RECOMBINANT STRAIN OF YARROWIA LIPOLYTICA IN SIMULTANEOUS BIOSYNTHESIS OF CITRATE AND INVERTASE FROM SUCROSE

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Abstract. The invertase positive Yarrowia lipolytica A-101-B56-5 strain was analyzed for simultaneous production of citric acid (CA) and invertase in four media of different composition. The analysis was carried out as bioreactor batch cultures with sucrose as carbon source. The highest CA concentration (157 g/L) was noted in medium A (250 g sucrose, 7.50 g NH₄Cl, 0.10 g KH₂PO₄, 5.00 g MgSO₄×7H₂O and 3.0 g yeast extract in 1 L). The yield of CA from consumed substrate reached 0.698 g/g, the volumetric productivity was 0.785 g/L/h and the specific production rate reached 0.018 g/g/h. In terms of invertase biosynthesis, the best medium was the rich medium D (150 g sucrose, 3.0 g yeast extract, 5.0 g malt extract and 5.0 g peptone in 1 L). The total invertase activity obtained in this medium reached 124 000 U/L. The final CA concentration in this medium was lower than in medium A, however, it presented the highest volumetric productivity (1.281 g/L/h), specific production rate (0.065 g/g/h) as well as the highest yield from biomass (4.704 g/g). This culture was also the shortest (72 h) among all tested processes. Surprisingly, the highest amount of secreted invertase (48.7%), calculated from the total invertase activity, was obtained for the process in medium A, and was six times higher than in the other media. This phenomenon was dependent on sucrose concentration, the accumulated biomass and the osmotic pressure of the medium.

Key words: invertase, citric acid, sucrose, Yarrowia lipolytica
INTRODUCTION

Invertase [EC.3.2.1.26] belongs to glycoside hydrolases that catalyze the breakdown of sucrose (table sugar) into glucose and fructose. Invertase is very important enzyme and is commonly used in preparation of creams, marzipan, candies containing liquefied sugar centers, chocolate covered cherries and pralines, artificial honey as well as in cosmetics as plasticizing agent [Nadeem et al. 2015]. It is also used to prevent the accumulation of sugar crystal, causing an unappreciable for consumer aspect of the final products. Nowadays the enzyme preparations present on the market are mainly isolated from Saccharomyces cerevisiae yeast [www.novozymes.com, www.ncbe.reading.ac.ok, www.dsm-foodspecialities.com] and some species of Aspergillus genus [Nadeem et al. 2015]. In the yeast S. cerevisiae invertase is encoded by several SUC genes and allows the yeast to use sucrose as carbon source [Naumov and Naumova 2010]. Among these genes, the gene SUC2 is the most important in invertase biosynthesis and secretion.

Wild strains of another yeasts species, Yarrowia lipolytica, are not able to assimilate sucrose as carbon source. To overcome this inability some new strains were constructed by genetic engineering. The SUC2 gene from S. cerevisiae was introduced into Y. lipolytica genome allowing to obtain invertase positive strains [Nicaud et al. 1989, Förster et al. 2007, Walczak et al. 2009, Lazar et al. 2013]. Some of these strains, derived from Polish strain Y. lipolytica A-101, were tested as potential producers of citrate and invertase from glucose, fructose, sucrose and glycerol [Lazar et al. 2011]. The strain Y. lipolytica A-101-B56-5 appeared to be a good producer of these two compounds from all tested substrates. A simultaneous production of CA and invertase appeared to be possible and economically justified. In one process two valuable products were secreted into the medium and if the residual yeasts biomass could be used as feed supplement, the proposed practice will be an environmentally friendly technology [Robak et al. 2013]. However an optimized medium composition for this process was still envisaged and tested [Żubrowski et al. 2013].

According to the preliminary study performed on 50 sucrose based media (designed in Design Expert 8 software and using surface response methodology) in microculture device, Bioscreen C, three media were selected for further bioreactor study [Żubrowski et al. 2013]. Additionally, a rich medium not tested before was analyzed. The aim of the current study was to validate the suitability of the chosen media for simultaneous CA and invertase biosynthesis by Y. lipolytica A-101-B56-5 strain in bioreactor processes.

MATERIAL & METHODS

Microorganism

The strain Y. lipolytica A-101-B56-5 belongs to the yeast collection of the Department of Biotechnology and Food Microbiology, Wroclaw University of Environmental and Life Sciences. The strain was obtained by transformation of the wild type A-101 strain with the expression cassette originating from pINA302 vector containing S. cerevisiae SUC2 gene under the control of inducible promoter of XPR2 gene [Walczak and Robak 2009]. The strain was stored on YPD agar plate at 4°C or for long storage in 25% glycerol solution at -80°C.
Media and cultures

Batch cultures, were carried out in 5 L stirred-tank reactors BIO-STAT B-PLUS (Sartorius, Frankfurt, Germany) with a 2 L of medium, at 30°C, 800 rpm and aeration rate 0.36 vvm. Four different media (A, B, C and D) which composition are detailed in Table 1 were tested. Cultures acidity was automatically controlled at pH 6.8 with 40% (w/v) NaOH. Cultures were conducted until the substrate got exhausted and lasted: 72 h in D medium, 120 h in the media B and C, and 200 h in A medium. Inocula consisted of 10% of total working volume and were prepared in 0.05 L of the same medium in 0.25 L flasks and cultivated at 28°C, during 48 h with 240 rpm on a rotary shaker (Elpan, Poznań, Poland).

Table 1. Media composition

<table>
<thead>
<tr>
<th>Medium</th>
<th>Sucrose [g/L]</th>
<th>NH₄Cl [g/L]</th>
<th>KH₂PO₄ [g/L]</th>
<th>MgSO₄.xH₂O [g/L]</th>
<th>YE [g/L]</th>
<th>Pepton [g/L]</th>
<th>ME [g/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>250.00</td>
<td>7.50</td>
<td>0.10</td>
<td>5.00</td>
<td>3.00</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>100.00</td>
<td>5.00</td>
<td>5.00</td>
<td>0.30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>100.00</td>
<td>5.00</td>
<td>0.10</td>
<td>3.00</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>150.00</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>3.00</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Yeast extract (YE), malt extract (ME) and peptone were obtained from Difco (Becton Dickinson Polska Sp. z o.o.). Sucrose and all chemicals were of analytical grade and were obtained from Sigma-Aldrich.

For the analysis, 25 mL samples were taken from the bioreactors and centrifuged 10 min at 5000 rpm using 3-16K centrifuge (Sigma, St. Louis, MO, USA). Supernatants and cells sediment were collected for CA, sugars and invertase activity measurements. The first samples (time 0) were taken 10 min after inoculation and regular samples were taken as indicated on the figures. Biomass determination and intracellular invertase extraction was proceeded according to method described previously [Lazar et al. 2011].

Sugar and acid measurements

In all samples CA, glucose, fructose and sucrose were determined by HPLC (UltiMate® 3000, Dionex – Thermo Fisher Scientific, UK) using Aminex HPX87H column coupled to a UV (210 nm) and RI detectors. The column was eluted with 0.01 N H₂SO₄ at room temperature and flow rate of 0.6 mL/min [Lazar et al. 2011]. Before the HPLC analysis samples were filtered on 0.45 μm pore-size membranes.

Invertase activity

Extra and intracellular invertase activity was measured in post-culture media and cell’s extracts (supernatants) as described by Lazar et al. [2011]. Briefly, enzymatic reaction was started by addition of 0.2 mL of the substrate (0.1 M sucrose in H₂O) to the mixture containing 0.1 mL of enzyme (diluted when needed), 0.1 mL of 0.1 M acetate buffer (pH 5.0) and 0.1 mL of H₂O. Reaction mixtures were incubated at 37°C for 10 minutes followed by inactivation of enzyme by the addition of 1.5 mL of DNS reagent. Afterwards samples were boiled (100°C) for 5 minutes, cooled down to room temperature and filled with H₂O to the final volume of 10 mL. Sample absorbance was measured at λ = 530 nm.
(Spectrophotometer, Marcel Media). One unit of activity (U) was defined as the amount of enzyme releasing 1 μmol of reducing sugars per minute in assay’s conditions.

RESULTS

The ability of \textit{Y. lipolytica} A-101-B56-5 strain to synthesize CA and invertase from sucrose in bioreactor bath cultures was tested in four media (A, B, C and D) which differed in carbon and nitrogen source concentration. Cultures were monitored for sugars concentration (sucrose, glucose and fructose) and lasted until the available carbon source got exhausted, except for medium B, where the available fructose was not consumed at all.

Sugar consumption and biomass accumulation

In the first medium (A) initial concentration of sucrose was 250 g/L. This disaccharide was hydrolyzed by invertase with the rate of 6.94 g/L/h and was very quickly (in 36 h) completely broken down into glucose and fructose (Fig. 1A). Despite the consumption of the available monosaccharides by yeast cells, their concentration was increasing during the first 30–38 h, reaching the level of 68 and 125 g/L for glucose and fructose, respectively. During 78 h yeasts metabolized only glucose. Fructose was not consumed, therefore its concentration was higher than those of glucose. The cells began to use fructose when glucose concentration dropped below 12 g/L. The observed final biomass concentration in this medium was 44 g/L.

In the second medium (B), the initial sucrose concentration was lower, 100 g/L and the hydrolysis rate was nearly half of that observed in medium A (3.57 g/L/h). However, due to lower amount of sucrose than in culture A, its complete hydrolysis was achieved in 28 h. During the first 30 h of the process concentration of glucose and fructose was increasing, reaching 30 and 50 g/L, respectively (Fig. 1B). In this culture yeast did not consume fructose at all. It is probably due to low nitrogen amount available for biomass formation, what resulted in slower sugar uptake. The concentration of glucose at 108 h was still above 12 g/L. A very slow but continuous biomass growth was observed until the end of the process, reaching 12 g/L of dry biomass.

In the third medium (C) the initial concentration of substrate was identical as in the medium B (100 g/L). Sucrose was hydrolyzed with a rate of 4.17 g/L/h and in 24 h all available substrate was completely hydrolyzed. Similar to the previous cultures, glucose was the preferred sugar and after 24 h the concentration of glucose and fructose reached 16 and 48 g/L, respectively (Fig. 1C). Glucose exhaustion was observed at 48 h of the process and at that time yeast began to metabolize fructose. This monosaccharide was completely consumed at 120 h. The growth of biomass was observed until 52 h of the culture and its final concentration reached 20.5 g/L.

In the fourth medium (D), the initial concentration of carbon source was 150 g/L. The rate of sucrose hydrolysis was very fast (12.5 g/L/h) and it was completed after 12 h (Fig. 1D). During the first 12 h yeast cells metabolized both monosaccharides at the same time and concentration was similar, 58 and 60 g/L for glucose and fructose, respectively. Between 12 and 36 h of the process only glucose was consumed. After complete glucose exhaustion (36 h), fructose began to be rapidly consumed, and at the end of the process its concentration was only 10 g/L (72 h). The biomass was growing until 40 h and its final concentration reached 19.6 g/L.
Recombinant strain of Yarrowia lipolytica...

Fig. 1. Biomass and substrates concentration during the culture of Y. lipolytica A 101 B56 5 in medium A, B, C and D (♦ sucrose, ■ glucose, ▲ fructose, × dry biomass)

Table 2. The rate of sugar utilization during the bioreactor cultures in sucrose based media

<table>
<thead>
<tr>
<th>Culture sucrose concentration</th>
<th>Time of culture [h]</th>
<th>Consumed saccharide</th>
<th>Consumption rate</th>
<th>Residual sugar (fructose) [g/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 250 g/L</td>
<td>0–36</td>
<td>sucrose</td>
<td>6.940</td>
<td>0.231</td>
</tr>
<tr>
<td></td>
<td>36–78</td>
<td>glucose</td>
<td>1.563</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>78–200</td>
<td>fructose</td>
<td>0.826</td>
<td>0.019</td>
</tr>
<tr>
<td>B 100 g/L</td>
<td>0–28</td>
<td>sucrose</td>
<td>3.570</td>
<td>0.238</td>
</tr>
<tr>
<td></td>
<td>28–120</td>
<td>glucose</td>
<td>0.275</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>28–120</td>
<td>fructose</td>
<td>Not consumed</td>
<td>Not consumed</td>
</tr>
<tr>
<td>C 100 g/L</td>
<td>0–24</td>
<td>sucrose</td>
<td>4.170</td>
<td>0.231</td>
</tr>
<tr>
<td></td>
<td>24–52</td>
<td>glucose</td>
<td>0.521</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>52–120</td>
<td>fructose</td>
<td>0.662</td>
<td>0.032</td>
</tr>
<tr>
<td>D 150 g/L</td>
<td>0–12</td>
<td>sucrose</td>
<td>12.500</td>
<td>1.563</td>
</tr>
<tr>
<td></td>
<td>12–35</td>
<td>glucose</td>
<td>2.273</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>35–72</td>
<td>fructose</td>
<td>1.111</td>
<td>0.057</td>
</tr>
</tbody>
</table>

[g/g/h]* calculated for the biomass present at the end of culture period
The rate of monosaccharides consumption by yeast varied from 0.275 to 2.273 g/L/h (Table 2). The slowest consumption rate was noted for glucose in medium B and the fastest in medium D, for the same sugar. The rate of sucrose hydrolysis ranged from 3.57 to 12.50 g/L/h. The specific rate of sucrose hydrolysis by *Y. lipolytica* cells was very similar in the media A, B and C (0.23–0.24 g/g/h) and was nearly seven times higher in medium D. It was most probably connected with the highest amount of extracellular invertase noted during the culture in the D medium.

**Citric acid biosynthesis**

Biosynthesis of CA was followed for 72 h in the D medium, 120 h in the media B and C and for 200 h in the A medium (Fig. 2). As mentioned before, the duration of the process was in function of substrate availability: in medium A, even at 200 h, fructose was still detected (~25 g/L); in medium C all sugars were consumed before 120 h of the process; and in the B medium the observed extremely slow glucose consumption let us decide to stop the experiment at the same time as in the medium C; whereas in the D medium the process was stopped when fructose concentration dropped below 10 g/L. In all media CA secretion began at about 24 h. The maximal concentration of CA was reached in the A medium (157 g/L). In the media B, C and D the accumulated level of CA was significantly lower (37.8, 66.7 and 92.2 g/L, respectively). According the rate of CA biosynthesis the best process took place in the D medium in which the volumetric productivity reached 1.281 g/L/h and the calculated specific productivity reached 0.065 g/g/h (Table 3). The yield of CA biosynthesis from the consumed substrate ($Y_{CA/S}$) was high and comparable in all four media (0.659–0.756 g/g).

![Citric acid biosynthesis by Y. lipolytica A-101-B56-5 in media: A (♦), B (■), C (▲) and D (×)](image)
Table 3. Parameters of CA biosynthesis by *Y. lipolytica* A-101-B56-5 from sucrose in media A, B, C and D

<table>
<thead>
<tr>
<th>Culture / duration / consumed sucrose</th>
<th>Parameters</th>
<th>X* [g/L]</th>
<th>CA* [g/L]</th>
<th>Y_{CA} [g/g]</th>
<th>Y_{CAX} [g/g]</th>
<th>Q_{CA} [g/L/h]</th>
<th>q_{CA} [g/g/h]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, 200 h, 225 [g/L]</td>
<td></td>
<td>44.0</td>
<td>157.0</td>
<td>0.698</td>
<td>3.568</td>
<td>0.785</td>
<td>0.018</td>
</tr>
<tr>
<td>B, 120 h, 50 [g/L]</td>
<td></td>
<td>12.1</td>
<td>37.8</td>
<td>0.756</td>
<td>3.124</td>
<td>0.315</td>
<td>0.026</td>
</tr>
<tr>
<td>C, 120 h, 100 [g/L]</td>
<td></td>
<td>20.5</td>
<td>66.7</td>
<td>0.667</td>
<td>3.254</td>
<td>0.556</td>
<td>0.027</td>
</tr>
<tr>
<td>D, 72 h, 140 [g/L]</td>
<td></td>
<td>19.6</td>
<td>92.2</td>
<td>0.659</td>
<td>4.704</td>
<td>1.281</td>
<td>0.065</td>
</tr>
</tbody>
</table>

*Mean value of 3 measurements

Abbreviations: X [g/L] – biomass concentration; CA [g/L] – citric acid concentration; Y_{CA} [g/g] – yield of CA from the consumed substrate; Y_{CAX} [g/g] – yield of CA from biomass; Q_{CA} [g/L/h] – CA productivity; q_{CA} [g/g/h] – CA specific production rate

**Invertase biosynthesis**

Secretion of invertase (extracellular invertase) by yeast began between fourth and sixth h in all analyzed media. The highest activity was detected in the D medium (~14 000 U/L) at the end of process (72 h). The lowest invertase activity was measured in the B medium (~ 1750 U/L). In media A and C secreted invertase was partially degraded during the culture. Higher enzymatic activity was noted between 70–95 h of the process (9500 U/L for A medium and 7000 U/L for C medium) than between 105–200 h (~4800 U/L for A medium and 6000 U/L for C medium).

The increase of intracellular invertase activity in yeast cells in all four media was noticed at the same time as the enzyme secretion (at about 4–6 h). Intracellular invertase activity was much higher than that measured for the extracellular enzyme. The maximal accumulation of enzyme was observed between 60–105 h of the culture in the media B, C and D. In the A medium the increase of intracellular invertase activity took place at 60 h and remained stable for the following 60 h (~10 000 U/L). The highest activity was detected in the D medium (110 000 U/L), however, quite high activity was measured also in the media C (35 000 U/L at 83 h) and B (25 000 U/L at 80 h).

The measured invertase activity differed significantly among all analyzed media and type of enzyme. It ranged from 1750 U/L to 110 000 U/L for extracellular invertase secreted in medium B and intracellular enzyme in medium D, respectively. The distribution of extra (EX) and intracellular (IN) invertase, calculated as % of total invertase activity, varied from 1:14.4 (6.5 and 93.5%) to 1:1.05 (48.7 and 51.3%) in the media B and A, respectively (Table 4). For EX invertase this value ranged from 6.5% in medium B to 48.7% in medium A and for IN invertase from 51.3% in medium A to 93.5% in medium B. The highest ratio of secreted to intracellular invertase (~50%) obtained in medium A, could be a result of high osmotic pressure of this medium. The value of this parameter in media B, C and D was similar (440–500 mOsmol/kg), however in medium A it reached more than 6 times higher value (3160 mOsmol/kg).

The best yield of invertase biosynthesis from sucrose (Y_{IS}) was measured for IN enzyme from yeast growing in the D medium (786 U/g of sucrose), whereas the same pa-
rameter for the EX enzyme was 4 times lower (100 U/g of sugar) (Table 4). The yield of invertase synthesized by 1 g of biomass (Y_{I/X}) ranged from 145 U/g (EX invertase in medium B) to 5612 U/g (IN invertase in medium D). Comparing all parameters of invertase biosynthesis in all four analyzed media, the best production of this enzyme was obtained in medium D, in which the specific rate of invertase biosynthesis (87.9 U/g) and the yield from substrate (886 U/g) as well as the total amount of enzyme (124 000 U/L) reached 3 to 6 times higher values than in the other media.

DISCUSSION

Medium composition is one of the key factors to design an efficient and cost-effective process. Our previous study focused on optimization of medium composition for simultaneous CA and invertase biosynthesis using *Y. lipolytica* A-101-B56-5 strain (having two copies of SUC2 gene). In a small scale processes, three different compositions of the production medium were chosen from 50 tested, as being promising for simultaneous biosynthesis of both metabolites [Zubrowski et al. 2013]. In the present study, these three media were analyzed in bioreactors bath cultures (A, B and C) under controlled process parameters. Additionally, previously not analyzed rich medium (D) was used in the current bioreactor experiment for comparison.

The highest CA concentration obtained in medium A (157 g/L) and D (92.2 g/L) as well as quite high yield from the consumed substrate in all tested media (0.659–0.756 g/g) are very promising results for establishing industrial scale processes. In the previous study with *Y. lipolytica* A-101-B56-5 strain and sucrose as carbon source lower CA concentration (57.15 g/L) with lower yield (0.60 g/g) were obtained [Lazar et al. 2011]. Förster et al. [2007] using German strain *Y. lipolytica* H222-S4 T5 (also having two copies of SUC2 gene) obtained higher concentration of CA (140 g/L) with a very high yield (0.820 g/g) using sucrose as a substrate. However, Moeller et al. [2013] with the same German strain, produced lower amount of CA (80 g/L), with slightly lower yield (0.57 g/g) but the volumetric productivity reached quite high rate (1.1 g/L/h). In this study higher rate of volumetric productivity was obtained in medium D (1.281 g/L/h). In one of the first study on CA biosynthesis by SUC+ strains of *Y. lipolytica* using molasses [Żarowska et al. 2001], the French *Y. lipolytica* W29 ura3-302 strain secreted lower amount of CA (50.2 g/L) with lower rate of volumetric productivity (0.37g/L/h) but the yield (0.61g/g) was similar to the one obtained in the present study. Considering the specific production rate of CA biosynthesis by SUC2 transformants, the highest value was obtained in the current study in the medium D (0.065 g/g/h). Additionally, in the same medium the highest enzyme activity was also measured (124 000 U/L). Taking into account all obtained results, medium D could be considered as a reference medium for CA and invertase biosynthesis. Considering the components of the medium it could be noted that the addition of peptone, malt and yeast extracts, as an additional source of nitrogen and vitamins resulted in higher biomass production combined with high invertase biosynthesis and quite good CA secretion. High invertase activity allows for high rate of sucrose hydrolysis. The highest value of this parameter was observed in medium D (12.5 g/L/h) and was much higher than observed by Lazar et al. [2011] and Moeller et al. [2012, 2013].
In the previous study on invertase biosynthesis by *Y. lipolytica* A-101-B56-5 the maximal invertase activity reached 3 253 U/L [Lazar et al. 2011]. In that study the maximum of activity was 23 times higher, however, it was still lower than the one obtained for *S. cerevisiae* GCA-II, 107 400 U/L [Ul-Haq and Ali 2005, 2007] and for *Aspergillus* spp. M1, 182 250 U/L [Patil et al. 2012]. For *Aspergillus* spp. M1 invertase biosynthesis, the addition of 1% of sodium nitrate increased more than twice the enzyme activity (233 270 U/L). In the superficial culture of immobilized cells of *Aspergillus japonicus*, the activity of invertase reached 42 860 U/L [Mussatto et al. 2009]. The bioreactor culture of *Y. lipolytica* A-101-B56-5 combined with membrane filtration, improved the final preparation of invertase, and the activity reached 1 849 210 U/L [Lazar et al. 2011]. However, this value is still much lower than the commercial enzyme available on the market. The enzyme activity of MAXINVERT liquid has 10 000 SU/mL which corresponds to 20 000 000 U/L (1SU = 2U). Notwithstanding, application of some downstream processes could significantly improve the final concentration of the enzyme and will be a subject of future studies.

**CONCLUSIONS**

The biosynthesis of invertase and CA depends highly on the medium composition. The best medium turned out to be composed of 150 g/L of sucrose, YE (3.0 g/L), ME (5 g/L) and peptone (5 g/L). The total invertase activity in this medium reached 124 000 U/L, whereas the obtained CA concentration was 92 g/L. This process reached the highest volumetric productivity, the specific production rate as well as the yield of CA from biomass. It was also significantly shorter than the other processes as it lasted only 72 h. However, the highest final concentration of CA was noted in medium A composed of 250 g/L of substrate, NH₄Cl (7.50 g/L), KH₂PO₄ (0.10 g/L), MgSO₄ ×7H₂O (5.00 g/L) and YE (3.0 g/L). This process, in turn, reached the lowest total activity of invertase obtained among all of the analyzed media. Surprisingly, in medium A the highest amount of secreted invertase (48.7%), compared to the total invertase activity, was observed. This phenomenon was most probably connected to the highest osmotic pressure in this medium. Taking into consideration all important criteria for simultaneous CA and invertase production, the rich medium (D) turned out to be a good compromise.

**REFERENCES**


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**REKOMBINOWANE SZCZEPY *YARROWIA LIPOLYTICA***

**W JEDNOCZESNEJ BIOSYNTEZIE CYTRYNIANU I INWERTAZY Z SACHAROZĄ**

**Streszczenie.** Inwertazododatni szczep *Yarrowia lipolytica* A-101-B56-5 analizowany był w kierunku jednoczesnej produkcji kwasu cytrynowego (CA) i inwertazy w czterech podłożach o różnym składzie. Analizę prowadzono metodą hodowli okresowych w bioreaktorach z wykorzystaniem sacharozę jako substratu. Najwyższe stężenie CA (157 g/l) uzyskano w podłożu A (250 g sacharosa, 7,50 g NH₄Cl, 0,10 g KH₂PO₄, 5,00 g MgSO₄×7H₂O i 3,0 g YE w 1 l). Wydajność tego metabolitu z wykorzystanego cukru wynosiła 0,698 g/g, objętościowa szybkość produkcji 0,785 g/l/h, a specyficzna szybkość produkcji 0,018 g/g/h. Z punktu widzenia produkcji inwertazy najlepszym podłoże okazało się bogate podłoże D (150 g sacharosa, 3,0 g YE, 5,0 g ME i 5,0 g pepton w 1 l). Całkowita aktywność inwertazy uzyskana w tym podłożu wynosiła 124 000 U/l. Koncowe stężenie CA w podłożu D było niższe niż w podłożu A, jednak proces ten charakteryzował się najwyższą objętościową szybkością produkcji tego metabolitu (1,281 g/l/h), najwyższą wartością szybkością produkcji (0,065 g/g/h) jak i najwyższą wydajnością z biomasy (4,704 g/g). Proces ten był również najkrótszy (72 godz.) spośród wszystkich przeprowadzonych procesów. Co zaską-
kujące, najwyższą zawartość inwertazy pozakomórkowej (48,7%) w porównaniu z całkowitą aktywnością inwertazy uzyskano w podłożu A. Wartość ta była sześciokrotnie wyższa niż w przypadku pozostałych podłoży. Zjawisko to zależe¬ne jest najprawdopodobniej od wyjściowego stężenia sacharozy, stężenia wyprodukowanej biomasy oraz ciśnienia osmo¬tycznego podłoża. Biorąc pod uwagę wszystkie kryteria dotyczące jednoczesnej produkcji CA i inwertazy, bogate podłoże D wydaje się być odpowiednim kompromisem.

Słowa kluczowe: inwertaza, kwas cytrynowy, sacharoza, Yarrowia lipolytica

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