Purified Butanol from Lignocellulose – Solvent-Impregnated Resins for an Integrated Selective Removal

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In traditional microbial biobutanol production, the solvent must be recovered during fermentation process for a sufficient space-time yield. Thermal separation is not feasible due to the boiling point of *n*-butanol. As an integrated and selective solid-liquid separation alternative, solvent impregnated resins (SIRs) were applied. Two polymeric resins were evaluated and an extractant screening was conducted. Vacuum application with vapor collection in fixed-bed column as bioreactor bypass was successfully implemented as butanol desorption step. In course of further increasing process economics, fermentation with renewable lignocellulosic substrates was conducted using *Clostridium acetobutylicum*. Utilization of SIR was shown to be a potential strategy for solvent removal from fermentation broth, while application of a bypass column allows for product removal and recovery at once.

Keywords: Biofuel, Biorefinery, Butanol, *Clostridium acetobutylicum*, Lignocellulose, Solvent-impregnated resins

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1 Introduction

The removal of butanol from fermentation broths is an ongoing target in downstream processing [1–3]. The major declared intent is to remove butanol in an in situ product recovery (ISPR) step, which reduces product inhibition and equipment costs in a single approach [4]. For this purpose, many standard operations in process engineering have been evaluated. These include (in descending order of energy consumption needed): distillation, pervaporation, perstraction, liquid-liquid extraction and adsorption [5]. The specific energy-content of butanol is 36 MJ kg^{-1}, hence, the recovery of the product should not extent the energy amount of the whole purification step [6]. Thus, adsorption and extraction processes are advantageous in terms of downstream energy demand. Nevertheless, the realization of solid-phase adsorption for product recovery might be accompanied by irreversible adherence of other components from the fermentation broth, and lead to unspecific binding [7, 8]. In extractive fermentations oleyl alcohol (OA) is usually regarded as nontoxic for the producing microorganisms. On the other hand it has only a moderate butanol distribution coefficient of 3.21–4.81 [9, 10]. Application of extractants with higher distribution coefficients in a system where the immiscible solvent is contacted with fermentation medium, conceivably results in depletion of fermentation performance [9].

A promising alternative for this dilemma is the usage of solvent impregnated resins (SIRs). SIRs are fusions of porous particles enclosing a liquid extractant realized by a physical impregnation technique. Its principle was originally introduced by Warshawsky for the selective copper extraction [11]. The technology can be viewed as an alternative adsorbent material, combining the advantages of liquid-liquid extraction and ion exchange separation [12]. These include selectivity and specific nature of the extractant and adopting continuous liquid-solid operation. A deceive advantage comparted to sole liquid-extraction is, that no emulsion is formed during operation [13] and thus phase separation processes are not necessary. Additionally, extractants with higher toxicity and distribution coefficients could be appropriate, since removal can be operated isolated from fermentation process using a bypass fixed-bed column.

In the past, SIRs have been considered for the extraction of other low concentrated aqueous solutions such as phenols, while increasing the volumetric production of *Pseudomonas putida* S12TPL fourfold [14]. For this reason, it seems reasonable to consider SIRs in solvent fermentation with clostridia, as well, reaching maximum solvent concentrations of 2 vol %.

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Besides various already established product removal technologies, there exist some further aspirations in order to obtain a commercially viable ABE (acetone-butanol-ethanol) process [15]. The required expenditure for fermentation substrates account to 63 % of the total ABE cost and are, therefore, directly linked to the profitability of the production plant [16, 17]. Shortly after the discovery of the ABE process in the last century, alternative renewable feedstocks were used as substrates for fermentation due to their prompt availability [18]. Nowadays, low-cost raw materials are widely seen as economic alternative substituting traditional substrates such as molasses or corn sugars due to their abundant occurrence [19]. In particular, agricultural residues, such as corn stalk, corn stover, rice bran, switchgrass or forestry residues from wood, were considered for ABE fermentation and might improve sustainability [20–23].

The aim of the work conducted here is the evaluation of SIRs technology for an integrated biobutanol removal, which to our knowledge is implemented for the first time. Besides, ABE fermentation using economical competitive concentrated beech wood hydrolysate was accomplished in batch mode with glucose and xylose as carbon source. In parallel fermentations, the process integration of SIRs in a fixed-bed column was evaluated. Accordingly, this study will give a very first insight to a promising alternative for in situ recovery during solvent fermentation of *Clostridium acetobutylicum* DSM 792.

2 Materials and Methods

2.1 Adsorber and Chemicals

Two adsorber resins were used for impregnation studies: Amberlite® XAD16N (XAD-16) and Dowex Optipore® L-493 (L-493), purchased from Sigma-Aldrich (Taufkirchen, Germany). *n*-Butanol, acetone, ethanol, acetic acid and butyric acid of highest purity available were purchased from VWR (Darmstadt, Germany). If not otherwise mentioned, all other chemicals were purchased from C. Roth (Karlsruhe, Germany). Different extractants were tested for this study: castor oil (Alfa Aesar), 1-decanol (Aldrich Chemistry), 1-hexanol (Merck KGaA), 1-octanol (Alfa Aesar), and oleyl alcohol (Merck KGaA).

2.2 Impregnation of SIRs

To remove impurities from adsorber resins, two cleaning cycles were applied. Therefore, 100 g particles (XAD-16 or L-493) were placed three times in an ultrasonification bath at room temperature for 10 min with 1 L demineralized (DI) water for salt removal. Afterwards, the particles were submerged three times with aqueous methanol in a ratio 1:1 to remove organics. Excess methanol was removed by drying the cleaned particles for at least 24 h at 50 °C. For impregnation studies, extractant was either diluted in concentrations of 300, 500, 700 g L^{-1} with methanol to decrease their viscosity or used undiluted. If not stated otherwise, cleaned particles were immediately contacted with impregnation solution in a ratio of 1:10 (v/v) and shaken in an incubator at 150 rpm. After 24 h the particles were filtered and washed with DI water to remove residual solvent amounts. A final drying step of at least 24 h at 60 °C ensured removal of residual methanol from particles. The extractant loadings (X_E) in g per $g_{Particle}$ were determined routinely by weighing the amount of particles before ($m_{particle}$) and after impregnation (m_{SIR}) according to Eq. (1). Samples of XAD-16 particles were taken after impregnation with oleyl alcohol for imaging by scanning electron microscopy (SEM).

$$X_E = \frac{m_{SIR} - m_P}{m_P} \tag{1}$$

2.3 Adsorption Equilibrium Experiments

Extractant uptake experiments were conducted in triplicates using 3–5 g SIR with different impregnations in 50 mL graduated conical polyethylene (PE) tubes. If not mentioned otherwise, mixtures were equilibrated at 37 °C in an overhead rotary shaker (Neolab) at 60 rpm with 20 mL model solution. After 24 h, supernatant samples were taken for gas chromatography (GC) analysis. The extraction performance was estimated by specific loading (L_i) and partitioning coefficients (K_i).

The specific loading (L) in extraction experiments was estimated for each component i of the model solution separately, for a particular resin fraction (X_r). For comparison, the calculations were based on the inserted dry particle amounts (m_P), determined in the extraction volume ($V_{E,aq}$),

$$X_r = \frac{m_P}{V_{E,aq}} \tag{2}$$

$$L_i = \frac{\left(\left[C_{i,B}\right] - \left[C_{i,E}\right]\right)}{X_r} \tag{3}$$

$$K_i = \frac{L_i}{\left[C_{i,E}\right]} \tag{4}$$

where $[C_{i,B}]$, is the concentration of the component at the beginning and $[C_{i,E}]$ after extraction. According to Tab. 1 different model solutions (A–G) were prepared with increasing complexity, in order to test the extraction performance. For repeated loading experiments of XAD-16-(OA), SIRs were equilibrated with a model solution of either 7.5 g L^{-1} or 15 g L^{-1}. Afterwards three additional medium exchanges (24 h, 48 h, 72 h) were conducted and relative increase of butanol loading was calculated, based on the initial equilibrium results.

Table 1. Model solutions (A-G) used for extraction experiments.

Component [g L^{-1}]	Model solution						
	A	B	C	D	E	F	G[a)]
Acetone	–	–	–	6	6	6	6
Butanol	15	10	5	12	12	12	12
Ethanol	–	–	–	2	2	2	2
Glucose	–	–	–	–	10	10	75
Acetic acid	–	–	–	–	–	5	5
Butyric acid	–	–	–	–	–	5	5
Media composition	–	–	–	–	–	PYX	P2[b)]

a) Corresponds to spent fermentation media with adjusted component concentration; b) composition: 1 g L^{-1} yeast extract and solution I–II (as described in method section).

2.4 Butanol Desorption and SIR Regeneration

A stainless-steel double jacket column (V = 500 mL, T = 37 °C) containing 300 g XAD-16-OA SIRs was used for desorption studies. Equilibration of the particles was conducted by circulating 2–5 L aqueous butanol solution of 15–20 g L^{-1} (T = 37 °C) through the column at a flow rate of 100 mL min^{-1}. Afterwards, the column was flushed with air to remove excess liquid. Regeneration was achieved by applying a vacuum (< 10 mbar) within the column. In several cycles, outgoing vapor was condensed, collected and removed from a cold trap, cooled with liquid nitrogen. These adsorption and desorption stages were repeated three times using the same SIRs. The obtained fractions were analyzed for their butanol content by GC.

2.5 Microorganism and Culture Media

ABE fermentation was conducted with *C. acetobutylicum* DSM 792 (DSMZ, Braunschweig, Germany). PYX medium containing 5 g L^{-1} trypticase peptone, 5 g L^{-1} meat peptone, 10 g L^{-1} yeast extract, 5 g L^{-1} *D*-glucose, 1 mg L^{-1} resazurin, 40 mL L^{-1} salt solution and 0.4 g L^{-1} *L*-cystein-HCl was used for reactivation of cells. 1 mL stock cultures, containing 30 % of glycerol, were prepared and stored at –80 °C. The stock culture was inoculated in 100 mL PYX for 24 h at 37 °C and used for inoculum of main culture. A concentrate of beech wood hydrolysate was produced at demand. Briefly, beech wood was pretreated by the Organosolv process and crude cellulose fibers were enzymatically decomposed. Conditions for the pretreatment were: 100 min reaction time at 170 °C with a solid-to-liquid ratio of 1:3 (ethanol/H_2O = 1:1; with 0.5 wt % H_2SO_4 as catalyst). The resulting concentrated sugar solution, consisting of glucose and xylose, was diluted with deionized water to a final concentration of approximately 60 g L^{-1} glucose and 20 g L^{-1} xylose, respectively. Additionally, the hydrolysate medium was supplemented with 1 g L^{-1} yeast extract, 2.2 g L^{-1} of ammonium acetate, 1 mL antifoam agent, 0.1 mL vitamin solution (I.) and 5 mL mineral solution (II.). Solution (I.) contained: 1 g L^{-1} *p*-aminobenzoic acid, 1 g L^{-1} thiamin and 0.01 g L^{-1} biotin. Solution (II.) was composed of 40 g L^{-1} $MgSO_4 \cdot 7\,H_2O$, 2 g L^{-1} $MnSO_4 \cdot H_2O$, 2 g L^{-1} $FeSO_4 \cdot 7\,H_2O$, and 2 g L^{-1} NaCl.

2.6 Fermentation Using Hydrolysate Medium and Butanol Removal

The fermentation of *C. acetobutylicum* was performed in bioreactors with a working volume of 1.3 L. Before autoclaving for 20 min at 121 °C, the bioreactor was sparged with oxygen free nitrogen for 15 min. Hydrolysate concentrate was filtered through a 0.2-µm vacuum filtration system (VWR, Darmstadt, Germany) and added aseptically after autoclaving. Fermentation was conducted at a temperature of 37 °C and a stirring rate of 150 rpm. Inoculation of the bioreactor was performed with 10 vol % of a freshly grown preculture. During fermentation, the butanol amount available on the SIRs was calculated by means of isotherm data using Freundlich isotherm model, as reported by Nielsen and Prather [24]. Together with the amount quantified in the broth, the total titer was calculated.

2.7 Analytical Procedures

Organic acids and solvents of model solutions and fermentation products were determined by GC (Clarus 500, PerkinElmer, Waltham, USA) using a flame ionization detector and an RTX-5-AMINE column (Restek, Bellefonte, USA). The flow rate for the helium carrier gas was 250 mL min^{-1} and the column was heated up to 180 °C. Glucose concentrations in the supernatant were measured by high performance liquid chromatography (HPLC) using a refractometric (RI) detector. Separation was carried out on a 300×8 mm ReproGel Ca^{2+} column (Dr. Maisch GmbH, Ammerbuch-Entringen, Germany) at 80 °C. Ultrapure water with a flow rate of 0.5 mL L^{-1} served as mobile phase. The injection volume was 20 µL.

3 Results

3.1 SIR Screening and Butanol Affinity

Impregnation of SIRs was conducted with five extractants, which were identified as potential candidates for butanol removal in liquid-liquid extraction on basis of their distribution coefficients and selectivity towards butanol [25]. As exploitable SIR matrix, initially two commercially available

adsorber particle types XAD-16 and L-493 were chosen. These were considered based on their given surface area and pore volume, respectively. On this account, the effective solvent amount available after the impregnation procedure was determined together with the calculated maximum theoretical loading per particle, based on the particle properties (Fig. 1).

Highest extractant loading was achieved with XAD-16 particles with X_r = 0.06 g L^{-1}. Regardless of the dilution, impregnation with oleyl alcohol resulted in stable extractant loadings between 1.63–2.30 g $g_{XAD\text{-}16}^{-1}$ and 0.94–1.45 g $g_{L\text{-}493}^{-1}$. Best loadings using castor oil were achieved for undiluted solvent (2.83 g $g_{XAD\text{-}16}^{-1}$). Impregnation with castor oil and oleyl alcohol with concentrations > 300 g L^{-1} resulted in nearly complete pore volume filling for both particles tested. With regard on the other extractants, octanol and 1-decanol showed the highest amounts with dilutions of 700 g L^{-1} for XAD-16. The level of extractant loading on L-493 particles can be ranked as follows octanol > decanol > hexanol. Yet, only 50 % of the theoretical loading (based on the pore volume) was reached, indicating an insufficient coating of the particles. As uniform approach and also due to handling reasons, for all further experiments, dilutions of extractant with methanol in end concentrations of 700 g L^{-1} extractant solvent was chosen.

Scanning electron microscope (SEM) images of XAD-16 particles show the appearance before and after impregnation with oleyl alcohol in Fig. 2. In particular at high resolutions (10 000–50 000×), a notable difference between the coated (Fig. 2d) and uncoated (Fig. 2b) particle surface is visible. On the one hand, the native particle shows a defined pore structure; on the other hand, the impregnated particle exhibits an extractant coating on its surface. In principle defects can be caused by swelling stress of the matrix during impregnation with the extractant solvent [26]. Nevertheless, such as cavities or microcracks on the surface of the particle do not appear after impregnation.

Hereinafter, loading capacities using aqueous butanol concentrations between (15–5 g L^{-1}) were evaluated for XAD-16 and L-493 particles impregnated with oleyl alcohol (Tab. 2). For a better comparability with non-impregnated resins, these values were referred to the particle dry mass. By impregnating XAD-16 particles having a surface area of 800 $m^2 g^{-1}$, butanol loadings between 25.4 and 87.3 mg $g_{XAD\text{-}16}^{-1}$ were measured. With regard to non-impregnated XAD-16 particles, the adsorption capacities found for butanol with XAD-16 (74 mg $g_{XAD\text{-}16}^{-1}$) are comparable to literature data with 75 mg g^{-1} [7].

Using L-493-(OA) SIRs, a maximum loading of 87.3 mg $g_{L\text{-}493}^{-1}$ was achieved. These particles disposed 94 % of the adsorption capacities compared to the plain L-493 particles found in this study. Hence, the substantial difference found for non-impregnated L-493 in literature (Tab. 2) might be caused by variations in experimental setup. Nevertheless, values found by Nielsen and Prather [24] are relatively exalted (175 mg g^{-1}) in contrast with values from Lee et al. [27] (80 mg g^{-1}), which are more within the range of the values found in this study (93 mg $g_{L\text{-}493}^{-1}$).

Butanol removal might also be conducted with other potential extractive candidates used in liquid-liquid extraction. These include other alcohols, aldehydes, or natural products such as oil. The adsorption capacities of further

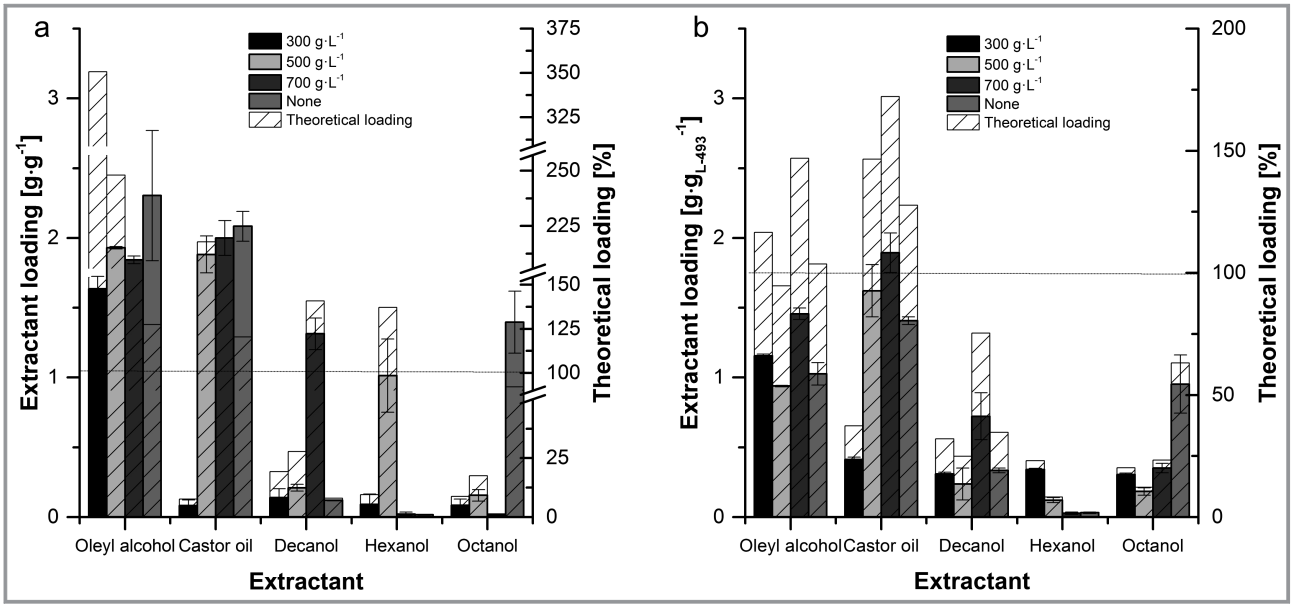

Figure 1. Extractant loading of XAD-16 (a) and L-493 (b) particles with different methanol-extractant dilution (none, 300, 500, 700 g L^{-1}). Oleyl alcohol (black), castor oil (light gray), 1-decanol (white), 1-hexanol (dark gray) and 1-octanol (light gray, dashed). Dashed bars indicate percentage of theoretical loading based on particle properties. Horizontal dotted line displays 100 % extractant loading. Error bars represent standard error from triplicate measurements.

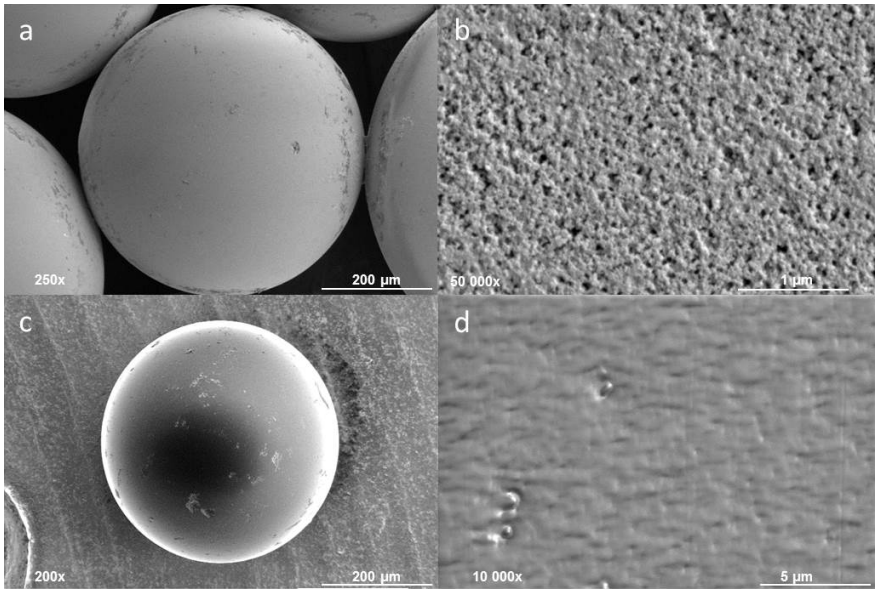

Figure 2. SEM image of XAD-16 particles before impregnation (a, b) and after impregnation with OA (c, d).

Table 2. Butanol loading capacities ($L_{Butanol}$) of adsorber particles (XAD-16 or L-493) in comparison to SIRs impregnated with oleyl alcohol

Particle/SIR	Butanol solution [g L^{-1}]	T [°C]	$L_{Butanol}$ [mg $g_{Particle}^{-1}$]	Volume [L]	Reference
XAD-16	9.2[a)]	34	75	1.0	[7]
	15	37	74 ± 1.16	0.02	this study
L-493	20	30-37	175	n.d.	[24]
	15	37	80	0.10	[27]
	15	37	92.6 ± 0.14	0.02	this study
XAD-16-(OA)	15	37	97.7 ± 0.45	0.03	this study
	10	37	65.2 ± 0.28	0.03	this study
	5	37	32.6 ± 0.06	0.03	this study
L-493-(OA)	15	37	87.3 ± 1.41	0.03	this study
	10	37	56.3 ± 1.09	0.03	this study
	5	37	25.4 ± 0.36	0.03	this study

a) Fermentation broth consisted of ABE components.

promising extractants such as decanol, octanol or the non-toxic castor oil are shown in Fig. 3. The extraction capacities with castor oil as extractant and XAD-16 particles are 26 % higher compared to impregnation of L-493. Butanol loading was the lowest for decanol-impregnated particles, with 42 and 47.5 mg $g_{XAD\text{-}16}^{-1}$.

Besides loading capacities, also the selectivity towards the target product is an important factor for a recovery system. This is especially the case for the complex intermediate and product composition of ABE fermentation broths. The ABE solvent production is characterized by two pH-dependent distinct phases: the acidogenesis and solventogenesis [28]. In the first phase, mainly carboxylic acids such as butyric and acetic acid are built up, while in the second phase the ABE production takes place. A loss of these intermediates or other essential substrate components must be avoided for a high yield process. For this reason, it must be assured that during extraction, high selectivity towards the target alcohol product is given. In order to evaluate the affinity towards media components, different model solutions were prepared with increasing complexity (Tab. 1). The results of the extraction are summarized in Tab. 3 and represented by specific loadings and partitioning coefficients of each solute within the tested SIRs. The affinity for either XAD-16 or L-493 impregnated with oleyl alcohol is highest for butanol, with all model solutions tested. Conceivably on account of solubility changes with increasing model solution complexity, the extraction with XAD-16-(OA) SIRs even shows a rise in butanol affinity resulting in maximum loading of 150.3 mg g^{-1} (solution G, Tab. 1). In contrary, with L-493-(OA) SIRs a maximum butanol adsorption of 111.4 mg g^{-1} is reached with ABE components and glucose (solution E) while the complex P2 medium (model solution G) decreases the adsorber capacity. The affinity towards glucose and organic acids stays low, especially using fermentation broth (< 8 %), which is favorable in fermentation approach.

3.2 Repeated Loading and Desorption of SIRs

In order to prove the ability of using SIRs repeatedly in consequent runs without intermediate regeneration steps, three additional medium exchanges (four runs) with two butanol concentrations of 7.5 g L^{-1} and 15 g L^{-1} were carried out (Fig. 4). Based on the assumption of using hydrolysate media from renewable raw materials during the fermentation with *C. acetobutylicum*, the lower initial concentration was chosen to represent previously published fermentation

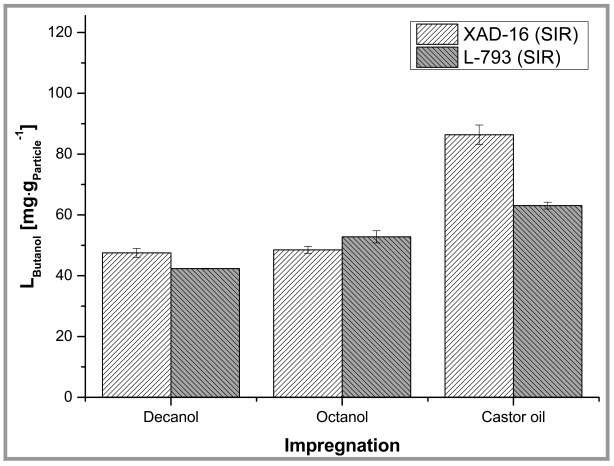

Figure 3. Butanol loadings using decanol, octanol, and castor oil for impregnation of XAD-16 (light-striped) and L-493 (dark-striped) particles.

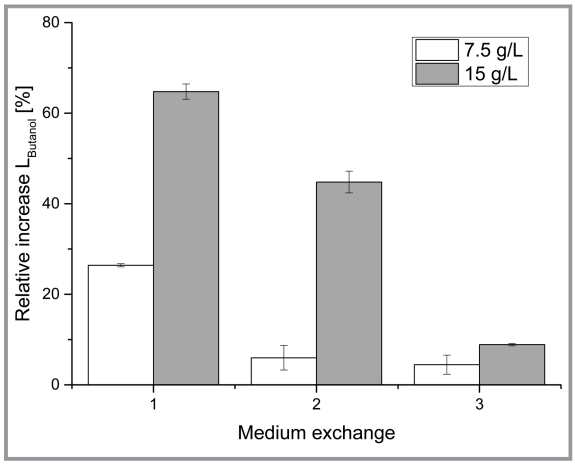

Figure 4. Reuse of XAD-16–OA SIR without regeneration for three extraction cycles. Solution of 7.5 g L^{-1} (white) or 15 g L^{-1} (gray) was changed after 24 h. Relative increase is calculated based on the maximum butanol loading reached before reuse of particles was initiated. Error bars represent standard error from triplicates.

results using beech wood hydrolysate (max. 8.1 g L^{-1}) [23]. However, in ABE fermentations with synthetic media, butanol concentrations up to 12–15 g L^{-1} are reported. These higher concentrations are of special interest as they are also regarded as the toxic level for the bacterial culture [18].

In Fig. 4, the relative increase of butanol loading of already saturated SIRs is depicted after three additional model solution exchanges, without intermediate regeneration steps of the adsorbers. With the low concentrated solution (7 g L^{-1}), it was possible to get an additional loading of 26 % during the first additional extraction cycle, while only a minor increase in butanol loading of 6 % and 4.5 %, respectively, was encountered in the following cycles. Considering higher initial concentration (15 g L^{-1}), an additional loading of 64 % was detected after the first medium exchange, while the following cycles showed an increase of 45 % and 9 %. Thus, larger initial butanol concentrations lead to higher additional butanol loading. Regarding the fermentation procedure, this extraction behavior implies a sequential removal process, which is only initiated after moderate levels of butanol have been generated by the clostridia.

The ability to recover butanol from the impregnated resin phase after a complete loading with butanol with the objective of subsequent reuse of XAD-16-(OA) SIRs was investigated using a butanol model solution. To adapt the bypass to the fermentation step and to avoid damage of the polymeric resins due to heat stress the column temperature was set to 37 °C during loading and unloading. The results of three load and unload cycles, gathered in 30 min interval fractions, are depicted in Tab. 3. Up to eight vapor fractions could be collected during the operating time. In the first load/unload cycle, desorption via vacuum regeneration resulted in eight fractions, with a total of > 86 % recovered butanol. In the following cycles a recovery of 73.6 % and 81.2 % was reached, indicating a proficient recovery. Additionally, it was found that within the first four intervals, already 73.3 % of the bound butanol could be recovered.

Table 3. Specific loadings and partitioning coefficients measured form different model solutions of XAD-16-OA and L-493-OA particles

SIR/Solution		Specific loading [mg $g_{particle}^{-1}$] : partitioning coefficient [$mg_{particle}^{-1} g^{-1} L^{-1}$]					
		Butanol	Acetone	Ethanol	Glucose	Acetic acid	Butyric acid
XAD-16-(OA)	D	52.3:5.9	16.0:3.0	0.6:0.3	–	–	–
	E	56.7:6.5	16.7:3.3	3.1:1.4	7.5:0.7	–	–
	H	137.7:16.6	15.4:3.9	2.5:1.4	18.6:2.0	6.8:0.0	20.7:5.5
	G	150.3:37.3	9.6:1.8	17.5:16.2	12.0:0.2	0.0	6.1:0.0
L-493-(OA)	D	40.7:4.4	3.5:0.7	1.2:0.6	–	–	–
	E	111.4:21.5	10.0:1.6	4.8:2.9	10.8:1.3	–	–
	H	52.2:7.2	4.1:0.7	1.9:6.0	10.3:2.4	0.0:0.0	17.4:4.5
	G	67.3:9.3	12.8:2.5	10.3:4.1	5.8:0.1	0.0:0.0	7.9:1.8

3.3 Fermentation with Product Removal Using Hydrolysate Medium

ABE fermentations were executed with *C. acetobutylicum* DSM 792 using a lignocellulose hydrolysate medium (Fig. 5). As baseline

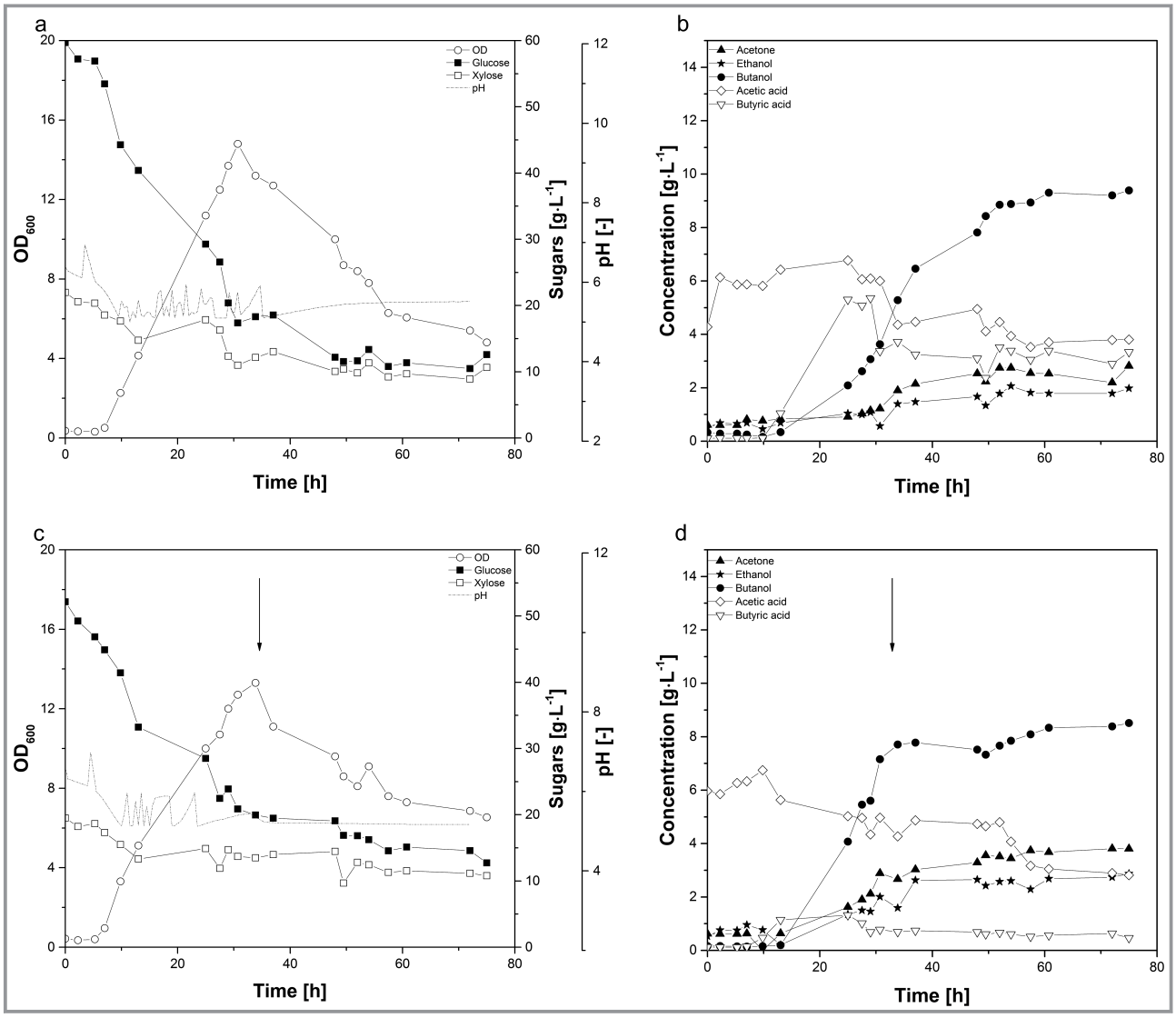

Figure 5. Beech wood hydrolysate ABE fermentation using *C. acetobutylicum* (DSM 792) without (a, b) and with in situ product removal with SIR bypass column (c, d). Left side: Concentration of glucose (solid squares) and xylose (open squares), optical density (open circles), pH (dashed line). Right side: Concentration of acetone (solid triangles), butanol (solid circles), ethanol (solid stars), acetic acid (open diamonds), butyric acid (open triangles). Arrow indicates start of circulation trough SIR bypass column.

control, the ABE fermentation was performed in batch mode without solvent removal. In a parallel approach, fermentation with a fixed-bed bypass column was evaluated. The product removal was performed with solvent-impregnated particles containing oleyl alcohol as extractant. In Fig. 5 (a, b) the course of the 75-h fermentation is given, while a summary of important process results can be found in Tab. 4.

After the initial lag-phase during the first 7 h, maximum cell growth was reached after 31 h, resulting in maximum optical density of 13.3. Subsequently, production of acetic and butyric acid was initiated, and after 52 h maximum titers of 7.96 and 3.5 g L^{-1} were detected. The high initial amounts of acetic acid are attributed to its formation during the pretreatment process of beech wood prior to enzymatic decomposition. While solvent formation began after approximately 25 h, maximum butanol concentration was reached after 75 h with 9.4 g L^{-1}. In addition, solvent titers of acetone and ethanol were 2.8 and 2.0 g L^{-1}, respectively, resulting in a total solvent concentration of 14.2 g L^{-1}. The total ABE yield corresponds to 0.24 g g^{-1}. At the beginning, the pH value of the hydrolysate medium was adjusted to 6.6, which was found to be a suitable initial value for cell growth in previous fermentation approaches. During the first 12 h, the pH dropped to 5, and was afterwards controlled by a PID-controller at this lower boundary in order to avoid an irreversible acid crash [29]. Due to the rise in solvent concentration after 40 h, also the pH increases.

Table 4. Comparison of batch ABE fermentation using hydrolysate medium with and without product removal using SIRs in a bypass column

Parameter	Batch	Batch+ SIRs[a)]
Butanol [g L^{-1}]	9.9	8.51
Yield (butanol) [g g^{-1}]	0.16	0.18
Initial glucose [g L^{-1}]	60.0	52.2
Final glucose [g L^{-1}]	12.6	12.7
Initial xylose [g L^{-1}]	22.0	19.5
Final xylose [g L^{-1}]	10.6	10.8
Glucose utilization [%]	79	76
Xylose utilization [%]	52	45

a) Amounts of butanol were calculated based on concentration found in the aqueous and on the SIR using isotherm data.

Glucose and xylose at concentrations of approximately 60 and 22 g L^{-1} were the available sugars in the hydrolysate medium. During the fermentation a decrease of sugars is apparent. The residual amounts were 12.6 and 10.6 g L^{-1}, respectively, which equals a sugar utilization of 79 % and 52 %, respectively.

The fermentation approach with an integrated bypass column containing 300 g SIRs impregnated with OA as extractant was used during batch fermentation, using the removal technique simultaneously with solvent production stage of the bacteria. The ABE fermentation with integrated product removal in Fig. 5 (c, d). shows a similar behavior in comparison to the control fermentation Fig. 5 (a, b). Shortly before removal was induced, maximum cell concentration was distinguishable with an OD of 13.3 g L^{-1}. Start of product removal was initiated during solventogenesis (t = 34 h), which was accompanied by a rise in pH, whereat pH 5.4 was the starting point. The focus of fermentation was set on butanol production and removal, using SIR in a bypass column. The final butanol titer in the aqueous phase after 75 h was 4.8 g L^{-1}. The total amount, including the extracted butanol, equaled 8.51 g L^{-1} corresponding to a yield of 0.18 g g^{-1}. Although solvent removal was accomplished, sugar consumption occurred similarly to control fermentation with final titers of 12.7 and 10.8 g L^{-1}, glucose and xylose, respectively. However, the sugar consumption rate was higher than that of the control batch fermentation, which are deduced to a reduction of butanol solvent stress, caused by the continual removal [30].

4 Discussion

In the work presented, a particle-based butanol extraction system using SIRs was established. It combines product loadings as known in an adsorption process [24] with the selectivity known by liquid-liquid extraction [31]. With this particle-based system, the major obstacles in conventional liquid-liquid solvent extractions, e.g., emulsion formation, separation of the fractions or slow mass transfer into the organic phase [32], could be overcome. Another significant advantage is that the amount of extractant used is negligible compared to the amount of aqueous phase, thus, separation can be achieved much faster. From the two examined adsorber resins, impregnation of XAD-16 particles resulted in the highest extractant loadings and butanol adsorption affinity using oleyl alcohol.

During the fermentation process, the toxicity of the solvent towards the microorganism used is of great importance. In fermentations with in situ extraction, high distribution coefficients and a reasonable biocompatibility are desired [33]. For this reason, extractants such as oils or short chained alcohols [9, 13, 31] have been evaluated. The usage of castor oil as extractant solvent was tested and a partitioning coefficient of 2.59 determined, which is comparable with literature data [13]. However, the performance of SIRs was inconsistent (data not shown). This could be attributed to variations in composition of fatty acids and TG, of this natural product in several batches for SIR preparation [34].

A correlation of diminishing biocompatibility contrary to growing k values are often made in literature [35]. From a process-specific point of view, high distribution coefficients are desired to minimize the amount of solvent needed. Usage of SIRs in an external separation column could ease this problem since the particles are not in direct contact with the fermenting organisms. On this account, also decanol (k = 6.2), octanol (k = 5.6–7.33) and hexanol (k = 9.91) [2, 25, 36] were tested as potential candidates. To facilitate the impregnation procedure and to increase the rate of mass transfer into the solvent, different dilutions of extractant, resulting in decreased viscosities, have been used. However, impregnation of L-493 particles has proven inadequate since the pore volume was only filled to 20–50 % with decanol and octanol. With hexanol, the least pore filling was achieved in between 5 to 20 %. This phenomenon was trackable for both adsorber species. This is why it can be assumed that maximum swelling during impregnation procedure of the polymer is not achieved, leading to insufficient extractant uptake [37].

In extractive bioconversion, negative ramifications concerning the competitive adsorption of nutrients, precursor metabolites and cells must be considered. Commercial XAD-4 particles were found to impair butanol production and growth of *C. acetobutylicum* due to adsorption of nutrients found in yeast extract [8]. So far, these negative effects have not been encountered when using SIRs in our experiments.

An increasing capacity of 35 % was observed when XAD-16 particles impregnated with oleyl alcohol were used with fermentation broth. The increase in the distribution coefficient for butanol after electrolyte addition has already

been reported for liquid-liquid extraction, and was ascribed to alterations of extraction equilibria, which changes the water activity [38].

It is reported that capacity reductions of up to 29–32 % occur compared to binary solution (butanol/water), if plain adsorber resins are implemented with fermentation broths, due to competitive adsorption of other broth components [24, 39]. Because of this, SIR application seems to be more reasonable for fermentation approaches. Besides, influence of sorption at different temperatures was neglected for SIR application, but it is probable that extraction capacities would increase further [40].

Butanol recovery was achieved very efficiently by vacuum application within the bypass column. During the regeneration, bound volatile organic components, as in this case butanol, are removed and condensated while the immobilized non-volatile extraction liquid remains in the pores of the polymeric particle. Similar approaches have been applied by the application of supported liquid membranes (SLM), where a combination of liquid-liquid extraction and pervaporation is used. For example, with a polypropylene membrane impregnated with oleyl alcohol, high selectivity (α = 180) and 100-fold compression of a 4 g L^{-1} butanol feed solution was possible [41]. Nevertheless, extractant loss through the membrane was encountered. In our approach no alteration in desorption performance was experienced so far. Muraviev assumed that in macroporous matrices, the impregnation solution can be immobilized in the pore and gel region of the polymer. In the first case, extractant can easily leach or be washed out from freshly prepared SIR, since extractant in the pore volume is only weakly retained in the polymer (capillary forces), while additional forces (e.g., π-π) between the extractant and the particle polymer network remain stable in terms of capacity for a long period in the latter case [37]. Moreover, attempts were made to increase the stability of sulfonate impregnated resins, which include conditioning in several cycles, post-impregnation by encapsulation and wet drying, where samples are kept in boiling water for several hours [26]. Although no extractant loss could be quantified so far, long-term stability test will be performed to address this question.

Nonetheless, it should be emphasized that the SIR regeneration process was completed within 4 h resulting in a mean recovery of 80 %. Beyond that, already after 2 h 73 % of the total butanol could be recovered. In comparison, adsorption columns using non-impregnated particles of Dowex Optipore SD-2 and L-493 particles resulted in similar recovery values (81–83 %) but much longer regeneration times [24, 42]. The total operating time was up to 6-fold higher in comparison to our SIR regeneration technique. In another study using L-493 particles, an ABE recovery of nearly 95 % was possible by desorption carried out using 140 °C saturated steam, significantly increasing the energy demand of regeneration in comparison the SIR process [43]. Looking at the low energy demand of the SIR process, we see no need for further change of the desorption step as, e.g., application of higher feed temperatures or longer operation time [44].

In most instances the collected vapor fractions displayed two distinct phases, an aqueous and an organic phase. The latter contained butanol at concentrations above the water solubility limit (7.7 wt %, 20 °C). In further purification steps, this preconcentrated butanol solution can be used to separate the fractions gravimetrically, resulting in a highly purified product. Hereby, a subsequent energy-intensive evaporation step can be circumvented. With the application of SIR, it was thus possible to demonstrate that the usage in a fixed-bed column allows simplified process integration without the need of collateral particle handling or transportation.

4.1 Fermentation

In order to address the fermentability of hydrolysate medium and its butanol outcome, ABE fermentations using beech wood as raw material were evaluated in batch mode. The total ABE titer that was achieved within 75 h fermentation time was 14.2 g L^{-1}. This is in the concentration range from reports of other groups, where pretreated woody biomass, such as wood pulp (8.98–17.73 g L^{-1}) or aspen wood chips (5.04–13.15 g L^{-1}) were investigated [45, 46]. In a previous approach using technical-grade beech wood hydrolysate medium created by enzymatic cellulose hydrolysis in a solid state reactor, total solvent titer of 15.1 g L^{-1} was achieved, while having higher initial acetic acid concentrations in the hydrolysate medium in laboratory scale preparation [23]. In the current study, we evaluated the usage of concentrated sugar solution from pretreated beech wood gained from pilot plant scale, which resulted in a sugar consumption of 79 % glucose and 52 % xylose, respectively. In comparison, an enzyme treated corn fiber hydrolysate (ETCFH) resulted in 8.6 g L^{-1} total ABE using *Clostridium beijerinckii* BA101 [47]. Nevertheless, the total ABE yield was higher (0.31 g g^{-1}) compared to our study (0.24 g g^{-1}).

It should be stated that the primary aim of this study was not an optimization of the hydrolysate fermentation but to implement a selective butanol removal with solvent impregnated particles. In this context, end-product inhibition during ABE fermentation is a major problem, which is why butanol must be removed from fermentation broths. Failing this will lead to reduction in phospholipid bilayer of *C. acetobutylicum* and subsequently higher membrane fluidity, which contrarily leads to losses in essential cellular functions such as, glucose uptake, ATP- and internal pH control [48]. In this study, the implementation of a bypass column into fermentation process was shown to be feasible allowing an effective coupling of butanol production and an integrated product removal step.

We calculated that an amount of 300 g SIRs, which corresponds to 106 g particles (dry basis), is sufficient for the downstream processing of 1 L of a 1.5 % butanol solution.

Retention of cells before feeding the suspension into the butanol removal bypass column was not necessary. The contact of cells with a fixed bed may result in a biomass concentration decrease due to shear stress and unspecific adsorption. Nevertheless, a decline in cell growth due to solvent removal through the column was not evident, which could be seen by stable values in optical density (Fig. 5). Even more, the final biomass concentrations were 30 % higher compared to the parallel control fermentation without butanol removal. Contrary, expanded-bed adsorption had a negative impact on growth of *C. acetobutylicum* ATCC 824, caused by continuous pumping and recirculation of the culture through an external column [42].

Although only simple batch fermentations were conducted, an increase of the butanol yield of 13 % from 0.16 to 0.18 $g\,g^{-1}$ was distinguishable using SIRs in a fixed-bed column. This was accomplished by alleviation of butanol concentration in the aqueous phase and, therefore, increasing the capacity for solute accumulation in the broth. In comparison to other batch approaches using synthetic medium, maximum butanol yield of 0.21 $g\,g^{-1}$ were achieved using biodiesel as extractant while in the control fermentation, without butanol removal a yield of 0.185 $g\,g^{-1}$ was reached [49].

Currently, fed-batch operation is evaluated, which is expected to increase overall ABE productivity from currently 0.29 $g\,L^{-1}\,h^{-1}$ during batch operation, as was already reported by different authors. For example, productivity increased by 400 % using integrated fed-batch fermentation with solvent removal compared to batch experiments [50]. In a study by Roffler a 1.7-fold increase in productivity was detected by pumping the broth through a Karr column [33].

In conclusion, this study was intended to prove the applicability of SIRs for solvent removal in ABE fermentation using beechwood hydrolysate. In small-scale experiments, stable butanol loadings were accomplished, using oleyl alcohol as extractant for SIR preparation. Furthermore, application of SIR in a fixed-bed column resulted in an integrated process where solvent removal and recovery could be performed in succession. Nevertheless, change of the fermentation mode should further increase efficiency.

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