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In Search of the Best Technological Solutions for Optimal Biobutanol Production: A Multi-Criteria Analysis Approach

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***Abstract* – Rising energy demands and the environmental impact of fossil fuel combustion have promoted a growing interest in alternative fuel sources. Biobutanol is a promising biofuel that can be used as a partial or complete substitute for petrol in unmodified internal combustion engines. It can be produced through a microbiological process called ABE fermentation. Currently, its production is uncompetitive in the market, but researchers are still working on solutions to improve the technology. This paper used a multi-criteria decision analysis method to evaluate different alternatives for biobutanol production: microorganism strain, agro-industrial waste substrate as process feedstock, bioreactor type and extraction method. It was determined that *C. beijerinckii* and *C. saccharoperbutylacetonicum* have great potential for being used for efficient biobutanol production. Cheese whey is a promising residue for being used in the fermentation medium. Other residues evaluated in the paper gained similar results as being "close to ideal". Fed-batch with immobilized cells was chosen as the most promising fermentation method. It showed the greatest prospects as an optimal way to produce butanol. And, finally, adsorption and liquid-liquid extraction methods were identified as the most promising for ABE product extraction in comparison to others. Identified combinations of optimal solutions for microorganisms, fermentation methods, substrates and extraction techniques should be further evaluated in the laboratory setting.**

***Keywords* – ABE fermentation; agro-industrial waste; bacteria; biobutanol; biofuel; bioreactors; butanol; Clostridium; MCDA; multi-criteria analysis; renewable fuels; residues; TOPSIS; waste biomass.**

1. INTRODUCTION

Growing demand for rapidly depleting fossil fuels, fuel price volatility and growing concerns about the negative effects of greenhouse gases (GHG) are creating an interest in alternative fuel sources [1]. Currently, bioethanol is the focus of attention. It is considered a substitute for gasoline or an effective admixture, but the scientific community has not lost interest in a more chemically efficient alternative – biobutanol [2]. Biobutanol is a more efficient alternative to petrol than bioethanol and can be used as a partial or complete substitute for petrol in unmodified internal combustion engines. It has a higher energy capacity, is less volatile, less hygroscopic, less corrosive, and more suitable for unmodified gasoline engines than ethanol [3]. It is more similar to gasoline in its characteristics [4]. As a result, butanol is superior as an alternative biofuel. Currently, butanol is mainly used to

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produce varnishes and enamels and is widely used as a solvent in the production of a variety of vitamins, antibiotics, and hormones [5]. Although currently most of the butanol is produced from fossil fuels, it is possible to produce it through a microbiological process called ABE fermentation (short for "Acetone-Butanol-Ethanol fermentation"), which was a widely used butanol production method in the first half of the 20th century. Some strains are capable of converting the generated acetone into isopropanol via IBE (Isopropanol-Butanol-Ethanol) fermentation route [4], [6].

Interest in ABE has been renewed with extensive research and development (R&D) projects to make biobutanol production economically viable. The reasons why ABE fermentation became uncompetitive 50 years ago are still unsolved and research is ongoing to improve the technology's economic performance and reduce the technology's dependence on food-quality agricultural products (corn, potatoes, wheat, sugarcane etc.). The main problems with ABE are high substrate (carbohydrate source) costs, low product concentrations (about 2 % of the total volume of the bioreactor), toxic effects of the products on bacterial growth and expensive extraction of the products from the post-fermentation substrate [7]. To solve these problems, researchers initially investigated how to improve bacterial butanol production efficiency, but even after more efficient strains were created, the product concentration per unit of volume remained unchanged. This was explained by the bacteria's low tolerance to the toxicity of the synthesized products. Consequently, active work is underway to improve the tolerance properties of Clostridium bacteria. Increasing our understanding of the biochemical, metabolic, and genetic pathways responsible for tolerance is required to increase tolerance to product toxicity. As work on these biological mechanisms is still incomplete, some research teams are developing ABE technology further by improving the fermentation methods used, combined with simultaneous product recovery and bacterial cell concentration increase [8]. Since the high cost of raw materials is considered one of the main barriers against commercial butanol fermentation [9], the use of different agro-industrial residues is evaluated and researched as the potential feedstock for the process [10]. Substrates such as cheese whey, food waste, residues from beverage production etc. can be applied as cheap raw materials for ABE technology [9], [10].

Sustainable production of biobutanol depends on the used feedstock source and its pre-treatment methods, selected enhancing strategy of microorganism strain, fermentation effectiveness, and solvent recovery techniques. This paper aims to find the optimal set of technological solutions to produce biobutanol from agro-industrial by-products in a cost-effective manner which would be also in line with circular economy principles. Identification of the optimal solution set for efficient biobutanol production was done for four groups of technological alternatives: microorganism strain, agro-industrial residue used as feedstock, cultivation technology and extraction methods. Identification was done by using multi-criteria decision analysis (MCDA).

2. METHODOLOGY

The research methodology includes MCDA (Multi-Criteria Decision Analysis) for which TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) is used for evaluation. The TOPSIS tool provides an optimal solution that evaluates which alternative is the closest to the ideal by calculating the relative closeness coefficient to the ideal solution (the closeness coefficient is always between 0 and 1, where 1 is the preferred action) [11]. The method aims to select the best alternative closest to the positive ideal. The implementation of TOPSIS distinguishes six main steps, from the creation of the matrix to

the calculation of the relative coefficient [11]. In this study, authors followed the methodology of performing TOPSIS previously described by Behzadian *et al.* [12].

Four different technology factors for biobutanol production were evaluated by the TOPSIS method – microorganism, substrate, fermentation technology and solvent extraction method. The methodologies algorithm is represented in Fig. 1.

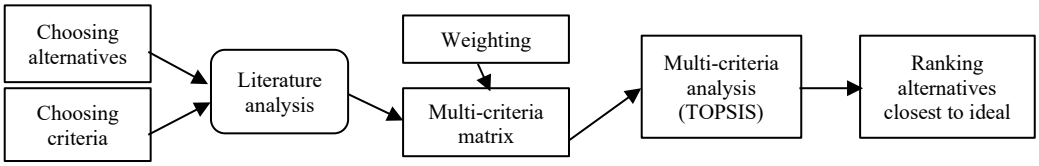

Fig. 1. Methodologies algorithm.

The alternatives and criteria used will be described, discussed and critically evaluated in further sections for each factor. Data obtained from the scientific literature was used to calculate normalized values. Appropriate assumptions were made if needed. Multi-criteria matrixes were based on expert evaluation. Experts were chosen from the research community of Riga Technical University, Institute of Energy Systems and Environment with expertise in microbiology and biotechnologies. Each expert gave the evaluation without consultation with another to provide a discrete individual evaluation. The weighted sum for all criteria in each analysis was 1.

Values used for performing MCDA were obtained from the scientific literature and are available in Annex A.

3. RESULTS

3.1. Evaluation of Microorganisms

Three alternative microorganisms were chosen for biobutanol production: *C. acetobutylicum*, *C. beijerinckii* and *C. saccharoperbutylacetonicum*. *C. acetobutylicum* and *C. saccharoperbutylacetonicum* are ABE-producing strains and *C. beijerinckii* is an IBE-producing strain [6]. The choice of strains to be included in this study was made based on previously conducted studies and reviews [13]. It is reported that Clostridium bacteria can utilize many carbon sources ranging from monosaccharides, oligosaccharides, and polysaccharides, including refined starches, pentoses, hexoses, and glycerol [10]. These strains were evaluated by seven criteria with weight provided by experts (see Table 1). Data used to characterize the selected strains is available in Annex A.

According to the expert evaluation, the most important criteria are the strain's ability to produce butanol in high amounts and high productivity. The strain tolerance to oxygen and cultivation temperature has the lowest importance.

It can be seen from the results of the MCDA that the strains *C. beijerinckii* and *C. saccharoperbutylacetonicum* have a great potential for being used in biobutanol production (see Fig. 2). These two species showed higher butanol and ABE production efficiencies than *C. acetobutylicum.* The later strain has shown inferior abilities if wild type strains of these species are compared [13]. If bioengineered *C. acetobutylicum* becomes very competitive with other *Clostridium* strains [13]. The reported ABE process time for *C. acetobutylicum* is longer than for *C. beijerinckii* and *C. saccharoperbutylacetonicum* (see Annex A).

TABLE 1. INDICATORS AND WEIGHTS USED IN MCDA OF MICROORGANISMS

Criteria	Unit of measure	Weight
Expected biobutanol concentration	g/L	0.19
Biobutanol yield	g/g gluc.	0.18
ABE/IB yield	g/g gluc.	0.15
Process time	h	0.15
Biobutanol productivity	g/L/h	0.17
Strain tolerance to oxygen	%	0.07
Optimal cultivation temperature	°C	0.09
	Σ	1.00

Successful production of biobutanol requires the cultivation medium to be flushed with nitrogen or the process will fail (or be inefficient) in case if strictly anaerobic strains are used. That is why the oxygen-tolerant strains could be superior and easier to manage in comparison to strictly anaerobic strains. It was found that *C. acetobutylicum* can tolerate 0–3 % of O_2 in a liquid medium [14] and gains better result in MCDA than the two other strains. No such information was found on other strains. However, it would be valuable to evaluate their tolerance to oxygen and whether it is possible to improve it without affecting butanol productivity: an oxygen-tolerant *C. beijerinckii* strain was used in aerobic conditions for butanol production, reported by Yao *et al.* [6], for example.

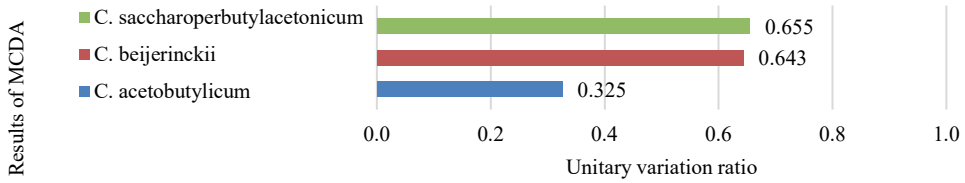

Fig. 2. MCDA results of optimal microorganism.

In addition, it was published, that improving medium composition by micronutrients and other chemicals could be beneficial to improve the strains' ability to tolerate butanol [15]–[18]. This property of the strains can also be improved via mutagenesis [18]–[20]. Liu *et al.* increased the strain's butanol tolerance by storing *C. acetobutylicum* in a butanol-glycerol solution for a year [21].

From a practical point of view, these three strains of microorganisms should be tested on a small scale in different conditions. It is possible to optimize the culture medium and find the best cultivation conditions for each strain. The possibility of improving each of the above-mentioned criteria has to be evaluated in practical tests and after that, strains could be compared with each other, and the best organism could be selected.

3.2. Evaluation of Waste Substrates for Fermentation

Different biodegradable agricultural or industrial residues can be used for microorganism cultivation. A lot of substrates have been studied [22]–[28] as a possible source for ABE production. Nine substrates were chosen as an alternative resource for biobutanol production for this MCDA: glycerol (from biodiesel production), apple and quince pomace, brewery residues (from beverage industries), straw hydrolysate (agricultural residue), molasses (sugar production residue), potato starch and pomace (from potato processing industries), rapeseed residue (from rape oil production) and liquid cheese whey (from cheese production).

These alternative substrates are quite different from each other. For example, starch, fruit pomace and spent grains have different structural sugar contents. Fruit wastes can be rich in simple sugars and fibre [29]. Molasses, cheese whey, some fruit wastes and straw hydrolysate can be classified as monosaccharides and disaccharides-rich sources, but potato pomace, brewery residues and spent grains are structural polysaccharides-rich sources [29]. It can be difficult for some microorganisms to use polysaccharides as feedstock if they cannot produce the necessary enzymes [30], [31]. Therefore, these substrates are often pre-treated before use to make them more readily available as a nutrient source for the microorganisms. The most common pre-treatment methods are acid hydrolysis and alkali hydrolysis, which have such disadvantages as extensive use of highly reactive chemicals, high energy requirements, long process time, and formation of inhibitors [4].

Substrate alternatives were evaluated according to eleven criteria with weight provided by experts (see Table 2). Values for such factors as carbon or nitrogen content, expected butanol yields, butanol production rate, availability, pre-treatment cost and transportation costs were obtained from published papers (see Annex A). Such criteria as shelf life and the energy required for storage were evaluated based on whether the substrate could be stored at room temperature, cold storage or freezing. Similarly, the seasonality of waste product generation was evaluated, considering whether the residue could be produced year-round or only during certain months. Therefore, for shelf life, waste product seasonality and energy needed for storing assumptions were made to assign a value.

TABLE 2. INDICATORS AND WEIGHTS USED IN MCDA OF SUBSTRATES

Criteria	Unit of measure	Weight
Substrate carbon or nitrogen content	%	0.08
Expected biobutanol concentration	g/L	0.13
Biobutanol yield	g/g gluc.	0.12
Biobutanol productivity	g/L/h	0.12
Substrate availability	million t/year	0.10
Shelf life	–	0.06
By-product seasonality	–	0.07
Storage cost	–	0.07
Substrate pre-treatment cost	EUR/t	0.09
Transportation costs	EUR/t/km	0.07
Substrates price	EUR/t	0.11
	Σ	1

The ability to generate higher butanol yields is the most important factor similar to the evaluation of microorganisms. The second place of importance takes substrate price and availability. It is worth noting that the need for specific storage conditions, such as refrigeration, could potentially increase both capital and operational costs.

MCDA analysis results are represented in Fig. 3.

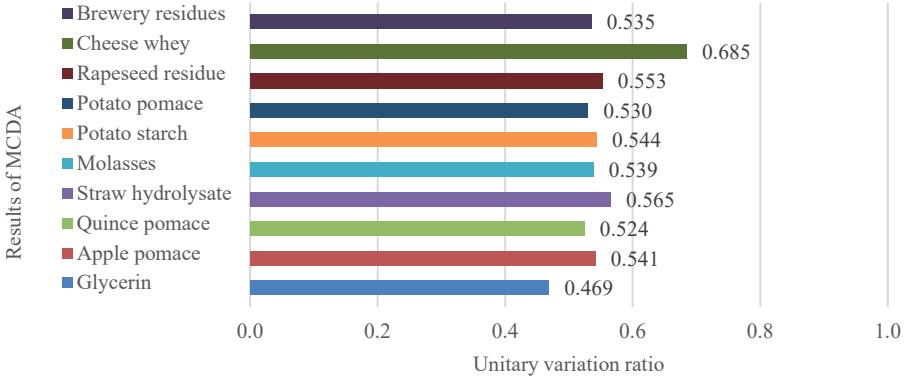

Fig. 3. MCDA results of optimal feedstock substrate.

The MCDA results show that cheese whey is one of the most promising for biobutanol production. High butanol yields have been reported when using this substrate [24], [32] and it is available in relatively large quantities [33]–[35]. Around 50 % of this residue is dumped into sewage systems or directly into water bodies, therefore it can have a negative effect on the environment [34]–[36]. So, using it for fermentation processes has some additional benefits like reduction of environmental pollution. It has a great potential to be used as a cheap resource for value-added product production as well. The issue with this resource lies in the space and energy requirements for storage. It requires immediate freezing or use upon acquisition to prevent spoilage.

High butanol yields have been reported when butanol-producing strains have been cultivated on such substrates as straw hydrolysate [37]. However, straw hydrolysate requires pre-treatment by steam explosion [37], which is more costly in comparison to the other pre-treatments (acidic, alkali or enzymatic hydrolysis) of biomass [38], [39]. Although experts prioritize factors such as overall butanol production and efficiency above mentioned substrate remains competitive with the other ones.

Pre-treatment and hydrolysis could be crucial for the utilization of materials with complex structures in fermentation processes [10]. Pomace, rape seed residues and brewery residues need to be pre-treated [28], [38], [40]. Therefore, the process could be costlier in comparison to the enzymatic hydrolysis. It could be beneficial to perform experiments where the substrate is used in sequential fermentation by fungi to reduce pre-treatment costs. The fungi cause extracellular depolymerisation of the substrate into oligomers and monomers during this process, which makes it more accessible to the microorganisms grown on this hydrolysate [41], [42]. This approach would be environmentally friendly and cost-effective and interest in this type of pre-treatment of biomass has grown in recent years [43], [44]. It is non-toxic, requires low energy amounts, does not generate inhibitors for downstream processes, and can be used to hydrolyse cellulose or to depolymerize and remove lignin [45] as well.

Seasonal residues have obtained lower MCDA results. The consistent availability of the required resource throughout the entire year can be crucial in the technological process. But, if the substrate is generated in large quantities and can be stored cheaply to meet production demands, then it should not be an issue.

Glycerol is known to be easily applicable as a substrate for microbial fermentations. In this assessment, it got the lowest weight in MCDA, probably, because its price has significantly increased in recent years. In addition, from an economic feasibility point of view, glycerol can be also used for the production of products with higher added value than biobutanol, e.g., single-cell protein [36], [46].

3.3. Evaluation of Fermentation Types

ABE fermentation can be performed using different feeding modes – batch, fed-batch and continuous. Clostridium cells can be either free-floating in the fermentation tank or immobilized. All these fermentation setup variations were subjected to comparative studies where the productivities and yields of these fermentation regimes were assessed [47]–[51] (see Annex A). The results of the studies were used in the TOPSIS analysis for butanol and ABE productivities and yields. It was decided not to use fermentation time as one of the criteria in the TOPSIS analysis because the factor of time relative to the final products output is already taken into account in the butanol and ABE productivity values. In addition, the comparison of fermentation time between batch, fed-batch and continuous fermentation types may not be accurate. The reason is because of fermentation times vary widely for each fermentation type (3 h for batch and 150 h for continuous) and all fermentation types are intended to be combined with one of the *in situ* product removal methods (see chapter 3.4.), therefore the productivity values are more appropriate.

The substrate and nutrients are added at the same time as the inoculant or shortly thereafter in batch fermentation. The process takes place under controlled conditions until the maximum concentration of the final product (in this case butanol) in the fermentation vessel is reached [52]. Batch fermentation is very widely used in breweries and the pharmaceutical industry due to the flexibility of batch fermentation, high product concentrations, reduced risk of contamination, low maintenance costs, and simple operation [48]. The only shortcoming is lower productivity: to achieve the same productivity as for continuous fermentation, the capital costs should be higher [52].

Fed-batch fermentation can be considered in part as an improved version of batch fermentation, designed to overcome the limitations of batch fermentation. In the fed-batch fermentation mode, when the culture reaches the exponential growth phase, it is fed with additional nutrients, which allows it to prolong the exponential phase and obtain higher product yields [53]. Since ABE fermentation can be inhibited both by the substrates used and by the products, fed-batch ABE fermentations are usually started with a low concentration of consumable substrate in the culture medium. As the culture consumes the substrate, additional substrate is added accordingly to avoid reaching an inhibitory substrate level. In addition, the periodic addition of the substrate in dosed amounts allows for control of the concentrations of ABE products and intermediates, which, in turn, prevents the toxic effect of catabolites on the culture [48].

Cell immobilization is recognized as an attractive technique for biobutanol production. Fermentation with immobilized cells means fixing or localizing the cells in a specific area of the vessel using various methods [54]. Cell immobilization allows to achieve higher cell concentrations, higher productivity, shorter fermentation periods, it provides wider opportunities for ensuring a continuous process, it allows to reduce the inhibitory effect of

substrates on the culture, reduces the inhibitory effect of final products, reduces the risk of contamination, and also reduces costs [55]–[60].

Six batch fermentation types were evaluated: with free cells, repeated batch fermentation with immobilized cells, fed-batch fermentation with free cells, fed-batch fermentation with immobilized cells, continuous fermentation with free cells and continuous fermentation with immobilized cells. The criteria for evaluation are presented in Table 3 with experts given weights.

TABLE 3. INDICATORS AND WEIGHTS USED IN MCDA FOR EVALUATING FERMENTATION TYPE

Criteria	Unit of measure	Weight
Butanol productivity	g/L/h	0.300
ABE productivity	g/L/h	0.170
Butanol yield	g/g gluc.	0.255
ABE yield	g/g gluc.	0.275
	Σ	1

According to experts given opinion butanol productivity and the quantity of butanol that can be gained have a greater importance than total ABE solution yields and productivity.

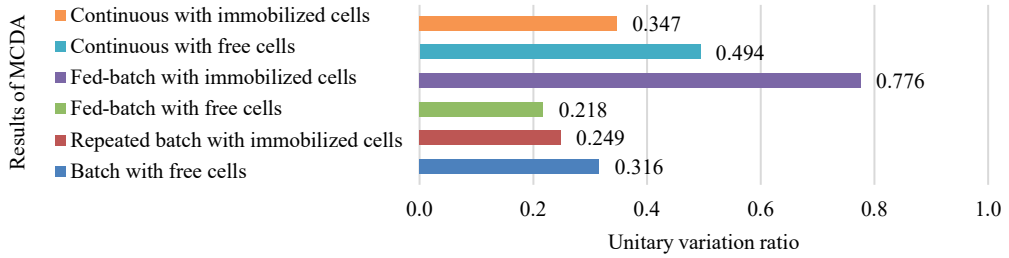

Fig. 4. MCDA results of optimal fermentation method.

The TOPSIS analysis showed that the best choice is fed-batch fermentation with immobilized cells (see Fig. 4). It was based on the evaluations provided by experts and the data reported in publications on the productivities and yields of each type of fermentation (see Annex A).

An ABE fed-batch fermentation is widely used and has often outperformed continuous fermentation in comparative studies. Nevertheless, several research groups have pointed out several problems when trying to ensure the stability of the continuous fermentation process [48], [49], [51], [54].

It should be emphasized that the analysis of fermentation methods did not take into account the costs of each method. This was not done because the available information on cost differences between various fermentation methods in the production of biobutanol was very limited and incomplete. The best examples of such fermentation methods were found in the pharmaceutical industry, but the highest working volumes of bioreactors in the examples given there were very small (up to 200 litres), while when assessing the costs of fermentation methods in the context of biobutanol production, the working volumes of bioreactors should be tens to hundreds of times larger. Overall, this factor highlights one of the shortcomings of this study, which should be further explored in future research.

3.4. Evaluation of Extraction Methods

Five alternative techniques were chosen for this MCDA to evaluate the extraction of ABE or IBE products (see Annex A). The alternatives were gas stripping, pervaporation, membrane distillation, adsorption and liquid-liquid extraction. Although reverse osmosis is mentioned as one of the methods for extracting butanol from the fermentation broth, it was not included in MCDA because of the lack of suitable data for further analysis. The five alternatives were evaluated by five criteria: technologies capital and operational costs (EUR/kg butanol), the recovery rate of butanol (g/L/h), energy consumption (MJ/kg butanol) and recovery efficiency (%). Experts evaluated weights for the mentioned criteria can be seen in Table 4. All the technologies can be combined with cultivation process according to published research, therefore that factor was not taken into account as criteria.

TABLE 4. INDICATORS AND WEIGHTS USED IN MCDA FOR EVALUATING RECOVERY TECHNOLOGIES

Criteria	Unit of measure	Weight
Capex	EUR/kg butanol	0.14
Opex	EUR/kg butanol	0.19
Recovery rate	g/L/h	0.23
Energy consumption	MJ/kg butanol	0.19
Recovery efficiency	%	0.25
	Σ	1

Experts gave the highest values to butanol recovery rate and efficiency. Operational costs and energy consumption for the process were evaluated as equally important and capital costs obtained the lowest importance. The results of TOPSIS analysis can be seen in Fig. 5.

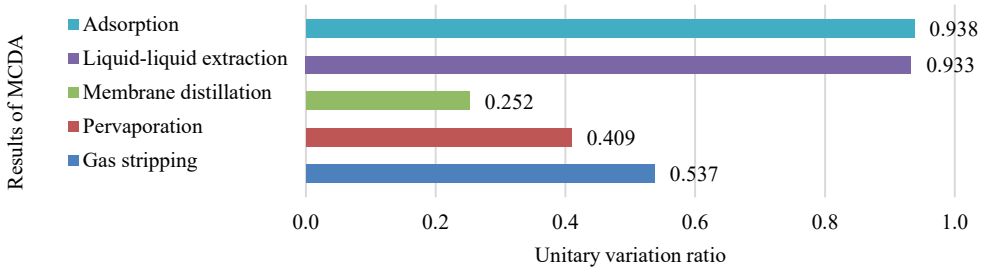

Fig. 5. MCDA results of optimal extraction method.

According to TOPSIS results adsorption and liquid-liquid extraction is almost ideal for butanol extraction according to the criteria. Both these technologies were reported by authors [61] as the most promising for butanol separation and purification.

Adsorption is a process in which components from the liquid or gas phase are absorbed by the solid phase and then are recovered from it. Adsorption could be characterized by several significant advantages such as very high selectivity of extraction and high concentration of butanol after desorption, and low energy consumption. At the same time, it has a number of disadvantages, which include some difficulties of applying the adsorbent in situ to a

fermentation broth (an intermediate step of separating the cells from the broth is necessary), poisoning of the adsorbent by impurities (cause degradation of adsorption properties) and adsorbing nutrients from fermentation broth [61]–[66]. If overcoming the specified shortcomings, it would be closest to ideal.

Liquid-liquid extraction is a process in which immiscible liquid phases are mixed and components from one liquid move to another (according to their solubility). It is characterized by a very high selectivity of the extraction process, and as a result, a high concentration of the final product. Disadvantages include difficulties in applying the technology directly to the substrate (since the ionic liquids used for extraction are usually toxic to cells), formation of emulsion and significant capital costs [61], [63], [64], [65]–[68]. According to literature data, liquid-liquid extraction could be competitive to adsorption if the needed energy for the extraction was lower.

Gas stripping is the third closest to the ideal extraction method according to the results. This process involves pumping gases through a fermentation broth. It is the simplest process from the technical implementation point of view. The most important advantages include the possibility of using culture-produced gases (CO_2, H_2), in situ appliance and extraction of butanol (which inhibits the growth of the cells) directly from a fermentation broth without harming the substrate. Disadvantages include very low extraction selectivity and significant energy consumption [61], [64], [66], [69]–[71]. If the technology required lower costs compared to other methods, lesser energy requirements and higher extraction efficiency it could become more competitive with adsorption and liquid-liquid extraction methods.

Membrane distillation obtained the lowest result and showed itself to be the furthest of ideal. In this process two liquid phases are separated by the membrane and components from one liquid move through it to the other. It is characterized by selectivity and the possibility of applying the process *in situ* to the substrate. However, the disadvantages include high cost, low chemical- and bio-resistance of membranes (which significantly affects their performance) and difficulties in their restoration [61], [63], [72], [73].

Pervaporation technology uses partial vaporization across a membrane to separate butanol from a multicomponent liquid mixture. Therefore, it has the same advantages and disadvantages as membrane distillation. Advantages include lower energy consumption in comparison to membrane distillation, and recent progress in the development of new more effective, renewable and cheaper membranes [61], [64], [74]–[76].

Whereas, developing new types of membranes is in progress, so both these technologies (membrane distillation and pervaporation) may become competitive with other ones over time. Moreover, depending on the initial conditions, the combination of different technologies (when disadvantages of one are covered by another) could be more effective than using one method of extraction.

4. CONCLUSION

The evaluation and MCDA analysis for a combination of four groups of technological alternatives for biobutanol production via microbiological fermentation was performed.

According to the TOPSIS results *C. beijerinckii* and *C. saccharoperbutylacetonicum* have a great potential for being used for developing biobutanol production technology. Combinations with appropriate substrates and supplements can result in high butanol yields. If needed, the strains can be improved by enhancing their butanol tolerance.

Cheese whey has many advantages for being used in ABE production. It was the closest one to the ideal for butanol production due to TOPSIS results. Other evaluated substrates had

a unitary variation ratio of around 0.52–0.54, which indicates that they have more or less equal prospects of being used to produce butanol.

Fed-batch fermentation with immobilized cells was the closest to the ideal. This process is widely used and has often outperformed continuous fermentation in comparative studies.

Adsorption and liquid-liquid extraction are almost ideal for butanol extraction according to the chosen criteria. Some of the extraction technologies have the potential to be improved and should be evaluated in the future again.

Following MCDA and TOPSIS analyses, we have identified several potential combinations of microorganisms, substrates, fermentation methods, and extraction techniques that could optimize biobutanol production. Each of these combinations requires thorough testing and evaluation, with the results of these tests becoming the focus of future research papers. It is also important to note that new potential combinations may emerge in the future, indicating that there is ample scope for enhancing the efficiency of biobutanol production.

ANNEXES

Available at: https://zenodo.org/records/10246077

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