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Enhanced Isopropanol-Butanol-Ethanol (IBE) production in immobilized column reactor using modified *Clostridium acetobutylicum* DSM792

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Highlights

- Metabolic engineering to convert acetone into isopropanol
- Continuous immobilized fermentation to validate industrial feasibility of strain
- Optimization of bioprocess to enhance the solvent yield and productivity
- Utilization of lignocellulosic spent liquor as a substrate for the fermentation

Abstract

The co-production of acetone during traditional butanol fermentation (acetone-butanol-ethanol) lowers the yield of alcohol biofuels. The present study was aimed to develop an improved *Clostridium acetobutylicum* strain with enhanced alcohol fuel production capability. The previously developed IBE producing *C. acetobutylicum* DSM792-ADH strain was further optimized to enhance the solvent productivity and yield. Immobilized cell column reactor was used to investigate the effect of dilution rate on IBE fermentation. Pure glucose, artificial sugar mixture and SEW spent liquor from spruce chips were used as substrates to study the behavior of the modified microorganism. The highest total solvent concentrations of approximately 10.60 g.l⁻¹, 10 g.l⁻¹ and 6 g.l⁻¹ were obtained when glucose, sugar mixture and SEW spent liquor were used as substrate respectively. The modified microorganism could effectively utilize the lignocellulosic biomass hydrolyzate (SEW spent liquor) to yield a solvent productivity of 1.67 g.l⁻¹.h⁻¹.

Keywords: Clostridia, column reactor, IBE, fermentation, isopropanol, immobilization, SEW liquor

1. Introduction

An increase in the atmospheric carbon dioxide level and limitedly available petroleum resources have drawn an attention to the production of biofuels and bulk chemicals from renewable resources. Although the use of bioethanol as an alternative fuel has been commercialized in several countries, its chemical properties do not replace gasoline copiously [1]. Advanced biofuels such as *n*-butanol, iso-butanol or iso-propanol, which involve higher carbon chains, are believed to circumvent the current problems of ethanol as an advanced biofuel [2].

An industrial production of acetone, butanol and ethanol (ABE) with *Clostridium spp.* is already in the progress [3-5]. However, the co-production of acetone in the ABE process is considered as un-desirable because of its corrosiveness to rubber engine parts and its poor fuel properties [2-6]. Some natural *Clostridium beijerinckii* strains [7] produce isopropanol, instead of acetone, together with butanol and ethanol (IBE) to produce alcohol biofuel mixture which is considered as a 'green solvent'. *C. beijerinckii* a natural IBE producer possess an additional primary-secondary alcohol dehydrogenase (ADH) that catalyzes the NADPH dependent reduction of acetone to isopropanol [8]. However, the IBE production with these natural isopropanol producers is still non-competitive to the industry (total solvent concentration of 5.87g.l⁻¹; and productivity of 0.12 g.l⁻¹.h⁻¹) in batch cultures [9].

Butanol is known to inhibit the metabolic pathways of butanol producing bacteria leading to lowered titer in the fermentation broth [1,3,10]. *C. acetobutylicum* and *C. beijerinckii* are recognized as high butanol producers [11]. However, some of them face the difficulties such as acid crash, degeneration of solvent producing capability, sporulation during solvent production, and a solvent intolerance [12]. Metabolic and genetic engineering strategies are continually being employed by several researchers to overcome the hurdles associated with the butanol fermentation

[13-15]. Besides, the development of a simple and an economic bioprocess is also desirable to maximize the butanol production as explained herein this study.

The genetic studies of *C. acetobutylicum* ATCC824 have been very well succeeded to enhance the ABE solvent yields [14-16]. Hence, this industrially feasible strain is highly interested to be transformed into an efficient IBE producer. ADH encoding gene from *C. beijerinckii* can be introduced into the *C. acetobutylicum* to reduce the acetone into isopropanol was targeted in present study. However, increase in the solvent titer and yields are some of the inevitable parameters that need to be addressed during the process economy of the IBE fermentation. Previous studies with the genetically modified strain *C. acetobutylicum* DSM792-ADH were carried out to enhance the solvent productivity and yield (unpublished data, supplementary). The present study is the continuation of our previous study to further optimize the fermentation process of *C. acetobutylicum* DSM792-ADH to enhance the solvent titer and yields.

A low solvent productivity in batch reactors with long lag phase and a strong product inhibition propelled the researchers to opt for continuous fermentations [1,17]. Besides, the cell immobilization technique in continuous fermentation is a promising method with many advantages over the conventional suspended cell fermentation process. The immobilized cells can maintain high viable cell densities in a reactor and eliminates the cellular lag phase to increase the productivity due to the possibility of sustaining higher dilution rates [18]. Moreover, the immobilized cell reactors might enhance the tolerance to adverse conditions such as butanol toxicity [19,20].

The choice of an economical feedstock is significantly affecting the process economy of the biofuels [21]. Lignocellulosic biomass is a multi-carbon-source feedstock containing significant fractions of pentose and hexose sugars which can be utilized as monosaccharides in fermentation. Lignocellulosic biomass being abundantly available in the nature offers great

potential for the production of biofuels with Clostridial strains. SO₂–ethanol–water (SEW) pulping is a hybrid of acid sulfite and organosolv pulping process and it is a promising pretreatment method for lignocellulosic biomass [22-24]. The use of SEW spent liquor for the production of solvents can promote the biorefinery concept which harvests and processes the trees for their subsequent use. Hardwood, softwood and recycled fibers can easily be fractionated with SEW method to produce a stream of fermentable sugars from the mixed feedstock with low costs. Hence, the spent liquor from SEW process was used in the present study to carry out IBE fermentation.

The present study was a continuation of our previous work (communicated for publication; supplementary) and was aimed to develop an effective IBE producer strain of *C. acetobutylicum* with its optimization of bioprocess. The fermentation process for the modified strain *C. acetobutylicum* DSM 792-ADH was optimized to enhance the IBE accumulation. The cells were immobilized on recyclable and biodegradable wood pulp fibers and the process with immobilized cells was further studied to improve the substrate consumption and solvent productivity. The performance of the immobilized cell reactor was investigated for the production of IBE solvents using glucose, sugar mixture and SEW spent liquor as substrates.

2. Materials and methods

2.1. Materials

All the nutritional components required for the fermentation were purchased from the local vendors from Finland [3]. *C. beijerinckii* NRRL B593 (synonym: DSM6423) and *C. acetobutylicum* DSM792 (synonym: ATCC 824) strains were procured from DSMZ (Germany). The SO₂–water–ethanol (SEW) spent liquor was obtained from the Department of Forest Products Technology, School of Chemical Technology, Aalto University, Finland.

2.2. Microorganism and medium

The culture was maintained on a reinforced clostridia medium (RCM) as explained earlier [4]. The production medium reported by Tripathi et al. [25] was modified to use a sugar mixture as a carbon source containing glucose, mannose, arabinose, galactose, and xylose as a replacement to sole glucose (60 g L⁻¹). The artificial sugar mixture equivalent to SEW spent liquor contained (g L⁻¹) glucose 32.73, mannose 14.78, arabinose 2.14, galactose 3.95, and xylose 6.41 to get total sugars to be 60 g L⁻¹. The medium was adjusted to pH 6.5 with HCl, if necessary and purged with nitrogen and autoclaved at 203.4 kPa (121 °C) for 20 min.

2.3. SEW spent liquor preparation and conditioning

The fractionation of spruce wood chips was carried out by using SEW method and the spent liquor obtained from it was processed further. The SEW spent liquor was produced and conditioned as reported by Sklavounos et al. [23]. The conditioning comprised of evaporation, steam stripping, liming and catalytic oxidation to get a fermentable liquor. The concentrations of fermentation inhibitors including acetic acid, formic acid, furfural and hydroxy methyl furfural (HMF) were below the lethal level for Clostridia [23]. The pH of the spent liquor was finally adjusted to 6.5 with Ca(OH)₂ before the addition of other nutritional components. The total sugar concentration in the final liquor was approximately 43.65 g.L⁻¹. The individual sugar concentrations were (in g.L⁻¹) glucose 12.4, mannose 16.88, galactose 4.18, arabinose 2.95 and xylose 7.24. The liquor was diluted to 4 times to further reduce the inhibitory components and was supplemented with other nutritional components as detailed in the previous section to make final sugar concentration to be 60 g.L⁻¹.

2.4. Bacterial plasmids and maintenance

Actively growing cells of *C. beijerinckii* were used for DNA extraction. The detailed procedures for plasmid construction, transformation and integration was explained in our previous study (unpublished data, supplementary) to construct a modified *C. acetobutylicum* DSM792-ADH.

Briefly, the harvested cells were used for DNA extraction by Wizard® Genomic DNA Purification Kit (Promega Corporation, USA). The plasmid DNA was isolated from *E. coli* and *C. acetobutylicum* transformants using QIAprep Spin Miniprep Kit (QIAGEN, USA). The *adh* gene was amplified from *C. beijerinckii* NRRL B-593 genomic DNA using P05 and P06 as primers. The ‘*adc* promoter-*adh*-*adc* terminator’ fragment was amplified from pADH, digested with NotI-NheI plasmid pMTL-JH16 and transformed into *E. coli* TOP10 to obtain pMTL-JH16-ADH. This pMTL-JH16-ADH plasmid was methylated in *E. coli* TOP10 prior to transformation of *C. acetobutylicum* DSM 792 as described previously.

2.5. Batch and continuous experiments

The primary experiments to study the growth and production behavior of the modified strain *C. acetobutylicum* DSM792-ADH were carried out as both batch and continuous experiments (supplementary). In order to improve the solvent productivity and to reduce the operational cost of the process, the continuous immobilized column experiments were performed.

2.6. Preparation of column reactors and operation

The previous study of *C. acetobutylicum* DSM792-ADH in continuous cell culture cultivation propelled us to perform the experiments with immobilized cell culture techniques in a column reactor to enhance the IBE productivity and yield. The use of immobilized cell reactors for the production of solvents has been studied earlier to enhance the solvent productivities [3,4,26]. The column reactors were prepared as reported by Bankar et al. [3]. The wood pulp fibers were rolled in a nylon mesh and inserted into the jacketed glass columns. The column consisted of 25 ml void volume. Further, the column was decontaminated with ethanol (70%) for 24 h. The inoculum was prepared as described previously [4] using activated spore culture in air-tight, anaerobic glass bottles and grown for 20 h at 37 °C. Ethanol in the column reactor was replaced with the production medium followed by the inoculation of 20 h old highly motile cells of *C. acetobutylicum* DSM792-

ADH. The fermentation was allowed to proceed in the batch mode for 24 h with the re-circulation to assist the cell growth and immobilization at 37 °C. Subsequently, the fermentation feed medium was continuously introduced from the bottom of the cell immobilized column at different dilution rates. The temperature of the column was maintained at 37 °C by continuously circulating warm water through the jacket. The dilution rate of immobilized cell reactor was varied between 0.25 and 2.0 h⁻¹. Samples for product analysis were taken after five working volume change for three consecutive days. The fermentation, samples were analyzed for biomass, acetone, isopropanol, butanol, ethanol, residual sugar and acids. The steady state was confirmed by the constant product values at specific dilution rates. The *C. acetobutylicum* DSM792-ADH fermentation was carried out in three sets of experiments viz. glucose as a substrate, sugar mixture as a substrate and SEW spent liquor as a substrate in addition to the standard production medium [3,4]. All the experiments were carried out at least in triplicate and results reported are average $\pm$ standard deviation of three values.

2.7. Determination of substrates and products

The solvents (*n*-butanol, isopropanol, acetone and ethanol) and acids (acetic acid and butyric acid) were quantified by gas chromatography (Hewlett Packard series 6890) [24]. Glucose, mannose, arabinose, galactose, and xylose were determined by high-performance liquid chromatography (Bio-Rad Laboratories, Richmond, CA) [3]. The bioprocess parameters including the solvent productivity and yield were calculated as explained earlier [3,4].

3. Results and discussion

The present study was a continuation of previous study with modified strain *C. acetobutylicum* DSM792-ADH to produce IBE as a ‘green’ solvent (unpublished data, supplementary). The acetone produced during the traditional ABE fermentation was preferentially converted into isopropanol to improve the process economy [1,6]. Hence, *C. acetobutylicum* which is a commonly used microorganism for ABE fermentation was engineered to convert acetone into

isopropanol by introducing the secondary alcohol dehydrogenase gene from *C. beijerinckii* NRRL B593 using allele-coupled exchange approach. *C. acetobutylicum* does not possess a gene for secondary alcohol dehydrogenase (*adh*) which is required to produce 2-propanol (isopropanol) from acetone [6,27]. *C. beijerinckii* NRRL B593 contain a NADPH dependent primary/secondary alcohol (isopropanol) dehydrogenase (EC 1.1.1.1) to produce a mixture of isopropanol and *n*-butanol. The *adh* gene of *C. beijerinckii* NRRL B593 was introduced into *C. acetobutylicum*-DSM792 chromosome to produce IBE as the mixture of solvents (supplementary).

Immobilized biomass with continuous cell cultures are reported to give high solvent productivities due to operation at high dilution rates and increased concentration of cells [28]. Hence, the strain *C. acetobutylicum* DSM792-ADH was immobilized on wood pulp fibers. The standard medium containing, pure glucose, sugar mixture and SEW spent liquor were used as substrates for solvent production.

3.1. IBE production with standard production medium

The continuous cultures with an immobilized biomass were expected to operate at high dilution rates which in turn results in higher solvent productivities [28]. Currently, agricultural immobilization materials which are recycled and reused are more common in use [26]. Wood pulp fibers were used in the present study as an immobilization material in a column reactor.

Single-stage chemostat cultivation experiments were performed previously with suspended cell culture technique (supplementary). The highest total solvents achieved with suspended cell culture technique when glucose was used as a substrate was found to be 11 g.l⁻¹. The highest possible productivity observed was 0.45 g.l⁻¹.h⁻¹ at the dilution rate of 0.075 h⁻¹ (supplementary, Fig. 3). The immobilized column reactor efficiency was investigated with respect to the higher dilution rate to attain the maximum solvent productivity and solvent concentration in the effluent stream. The present immobilized column reactor was operated at highest sustainable

dilution rate of 2 h⁻¹. The maximum overall total solvent concentration of 10.60 g.l⁻¹ (acetone, 1.80 g.l⁻¹; isopropanol, 1.55 g.l⁻¹; butanol, 6.19 g.l⁻¹; and ethanol, 1.05 g.l⁻¹) was observed at a dilution rate of 0.25 h⁻¹ (Fig. 1, Fig. 2). At this dilution rate the solvent productivity observed was 2.65 g.l⁻¹.h⁻¹. The maximum total solvent productivity observed was around 5.4 g.l⁻¹.h⁻¹ at a dilution rate of 0.75 g.l⁻¹.h⁻¹, which was much higher than with a suspended culture in a chemostat. The conversion of acetone into isopropanol was also found to be close to 50 % similar to earlier studies (Table 1; Supplementary Table 3). However, the ratio of ethanol and butanol production during the fermentation was consistent throughout all the dilution rates studied. Almost 72 % of the glucose was utilized at the dilution rate of 0.25 h⁻¹ (Table 2). The maximum solvent yield was found to be 0.34 g.g⁻¹ when glucose was used as a substrate (Fig. 2).

The results presented in this study are in accordance with the natural producer of isopropanol (*Clostridium beijerinckii*). Previous studies with *C. beijerinckii* DSM 6423 showed the maximum solvent production to be 11.99 g.l⁻¹ with almost 68 % glucose utilization. The highest solvent productivity observed was 5.58 g.l⁻¹.h⁻¹ at a dilution rate of 1.50 h⁻¹ [26]. The results observed in the present study with glucose as a substrate are competitive enough to check its superiority over the natural producer. Hence, further studies with the sugar mixture and SEW liquor as substrates were carried out to observe the behavior of modified strain *C. acetobutylicum* DSM792-ADH in immobilized reactors.

3.2. IBE production with artificial sugar mixture

The efficiency of modified strain to utilize the pentose and hexose sugars was analyzed with the use of artificial sugar mixture containing sugar concentration equivalent to SEW spent liquor. The total sugar concentration was adjusted to 60 g.l⁻¹ with the addition of pure glucose; for better comparison and understanding with other substrates. The results obtained with the sugar mixture as a substrate when *C. acetobutylicum* DSM792-ADH was immobilized on the wood pulp

column are presented in Fig. 1. A typical declining trend of solvent production with subsequent increase in the dilution rate was observed. The highest concentration of total solvents produced was approximately 10 g.l⁻¹ (acetone, 1.63 g.l⁻¹; isopropanol, 1.48 g.l⁻¹; butanol, 5.89 g.l⁻¹; and ethanol, 0.99 g.l⁻¹) at a dilution rate of 0.25 h⁻¹. A significant increase in solvent productivity from 2.5 g.l⁻¹.h⁻¹ to 4.5 g.l⁻¹.h⁻¹ was observed when the dilution rate was increased from 0.25 h⁻¹ to 0.75 h⁻¹. The previous studies with *C. acetobutylicum* DSM792-ADH when sugar mixture was used as a substrate resulted in the highest solvent productivity to be 0.34 g.l⁻¹.h⁻¹ in suspended cell culture chemostat (unpublished data, supplementary). The increase in the solvent productivity upto 4.5 g.l⁻¹.h⁻¹ with the immobilized cell reactor with using sugar mixture as a substrate suggested the scaling up possibilities of this technology. Moreover, the solvent yield was stable around 0.25 g.g⁻¹ for all the dilution rates (Fig. 2). A notable isopropanol production until the dilution rate of 1.0 h⁻¹ was observed with more than 40 % conversion of acetone (Table 1). The IABE (isopropanol:acetone:butanol:ethanol) ratio also remained constant for all the dilution rates; once again proved the effectiveness of the modified strain at higher dilution rates. Table 2 depicts the consumption of individual sugar components of the mixture. The sugar consumption was comparable with the experiments carried out with pure glucose as a substrate. Glucose utilization was almost complete below the dilution rate of 0.5 h⁻¹. The consumption of other sugars dropped significantly with increase in the dilution rate. However, the consumption of mannose and xylose was significantly higher than arabinose and galactose. The consumption of glucose was found to be independent of the other sugars present in the feed. This could be due to the large amount of glucose compared with the other sugars, as well as its status as the most preferred sugar for *C. acetobutylicum* [29, 30].

3.3. IBE production with SEW spent liquor

The compatibility of *C. acetobutylicum* DSM792-ADH to utilize the pentose and hexose sugars from the artificial sugar mixture invigorated us to carry out the experiments with

SEW spent liquor as a substrate. The fermentation inhibitors such as furfural, hydroxymethyl furfural, acetic acid, formic acid and soluble phenolic compounds which were expected to form during the pretreatment process of spruce wood chips were conditioned with subsequent steps [23,24]. The effect of dilution rate on the production of IBE solvents is shown in Fig. 1. The highest concentrations of total solvents produced were around 5.95 g.l⁻¹ (acetone, 0.93 g.l⁻¹; isopropanol, 0.79 g.l⁻¹; butanol, 3.57 g.l⁻¹; and ethanol, 0.66 g.l⁻¹) at a dilution rate of 0.25 h⁻¹. The column reactor was operated well below the dilution rate of 0.5 h⁻¹ and resulted in the highest solvent productivity to be 1.67 g.l⁻¹.h⁻¹. The dilution rate above 0.5 h⁻¹ resulted in substantial decrease in the solvent productivity as well as sugar consumption (Table 2). This may be because of the shorter residence time and limited availability of glucose in the medium. The sugar consumption trend was similar to that with the trend observed during the artificial sugar mixture as a substrate. Glucose was the most preferred sugar followed by mannose and xylose. The importance of excess availability of fermentable sugars in the broth was reported for both the onset and the maintenance of solvent production [26]. The IABE ratio also remained constant throughout all the dilution rates. Besides, the conversion of acetone into isopropanol was found to be in the range of 43 – 48 % (Table 1).

The use of column reactors for continuous solvent production has been studied by several researchers using different immobilization materials and substrates [31-33]. However, the use of SEW spent liquor for the production of IBE solvents with modified strains has not been reported previously. Besides, the reports on the use of immobilized column reactors for IBE production are also limited and substandard to the present study after considering the process economy in primacy. The use of a packed bed reactor containing Ca-alginate immobilized *C. beijerinckii* cells for continuous IBE fermentation has been tried and resulted in highest solvent production and productivity to be 0.88 g.l⁻¹ and 0.8 g.l⁻¹.h⁻¹ [34]. When glucose and xylose were used as the substrates for IBE production with *C. beijerinckii* B593 the productivity and yield were found to be 0.16 g.l⁻¹.h⁻¹ and 0.32 g.g⁻¹ respectively [35]. The engineered strain in present study

resulted in superior solvent productivity and yield with other recent studies (7.96 g.l⁻¹ and 0.29 g.g⁻¹) with pure glucose as substrate [2,35].

The consistent IABE ratio throughout all the dilution rates even with different substrates, confirms that the genetic manipulations in the modified organism are not dependent on the physiological conditions of the fermentation. However, the continuously changing solvent yields based on different substrate consumption were not dependent on the genetic modifications, but rather on the physiological conditions of the culture. Although the solvent productivity was increased significantly with the use of immobilized column reactor of *C. acetobutylicum* DSM792-ADH, the complete utilization of substrate with higher solvent productivity seems to be challenging. Further efforts to recirculate the unutilized sugars back into the reactor or to introduce parallel multistage reactors to utilize the sugars maximally are in progress. The *in-situ* solvent removal system as described in previous studies [3] can also be tried to enhance the solvent yield and productivity. Besides, the complete conversion of acetone into isopropanol was not achieved in this study. Since the secondary alcohol dehydrogenase is NADPH dependent enzyme, increased availability of the cofactor may further improve the conversion efficiency.

3.4. Techno-economic analysis of butanol production with SEW spent liquor

The study with SEW spent liquor as a substrate includes some assumptions for techno-economic analysis of the process (hemicellulose sugar losses during conditioning 10%; enzymatic hydrolysis yield 90 % at 20 % (w/w) consistency; fermentation yield to be 0.32 g.g⁻¹ corresponding roughly to 85 % (w/w) sugar conversion; maximum conversion of glucose and mannose 100 %; xylose 70 %; galactose and arabinose 0%). A process flow-sheet model was developed with Aspen 8.0 to calculate the detailed material balance including recycled stream. The total heat requirement for fractionation, ethanol-SO₂ recovery, butanol extraction, etc., was estimated by simulation to be 7.1 GJ /dry ton of feedstock. Energy in recovered lignin, biogas and

hydrogen gas was sufficient to satisfy the heat plus steam requirement of the process. The energy balance calculated for biomass per year is shown in table 3. Moreover, the cost profit analysis of this process with 700 kt softwood biomass resulted in 90 kt butanol per annum with overall profit to be 5 M€/annum (data not shown). Besides, the payback time on sales revenue was estimated to be 6 years. The profitability of present process can further be increased by producing esters, butyl butyrate and butyl acetate from the acids and butanol.

4. Conclusions

An engineered *C. acetobutylicum* DSM792-ADH was capable of substantially converting acetone into isopropanol. The immobilized cell column reactor was found to be promising fermentation technique to enhance the solvent productivities. The lowered IBE titers when non glucose sugars were used at higher dilution rates confirmed the dependency of the process on physiological parameters instead of genetic parameters. Besides, the effective use of sugar mixture and SEW spent liquor by *C. acetobutylicum* DSM792-ADH made it suitable for a cost effective industrial application. However, the complete utilization of sugars during the fermentation and complete conversion of acetone into isopropanol are further challenges that need to be addressed.

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Figure legends

Fig. 1. Effect of dilution rate on solvent production with different substrates in continuous immobilized cell culture of *C. acetobutylicum* DSM 792-ADH

Fig. 2. Solvent productivity, percent acetone conversion, solvent yield and total solvents produced in continuous immobilized cell culture of *C. acetobutylicum* DSM 792-ADH

Fig. 1. Effect of dilution rate on solvent production with different substrates in continuous immobilized cell culture of *C. acetobutylicum* DSM 792-ADH

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Table 1 IABE ratio and percent acetone conversion into isopropanol during the continuous immobilized cell column fermentation by using *C. acetobutylicum* DSM792-ADH

Glucose			Sug Mix.		SEW liquor	
Dilution rate (h ⁻¹)	IABE ratio	% acetone conversion	IABE ratio	% acetone conversion	IABE ratio	% acetone conversion
0.25	1.70:1.46:5.84: 0.99	46.24	1.63:1.48:5.90:0.99	47.58	1.57:1.32:6.00:1.10	45.76
0.50	1.58:1.54:5.88: 1.00	49.29	1.70:1.60:5.78:0.92	48.41	1.59:1.39:6.03:0.98	46.62
0.75	1.71:1.54:5.83: 0.92	47.40	1.70:1.24:6.02:1.05	42.14	1.52:1.34:6.08:1.06	46.88
1.00	1.86:1.23:5.98: 0.93	39.78	1.83:1.23:5.94:1.00	40.09	1.60:1.21:6.11:1.08	43.15
1.25	1.91:1.15:6.06: 0.88	37.50	1.78:1.25:6.01:0.96	41.17	-	-
1.50	1.95:1.03:6.07: 0.96	34.56	1.57:1.39:6.03:1.01	46.91	-	-
1.75	1.86:1.01:6.04: 1.09	35.16	1.30:1.01:6.27:1.42	43.67	-	-
2.00	1.83:0.73:6.24: 1.19	28.56	-	-	-	-

Table 2 Overall utilization of sugars during the continuous production of IBE solvents using the immobilized column reactor by *C. acetobutylicum* DSM792-ADH

Dilution rate (h ⁻¹)	Glucose (g/l)			Mannose (g/l)		Xylose (g/l)		Galactose (g/l)		Arabinose (g/l)		Total sugar utilization (%)		
	Pure Glucose	Sugar mixture	SEW liquor	Sugar mixture	SEW liquor	Sugar mixture	SEW liquor	Sugar mixture	SEW liquor	Sugar mixture	SEW liquor	Pure Glucose	Sugar mixture	SEW liquor
Initial sugars	58	32.72	28.75	14.78	16.88	6.41	7.24	3.95	4.18	2.14	2.95	100	100	100
0.25	41.48	32.03	26.71	4.38	4.79	2.54	2.61	0.34	0.23	1.06	0.92	71.52	69.57	60.80
0.50	32.50	30.46	22.68	2.65	3.09	1.16	1.36	0.22	0.17	0.85	0.68	56.03	60.92	48.26
0.75	22.72	25.48	15.69	0.95	0.75	0.85	0.16	0.12	0.04	0.15	0.26	39.17	47.47	29.11
1.00	14.38	15.95	6.66	0.65	0.36	0.60	0.00	0.06	0.00	0.05	0.00	24.79	29.83	12.10
1.25	9.47	9.47	0.00	0.35	0.00	0.36	0.00	0.00	0.00	0.00	0.00	16.32	17.55	0.00
1.50	5.87	5.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.12	9.33	0.00
1.75	3.77	1.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.49	2.15	0.00
2.00	2.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.29	0.00	0.00

1 **Table 3** Energy balance for 700 k ton Biomass per year

Input	MW	Biomass feed energy (%)
Dry Biomass	436	100
Products		
Butanol	107.30	24.6
Acetone	19.80	4.50
Ethanol	3.80	0.90
Acetic acid	19.80	4.50
Butyric acid	26.60	6.10
Hydrogen	24.80	5.70
Biogas	19.90	4.60
Lignin	154.30	35.40
Fiber Residue	18.10	4.20
Heat consumers		
SEW liquor heating	11.60	2.70
Sew liquor evaporation	23.10	5.30
Steam stripping	11.60	2.70
Sew liquor concentration	6.90	1.60
Extraction medium reg.	53.20	12.20
Solvent distillation	69.40	15.90
Total heat demand	175.90	40.30
Fuel H ₂ , CH ₄ , lignin, fiber res.	217.20	49.80

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