Influence of Carbon Dioxide and Nitrogen Source on Sustainable Production of Succinic Acid from Miscanthus Hydrolysates

Mariusz Kuglarz and Monika Rom

Abstract—This study presents sustainable succinic acid production from lignocellulosic hydrolysates, using A. succinogenes, which consumes CO$_2$ during succinic fermentation. Optimal combination of carbon source (gaseous and/or solid MgCO$_3$ 15-30 g/dm$^3$) and nitrogen source (mixtures of yeast extract and hydrolyzed rapeseed cakes: 100:0, 80:20, 60:40, 50:50 and 40:60 % w/w) were experimentally determined. Obtained results stated that simultaneous addition of gaseous CO$_2$ and MgCO$_3$ (20-30 g/dm$^3$) resulted in the highest sugar conversion (89-95%) and succinic yields (66-74%). Additionally, CH$_4$ content in biogas, used as CO$_2$ source, was increased by 21-22% and reached 91-92% after succinic fermentation with addition of MgCO$_3$ (20 g/dm$^3$). The course of succinic fermentation confirmed that 50% of nitrogen dosage can be added as rich in amino acids rapeseed cakes, originated from biodiesel production. Succinic fermentation with gaseous CO$_2$ as the only CO$_2$ or after replacing more than 50% of nitrogen dosage as rapeseed cakes, resulted in increased acetic and formic acids production (by-products of succinic fermentation). Optimal conditions of succinic fermentation (CO$_2$ source and dosage, waste nitrogen source) identified in this study can pave the way towards sustainable production of succinic acid from lignocellulosic biomass.

Index Terms—Succinic acid, biomass, hydrolysates after pretreatment, nitrogen source, carbon dioxide, biogas.

I. INTRODUCTION

Green technology is becoming a driving force in chemical industry because of the current need to decrease environmental impacts, caused by petrochemical industry. Succinic acid in particular, can be used as precursor for the production of biodegradable polymers, food, fine chemicals, green solvents and pharmaceuticals [1]. Succinic acid, also known as amber acid or butanedioic acid, has been recognized as one of the top twelve building-blocks of biobased chemicals [2]. Currently, this compound is mainly produced by Actinobacillus succinogenes from lignocellulosic biomass using waste CO2 (0395/IP2/2016/74) (public funds for science in the years 2016–2019).

Among number of bacterial strains able to generate succinic acid through anaerobic fermentation, Actinobacillus succinogenes is considered as one of the most promising ones for industrial applications [5], [6], mainly due to its ability to ferment wide range of carbon sources, i.e. glucose, xylose, arabinose, galactose etc. [5]. However, effective production of succinic acid (building block chemical) from lignocellulosic hydrolysates, is strictly connected with the amount of sugars released during biomass hydrolysis (pretreatment). According to the current state of knowledge, pretreatments with organic solvents are very effective in lignocellulosic biomass hydrolysis and lead to smaller sugar losses compared to most commonly used acid-based, alkali or oxidative methods [7]-[10]. Hydrolyzed biomass is converted into succinic acid through microbial fermentation (including Actinobacillus succinogenes) and carbon dioxide availability and nitrogen source constitute the most important parameters influencing effectiveness of succinic fermentation (sugar conversion rates, succinic yields). In particular, CO$_2$ supply is the crucial factor determining succinic yield, the ratio of succinic acid to other by-products as well as the degree of sugar utilization. Besides carbon source, nitrogen source and its dosage have a direct impact on the economy of succinic fermentation. In most cases, yeast extract, being rich in amino acids, vitamins and trace elements is considered as favorable nitrogen source in fermentation processes. However, yeast extract is treated as one of the most expensive medium component and sustainable succinic acid production should eliminate or decrease its usage, by application of waste products, rich in amino acids.

This study presents sustainable succinic acid production from hydrolysates after glycerol-based pretreatment, using A. succinogenes 130Z (ATCC 55618). The aim of this study was to analyze the influence of carbon dioxide and nitrogen source on the effectiveness of succinic fermentation. Optimal combination of carbon source (gaseous and solid MgCO$_3$) and nitrogen source (mixtures of yeast extract and rapeseed cake) were experimentally determined. According to best of our knowledge, this is the first study evaluating the usage of biogas after co-digestion processes (CO$_2$ source) and rapeseed cakes (nitrogen source) for the production of succinic acid from real hydrolysates, obtained from lignocellulosic biomass.

II. MATERIALS AND METHOD

A. Feedstock

Hydrolysates (35.6 ± 0.7 g-glucose/dm$^3$ and 8.25 ± 0.3
g-xylose/dm³) obtained after biomass pretreatment with glycerol-based method at optimized conditions (80% w/w of waste glycerol after biodiesel production + 1.25% w/v H₂SO₄ as a catalyst) and subsequent hydrolysis with the most effective enzymatic mixture (10% w/w Cellic® CTec2, 5% w/w β-Glucanase and 1% w/w Cellic® HTec2) were used as feedstock for succinic acid production. Pretreatment conditions and dosage of enzymatic cocktails were established during our previous studies [11].

B. Procedure of Succinic Fermentation

Before fermentation, hydrolysates were autoclaved at 121°C for 20 minutes as well as mixed with experimental medium (nutrients) at 75:25 (v/v), which was selected as the most optimal ratio, based on our previous studies [12]. Succinic fermentation was conducted in triplicates, using identical 3 dm³ fermenters (Sartorius BIOSTAT Aplus, Germany) with an initial working volume of 1.0 L, operated at 37 °C and 150 rpm for 48 h. In each case, about 5% (v/v) of exponentially growing inoculum was added. The strain of A. succinogenes 130Z (DSM 22257) was obtained from DSMZ and was stored in glycerol at -80 °C until used. Seed culture medium was composed of (g/dm³): glucose (10.0), yeast extract (5.0 or 10.0), NaHCO₃ (10.0), NaH₂PO₄·2 H₂O (9.6), K₂HPO₄·3 H₂O (20.3). During start-up of the process, N₂ gas was used to create anaerobic conditions in fermenters. Prior to start of batch fermentation, pH was adjusted to 6.8 with 50% H₂PO₄ and 0.05 mL of sterile Antifoam 204 was added. In case of pH decrease below 6.8 during fermentation due to acid production and insufficient carbonate–bicarbonate buffering capacity, NaOH solution (8 M) was added.

Two sets of succinic fermentation were conducted. Firstly, the influence of carbon dioxide source and dosage were taken into account. Solid MgCO₃ (15-30 g/dm³) and biogas (containing 75% of CH₄ and 25% of CO₂) were used as carbon dioxide. The biogas originated from our previous sewage sludge and kitchen biowaste co-digestion experiments [13]. This part of experiment was conducted using 5:1 gas-liquid ratio and atmospheric pressure (101.3 kPa). In order to increased CO₂ solubility, the process was conducted at increased pressure, i.e. (140 kPa). For monitoring the pressure inside fermenters, a pressure gauge was mounted with a relief valve. The biogas was recirculated during fermentation and changes in CH₄ and CO₂ concentrations were recorded. Considering the fact that gaseous CO₂ exhibits a relatively poor solubility, biogas (containing CO₂) was supplemented with solid MgCO₃ (15-30 g/dm³).

During the second set of succinic acid production, the impact of nitrogen source (yeast extract and/or rapeseed cakes after biodiesel production) was taken into account. The rapeseed cake dry matter (d.m.) content amounted to 950±20 g/kg and the main components of this substrate included: total protein 340±25 g/kg, cellulose 148±10 g/kg, hemicellulose 54.0±5.0 g/kg, insoluble lignin 150±8.5 g/kg, ash 48.2±2.5 g/kg. Total amino acid content in cakes before hydrothermal processing amounted to 310±10 g/kg. The hydrothermalization of rapeseed cake was performed in a batch reactor (Minotavr-1), at 210-220 °C, which is in consistent with previous studies on effective amino acids conversion from rapeseed cakes [14]. The fermentation was conducted using the following ratios of yeast extract and rapeseed cake (% w/w): 100:0, 80:20, 60:40, 50:50 and 40:60. Nitrogen dosage (yeast extract and/or rapeseed cakes) amounted to 5 and 10 g/dm³, which is in the range commonly used for succinic fermentation, using A. succinogenes [3], [11]-[13].

One milliliter samples were taken periodically (after 0, 3, 6, 12, 15, 18, 24, 30, 36 and 48 h) and used for analysis of sugars (glucose, xylose), and acids (succinic-, acetic- and formic). Succinic acid yield (YSA) was calculated as the amount of succinic acid (g/dm³) obtained per 1 g/dm³ of sugars (glucose + xylose) consumed. Sugar utilization was calculated as the difference between initial sugar content (g/dm³) and sugar content after succinic acid production (g/dm³).

C. Analytical Methods

Concentrations of sugars and organic acids (succinic, acetic, formic) were measured by using high performance liquid chromatography HPLC (Agilent 1260 Infinity, Germany) equipped with a Bio-Rad Aminex HPX-87H column at 63 °C and ultraviolet (UV) and refractive index (RI) detector (67162A, Germany), using 4mM H₂SO₄ as eluent at 0.6 mL/min flow rate. The pH was measured using a standard pH meter. All chemicals used in this study were of analytical grade. Dissolved carbon dioxide content in fermentation broths was determined using an InPro 5000i sensor coupled with Mettler Toledo (M400).

All results are presented as average values were compared statistically. One-way ANOVA test followed by HSD tests were used for multiple comparisons between samples, with the level of significant set at 0.05. The same letters represent data equivalent statistically (p > 0.05).

III. RESULTS AND DISCUSSION

A. Succinic Fermentation Using Different Carbon Sources

Carbon dioxide constitutes one of the major substrates used for biosynthesis of succinic acid, while, CO₂ source and dosage influence the metabolic flux as well as effectiveness of succinic acid production [15]. Firstly, the succinic acid fermentation was conducted, using gaseous CO₂ (biogas containing 75% CH₄ and 25% CH₆). The process conducted at atmospheric pressure (101.3 kPa, CO₂ partial pressure 40 kPa) allowed to utilize about 55% of available sugars (glucose and xylose) and resulted in a relatively low succinic yield - 54% (0.54 g-succinic acid/g-sugar consumed (Table I, Figure 1). Process conducted at increased pressure, i.e. 140 kPa (CO₂ partial pressure of 56 kPa) and thus at higher CO₂ solubility [6], had no significant impact on sugar utilization, whereas, succinic yield was increased by 7% (Table I). These succinic yields are slightly lower than yields previously reported, using model biogas mixture (60% CH₄ and 40% CO₂). However, the current study was based on biogas containing significantly lower CO₂ content (25% vol.) compared to previous studies [6].

In many previous studies on bio-succinic acid production, salts (MgCO₃, NaHCO₃ or CaCO₃) are used as carbon source. In particular, MgCO₃ was identified as the most effective CO₂ supplier and pH control agent [4], [16].

In these cases, the highest succinic titers (22-23 g/dm³) and yields (74-76%) were obtained after addition of 25-30 g/dm³...
MgCO₃ as carbon dioxide source (data equivalent statistically, data statistically equivalent, p > 0.05) (Table I). It should also be highlighted that the degree of sugar conversion and succinic yields were improved by 67-70% and 37-40%, respectively compared to the process with biogas as the CO₂ source (Fig. 1). Significantly lower succinic titers and yields were obtained for MgCO₃ dosage of 15-20 g/dm³, which proved that availability of CO₂ plays a crucial role during succinic acid biosynthesis and promotes the carbon flow towards SA production branch of TCA (tricarboxylic acid) cycle [3], [4].

<table>
<thead>
<tr>
<th>CO₂ source</th>
<th>Sugar util. (%)</th>
<th>Succinic yield (%)</th>
<th>Byproducts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogas (101.3 kPa)</td>
<td>54.7±3.5c</td>
<td>54.4±2.5de</td>
<td>34.5±1.4a</td>
</tr>
<tr>
<td>Biogas (140 kPa)</td>
<td>55.7±2.0c</td>
<td>58.1±2.1cd</td>
<td>36.6±2.1a</td>
</tr>
<tr>
<td>MgCO₃ 15 g/dm³</td>
<td>74.2±2.6b</td>
<td>48.6±4.7e</td>
<td>27.5±1.4b</td>
</tr>
<tr>
<td>MgCO₃ 20 g/dm³</td>
<td>80.2±4.6b</td>
<td>63.1±2.2cd</td>
<td>28.5±1.5bc</td>
</tr>
<tr>
<td>MgCO₃ 25 g/dm³</td>
<td>91.4±0.9a</td>
<td>74.3±2.4ab</td>
<td>24.7±1.2cd</td>
</tr>
<tr>
<td>MgCO₃ 30 g/dm³</td>
<td>93.2±0.8a</td>
<td>76.1±2.5a</td>
<td>24.2±1.2cd</td>
</tr>
<tr>
<td>CO₂+15 g/dm³ MgCO₃</td>
<td>79.3±3.8b</td>
<td>55.1±3.6bc</td>
<td>29.8±1.3b</td>
</tr>
<tr>
<td>CO₂+20 g/dm³ MgCO₃</td>
<td>89.3±1.5a</td>
<td>65.7±3.0bc</td>
<td>25.8±1.4cd</td>
</tr>
<tr>
<td>CO₂+25 g/dm³ MgCO₃</td>
<td>94.0±1.3a</td>
<td>72.8±4.3ab</td>
<td>25.9±1.3cd</td>
</tr>
<tr>
<td>CO₂+30 g/dm³ MgCO₃</td>
<td>94.2±1.9a</td>
<td>73.7±5.9ab</td>
<td>27.0±1.1cd</td>
</tr>
</tbody>
</table>

During the second part of the experiment, simultaneous effect of gaseous CO₂ and solid MgCO₃ on the performance of succinic fermentation, was taken into account. When MgCO₃ was added with the supply of gaseous CO₂, the following compounds: CO₂, HCO₃⁻ and CO₃²⁻ would become in the equilibrium in the fermentation broth [15]. The highest succinic titer and yield amounted to 22-23 g/dm³ and 73-74%, respectively and these values were obtained for MgCO₃ dosage of 25-30 g/dm³ (300-400 mM CO₂) (data equivalent statistically, data statistically equivalent, p > 0.05) (Table I). Slightly lower succinic yields were recorded for MgCO₃ dosage of 20 g/dm³ (Table I). In these conditions, the dosage of carbon sources significantly exceeded the maximum CO₂ solubility (139 mM) [15]. This proves that high succinic yields are obtained in conditions of excess CO₂ content. This is in accordance with previous reports stating that the highest succinic yields (69-71%, 0.69-0.71 g/g) were obtained for carbonate dosage of 300-500 mM [17]. Obtained succinic yields are within the values most commonly reported for lignocellulosic hydrolysates [2], [12]. It should be highlighted that maximal theoretical molar yield of succinic acid per mole of glucose corresponds to 1.71 (12/7) (Eq. (1)) and this equation equals to 1.12 g/g when expressed as a mass unit. This theoretical maximal yield would be achieved anaerobically and represents a theoretical situation where the microorganisms do not use substrate for growth, maintenance or an external electron acceptor.

\[ C_6H_{12}O_6 + \frac{6}{7} CO_2 \rightarrow \frac{12}{7} C_4H_6O_4 + \frac{6}{7} H_2O \]  

(1)

Based on our results, it can be stated that the availability of CO₂ is crucial for the bio-succinic acid production, and A. succinogenes consumes CO₂ during fermentation. The stoichiometric reaction cited above suggests that 1 kg of succinic acid will utilize 0.37 kg of CO₂. Therefore, it is evident that bio-succinic acid production could contribute to the abatement of CO₂ emissions. An additional purpose of using CO₂ for biosynthesis of succinic acid was to purify the biogas used as CO₂ source. CH₄ content in biogas after succinic fermentation with gaseous CO₂ source increased by 10-11% and reached the level of 82-84%±1.5%. There was no a significant impact of increased CO₂ pressure on the final CH₄ content in biogas (88±1.5%) (Table II). An increase of CH₄ content from 85% to 95% was reported in previous studies [6]. However, as previously mentioned, these authors used biogas containing significantly higher CO₂ content (40% vol.) compared to present study (25% vol.). As regards the simultaneous usage of gaseous CO₂ and MgCO₃ (dosage of 25-30 g/dm³), it was observed that CH₄ content after succinic fermentation, was not significantly increased (76-77% vol.). Such conditions reflected high CO₂ content, originated from carbonate. Whilst, the CH₄ content in biogas increased by 21-23% with a lower dosage of MgCO₃ (15-20 g/dm³) (Table II). In these cases, the final CH₄ content in biogas treated is in consonance with values possible to obtain via commercially available purification methods, e.g. biogas treatment with water scrubbing or using chemical scrubbers with amine solutions [18].

All in all, these results proves the positive impact of simultaneous addition of gaseous CO₂ and solid MgCO₃ on the course of succinic fermentation, both in regards of succinic yield and biogas purification (Table II). What is more, fermentation with simultaneous CO₂ source (biogas/MaCO₃) resulted in almost 90% sugar utilization, with complete glucose consumption and about 50%
bioconversion of xylose into carboxylic acids. Whereas, the process with biogas as the only CO\textsubscript{2} source resulted in significant residual glucose and almost 70% of initial xylose present in the solution (Fig. 1). This proves that *Actinobacillus succinogenes* prefers glucose as substrate rather than C\textsubscript{6} sugars (e.g. xylose) [11], [12].

**TABLE II: INFLUENCE OF SUCCINIC FERMENTATION ON BIOGAS COMPOSITION**

<table>
<thead>
<tr>
<th>CO\textsubscript{2} source</th>
<th>CH\textsubscript{4} (% vol.)</th>
<th>CO\textsubscript{2} (% vol.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biogas (101.3 kPa)</td>
<td>76.3±2.0</td>
<td>21.8±1.8</td>
</tr>
<tr>
<td>Biogas (140 kPa)</td>
<td>82.1±2.0</td>
<td>15.4±1.8</td>
</tr>
<tr>
<td>Biogas (140 kPa) + 15 g/dm\textsuperscript{3} MgCO\textsubscript{3}</td>
<td>91.8±2.2</td>
<td>5.17±1.2</td>
</tr>
<tr>
<td>Biogas (140 kPa) + 20 g/dm\textsuperscript{3} MgCO\textsubscript{3}</td>
<td>91.3±2.0</td>
<td>5.97±1.3</td>
</tr>
<tr>
<td>Biogas (140 kPa) + 25 g/dm\textsuperscript{3} MgCO\textsubscript{3}</td>
<td>78.8±2.5</td>
<td>17.1±2.0</td>
</tr>
<tr>
<td>Biogas (140 kPa) + 30 g/dm\textsuperscript{3} MgCO\textsubscript{3}</td>
<td>77.5±2.1</td>
<td>17.3±2.0</td>
</tr>
</tbody>
</table>

Depending on succinic fermentation conditions, including type of CO\textsubscript{2} source and its dosage, other metabolites, such as acetic, formic, lactic acid or ethanol, can be produced in different amounts [19]. In the present study, acetic- and formic acid were produced as the main fermentation by-products. In the initial growth phase, succinic, acetic and formic acids were simultaneously produced. It was noticed that during succinic fermentation with biogas as the only CO\textsubscript{2} source; by-products (acetic and formic acids) content significantly exceeded 30% of total fermentation products (succinic, acetic and formic acid) (Table I).

As compared to the succinic acid, which is formed via C\textsubscript{4} pathway, i.e. carboxylation of phosphoenolpyruvate and NADH (nicotinamide adenine dinucleotide) consumption within the reductive branch of TCA cycle, - acetic and formic acids are formed via C\textsubscript{1} pathway [20]. By-products concentration (acetic and formic acids) during fermentation with MgCO\textsubscript{3} or MgCO\textsubscript{3}+biogas was stable and did not exceed 30% of total fermentation products (succinic, acetic and formic acid, Table I). This showed that applied CO\textsubscript{2} concentration was enough to increase C\textsubscript{1} flux towards higher succinic acid production.

B. Succinic Fermentation Using Different Nitrogen Sources

Fermentation with yeast extract as a nitrogen source started without any lag phase and higher values of sugar utilization and succinic titers were obtained after application of higher yeast extract dosage (i.e. 10 g/dm\textsuperscript{3}) compared to dosage of 5 g/dm\textsuperscript{3} (Table III). This shows that yeast extract is a favorable component of medium, used for bio-succinic synthesis. However, yeast extract is considered as one of the most expensive medium component (200 USD/kg of extract suitable for microbial growth) [21]. Based on the results obtained, it was concluded that fermentation assays containing mixtures of yeast extract/rapeseed cakes, in which at least 50 % of yeast extract was present, also started without any lag phase. In such cases, sugar utilization and succinic yields amounted to 84-87% and 67-68% (Table III) (data statistically equivalent, p > 0.05). This is in accordance with previous studies stating that rapeseed meal (e.g. pretreated by dilute acid method and hydrolysed with pectinase) can be used as carbon and nitrogen source, for production of succinic acid by *A. succinogenes* [22]. Significantly higher residual sugar contents and lower succinic yields were obtained after application of more than 50% of rapeseed cakes as nitrogen dosage (Table III), which proves that it is difficult to completely replace yeast extract in the original fermentation medium, by rapeseed cakes as nitrogen sources.

Yeast extract, being rich in amino acids, vitamins and trace elements is considered as favorable nitrogen source in fermentation processes.

**TABLE III: INFLUENCE OF NITROGEN SOURCE ON SUCCINIC ACID PRODUCTION**

<table>
<thead>
<tr>
<th>YE:RK (% w/w)</th>
<th>Sugar util. (%)</th>
<th>Succinic acid (% yield)</th>
<th>Byproducts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100:0\textsuperscript{a}</td>
<td>81.7±3.0ab</td>
<td>14.6±1.0b</td>
<td>54.5±1.8b</td>
</tr>
<tr>
<td>100:0\textsuperscript{b}</td>
<td>89.3±1.5a</td>
<td>19.2±1.4a</td>
<td>65.2±3.2a</td>
</tr>
<tr>
<td>80:20\textsuperscript{b}</td>
<td>86.8±1.7a</td>
<td>19.3±0.6a</td>
<td>67.7±1.7a</td>
</tr>
<tr>
<td>60:40\textsuperscript{b}</td>
<td>86.8±1.8a</td>
<td>19.2±1.2a</td>
<td>67.1±3.4a</td>
</tr>
<tr>
<td>50:50\textsuperscript{b}</td>
<td>83.8±1.7a</td>
<td>18.8±1.0a</td>
<td>68.3±2.9a</td>
</tr>
<tr>
<td>40:60\textsuperscript{b}</td>
<td>74.6±3.5b</td>
<td>15.4±1.0b</td>
<td>61.9±3.2ab</td>
</tr>
</tbody>
</table>

(a - total nitrogen source of 5 g/dm\textsuperscript{3}, b - total nitrogen source of 10 g/dm\textsuperscript{3}, YE – yeast extract, RK - rapeseed cakes, average values n=3, ± standard deviation, the same letters represent data equivalent statistically p > 0.05)

Succinic fermentation with addition of more than 50% of rapeseed cakes resulted in increased production of acetic and formic acids (by-products of succinic fermentation) (Fig. 2). What is more, succinic production started with a 6 h lag phase after addition of 60% nitrogen dosage as rapeseed cakes (Fig. 2). During the first hours of fermentation, by-products (acetic and formic acids) significantly exceeded succinic production. Such disturbances in succinic fermentation were not
observed after addition of ≤ 50% nitrogen dosage as rapeseed cakes. After 18-24 h of the process, the production of acetic and formic acids ceased, which is connected with low cell-biomass generation during stationary phase and consequently lower energy requirements [3]. In this case, carbon flux shifts towards succinic acid production (Fig. 2). It should be mentioned that increased by-products concentration in fermentation broths influences negatively the final product (succinic acid) separation. It is estimated that downstream processing of succinic acid can make more than 50-60% of total costs and is attributed to recovery and refining [6]. Acetic and formic acids can be effectively removed via vacuum distillation as their boiling points are significantly lower than boiling point of succinic acids [23].

Based on the results presented, it can be stated that rapeseed cakes can be used as component of fermentation medium and partially (≤ 50%) replace yeast extract. It is in accordance with other reports stating that alternative nitrogen sources are not able to completely eliminate yeast extract during succinic fermentation, e.g. by addition of corn steep liquor (CLS). In this study, it was found that this substrate could not completely replace enriched yeast extract without adding additional ingredients in order to meet physiological demands of A. succinogenes [24].

IV. CONCLUSION

The results obtained in this study clearly confirmed that hydrolysates obtained from Miscanthus×giganteus after glycerol pretreatment can be considered as a promising feedstock for succinic acid production. The study focused on two the most important process parameters, which influence succinic yields and sugar conversion rates: carbon dioxide availability and nitrogen source.

1) These results proved the positive impact of simultaneous addition of gaseous CO₂ (biogas) and solid MgCO₃ on the course of succinic fermentation and succinic yield.

2) Costly yeast extract used during succinic fermentation can be successfully replaced (≤ 50% of its dosage) by rapeseed cakes, i.e. a residual material from pressing of oil from rapeseed.

However, the complex composition of oil cakes requires further research to confirm which component (of organic nitrogen) has the most significant impact on the effectiveness of succinic acid and other metabolites production. Further research should also include an effective concept of succinic broths purification and downstream processes, which would allow to obtain succinic acid (> 98%) suitable for further chemical transformations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

M. Kuglarz conducted the research on succinic fermentation using different carbon and nitrogen sources as well as analyzed hydrolysates and final products in succinic broths, using HPLC method. M. Kuglarz also prepared the manuscript for publication; M. Rom prepared hydrolysates for succinic fermentation, participated in hydrolysates analyses (carbohydrate, final products) and developed statistical evaluation of obtained results. All authors had approved the final version.

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