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FED-BATCH SIMULTANEOUS SACCHARIFICATION AND ETHANOL FERMENTATION OF NATIVE CORN STARCH

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Background. The most important innovations in boethanol production in the last decade were: simultaneous saccharification and fermentation processes (SSF), high gravity fermentation, the use of new enzyme preparation able to hydrolyse native granular starch and construction of genetically modified strains of microorganisms able to carry out simultaneous production of hydrolytic enzymes and fermentation of C6 and C5 sugars. The aim of this study was to assess the efficiency of ethanol fermentation using new type of amylolytic enzymes able to hydrolyse native corn starch in a SSF process.
Material and methods. The simultaneous saccharification and fermentation of raw corn flour by fed-batch processes using *Saccharomyces cerevisiae* strain Red Star Ethanol Red and Stargen 001 enzyme preparation was performed. As experimental variable were investigated: fermentation temperature (35-37-40°C), rate of mash stirring (100 and 200 rpm), fermentation time (0-92 h) and dosage of corn flour (different portion and different time).
Results. It was found that optimal temperature for fed-batch SSF process was 37°C at initial pH of 5.0. However, the yeast intensively fermented the saccharides also at 40°C. The fermentation stirring rate has significant effect on starch utilization and fermentation production. The prolongation of fermentation time over 72 h has no substantiation in additional ethanol production. In all experimental fermentations the level of produced organic acids was very low, significantly below toxic concentration for the yeast.
Conclusions. It was stated that the use of new method of starch raw material preparation resulted in satisfied fermentation yield and allowed to reduce energy requirements for starch liquefaction.

Key words: ethanol, fed-batch fermentation, SSF, *Saccharomyces cerevisiae*, Stargen 001

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INTRODUCTION

Biomass has been recognised as a major renewable energy source to substitute partially fossil fuel sources [Demirbas 2007]. The use of plant material for fuel production has many advantages. First, it allows to reduce carbon dioxide emission by sequestration of carbon into growing plants used as feedstock for biofuel production [Kheshgi and Prince 2005]. Second, it appears to have a significant positive effect on rural region development and contributes to sustainability of modern agriculture. Third, the production of biomass can be developed in the future, creating new agricultural business. From economical point of view, a saccharide plays crucial role, especially sugar cane, starch and lignocelluloses, and plant oils, mainly rapeseed, canola, soya and palm oils.

Up-today, the main raw material for liquid fuel production is starch. It is commonly used in many countries on industrial scale and for long time it will still be the most important feedstock for this application. The development of second generation biofuels produced from cellulose is foreseen in the next decade. However, the production of bioethanol from starchy materials will still be continued.

The technology of starchy materials processing for ethanol production is well--known, but all the time intensive investigations are carried out in order to improve the technology and economy of the production. In the last decade several novel amylolytic preparations were introduced into the market. There is also a progress in fermentation technology observed. One of the most important innovations was a simultaneous saccharification and fermentation (SSF) what allows to reduce investment costs, simplify technological operation, shorten fermentation process and increase mash gravity [Roy et al. 2001, Kroumov et al. 2005, Suresh et al. 1999, Nikolic et al. 2009, Srichuwonga et al. 2009].

Many SSF processes are operated in a fed-batch mode to regulate substrate concentration in the bioreactor [Lee et al. 1999, Carvalho et al. 2003, Wang and Shyu 1997, Ozmihci and Kargi 2007]. This type of fermentation is especially useful when culture medium generates high osmotic pressure, substrate and product inhibition, catabolite repression, and auxotrophic mutation. For this reason fed-batch operation offers advantage over batch and continuous operations. Fed-batch culture can be performed by adding a concentrated substrate at either constant or variable rates what results in a large fluctuation in substrate concentration and, therefore, makes intervals in growth rate and microorganism metabolism. A dosage of main substrate in some portions allows to keep this parameter at limited values and reduce the inhibition of cell growth. For this reason the replacement of batch process by fed-batch process is suggested. However, fed-batch fermentation is rarely used for ethanol production [Lu et al. 2003, Ferrari et al. 1994, Ozmihci and Kargi 2007, Roukas 1996].

Roy et al. [2001] analysed the performance of simple batch saccharification of starch and simple batch lactic acid fermentation versus fed-batch simultaneous saccharification and fermentation and they derived the conclusion that performance of fed-batch process cannot be improved while the batch saccharification performed in optimal conditions shows a stoichiometric yield. The main advantage in fed-batch process is better productivity of final metabolite and extended productive phase of the batch process.

The further important improvement in the SSF process is applying new type of amylolytic enzymes able to hydrolyse granular non-cooking form of starch. In recent years there have been numerous research works in this field conducted [Sarikaya et al. 2000,

Goyal et al. 2005, Mitsuiki et al. 2005, Tester et al. 2006], but the most important fact was market introduction of amylolytic preparation Stargen®, developed by company Genecor [Genecor lauches... 2005, Enzyme firms... 2005, Shariffa et al. 2009, Kumar 2008]. It is advised for industrial applications in bioethanol production [Kumar 2008, Mitchinson 2008]. The preparation contains two amylolytic enzymes: an α-amylase from *Aspergillus kawachi* expressed in *Trichoderma reesei* and a glucoamylase from *Aspergillus niger*. These enzymes work synergistically below the gelatinization temperature. According to Shariffa et al. [2009] the glucoamylase of Stargen 001 origin is able to drill sharp and deep pinholes, whereas α-amylase widenes the pinholes. This synergistic action enhances the release of glucose molecules from granular starch. Williams [2006] reported that Stargen 001 preparation had been tested with several companies over the world. The preparation can replace traditional enzyme preparations hydrolysing high--temperature-cooking starch according commonly used technology. Additionally, this preparation is active at fermentation pH and temperature. The omission of energy consuming process of thermal liquefaction allows to reduce operational costs. Generally, the action of amylolytic enzymes on granular starch is not efficient enough and results in slow and only partial macromolecule hydrolysis and very poor product gain.

In the literature, there are also reports on direct ethanol production in a single step using low-temperature-cooked corn starch instead of soluble starch using genetically modified strains displaying ether glucoamylase or α-amylase or the both enzymes simultaneously [Chen et al. 2008, Altintas et al. 2002, Oh et al. 1998, Ülgen et al. 2002]. Majority of genetic modifications concern introduction of genes coding fungal or bacterial amylases and glucoamylases into the genomes of ethanologenic microorganisms. This research is strongly progressing and every year there are new data published. However, there are only few pilot plant or industrial applications. It is clear that further development will be focused on the use of recombinant strains able to produce amylolytic enzymes to hydrolyse granular starch and ferment C6 and C5 sugars in the SSF processes.

The use of "cold" raw material processing can cause microbiological problems with bacterial contamination. To avoid this danger, the ethanol production facilities use antibiotic and aseptic substances, but it is unacceptable in many countries. For this reason new methods of grain decontamination are demanded.

This work was undertaken in order to investigate a zero-discharge system for long--term production of ethanol by an improved technology including the simultaneous saccharification and fermentation process using an enzyme preparation hydrolysing native granular corn starch and stillage recycling. In this study, the kinetics and yields of ethanol production, yeast cell growth characteristics, impurities accumulation and long-term process stability were taken into consideration. The process was carried out in laboratory bioreactor scale.

MATERIAL AND METHODS

Microorganism

An industrial strain Ethanol Red of the yeast *Saccharomyces cerevisiae*, in a dried form, obtained from Lesaffre Company (France) was used for the fermentation. The yeasts were stored at 4°C.

Starch material

Raw corn flour obtained from the BioCorn (Zębice, Poland) was used in the experiments. The flour had granulation below 250 μm and it contained 84.2% of starch calculated per dry matter.

Enzymes

The hydrolysis of native starch was carried out using Stargen 001™ preparation, produced by Genecor International, B.V. (Genencor International, Palo Alto, CA, USA). The preparation has pH optimum at 4.0-4.5 and recommended temperature of 20-40°C [Shariffa et al. 2009]. Additionally, a fungal acid protease GC 106 from *Aspergillus niger* produced by the same company was also employed.

Simultaneous saccharification and fermentation

The SSF medium consisted of aqueous suspension of raw corn flour. The substrate was added in two portions: the first at the fermentation start (time zero), and second after 24 h of fermentation. In variant no 3 corn flour was added in three portions: 1/2 at the fermentation start, 1/4 after 24 h, and 1/4 after 48 h of fermentation. All experiments were conducted in a stirred 5 l bioreactor BioFlo III (New Brunswick, USA) with working volume of 4 l. The conditions of the SSF process are described in Table 1. The pH values were adjusted with 10% H_2SO_4 and 20% NaOH. The liquefaction and saccharification of starch was carried out by adding amylolytic preparation Stargen 001 and protease GC106 in amount 2.0 g/kg and 30 μl/kg, respectively. The slurry was inoculated with 0.5 g/l dried yeast Ethanol Red. In experimental variant no 8 no yeast was added and fermentation was performed using autochthonous microflora of corn. The repeated batch SSF fermentations were performed in anaerobic non-sterile conditions. During the fermentation the samples from the bioreactor were collected for chemical and microbiological analysis. All experiments were performed in triplicate.

Table 1. Conditions of simultaneous saccharification and fermentation fed-batch processes

Variant	First dosage	Second dosage	Temperature °C	Yeast g/l	Stirring rpm	Time h
A	2/3	1/3	35	0.5	200	96
B	1/2	1/4+1/4*	35	0.5	200	72
C	2/3	1/3	37	0.5	200	72
D	2/3	1/3	37	0.5	100	72
E	2/3	1/3	40	0.5	200	72

*1/4 after 24 h, and 1/4 after 48 h.

Chemical analysis

Samples withdrawn from either fermentation or SSF medium were centrifuged at 10 000 rpm for 15 min and the supernatants were used for chemical analyses. They were

performed by using an high-performed liquid chromatograph MERCK HITACHI (auto-sampler Merck Hitachi L-7250, pump Merck Hitachi L-7100) with refractive index detection (Merck Hitachi L-7490). The samples from the fermentations were analysed with respect to glucose, disaccharides, ethanol, glycerol, acetic and lactic acid on an Aminex HPX87H column (300 × 7.8 mm; Bio-Rad). As an eluent 0.005 M sulphuric acid at a flow rate 0.6 ml/min was used. The analyses were performed at 30°C. The samples were 10-fold diluted, filtered through 0.22 μm filter (Millex-GS Millipore) and placed on the column in amount of 30 μl.

The concentration of reducing sugars was determined by the 3.5 dinitrosalicylic acid method according to Miller [1959]. The optical density was analysed at 530 nm using spectrophotometer Analityk Jena Specord 50 (Germany).

The starch amount was estimated by an enzymatic methods according to Holm et al. [1986]. The analyses based on the enzymatic hydrolysis of starch to reducing sugars assayed by DNS method. For this purpose two preparation was used: liquefied α-amylase Spezyme Ethyl produced by a recombinant strain *Geobacillus stearothermophilus*, and saccharified glucoamylase Fermenzyme L-400® originated from *Aspergillus niger*, both offered by Genencor International. The dry matter of maize flour and the fermentation slurry were determined by drying at 105˚C for constant weights.

Yeast cell density and viability

The cell densities were analysed microscopically at 40x magnitude using a haemocytometer. The cell viability was determined by staining with 0.01% methylene blue.

RESULTS

In the present work the following experimental variables were taken into account: the fermentation time, temperature and pH of the mash, stirring rate of the mash, and the moment and amount of substrate dosage. It was set up on the base of the literature, that all the mentioned parameters should influence the course of simultaneous saccharification and fermentation of corn starch. The results of these experiments are presented in Table 2 which includes five fermentation variations, each performed in triplicate.

According to the Genecor's information, the enzymatic preparation Stargen 001, which was used in this work, shows liquefying activity of α-amylase and saccharyfying activity of glucoamylase. Our previous studies on optimization of the SSF batch process with the use of this enzyme preparation showed that the most effective conditions for simultaneous saccharification and fermentation are pH 5.0, Osage of 2 g enzyme/kg of starch and an initial mash density of 25% w/v (paper in review).

Taking into consideration the fermentation rate, a crucial parameter for ethanol production is mash temperature. This parameter affected both enzyme activity and yeast growth. All offered commercial enzyme preparation demonstrated their amylolytic activity at temperature over 50°C. However, many of these are able to initiate hydrolysis of starch at temperature below 30°C. It concerns also Stargen preparation which hydrolyses the starch till temperature reaches 20°C [Shariffa et al. 2009]. Meantime, all applied industrial strains of *Saccharomyces cerevisiae* demonstrated their temperature optima for the growth at 30-35°C. In the conditions of simultaneous saccharification and fermentation, when temperature should be fitted for yeast growth and metabolism,

Table 2. Results of fed-batch fermentation performed at different SSF conditions

Variant	Time h	pH	Dis g/l	Glu g/l	Fru g/l	LA g/l	AA g/l	Glic g/l	EtOH g/l	Starch g/l	Viab %	Cells 10^8 CFU ml^{-1}
A	Conditions: time 96 h, pH 5.0, 200 rpm, 35°C, starch dosage 2/3 + 1/3											
	0	5.0	0.2	16.9	1.6	0.4	0.o6	0.7	0	143	100	0.26
	24	3.16	0.8	37.6	0.4	0.7	0.04	5.3	3.8	80	100	2.02
	24	3.85	0.6	39.5	0.6	0.6	0.05	4.5	3.07	140	100	2.05
	48	3.46	1.3	32.6	0.5	1.6	0.05	7.9	7.4	48	100	2.26
	72	3.52	1.3	14.3	0.3	2.2	0.09	9.9	9.8	17	99	2.20
	96	3.49	1.2	15.1	0.03	2.6	0.1	10. 6	10.4	20	99	2.21
B	Conditions: time 96 h, pH 5.0, 200 rpm, 35°C, starch dosage: 1.2 + 1/4 + 1/4											
	0	3.64	0	18,9	0.6	0,6	0.05	0.7	0	149	100	0.23
	24	3.83	1.3	49,5	0.3	0,5	0.1	5.8	4.3	82	100	2.23
	24	3.63	0.9	44,5	0.5	0,5	0.08	4.7	3.4	107	100	2.21
	48	3.79	1.4	32,8	0.4	0,7	0.1	8.2	6.3	40	100	2.34
	48	3.81	1.3	35,5	0.7	0,6	0.1	7.8	6.4	80	100	2.33
	72	3.94	1.3	36	0.5	0,8	0.2	9.6	8.13	55	98	2.31
	96	3.95	1.7	33,9	0.2	1	0.3	10	8.7	43	98	2.33
C	Conditions: time 72 h, pH 5.0, 200 rpm, 37°C, starch dosage: 2/3 + 1/3											
	0	5.0	0.3	11.3	0.5	0	0.03	0,5	0	156	100	0.23
	24	2.92	1.3	14.1	0.2	0.9	0.05	6	5.13	47	100	2.23
	24	3.48	1	14.5	0.4	0.8	0.06	5.8	4.34	121	100	2.21
	48	3.16	2.2	18.6	0.3	1.2	0.1	9.3	8.52	38	99	2.34
	72	3.17	2.3	5.2	0.3	1.4	0.1	11.2	10.5	10	99	2.33
D	Conditions: time 72 h, pH 5.0, 100 rpm, 37°C, starch dosage 2/3 +1/3											
	0	5.0	0.3	10.7	0.5	0.4	0.05	0.6	0	151	99	0.63
	24	3.65	0.8	44.7	0.3	6.3	1.6	3.8	2.4	92	98	2.01
	24	3.79	0.9	45	0.6	5.8	1.4	3.5	2.05	158	99	2.19
	48	3.63	1.4	92.2	0.6	11	3	2.9	2.44	155	97	2.24
	72	3.64	1.9	110	0.5	12.4	4	2.2	2.46	154	99	2.22
E	Conditions: time 72 h, pH 5.0, 200 rpm, 40°C, starch dosage 2/3 + 1/3											
	0	5.0	0.4	11.2	0.47	0	0	0.92	0	152	98	0.81
	24	3.75	1.2	28.7	0.39	1.16	0.08	5.61	47.4	76	85	1.87
	24	3.80	1.8	29.8	0.58	1.15	0.08	6.11	46.8	128	85	1.87
	48	3.10	1.9	21.7	0.46	1.4	0.2	8.5	77.9	56.1	82	1.81
	72	3.12	2.0	18.0	0.41	1.47	0.27	9.25	90.5	14.3	82	1.79

Abbreviations: Var – variant, Dis – disaccharides, Glu – glucose, Fru – fructose, LA – lactic acid, AA – acetic acid, Glic – glycerin, EtOH – ethanol, Viab – yeast viability, Cells – cell density.

the starch hydrolysis is the carried out hydrolysis is slower than that concluded from catalytic performance of enzymes used in experiments. However, a foundation is reasonable that the higher growth temperature of the yeast, the faster courses hydrolysis of starch. As a consequence, thermotolerant strains of *S. cerevisiae* are demanded by industry. In our study, we have chosen Red Star Ethanol Red strain. According to the data presented by the producer (Lasaffre Group), it is able to growth at 30-40°C and is resistant to ethanol concentration up to 18% v/v [www.fermentis.com]. In the present work, the fermentation performance of this strains at temperature up to 40°C was investigated. According to the data published, the yeast *Saccharomyces cerevisiae* can grow up to 40°C, but at 42°C their growth is stopped followed by mass death of cells at 43°C [Mensonides et al. 2002].

The experiments concerning an effect of temperature on the SSF process included three values: 35°C, 37°C and 40°C. The data presented in Table 2, Variant A, C and E, show the results of the SSF processes carried out at different temperatures. The final ethanol concentrations in the mashes after 72 h of fermentation process conducted at 35°C and 37°C amounted 9.8%w/v and 10.4% w/v, respectively. It means that fermentation performed at temperature 37°C was more efficient. without any significant decrease of starchy substrate utilization. This conclusion was confirmed by the analysis of other fermentation parameters, i.e. a high degree of starch utilization. The fermentation yield of fermentation carried out at 35°C and 37°C reached 0.473 and 46.0, what corresponded to 92.6 and 90.0% of theoretical yield. Unexpectedly, very good results were obtained in fermentations performed at 40C. The final ethanol concentration at this temperature was 90.5 g/l and utilization of starch reached 90.6%. The ethanol yield amounted 85.2% of theoretical yield. These yields can be considered as very satisfactory. Thus, the fermentation performed at 37°C resulted in higher final concentration of ethanol, but a little lower yield. It should be also stressed that the yeast strain is thermotolerant and very efficient at so high fermentation temperature. The yeast count confirmed an high thermotolerance of Ethanol Red strain. At the temperature 35°C and 37°C, the cell viability reached 99-100%. A significant reduction of cell viability was observed only at 40°C.

On the basis of industrial experience, it is well-known that during intensive fermentation course a large quantity of heat is generated because of the exothermal character of this reaction. In consequence, the temperature of mash increases and large amount of foam is produced. Extremely, the loss of fermenting mash through splashing can be observed. This phenomenon is observed especially in the summer time when temperature of the facility increases. To avoid this problem, the initial temperature of the mash is kept at 26-28°C and small quantity of antifoam is needed. However, it should be stressed that enhanced temperature of fermentation is advantageous because the requirements of energy for ethanol distillation is smaller.

Traditional starch substrate processing includes gelatinization and liquefaction of starch at high temperature, usually over 100°C, using jet-cooker. At these conditions, a dramatic reduction of microorganisms occurs in the mash, what efficiently eliminates the danger of the development of mash contamination. Because the production facilities work in non-sterile conditions, a small permanent contamination is tolerated and it did not required any special intervention. However, many facilities use the antibiotics and antiseptics to protect the process against bacterial contamination.

The situation changes dramatically when the raw material is not cooked before fermentation process. A cold processing of starch in simultaneous saccharification and

fermentation increases a risk of serious contamination of the mash. It was decided to verify an hypothesis that the risk of contamination can be reduced by lowering initial pH value of fermentation.

Taking into account final concentrations of lactic and acetic acids, amounted about 10-11 g/l and 0.1 g/l respectively, it can be ascertained that these concentrations were considerably below toxic levels. Narendranath et al. [2001] reported that the inhibition of growth of yeast culture occurred at concentration of acetic acid over 0.5% w/v and lactic acid over 2.5%. However, the toxic effect of these acids are synergic. In the fermented mash glycerol was also detected and its concentration was up to 1% w/v, that indicates lack of excessive osmotic stress in the mash during the fermentation process. According to expectations, the concentration of this metabolite grew till the end of fermentation, simultaneously with an increase of ethanol concentration. Regarding low concentration of glucose and maltose in the mashes, ethanol molecules can be considered as a main factor influencing osmotic pressure in the mash.

In fed-batch fermentation one of the most important factors affecting fermentation course is the rate of substrate dosage. Generally, it is well-known that it is better to start with low glucose concentration and afterwards supplement the medium with further portion of substrate. The optimisation of fermentation feeding with substrate is a complex problem [Arpornwichanop and Shomchoam 2007, Lee et al. 1999, Xiong and Zhang 2005]. Most fed-batch bioreactors are currently operated at constant feeding what is difficult to realise in small distillery facilities, especially using powdered insoluble substrates. In present work, we applied much more simple dosage schema which is easy to conduct in a typical small distillery. There were chosen two experimental variants. In the first variant, the first part of corn flour (2/3) was introduced at the beginning of the fermentation. This way, we obtained a mash with initial concentration of about 17% w/v. After 24 h of fermentation, the second part of flour, amounted to 1/3 of the whole, was added to the mash. The results of this fermentation are presented in Table 2 variant C. In the second experimental variant, the whole amount of flour was divided into three parts: 1/2, 1/4 and 1/4 of the whole. The first part of flour was introduced into to water at the beginning of process, what resulted in the mash concentration of about 12.5% w/v, the second part was added after 24 of fermentation, and the last part after 48 h of fermentation process. The results of this experiment are presented in Table 2 variant B. Thus, the fermentation processes were conducted under a large interval of substrate concentration in the mashes.

The data show that the application of two dosages was much better than the application of three dosages. After 72 h of fermentation, when two portion of flour was added, the final concentration of ethanol was 10.5% w/v, whereas at three dosages, the final concentration was only 8.1% w/v. Comparing the both experiments, we observed also a lower utilization of starch and decrease of yeast viability. Summarising, the results of these experiments show that better fermentation efficiency was obtained when a bigger dose of flour was introduced at the beginning of the fermentation and the smaller dose after 24 of fermentation.

A next factor taken into consideration was fermentation time. Usually, a standard fermentation time ranged from 48 h to 72 h. Regarding resistance of starch to enzymatic hydrolysis without previous cooking, we prolonged the fermentation time up to 92 h.

As is shown in Table 2 Exp. A the prolongation of fermentation time over 72 h gave only small increase of ethanol concentration and small utilization of starch. It can be

considered that the gain of ethanol during last day of fermentation did not cover the entire costs of the process continuation. This estimation is confirmed by a decrease in volumetric average productivity of ethanol from 1.4 g/lh to 1.1 g/lh between 72 h and 92 h of fermentation.

In the frame of the study on fed-batch SSF process an effect of mash stirring on fermentation course was investigated. This factor was considered as important. When the hydrothermal liquefaction of starch is omitted, the viscosity of the mash is much lower than in the mash produced according to traditional technology. As a consequence, the large particles of corn flour quickly sediment at the bottom of vessel. They form a dense wet cake what worsen the contact of enzymes and yeast cells with the substrate particles. The bubbles of carbon dioxide make this cake structure less compact, but they are not able to mix the sediments over the whole volume of bioreactor. Apart from improving of mash homogeneity, stirring causes intensive friction of solid particles, what reduces the particle diameters and makes easy the contact of enzymes with starch granules. Taking into account the results of variants C and D, it can be ascertained that the stirring at 100 rpm did not guarantee a proper homogeneity of the mash. In a bottom part of the bioreactor the density of the mash was significantly higher than that in the upper part. Moreover, a part of flour formed sediment at the bottom below turbine of stirrer. A lack of homogeneity strongly affected the fermentation performance. The final concentration of ethanol in mash, when stirring rate was 100 rpm, amounted only to 2.4% w/v, whereas, the value of the same parameter, when stirring rate 200 rpm, reached 10.5% w/v. It means that efficiency of the SSF process was four times higher at a higher stirring rate. The results show that the fermentation was conducted only during the first 24 h, when mash density was below 17% w/v. The introduction of second dose of flour caused an increase of viscosity what worsened the mash homogeneity and fermentation stopped. It is visible in the dynamic of ethanol production and starch utilization by the yeast.

In the literature there are only few reports on fed-batch fermentation using soluble substrates as molasses and glucose [Alfenore et al. 2004, Carvalho et al. 2003, Echegaray et al. 2000, Oh et al. 1998] but no report on the fed-batch process using native starch hydrolysing enzymes was published up-today. This work did not resolve the problem of process optimisation but showed the parameters which influenced the fermentation performance.

CONCLUSIONS

In this study it was ascertained that the course of fed-batch SSF process is influenced by all the studied factors, especially by fermentation temperature, agitation rate of the mash and the mode of starch dosage. It was found that the use of Stargen 001 preparation allows efficient hydrolysis of native starch without previous cooking. In spite of low temperature of processing the bacterial contamination was very low. The concentrations of carboxylic acids were below the toxic levels. The data achieved in this study showed good effectiveness of new starch substrate preparation and satisfactory ethanol yields. Moreover, this technology allows reduction of energy requirements for bioethanol production.

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JEDNOCZESNA HYDROLIZA I FERMENTACJA ETANOLOWA NATYWNEJ SKROBI KUKURYDZIANEJ METODĄ OKRESOWO-DOLEWOWĄ

Wstęp. W ostatnim dziesięcioleciu najważniejszymi innowacjami w dziedzinie produkcji bioetanolu był proces jednoczesnej hydrolizy polisacharydów i fermentacji (SSF), opanowanie metody fermentacji gęstych zacierów, wprowadzenie nowych preparatów enzymatycznych zdolnych do hydrolizy natywnej skrobi oraz opracowanie mikroorganizmów modyfikowanych genetycznie zdolnych do jednoczesnej produkcji enzymów amylolitycznych i fermentacji cukrów pięcio- i sześciowęglowych. Celem podjętych badań było określenie efektywności fermentacji etanolowej z użyciem nowych enzymów hydrolizujących natywną skrobię w procesie SSF prowadzonym metodą okresowo-dolewową.
Materiały i metody. Przeprowadzono jednoczesną hydrolizę enzymatyczną i fermentację etanolową (SSF) metodą okresowo-dolewową, stosując natywną mączkę kukurydzianą oraz drożdże gorzelnicze *Saccharomyces cerevisiae* szczep Ethanol Red i preparat amylolityczny Stargen 001. Zmiennymi doświadczalnymi były temperatura fermentacji (35-37-40°C), szybkość mieszania zacieru (100 i 200 obr/min), czas fermentacji (0-92 h) i dawka mączki kukurydzianej (różne dawki i różny czas wprowadzania dawki).
Wyniki. Stwierdzono, że optymalną temperaturą prowadzonego procesu było 37°C i początkowe pH zacieru 5.0, jakkolwiek drożdże fermentowały intensywnie cukry nawet w temperaturze 40°C. Istotny wpływ na wykorzystanie skrobi i wydajność etanolu miała szybkość mieszania zacieru. Wydłużenie czasu fermentacji ponad 72 h nie przekładało się na znaczące zwiększenie ilości wytworzonego etanolu. We wszystkich eksperymentach końcowe stężenie kwasów organicznych było bardzo małe, znacznie poniżej stężenia toksycznego dla drożdży.
Wnioski. Stwierdzono, że zastosowanie nowej metody przygotowania surowca skrobiowego pozwoliło na uzyskanie dobrej wydajności fermentacji i pozwoliło na zmniejszenie zapotrzebowania na energię niezbędną do upłynnienia skrobi.

Słowa kluczowe: etanol, fermentacja okresowo-dolewowa, SSF, *Saccharomyces cerevisiae*, Stargen 001

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