Project Number: 640462

Start date of the project (duration): 1st May 2015 (36 months)

BUTANEXT

Next Generation Biobutanol

D3.5

Performance data with VITO fed-batch & continuous ISPR system on pure and cellulosic sugar. Butanol productivities > 0.75g/L/hr

An evaluation document between fed-batch and continuous operation.

Organisation name of lead contractor: VITO NV

Other organisations involved:

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Table of Contents

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1 EXECUTIVE SUMMARY

In work package 3, VITO thoroughly evaluated the difference in performance between fed-batch and continuous fermentations coupled to several organophilic pervaporation membranes. The cells were freely suspended and the in-house designed and constructed organophilic pervaporation units were directly coupled to the fermentors. The developed methodology in reporting allows an objective comparison between fed-batch and continuous operations.

It was found that while both fed-batch and continuous conditions were compatible with organophilic pervaporation operations, continuous conditions have the edge over (fed-)batch conditions in terms of labour intensity, water consumption, solvent concentration in the permeate, volumetric productivity and base consumption.

Of the tested pervaporation membranes, one was identified with total fluxes of 0.9 – 1.39 $kg.m^{-2}.h^{-1}$ and solvent concentrations of 123 – 247 $g.kg^{-1}$ (in continuous mode) depending on residual solvent concentrations in the fermentor. Total fluxes depended on solvent concentration in the fermentor. An irreversible flux decline, i.e. membrane fouling, was not observed.

Solvent (acetone-butanol-ethanol) productivities up to 0.97 $g.L^{-1}.h^{-1}$ were obtained in continuous conditions. While glucose was completely consumed, a trade-off between xylose utilization and productivity was shown: the higher the productivity, the lower the xylose utilization. Therefore, an economic optimum has to be established between fermentor size (with an impact on capital costs) and substrate costs (as defined by carbohydrate utilization).

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2 GLOSSARY

ABE	Acetone-Butanol-Ethanol
A	acetone
B	butanol
D	dilution rate [h^{-1}]
E	ethanol
J	flux [$g.m^{-2}.h^{-1}$]
P	solvent productivity [$g.L^{-1}.h^{-1}$]
S	glucose consumption [$g.L^{-1}.h^{-1}$]
t_r	residence time [h]
x	mole fraction in feed [-]
Q_E	effluent flow rate [$kg.h^{-1}$]
Q_P	pervaporation flow rate [$kg.h^{-1}$]
y	mole fraction in vapour [-]
$Y_{P/S}$	solvent yield [$g_{solvents}.\ g_{glucose}^{-1}$]

Greek symbols:

α	separation factor (-)
β	enrichment factor (-)

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3 INTRODUCTION

In a conventional ABE fermentation, acetone, butanol and ethanol are separated and purified from the fermentation broth using steam stripping followed by distillation. The relatively low product titers (~2% solvents) due to severe product inhibition lead to high steam consumption and high waste water volumes per kilogram solvent (Ni and Sun 2009).

Integration of the fermentation with organophilic pervaporation leads to significant reductions in steam consumption and waste water production per kilogram solvent (Van Hecke et al. 2016).

In work package 3, VITO thoroughly evaluated the difference in performance between fed-batch and continuous fermentations coupled to organophilic pervaporation membranes. The cells were freely suspended and the in-house designed and constructed organophilic pervaporation units were directly coupled to the fermentors. Several organophilic membranes were compared as well. Simulated lignocellulosic feedstocks contained glucose and xylose in a 2:1 ratio as typically found in wheat straw hydrolyzates (Novy et al. 2014). Subsequently, wheat straw hydrolyzates were used as feed in continuous mode to investigate its influence on pervaporation and fermentation performance.

4 MATERIALS AND METHODS

4.1.1 Cultivation & media composition

Several microbial strains (GBL 6046 / GBL6209 / GBL6225) available from deliverable 3.1 and provided by GBL were utilized in work package 3 by VITO.

The culture medium used in the fermentor at the start of the experiment contained (for 1L) 2.5 g yeast extract, 2.5 g tryptone, 0.5 g $(NH_4)_2SO_4$, 0.025g $FeSO_4.7H_2O$, 40 g glucose, 20g xylose. The feed contained (for 1L) 2.5 g yeast extract, 2.5 g tryptone, 0.5 g $(NH_4)_2SO_4$, 0.025 g $FeSO_4.7H_2O$, 100 g glucose, 50 g xylose.

Tests on wheat straw hydrolyzate were executed in a one-stage continuous fermentation mode.

4.1.2 Fermentation and pervaporation

Three modes of operation were tested:

1. Fed-batch one-stage operation coupled to organophilic pervaporation
2. One-stage continuous operation coupled to organophilic pervaporation
3. Two-stage continuous operation coupled to organophilic pervaporation.

Fermentors were equipped with oxygen sensors and pH probes (Applikon Biotechnology, Schiedam, The Netherlands). All sensors were connected to an ez-control (Applikon Biotechnology, Schiedam, The Netherlands) which was interfaced with a personal computer containing the supervisory control and data acquisition software (BioXPert V2, Applikon Biotechnology, Schiedam, The Netherlands). Peristaltic pumps (Watson-Marlow 520U, Cornwall, UK) were used to add the medium to the fermentors and to remove fermentation broth from the fermentors. Nitrogen was sparged prior to inoculation. The in-house

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developed and assembled pervaporation unit consisted of one to two membrane modules (Pervatech, Enter, the Netherlands) connected in series with a total membrane exchange area of 0.009 – 0.018m². A permeate pressure of 20 mbar was established using a membrane vacuum pump (SC920, KNF Neuberger GmbH, Freiburg, Germany). A Watson-Marlow 620U peristaltic pump was used to recirculate the fermentation broth from the first fermentor over the pervaporation modules. The pervaporation unit was automatized using LabVIEW (National Instruments, Austin, Texas, USA).

4.1.3 Analysis

Xylose (16.0 min retention time) and glucose (14.5 min retention time) were determined by high performance anion exchange chromatography using a Dionex CarboPac PA1 column (2.5 m * 4 mm) with pulsed amperometric detection (Dionex ICS-5000 DC, Thermo Fischer Scientific, Waltham, Massachusetts). Column temperature was 25°C while the mobile phase consisted of 92.7 % demineralized water and 7.3% of a 250 mM NaOH solution. Volatile fatty acids and solvents were analysed as described previously by Van Hecke *et al.* (2013).

4.1.4 Calculations

The calculation procedures allow a direct comparison of the obtained parameters in the different modes of operation. The productivity numbers cannot be compared with the productivity numbers reported by Green Biologics. The calculation of productivity by GBL relies on the “average fermentor volume” and does not take into account the volume in their filtration loop. Only when the “final reactor volume including volume in the filtration loop” is used in productivity calculations by Green Biologics, a valid comparison of productivity with VITO’s technology is possible.

5 RESULTS

5.1 One-stage fed-batch tests

Table 1 shows an overview of the results obtained in fed-batch mode integrated with organophilic pervaporation. Figure 1 shows a (simplified) scheme of the experimental set-up. The pH setpoint was fixed at 5.3 as suggested by GBL.

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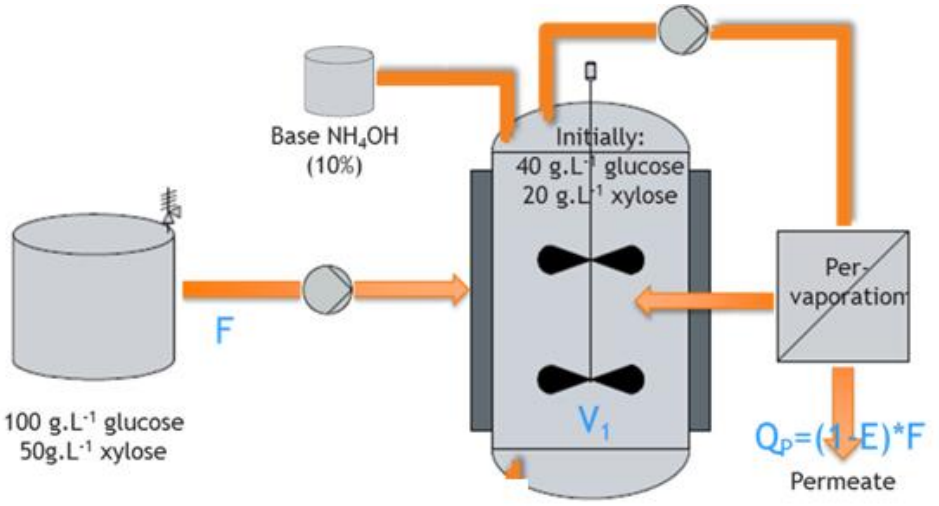

Figure 1: Fed-batch fermentation coupled to organophilic pervaporation

The accumulated solvent concentration[1] was rather limited (18.4 – 28.4 g.L^{-1}) due to the low initial carbohydrate concentrations (30 - 60 g.L^{-1}; initial volume 3L) and especially due to the limited addition of feed by the very nature of a fed-batch operation (final volume was around 5.5L-7.3L). The solvent productivity ranged between 0.13 & 0.43 g.L^{-1}.h^{-1} and lead to xylose utilization of 99% to 48%. Glucose was fully consumed in all cases. Complete xylose utilization could be shown, but only at industrially irrelevant productivities. Hence, a trade-off between productivity and xylose utilization has to be observed as also shown in fermentations using *Clostridium acetobutylicum* ATCC 824 (Van Hecke et al. 2016).
Organophilic pervaporation membranes Y and Z were utilized in the experiments. Fluxes of 0.9 – 1.6 kg.m^{-2}.h^{-1} were obtained using membrane Z which is a significant improvement in comparison to the fluxes obtained with membrane Y (0.36 – 0.58 kg.m^{-2}.h^{-1}; also see table 1). For both membranes it was observed that the flux depended on the solvent concentration in the fermentor. Higher fluxes lead to decreased capital investments, making the process more attractive from an economical point-of-view. An irreversible decline in flux in function of time, i.e. fouling was not observed in the experiments.

Table 1: Overview of fed-batch experiments. PV = Pervaporation membrane.

Test	Strain	Duration (h)	PV type	Productivity (g.L^{-1}.h^{-1})	Xylose utilization (%)
T005	GBL6046	166	Y	0.13	99%
T007	GBL6046	170	Z	0.15	94%
T008	GBL6046	143	Z	0.30	89%
T013	GBL6209	61	Z	0.43	48%

[1] the average solvent concentration from the fermentor (at cultivation time t) and all permeate obtained (at cultivation time t).

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5.2 Continuous fermentations

Continuous fermentation technology offers advantages in comparison to (fed-)batch technology especially for bulk chemicals, where a small decrease in production price will lead to an important competitive advantage (Leib et al. 2001; Van Hecke et al. 2014). When operated in a stable manner during a prolonged period of time, it leads to reductions in fermentor size for the same production capacity due to reductions in downtime and by avoiding the long lag phase of microbial cultivations (Maxon 1955). Continuous fermentations can be run in one or multiple stages. In VITO, one and two stage operations were studied. Initial attempts at a pH setpoint of 5.3 in continuous mode (similar as in fed-batch fermentations) lead to high butyrate and acetate concentrations. The problem was solved by fixing the pH at 4.8 in continuous mode which also lead to a decrease in ammonium hydroxide consumption.

5.2.1 One stage operation

Typically, a fermentor is switched from fed-batch to continuous mode once the desired working volume is reached. On an industrial scale the working volume is preferably as close as possible to the maximum working volume. Figure 2 shows a (simplified) scheme of the experimental set-up used in continuous mode.

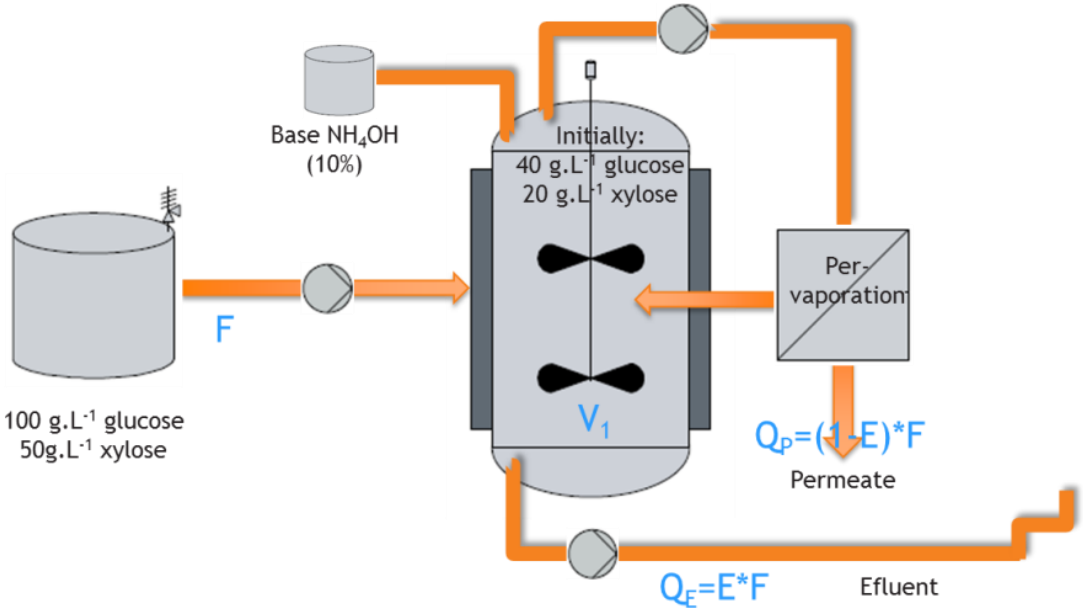

Figure 2: Continuous ABE fermentation coupled to pervaporation

Table 2 shows the overall results of a series of continuous fermentations. The accumulated solvent concentrations (33.9 – 47.1 $g.L^{-1}$) were significantly higher compared to fed-batch operations due to the continuous feeding at high carbohydrate concentrations (~150 $g.L^{-1}$). Hence, the water balance of a continuous process is more attractive than that of a fed-batch process leading to less waste water production per kg solvents in continuous conditions. The productivity in continuous conditions ranged from 0.28 to 0.97 $g.L^{-1}.h^{-1}$, higher than obtained in fed-batch mode. High xylose utilization was only observed at low solvent productivities. It is expected that higher productivities can be obtained by increasing the dilution rate further. However, xylose utilization would decrease further, leading to an undesired increase in feedstock costs. Due to the automated set-up and reductions in fermentor cleaning and manipulations,

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manual labour and supervision of the continuous set-ups was negligible in comparison to fed-batch experiments.

Table 2: Overview of one-stage continuous experiments. PV = Pervaporation membrane.

Test	Strain	Duration (h)	PV type	Productivity ($g.L^{-1}.h^{-1}$)	Xylose utilization (%)
T010 zone I	GBL6046	212	Z	0.28	84%
T010 zone II	GBL6046	138	Z	0.32	84%
T010 zone III	GBL6046	53	Z	0.52	55%
T013	GBL6209	110	Z	0.97	40%
T015 zone I	GBL6209	217	Z	0.65	55%
T016 zone I, F1	GBL6225	343	Z	0.77	59%

5.2.2 Two stage operation

Due to the solvent recovery in a one-stage fermentation by organophilic pervaporation, solvent concentrations in the fermentor are lower than can be maximally obtained (~20 $g.L^{-1}$). Furthermore, while glucose was completely converted in a one-stage fermentation, xylose was not. Therefore, sending the effluent from this first fermentor to a second fermentor might lead to two attractive features: a higher utilization of xylose and a higher solvent concentration to be sent to the downstream processing or end-of-pipe treatment (i.e. steam stripping). High xylose utilization leads to lower substrate costs and introduction of a stream containing a higher solvent concentration in the steam stripper decreases the steam consumption per kg solvent.

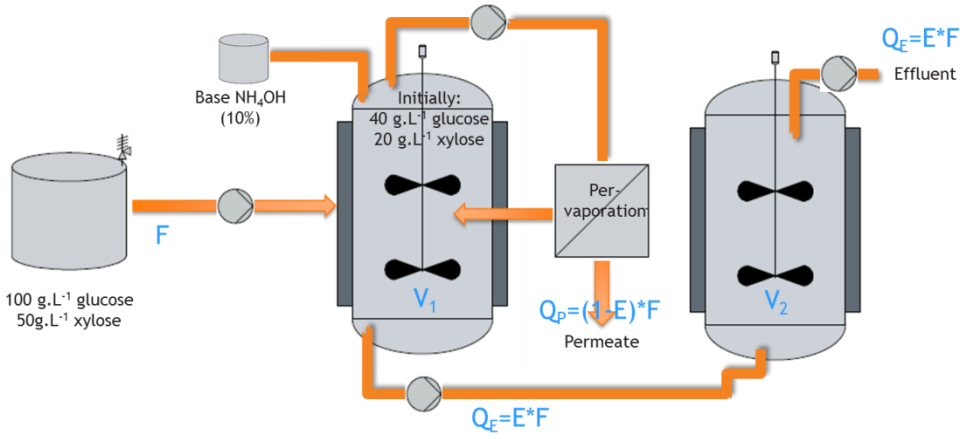

Figure 3: Schematic test set-up of the two-stage continuous fermentor experiment

This methodology paid off in terms of improved xylose conversion (from ~59% to ~72%). However, solvent productivities in the second fermentor were low in comparison with the productivities obtained in the first fermentor. Overall xylose utilization increased to 72-74% in the two-stage experiment in comparison with a xylose utilization of 59-61% in the first fermentor. Solvent productivities of 0.74-0.77 $g.L^{-1}.h^{-1}$ were obtained in the first fermentor while overall solvent productivities (in the combined two-stages) were ~0.34 $g.L^{-1}.h^{-1}$. The trade-off between solvent productivity and xylose utilization is illustrated again and shows that efforts have to be undertaken to improve xylose utilization rates further.

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5.3 Use of wheat straw hydrolyzates

In a one-stage continuous experiment, the use of wheat straw hydrolyzate as substrate for the ABE fermentation was investigated. The first batch of wheat straw hydrolyzate received by VITO from CENER contained around 90 $g.L^{-1}$ of total sugars. The wheat straw was hydrolyzed using MetGen's enzymes. In subsequent hydrolyzate production rounds, a higher carbohydrate concentration is expected which will positively influence the water balance of the process.
In a first experiment, it was shown that membrane fouling was not an issue when using wheat straw hydrolyzates. It was also shown that glucose was completely converted in a one-stage continuous conversion while xylose was not, as expected from the earlier tests using simulated C5/C6 carbohydrate mixtures. This initial test was encouraging and a subsequent test was performed to better assess the technology.

In the second test, the system was started up in batch mode and switched to fed-batch after 21h and to continuous mode after 92h. As long as glucose was depleted, feed flow was gradually increased with the aim to reach a flow of 2.4 $mL.min^{-1}$ (corresponding to a solvent productivity of 0.75 $g.L^{-1}.h^{-1}$) and run the fermentor at steady-state during at least 3-5 residence times. However, after 341h of operation and at a flow of 1.87 $mL.min^{-1}$, glucose concentrations increased significantly. Changes in operational conditions did not allow to improve sugar consumption and the experiment was therefore stopped after 460h. Because it seems that the productivity target is too high for this challenging substrate, it was agreed to schedule additional experiments on hydrolyzates to determine the feasible operational limits. Current expectations are that productivities of 0.5 $g.L^{-1}.h^{-1}$ can be reached.

6 DISCUSSION

Table 3 shows a summary of experimental results from fed-batch, one-stage and two-stage continuous experiments. Results marked in green are considered as excellent, results in orange as mediocre, and in red as to be improved considerably. The continuous 1 stage experiments score well in terms of accumulated solvent titer, productivity, flux, yield, and solvent concentration in the permeate. As discussed above, xylose utilization is near completion only at industrially irrelevant productivities. This is also illustrated in figure 4 where solvent productivity is plotted in function of xylose utilization for the different bacterial strains that have been tested. In short, the higher the productivity, the lower the xylose utilization. It is expected that the productivity can be increased by further increasing the dilution rate during continuous operations. However, this will lead to unacceptably low xylose utilization and was therefore not explored further.

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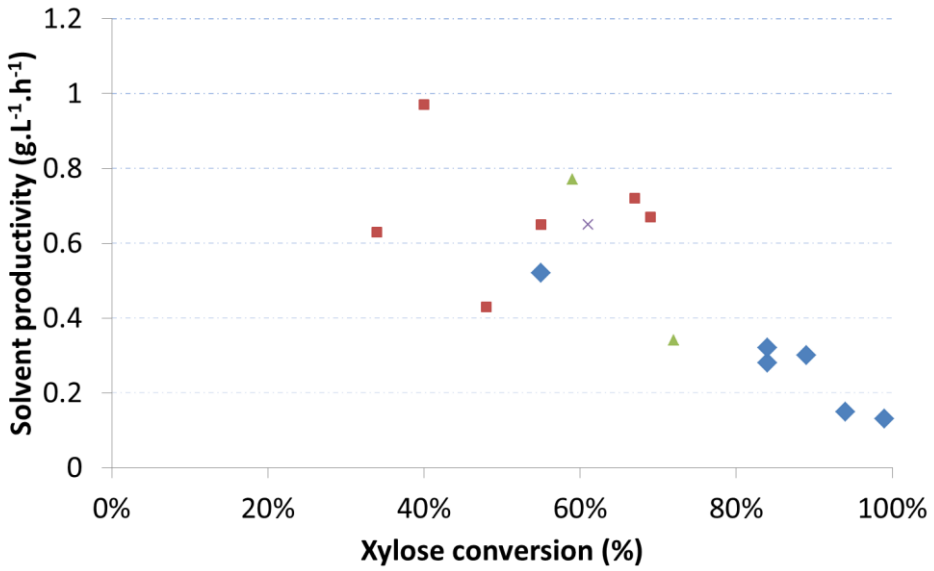

Figure 4: Solvent productivity in function of xylose conversion. Four strains were used in the experiments: ◆GBL6046; ■GBL6209; ▲GBL6225; ×ATCC824.

Table 3: Summary of experimental results.

Test number and code	Concept	Type	Duration (h)	Membrane type	Productivity ($g.L^{-1}.h^{-1}$)	Xylose utilization (%)
T005	Fed-batch	GBL6046	166	Y	0.12	99%
T007	Fed-batch	GBL6046	170	Z	0.15	94%
T008	Fed-batch	GBL6046	143	Z	0.30	89%
T013	Fed-batch	GBL6209	61	Z	0.42	46%
Prior results	Continuous 2-stage[2]	ATCC824	192	Y	0.65	61%
T010 zone I	Continuous 1-stage	GBL6046	212	Z	0.26	84%
T010 zone II	Continuous 1-stage	GBL6046	138	Z	0.32	84%
T010 zone III	Continuous 1-stage	GBL6046	53	Z	0.52	55%
T012 zone II,	Continuous 2-stage	GBL6209	300	Z	0.63 [3]	54%
T012 zone III,	Continuous 2-stage	GBL6209	194	Z	0.67 [3]	69%
T012 zone IV	Continuous 2-stage	GBL6209	125	Z	0.72 [3]	67%
T013	Continuous 1-stage	GBL6209	110	Z	0.97	40%
T015 zone I	Continuous 1-stage	GBL6209	217	Z	0.65	55%
T016 zone I, F1	Continuous 2-stage	GBL6225	343	Z	0.77 [3]	59%
T016 zone I, overall	Continuous 2-stage	GBL6225	343	Z	0.34	72%

[2] Van Hecke W, Vandezande P, Dubreuil M, Uyttebroek M, Beckers H, De Wever H. 2016. Biobutanol production from C5/C6 carbohydrates integrated with pervaporation: experimental results and conceptual plant design. J Ind Microbiol Biotechnol 43(1):25-36.

[3] In first fermentor

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7 CONCLUSIONS

While both fed-batch and continuous conditions were found to be compatible with organophilic pervaporation operation, continuous conditions clearly have the edge over fed-batch conditions in terms of labour intensity, water consumption, obtained concentrations of solvents in the permeate, productivity and base consumption.

The highest obtained experimental solvent (acetone-butanol-ethanol) productivity was 0.97 $g.L^{-1}.h^{-1}$ and was demonstrated in continuous mode during 110h. A trade-off between xylose utilization and productivity was shown: the higher the productivity, the lower the xylose utilization. This implicates that an economic optimum has to be established between fermentor size (as defined by productivity) and substrate costs (as defined by xylose utilization).

Due to the improvements obtained in continuous mode in comparison to fed-batch mode, we advise to run the pilot tests in a one-stage continuous mode using pervaporation membrane Z.

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