce installed CAPEX by an estimated of 2.2 bln (USD) and lower overall production costs by 60 %. This case study demonstrates how FAST technology intensifies biomanufacturing by alleviating the toxic effect of butanol in long process durations.

CONCLUSIONS

This case study shows that FAST can enable more bioprocesses to reach commercial production unlocking markets previously unattainable for existing and novel products.

FAST:

-Maintains high productivity rates for long process durations by removing the toxic product effects and increasing product flux from the cell to the broth.

-Concentrates the product in a continuous organic phase, resulting in significantly reduced process streams.

-Simplifies complex downstream processing by enabling continuous manufacturing and concentrating the process stream.

- Reduces the working volumes of both upstream and downstream unit operations for a given production capacity by several fold.

FAST is Ready at Demonstration Scale

@

Biobase Europe Pilot Plant in Ghent, BE

Limited slots available in Q2, 2022 for process development or commercial sample generation.

Contact **info@dab.bio** to find out how FAST can lower your production costs.

*This research was supported by the Dutch Ministry of Economic Affairs through the Netherlands Enterprise Agency (RvO).
*We thank our project partners WFBR and Biocleave for their contribution.
*This project was funded under the name DEI+FASTEST, grant number DEI2719024.