Integration of ABE Solvents Fed-batch Biosynthesis with their Recovery by Gas-stripping

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The biosynthesis of ABE (acetone, butanol, ethanol) solvents with Clostridium acetobutylicum occurs due to its growth in a glucose substrate medium. Clostridial ABE production is commonly conducted in bioreactors at a pH value of 4.5 and temperatures ranging between 36 and 38°C. Aiming at diminishing the butanol inhibitor effect on Clostridium acetobutylicum bacterial system, a controlled removal of solvents from reaction medium can be used for batch and fed-batch bioreactors. An experimental study of solvents removal by gas-stripping was performed in order to estimate the kinetics of solvents stripping from fermentation broth. A mathematical model was applied to solve an optimization case, i.e. to maximize the solvents production in a bioreactor which operates sequentially in batch, fed-batch, and fed-batch with gas-stripping system. An optimization function depending on the feed flow rate, substrate concentration in the reactor feed, switching time from batch to fed-batch system, and stripping start time was defined and evaluated.

Keywords: biosynthesis, ABE solvents, Clostridium acetobutylicum, modelling, gas-stripping, process optimization

Replacing of fossil fuels by bioenergy carriers is a major research direction in biotechnology [1-3]. Near to bioethanol and biodiesel, which are commonly used, the biobutanol is a promising biofuel in the future. Until the middle of the last century, acetone-butanol-ethanol (ABE) anaerobic fermentation in the presence of Clostridium genus bacteria, e.g. C. acetobutylicum, C. beijerinckii, C. saccharoperbutylicum, was applied on industrial scale to synthesize the biobutanol [4]. Clostridial fermentation consists of an acidogenesis phase, wherein the bacteria produce acetic acid (AA), butyric acid (BA), CO₂, and H₂ from a glucose substrate, followed by a solventogenesis phase, wherein the acids are converted into acetone (A), butanol (B), and ethanol (E), usually in 3:6:1 molar ratio [3]. Glucose substrate is used for bacterial cell growth, for acids production during the acidogenesis phase, and for acids conversion into ABE products during the solventogenesis phase [5-7]. Due to a high substrate cost, the industrial application of butanol fermentative biosynthesis was restrained for a long time. Recently, due to oil crisis and increasing demand for alternative fuels, researchers and industrials have again paid attention to clostridial ABE fermentation [8]. Starchy and lignocellulosic wastes, e.g. molasses, straws, corn stovers/cobs, spoiled grains/fruits, forest residues, are glucose precursors which are widely available at low cost [9,10].

ABE fermentation can be performed in batch, fed-batch or continuous reactors. Batch fermentation is the simplest biosynthesis way but its productivity is limited by substrate and product inhibition. Fed-batch fermentation is adopted to avoid substrate inhibition and achieve a high cell density. Butanol production has a strong inhibitory effect on cell growth, leading to low levels of cell density and butanol yield in the fermentation broth [3,8]. This product inhibition can be diminished by coupling the synthesis bioreactor with a system of butanol removal from the reaction medium. Various methods have been employed to remove the butanol produced by fermentation, e.g. liquid-liquid extraction [11-13], gas-stripping [14,15], membrane pervaporation [16-18], adsorption [19], perstraction [20]. Applying one of these separation techniques, a significant improvement in solvents production can result. For instance, ABE biosynthesis in the presence of Clostridium beijerinckii BA 101 led to a yield of solvents to glucose of 0.47 g/g and an ABE productivity of 1.16 g/(Lhr) in a fed-batch reactor with gas-stripping compared to values of 0.39 g/g and 0.29 g/(Lhr) in the fed-batch reactor without gas-stripping [14].

ABE biosynthesis modelling has been used for: i) quantitative characterization of intricate biochemical process describing the dynamics of glucose transformation in ABE, CO₂, and H₂; ii) design/simulation/optimization of ABE synthesis reactor; iii) design/simulation/optimization of ABE synthesis reactor coupled with ABE separation system; iv) analysis of final reaction mass processing [21]. The present paper has aimed at optimizing the operation of a fed-batch reactor with gas-stripping of reaction products in order to maximize the amount of ABE solvents produced by clostridial fermentation using Clostridium acetobutylicum. Biochemical process kinetics in ABE synthesis reactor was predicted based on a model developed by Volesky and Votruba [22], whereas gas-stripping kinetics of volatile species from reaction medium was experimentally estimated.

Model of fed-batch reactor with gas stripping of volatiles

A structured growth model was adopted to simulate the dynamics of clostridial ABE synthesis using glucose substrate (S) in a fed-batch bioreactor with gas-stripping [22]. According to this model, the specific growth rate of biomass (X), μ, can be expressed depending on dimensionless cellular RNA (ribonucleic acid) concentration, y, considered as a marker of culture physiological state, by correlation (1), wherein cRNAmin is cellular RNA concentration at μ=0.
Physical model and simplifying assumptions

The physical model used to describe ABE biosynthesis in a mechanically stirred bioreactor is schematically represented in figure 1. A sequential operation of the bioreactor (1) was selected, i.e. batch mode in the first sequence, fed-batch in the second one and fed-batch with gas-stripping of volatile species in the last sequence. The batch operation was switched to fed-batch at a switching time, \( \tau_{\text{sw}} \) (hr). The fermentation broth volume, \( V \) (L), was steadily increased in fed-batch system by feeding a volumetric flow rate, \( F \) (L/hr), of glucose substrate at a concentration \( c_{S} \) (g/L). The fed-batch mode turned into fed-batch with gas-stripping at a stripping start time, \( \tau_{\text{str}} \) (hr). A volume of stripping gas, \( V_g \) (L), was circulated by pump (4) at a \( G_v \) (L/hr) volumetric flow rate through the system consisting of bioreactor (1), condenser (2) and heater (3). Vapour of A, B, E, and water (W) as well as gases, i.e. \( \text{CO}_2 \) and \( \text{H}_2 \), removed from fermentation broth by gas-stripping, were cooled in the cooler (2) at a \( t_c \) temperature, producing a vapour partial condensation. The stripping gas containing uncondensed vapour, \( \text{CO}_2 \), and \( \text{H}_2 \) was heated in the heater (3) at the fermentation temperature, \( t_f \), and further fed in the bioreactor (1).

![Diagram of fed-batch reactor with gas-stripping](image)

Other assumptions considered for fed-batch with gas-stripping running are as follows: i) the stripping gas leaves the cooler (2) saturated with vapour at a concentration \( c_{i} \), \( i = \text{A, B, E, W} \); ii) carbon dioxide and hydrogen accumulate in the stripping gas at a concentration \( c_{i} \), \( i = \text{CO}_2, \text{H}_2 \), respectively; iii) characteristic gas-liquid equilibrium of species A, B, E and W in fermentation broth is expressed by distribution coefficient, \( k_{di} \); iv) mass transfer resistance of A, B, E, \( \text{CO}_2 \), and \( \text{H}_2 \) in fermentation broth is located in liquid film surrounding the gas bubble, whereas that of water (W) is located in gas phase. According to these assumptions, the mass flow rate of \( i \) component vaporized inside the bioreactor, \( n_{i} \) (g/hr), is expressed by equation (2), wherein \( c_{i} \) (g/L) is concentration of \( i \) compound in fermentation medium, \( c_{i} \) (g/L) concentration of \( i \) component in stripping gas, and \( k_{a} \) (hr\(^{-1}\)) volumetric mass transfer coefficient in liquid film. The concentration of \( i \) component in recycling gas was estimated depending on masses of condensed compound, \( m_{i} \) (g), and \( A, B, C \) Antoine constants by relation (3) derived from Raoult law.

\[
\mu = 0.56(y-1) = 0.56 \left( \frac{c_{\text{sp}}}{c_{\text{sp,sp}}} - 1 \right) \quad (1)
\]

\[
n_{i} = \nu k_{a}[c_{i} - k_{a}c_{i}(t_{j})] \quad i=A, B, E, \text{CO}_2, \text{H}_2 \quad (2)
\]

\[
c_{i} = \frac{m_{i}}{RT \sum_{i=1}^{n} m_{i}} \quad i=A, B, E \quad (3)
\]

Equations and restrictions system

The mathematical model characterizing the bioreactor dynamics was based on equation (4) of \( i \) species mass balance in fermentation broth.

\[
d(V_{i}) \quad \frac{dV}{d\tau} = VR + Fc_{SP} - n_{i} \\
\quad \quad i=\text{X, S, A, B, E, AA, BA, CO}_2, \text{H}_2 \quad (4)
\]

According to relations (1)-(4), simplifying assumptions as well as expressions of rate of \( i \) species generation/consumption, \( R \) (g/hr), reported in the related literature [22], characteristic mathematical model of ABE biosynthesis in a fed-batch bioreactor with solvents gas-stripping consists of the following system of equations:

\[
\frac{dV}{d\tau} = \frac{1}{\rho_{A}} \frac{dm_{A}}{d\tau} - \frac{1}{\rho_{B}} \frac{dm_{B}}{d\tau} - \frac{1}{\rho_{E}} \frac{dm_{E}}{d\tau} - \frac{1}{\rho_{W}} \frac{dm_{W}}{d\tau} \quad (5)
\]

\[
\frac{dy}{d\tau} = \left[ k_{1} \frac{c_{y}}{c_{1} + c_{n}} - 0.56(y-1) \right] y \quad (6)
\]

\[
\frac{dc_{X}}{d\tau} = 0.56(y-1)c_{X} - k_{4}c_{X}c_{E} - \frac{F}{V}c_{X} \quad (7)
\]

\[
\frac{dc_{S}}{d\tau} = -k_{3}c_{S}c_{X} - k_{4}c_{S}c_{X} - \frac{F}{V}(c_{SP} - c_{S}) \quad (8)
\]

\[
\frac{dc_{BA}}{d\tau} = k_{5} \frac{k_{4}c_{X}}{c_{1} + c_{n}} - \frac{F}{V}c_{BA} - \frac{c_{BA}}{c_{BA} + k_{6}}c_{BA} \quad (9)
\]

\[
\frac{dc_{DD}}{d\tau} = k_{7} \frac{c_{S}}{c_{1} + c_{n}}c_{X} - k_{8}c_{DD} \quad (10)
\]

\[
\frac{dc_{AA}}{d\tau} = k_{9} \frac{c_{S}}{c_{1} + c_{n}}c_{X} - k_{10}c_{AA} - \frac{F}{V}c_{AA} \quad (11)
\]

\[
\frac{dc_{EE}}{d\tau} = k_{11} \frac{c_{S}}{c_{1} + c_{n}}c_{X} - F \frac{c_{EE} - k_{12}}{c_{1} + c_{n}} \quad (12)
\]

\[
\frac{dc_{GG}}{d\tau} = k_{13} \frac{c_{S}}{c_{1} + c_{n}}c_{X} - F \frac{c_{GG} - k_{14}}{c_{1} + c_{n}} \quad (13)
\]
Model restrictions imposed by operation mode, i.e. batch, fed-batch, and fed-batch with gas-stripping, are as follows:

\[
\frac{dc_{CO_2}}{dt} = k_{CO_2} \frac{c_{CO_2}}{c_x + k_{CO_2}} x - \frac{F}{V} c_{CO_2} - k_{CO_2} \alpha (c_{CO_2} - k_{CO_2} c_{CO_2}) \tag{14}
\]

\[
\frac{dc_{H_2}}{dt} = k_{H_2} \frac{c_{H_2}}{c_x + k_{H_2}} x + k_{H_2} \alpha c_{H_2} x - \frac{F}{V} c_{H_2} - k_{H_2} \alpha (c_{H_2} - k_{H_2} c_{H_2}) \tag{15}
\]

\[
\frac{dc_{CO_2}}{dt} = k_{CO_2} \alpha V \left( c_{CO_2} - k_{CO_2} c_{CO_2} \right) \tag{16}
\]

\[
\frac{dc_{H_2}}{dt} = k_{H_2} \alpha V \left( c_{H_2} - k_{H_2} c_{H_2} \right) \tag{17}
\]

Optimization case

The mathematical model can be applied to solve some optimization problems, e.g. to maximize ABE solvents production. This problem requires obtaining a set of values for initial volume of fermentation broth, \( V_0 \), feed volumetric flow rate, \( F \), feed substrate concentration, \( c_{SF} \), switching time from batch to fed-batch operation, \( \tau_{sw} \), and stripping start time, \( \tau_{str} \), for which a maximum amount of solvents can be produced.

The carbon mass balance in desirable compounds (A, B, E) and undesirable ones (S, X, AA, BA, CO_2, H_2) was used to formulate a function to be maximized. This function is expressed by relation (25), wherein \( f_{subscripts} \) refer to the final process state. Terms coefficients values in correlation (25) were calculated as ratio between \( i \) species molecular mass, \( M_i \) (g/mol), and carbon atoms mass in \( i \) species molecule, \( M_{ci} \) (g/mol).

\[
F_{w}(V_0, F, c_{SF}, \tau_{sw}, \tau_{str}) = 0.62 m_w + 0.65 m_s + 0.52 m_a + 0.62 \left( c_w V_{w} + \frac{F}{V} c_w \right) + 0.65 \left( c_w V_{w} + \frac{F}{V} c_w \right) + 0.52 \left( c_w V_{w} + \frac{F}{V} c_w \right) - 0.40 \frac{m_a}{V} - 0.40 \frac{m_a}{V} - 0.53 \frac{m_a}{V} \tag{25}
\]

According to data reported in a previous paper, the feed flow rate is linked to the initial fermentation broth volume, i.e. \( F = \epsilon V_0 \) [21]. Consequently, the optimization function defined by correlation (25) depends on four independent variables, namely \( F, c_{SF}, \tau_{sw} \), and \( \tau_{str} \).

Estimation of model parameters

An accurate estimation of species volumetric mass transfer coefficient as well as of kinetic and Monod constants is essential to solve the model. In order to predict the volumetric mass transfer coefficient characterizing the liquid-gas mass transfer of A, B, E, W, CO_2, and H_2 in the bubble bioreactor operated in fed-batch with gas stripping mode, an experimental procedure was developed for butanol (B) transfer, whereas specific correlations were adopted for the other compounds.

For water (W) vaporization in the stripping gas, the gas phase diffusion was considered as rate-determining step. The relation (26), describing the dependence of water volumetric mass transfer coefficient, \( k_{w,a} \), on stripping gas velocity, \( \nu_{w} \), was selected based on data reported in the related literature [23,24].

\[
k_{w,a} = 0.012 + 0.6w \ s^{-1} \tag{26}
\]

For ABE solvents, CO_2, and H_2, the species diffusion in the liquid film surrounding the gas bubble was assumed as rate-determining step. An experimental research using a dynamic method, based on measurements of butanol concentration in a liquid phase when a stripping gas was passed through this, was developed to estimate the butanol volumetric mass transfer coefficient, \( k_{L,a} \). Butanol stripping from a volume \( V = 1 \) L of glucose aqueous solution (20 g/L glucose) using nitrogen and air as stripping agents was studied in a stirred tank bioreactor (Biostat Aplus Sartorius) at 30°C. No differences were obtained between nitrogen or air stripping runs. A refractometric method was applied to determine the butanol concentration in the liquid phase, \( c_{B} \).

The tank bioreactor equipped with a six-blade disk impeller was characterized by the following geometrical simplexes: \( D/d = 3, H/d = 2, h/d = 0.6 \), wherein \( D = 0.15 \) m is tank diameter, \( d = 0.052 \) m stirrer diameter, and \( h = 0.03 \) m distance between stirrer inferior part and tank bottom.

The experiments were conducted at three levels of process independent variables (factors), i.e. butanol initial concentration in liquid phase, \( c_{L,0} \), stirring speed, \( n \), and dynamic stripping gas intake, \( c_{d,g} \) (table 1). Dynamic stripping gas intake, \( c_{d,g} \), was obtained as ratio between volumetric flow rate of stripping gas, \( G_v \), and liquid phase volume, \( V \). Values of butanol initial concentration in liquid phase of 10, 15, and 20 g/L were selected in order to reproduce characteristic concentration levels of ABE synthesis bioreactor [21].

Some experimental curves describing butanol dynamics in batch with gas-stripping bioreactor under different operational conditions are depicted in figure 2. For each experiment conducted according to factors level summarized in table 1, the butanol volumetric mass transfer coefficient, \( k_{L,a} \), was obtained by minimizing the function defined by relation (27), wherein \( N \) is
measurements number performed in an experiment. Estimated values of \((k_{mB})_{\text{exp}}\) listed in Table 1 highlight an increase in mass transfer coefficient with stirring speed, \(n\), and stripping gas intake, \(C_{\text{dsg}}\), as well as a very slight dependence on butanol initial concentration in the liquid phase, \(C_{\text{B0}}\). Experimental data processing of \((k_{mB})_{\text{exp}}\) dependence on \(n\) and \(C_{\text{dsg}}\) led to correlation (28) which is based on dimensional analysis theory and it is usually applied in the related studies [25]. Experimental and predicted values of butanol volumetric mass transfer coefficient shown in figure 3 emphasize a good reproducibility of experimental data given in Table 1 by correlation (28).

\[
F[k_{mB}]_{\text{exp}} = \sum \left( C_{\text{Bout}} - C_{\text{Bin}} e^{-T_{\text{stir}}/k_{mB} n} \right) \right] \tag{27}
\]

\[
k_{mB} = 0.215 C_{dsg}^{0.444} n^{1.299} \tag{28}
\]

Volumetric transfer coefficients of acetone and ethanol, \(k_{mA}\) and \(k_{mA}\), were estimated by correlations (29), based on the renewal theory of interphase species transfer, wherein values of species diffusion coefficients in the liquid phase were as follows:

\[
D_lA = 11.64 \times 10^{-10} \text{ m}^2/\text{s}, \quad D_lB = 9.32 \times 10^{-10} \text{ m}^2/\text{s}, \quad D_lE = 10.5 \times 10^{-10} \text{ m}^2/\text{s}.
\]

Values of kinetic and Monod constants recommended for fed-batch ABE biosynthesis model are summarized in Table 2 [22].

### Results and discussions

Aiming at establishing the optimization function maximum and revealing the process specificity for ABE synthesis in fed-batch with gas-stripping (FBGS) system, simulations were conducted according to estimated model parameters and input data summarized in Table 3.

### Simulation results

Some simulation results, expressed as dynamics of solvents and biomass concentration in bioreactor as well as condensed solvents and water concentration in cooler under various operational conditions, are presented in figures 4 and 5. A decrease in solvents content in bioreactor and an increase in their amount in the cooler is observed at \(\tau > \tau_s\). ABE biosynthesis coupled with gas-stripping exhibits two major advantages: i) a diminishing of butanol toxic effect on the bacterial system (fig. 4) and ii) a high proportion of butanol in the final condensate, i.e. about 44% (fig. 5).

### Table 1

<table>
<thead>
<tr>
<th>(C_{\text{B0}}), g/L</th>
<th>(C_{\text{dsg}}), min</th>
<th>(n), rpm</th>
<th>250</th>
<th>500</th>
<th>800</th>
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<td>1.205</td>
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<td>4.028</td>
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<tr>
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<td>1.956</td>
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<td>3</td>
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### Table 2

<table>
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<th>Value</th>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
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</thead>
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<td>(k_{mB})</td>
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<td>(k_{mB})</td>
<td>%</td>
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<td>(k_{mA})</td>
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<td>%</td>
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<td>(k_{mA})</td>
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<td>0.0255</td>
<td>(k_{mB})</td>
<td>%</td>
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</tr>
<tr>
<td>(k_s)</td>
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<td>0.6764</td>
<td>(k_{mB})</td>
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<td>(k_{B0})</td>
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<td>(k_{mB})</td>
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<td>(k_s)</td>
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<td>(k_{mB})</td>
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<td>(k_{B1})</td>
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<tr>
<td>(k_{B2})</td>
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<td>(k_{mB})</td>
<td>hr^{-1}</td>
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<tr>
<td>(k_{B3})</td>
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<td>0.1360</td>
<td>(k_{mB})</td>
<td>hr^{-1}</td>
<td>0.5</td>
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</table>

\[D_{iA} = 11.64 \times 10^{-10} \text{ m}^2/\text{s}, \quad D_{iB} = 9.32 \times 10^{-10} \text{ m}^2/\text{s}, \quad D_{iE} = 10.5 \times 10^{-10} \text{ m}^2/\text{s}.
\]

\[
k_{mA} = k_{mB} \left( \frac{D_{iB}}{D_{iA}} \right)^{0.5} \tag{29}
\]

### Table 3

<table>
<thead>
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<th>Type</th>
<th>Name</th>
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<tr>
<td></td>
<td>Stripping start time</td>
<td>(\tau_p)</td>
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<td></td>
<td>Dynamic stripping gas intake</td>
<td>(C_{\text{dsg}})</td>
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<td></td>
<td>Speed of liquid mixing</td>
<td>(n)</td>
<td>240, 960</td>
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<td></td>
<td>Feed flow rate</td>
<td>(F)</td>
<td>0.005, 0.01, 0.025, 0.05, 0.1</td>
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<td></td>
<td>Feed glucose concentration</td>
<td>(C_{\text{GF}})</td>
<td>20, 50, 80, 100, 120</td>
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<td>Glucose concentration</td>
<td>(C_{\text{G}})</td>
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<td>Biomass concentration</td>
<td>(C_{\text{B}})</td>
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<td>Butanol concentration</td>
<td>(C_{\text{B}})</td>
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<td></td>
<td>Acetone concentration</td>
<td>(C_{\text{A}})</td>
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<td>Ethanol concentration</td>
<td>(C_{\text{E}})</td>
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<td>Butyric acid concentration</td>
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<td>Acetic acid concentration</td>
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<td></td>
<td>Hydrogen concentration</td>
<td>(C_{\text{H}})</td>
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<td>Marker concentration</td>
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<td></td>
<td>Fermentation broth volume</td>
<td>(V_0)</td>
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Fig. 2. Dynamics of butanol (B) stripping by air in Biostat Aplus bioreactor.
Plots in figures 6-9, showing the effect of stripping start time and stripping duration on the both process dynamics, i.e. biosynthesis and solvents removal from the fermentation broth, emphasize the following aspects: i) estimated values of water volumetric mass transfer coefficient, $k_{gA}$, of 0.65 h⁻¹ impose a gas bubbling superficial (fictive) velocity over 1 m/s, determining intense gas bubbling and stripping, respectively; ii) a rapid removal of solvents from the reaction broth occurs for all values of stripping start time and stripping duration; iii) the biochemical process remains active during the stripping and after its stopping (fig. 6); iv) the acetone production increases over that of butanol after stripping stopping (figs. 6 and 7); v) the solvents
concentration in the fermentation broth decreases rapidly in the first 5 h from the stripping start (figs. 6 and 7), whereas the condensed solvents and water mass in the cooler increases significantly in the same period (figs. 8 and 9).

The effect of feed flow rate, $F = \epsilon V_0$, on butanol, substrate and biomass dynamics is shown in figures 10 and 11. Plots in figure 10 reveal an increase in butanol concentration in final fermentation broth as well as in condensed butanol mass in cooler with feed flow rate increasing, the effect being significant at $\epsilon \geq 0.025$ hr$^{-1}$. Referring to the influence of gas-stripping and feed flow rate on substrate and biomass dynamics (fig. 11), it can be noticed that, during the stripping process (which lasts from $\tau_{sw}$ = 25 hr to 35 hr), the substrate concentration decrease from 5-35 g/L to 0.5-22 g/L, whereas the biomass concentration increases from 0.8-2 g/L to 2-3.5 g/L, the effect being significant at $\epsilon \geq 0.025$ hr$^{-1}$. The results are in good agreement with those reported in other studies [8,15,26,27].

**Statistical analysis of optimization case**

Graphic representation in figure 12, showing the variation of optimization function defined by correlation (25), $F_{opt}(\tau_{sw}, \tau_{str}, \tau_{sw})$, depending on feed flow rate, $F$, and feed substrate concentration, $c_{sw}$, highlights an optimal response surface in the field determined by $F \in (0.09 V_0, 0.14 V_0)$ L/hr and $c_{sw} \in (100, 150)$ g/L.

A data statistical analysis based on experimental design was performed in order to build a hyper surface for $F_{opt}$ [28]. According to the procedure recommended for a $2^4$ factorial experiment, coded (dimensionless) values of factors were determined depending on their natural values using equations system (30)-(33), wherein $F_0 = 0.075 V_0$/L/hr, $c_{sw} = 90$ g/L, $\tau_{sw} = 10$ hr, and $\tau_{str} = 30$ hr represent values...
of process factors within experimental plan centre \((c)\). Coded and natural values of process factors as well as predicted values of optimization function are summarized in table 4. Table 5 Coefficients values of correlation (34), identified based on design matrix presented in table 4, are listed in table 5.

\[
\begin{align*}
\beta_{c} &= 0.296 \\
\beta_{p} &= 2.935 \\
\beta_{r} &= 1.708 \\
\beta_{f} &= 0.861 \\
\beta_{p} &= 1.039 \\
\beta_{p} &= 0.286 \\
\beta_{r} &= 0.010 \\
\beta_{f} &= 0.488 \\
\beta_{f} &= 0.174
\end{align*}
\]

The positive effect of gas-stripping coupling to ABE biosynthesis was analyzed based on graphic representations obtained by process simulation under different operational conditions. A procedure to build a hyper surface \(F_{opt}(F, c_{SF}, \tau_{sw}, \tau_{str})\) was developed based on experimental design.

\[
F_{opt}(x_1, x_2, x_3) = \frac{7.187 + 2.935 x_2 - 1.708 x_3 - 0.861 x_1 - 1.039 x_1 - 0.488 x_1 - 0.598 x_1 - 0.938 x_2 - 0.268 x_3 - 0.035 x_4 - 0.010 x_5 - 0.010 x_6}{10}
\]

### Conclusions

A mathematical model was developed for the coupled processes of fed-batch ABE solvents biosynthesis in *Clostridium acetobutylicum* culture on glucose substrate and gas-stripping of solvents from reaction broth. The concentration profiles for different species in the fermentation broth and in the solvents separator were predicted based on this model. An optimization case consisting of solvents production maximization was studied. The feed flow rate, \(F\), substrate concentration in the reactor feed, \(c_{SF}\), switching time from batch to fed-batch system, \(\tau_{sw}\), and stripping start time, \(\tau_{str}\), were characteristic manipulated variables of an optimization function, \(F_{opt}\), which was defined and evaluated. An experimental investigation concerning the gas-stripping of acetone, butanol and ethanol from reaction broth was performed to estimate the stripping kinetics, i.e. to determine the species volumetric mass transfer coefficient in liquid phase. The positive effect of gas-stripping coupling to ABE biosynthesis was analyzed based on graphic representations obtained by process simulation under different operational conditions.

A procedure to build a hyper surface \(F_{opt}(F, c_{SF}, \tau_{sw}, \tau_{str})\) was developed based on experimental design. Non significant coefficients of optimization function, \(F_{opt}\), were identified by Student test. Three factors and four interactions described by correlation (34) can be written in simplified canonical form (35), which emphasizes the following aspects: i) all process factors participate to the state of optimization function; ii) the dimensionless feed substrate concentration, \(x_p\), switching time, \(x_r\), and stripping start time, \(x_s\), present a direct and an interaction influence on \(F_{opt}\) state; iii) high level of \(x_p\) along with low levels of \(x_r\) and \(x_s\) lead to a high value of optimization function, \(F_{opt}\). An increase in optimization function values with switching time and stripping start time decreasing is shown by graphic representation in figure 13.

\[
F_{opt}(x_1, x_2, x_3, x_4) = \frac{7.187 + 2.935 x_2 - 1.708 x_3 - 0.861 x_1 - 1.039 x_1 - 0.488 x_1 - 0.598 x_1 - 0.938 x_2 - 0.268 x_3 - 0.035 x_4 - 0.010 x_5 - 0.010 x_6}{10}
\]
of factors have determined the final expression of optimization function. This correlation could be applied to establish characteristic factors values of a laboratory or industrial bioreactor for clostridial ABE biosynthesis operating in fed-batch with gas-stripping system.

References
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