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Use of *Saccharomyces cerevisiae* and *Zymomonas mobilis* for bioethanol production from sugar beet pulp and raw juice

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Biofuels have received great attention as an alternative energy source mainly due to limited oil reserves. Bioethanol can be produced from wide range of raw materials like starch, sucrose and cellulosic based sources. Sugar beet and raw juice, as its intermediate product, constitute very profitable substrates for bioethanol production, considering content of easy available fermentable sugars. In this study, sugar beet pulp and raw juice were fermented with *Saccharomyces cerevisiae* distillery yeasts and bacterium *Zymomonas mobilis*. Different medium dilution rate as well as yeasts preparations (Fermiol, Safdistil C-70) were investigated. Fermentation was run for 72 h at 30°C. Quality of obtained raw distillates was evaluated using GC method. *S. cerevisiae* distillery yeasts turned out to be more favourable microorganism than bacterium *Z. mobilis* for sucrose material fermentation. The ethanol yield obtained from sugar beet pulp and raw juice was 84 and 95% of theoretical yield, respectively. Fermentation of sugar beet raw juice obtained by pressing without enzymatic treatment yielded higher ethanol efficiency as compared to raw juice pressed with enzyme. Dilution ratio 1:1 for fermentation medium appeared to be profitable for effective fermentation process.

Key words: Sugar beet roots, raw juice, fermentation, bioethanol, *Saccharomyces cerevisiae*, *Zymomonas mobilis*.

INTRODUCTION

Seventy percent of the total world ethanol production is utilized as addition to fuel oils; the rest is used by the food (19%) and chemical (11%) industries (Cibis et al., 2006; Dreszer et al., 2003; Kowalewska and Broda, 2009). The main reason why biofuel is of great interest is the fact that existing fossil sources of energy are more and more limited. Special attention is focused on bioethanol as a renewable energy produced from renewable sources as well as on non-waste fermentation technology (Kowalewska and Broda, 2009; Grajek et al., 2008; Lin and Tanaka, 2006; Patrascu et al., 2009).

Bioethanol production is done over the world from different initial starting materials which are classified into three main types: sugars, starch and cellulose. Starchy materials, like corn, wheat, triticale must be first hydrolyzed to fermentable sugars. Cellulose materials like agriculture residues and wood must be pretreated to alter the structure and composition, and improve the enzymatic hydrolysis. The cellulose hydrolysis is much slower than the enzymatic degradation of other sugars (Lin and Tanaka, 2006; Sanches and Cordona, 2008). For this reason, starch and sugar materials have the main

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applicability for ethanol production.

An advantage of sugar materials, like sugarcane, sugar beet or molasses is that they can be converted into ethanol directly, without additional technological operations (Leiper, 2006; Patrascu et al., 2009). Sugar beets can be processed to spirit using at least two methods: one consisting of the fermentation of juice produced from sugar beets by pressing and the other consisting of the fermentation of liquid mass, obtained from whole sugar beet roots (Ogbonna et al., 2001; Balcerek and Pielech-Przybylska, 2008). The argument supporting purposefulness of sugar beet as raw material for bioethanol production is also its high fertility and high ethanol yield (Dreszer et al., 2003; Stecka, 2006).

Although the process of spirit biosynthesis by yeast is relatively well-known, it is essential to optimize the bioethanol production from sugar beet roots, that is, select proper fermentative microorganism, develop fermentation conditions and select the most profitable and easy handle medium for fermentation.

Bioethanol production in sugar industry concerns not only sugar beet roots but also raw and thick juice (Ranković et al., 2009; Dodić et al., 2009). Raw juice contains about 15 to 20% dry solids. Sugars constitute 85 to 90% of dry matter. This fact makes the raw juice to be used straightaway for fermentation which makes this material very profitable for ethanol production. The only disadvantages of raw juice, as well as sugar beet roots, are low storability and easy decomposition by the action of microorganisms. That is why the raw juice is often submitted to evaporation for a high sugar concentration to reduce volume and inhibit microbial growth. So despite the fact that some research on production of ethanol from sugar beet processing intermediated has been done, sugar beet pulp which is a cellulosic by-product of sugar production plants, represent still an interesting feedstock for second generation ethanol production.

The present work was focused on effective utilization of sugar beet pulp and raw juice for bioethanol production, processing in batch culture by free *Saccharomyces cerevisiae* and *Zymomonas mobilis* cells, depending on different fermentation parameters. Finally, the influence of selected fermentation parameters on ethanol efficiency and its composition was determined.

MATERIALS AND METHODS

Biological material

The experimental material consisted of sugar beet pulp obtained after grinding of sugar beet roots, and raw juice was obtained by pressing on a laboratory-scale, with and without enzymatic treatment. Sugar beet roots are characterized with high pectin content. Therefore, it was advisable to use hemicellulolitic enzymes which support the pressing process by pectin chains degradation which enables the increase of the yield of juice obtained from the pulp. Preparations using pulp maceration constitute pectinolases, hemicellulases and cellulases enzymes composition. The efficiency of obtained juices was 65 and 73% (% w w^{-1}) for non enzymatic and enzymatic treatment, respectively. Sugar beet roots were obtained from domestic sugar factory in Opalenica, Poland and came from the 2011/2012 campaign.

Microorganisms

Microorganisms used in this study were dry alcohol yeast *S. cerevisiae*, preparation of Safdistil C-70 and Fermiol (Lesaffre, Fermentis, France) as well as *Z. mobilis* 3881 (from Czech Culture Collection in Brno). Yeasts were used at the amount of 0.5 g kg^{-1} mash in the form of yeast milk. Inoculum of bacterium *Z. mobilis* was added to the fermentation medium in the amount of 10.0% (v v^{-1}).

Fermentation process

The fermentation process was run in Erlenmeyer flasks of 300 ml. Sugar beet pulp was prepared for fermentation by mixing with distilled water in the proportion of 1:1 and 1:0.75. Whereas, raw juice was fermented directly without dilution but raw juice pressed with enzyme treatment was fermented both directly and diluted 1:1. The pH of the media was adjusted to 5.5 with 2 M H_2SO_4 before fermentation. The sugar beet pulp as well as juices were pasteurized on a boiling water bath for 15 min before fermentation. To increase the efficiency of ethanol production (through the hydrolyze of non sucrose polysaccharides- cellulose substrates), additionally, enzymatic preparation Optimash (xylanase and cellulase) (Genencor International), in the amount 0.08 ml kg^{-1}, was applied 15 min before the yeast. To assure a sufficient nitrogen and phosphate source, the fermentation media were enriched with diammonium phosphate, in the amount of 0.4 g l^{-1}. Fermentation was run for 72 h at 30°C in stationary culture.

Analytical methods

The characteristics of raw material were determined using standard methods: dry matter by drying method (Krełowska-Kułas, 1993) and reducing sugars according to Miller (1959). Analysis of sucrose content was realized using polarimetric method (Krełowska-Kułas, 1993). Moreover, ethanol in mash was determined by areometric method after distillation. The ethanol yield was expressed as ethanol % (v v^{-1}), % of theoretical yield, L from 100 kg sucrose and L of ethanol obtained from 100 kg sugar beet roots. The composition and purity of the obtained raw distillates were checked on a Hewlet Packard HP gas chromatography, using Supelcowax - 10 column and a FID detector.

Statistical analysis

All experiments were carried out in triplicates. The results were statistically tested by analysis of variance (ANOVA) using Statistica 6.0 ($\alpha = 0.05$); to compare the significance of differences between samples.

RESULTS AND DISCUSION

The study shows the chemical composition of raw material and ethanol production from sugar beet pulp and juice obtained from sugar beet roots. The results of raw material characteristic are shown in Table 1. Sugar beet roots were characterized with sucrose content as compared to Henke et al. (2006).

Table 1. Composition of raw materials.

Component	Dry matter (%)	pH	Reducing substances (mg ml^{-1})	Sucrose	
				%	% d. m.
Sugar beet roots	21.93	6.80	8.92	15.34	69.95
Raw juice	22.87	6.38	1.24	16.56	72.41
Raw juice pressed enzymatic	21.00	4.47	53.13	15.12	72.00

The coefficient of variation was below 5% in all cases.

Table 2. Ethanol yield from sugar beet pulp fermentation with *S. cerevisiae* (Safdistil C-70 and Fermiol) and bacterium *Z. mobilis* 3881 (30°C, 72 h).

Dilution	pH after fermentation	Ethanol yield				Reducing substances in stillage (mg ml^{-1})
		% v v^{-1}	L 100 kg^{-1} sucrose	Theoretical yield (%)	L 100 kg^{-1} sugar beet	
Safdistil C-70						
1 : 1	4.17	5.05	57.61	84.52 ^{d}	8.83	0.76
1 : 0.75	4.19	4.17	54.35	79.74 ^{b}	8.33	0.80
Fermiol						
1 : 1	4.19	4.34	56.52	82.92 ^{c}	8.67	0.66
1 : 0.75	4.08	4.76	54.35	79.74 ^{b}	8.33	0.64
Zymomonas mobilis 3881						
1 : 1	3.57	3.47	45.29	66.44 ^{a}	6.94	10.93
1 : 0.75	3.57	4.03	46.02	67.51 ^{a}	7.06	11.25

The coefficient of variation was below 5% in all cases. Means within column with different letters are significantly different ($p < 0.05$).

Raw juice was characterized with reducing substances in the amount of 1.24 mg ml^{-1} and enzymatic pressed raw juice in the amount of 53.13 mg ml^{-1}. The high content of reducing sugars in enzymatic pressed raw juice may results from the fact that during raw juice pressing with the support of enzymes, partial sucrose inversion occurred (Table 1). pH value detected for sugar beet pulp was 6.80 and for raw juice, it was 6.38. Raw juice pressed with enzyme treatment was characterized with lower pH 4.47, which was the effect of pH required for the used enzymes.

Although sugar beet fermentation process is commonly known, it is very important to create such parameters to obtain economic ethanol production. The research comprised the selection of the most effective microorganism, fermentation medium density as well as kind of sugar medium for alcohol fermentation process intensification.

Among commercial preparations of distillery yeasts *S. cerevisiae* fermenting selected media, there are yeast preparations preferred, for example sugar medium, like Safdistil C-70 which is available. The industrial specification of Safdistil C-70 preparation (Lesaffre) confirms its use for non starchy materials fermentation. First stage of the present research was to evaluate which yeasts preparation (Fermiol or Safdistil C-70) was the most profitable for sugar beet fermentation.

The experiments were conducted on sugar beet pulp applying dilution of the medium 1:1 and 1:0.75. Dilution was necessary because of the dense structure of sugar beet pulp preventing access of yeasts to the fermenting sugars. It was observed that there was correlation between density of pulp mash and ethanol yield (Table 2). Sugar beet pulp fermentation, both for Safdistil C-70 and Fermiol preparation, showed that lower mash density gave importantly ($p<0.05$) higher ethanol yield, which was also stated by Balcerek and Pielech-Przybylska (2008). Fermentation process with Safdistil C-70 on sugar beet pulp diluted 1:1 with distilled water caused the obtaining of the highest ethanol yield (84.52%), theoretical yield (Table 2). The above research was comparable with the yield obtained by Icöz et al. (2009). Whereas, the Fermiol preparation application for sugar beet pulp fermentation caused the obtaining of the highest (82.92%) theoretical ethanol yield (Table 2). As seen in the result of the research, the Safdistil C-70 yeast preparation was selected for further fermentation processes of raw juice. Patrascu et al. (2009) also achieved higher ethanol productivity with the strain Safdistil C-70 from molasses in compareson with other strains used.

The next stage of experiment was to compare microorganisms effectiveness in sugar beet pulp fermentation.

Table 3. Ethanol yield from sugar beet raw juices fermentation with *S. cerevisiae* (Safdistil C-70) and *Z. mobilis* 3881 (30°C, 72 h).

pH after fermentation	Ethanol yield				Reducing substances in stillage (mg ml^{-1})
	% v v^{-1}	L 100 kg^{-1} sucrose	Theoretical yield (%)	L 100 L^{-1} juice	
S. cerevisiae					
Raw juice pressed without enzymatic treatment					
3.71	10.77	65.05	95.42^{e}	10.77	5.70
Raw juice pressed with enzymatic treatment					
3.66	4.90	32.41	47.55^{b}	4.90	26.31
Raw juice pressed with enzymatic treatment, diluted 1:1					
3.66	4.40	58.20	85.39^{d}	8.80	3.55
Z. mobilis					
Raw juice pressed without enzymatic treatment					
4.16	7.40	44.69	65.56^{c}	4.44	12.44
Raw juice pressed with enzymatic treatment, diluted 1:1					
3.91	0.20	2.65	3.88^{a}	0.28	44.35

The coefficient of variation was below 5% in all cases; Means within column with different letters are significantly different ($p<0.05$).

Bacterium *Z. mobilis* 3881 was used to ferment sugars included in the pulp, and importantly, ($p<0.05$) lower ethanol yield (67.51%) theoretical yield was obtained (Table 2). Besides, bacterium gave the lowest ethanol yield from sugar beet pulp; it was decided to use them in juice fermentation, and it was an easier medium to handle, which could be a profitable agent for *Z. mobilis*.

Ethanol fermentations were conducted using sugar beet raw juices: pressed without and with enzymatic treatment, as the next stage of the research. Selected Safdistil C-70 yeast preparation showed 95.42% theoretical yield for raw juice fermentation. The fermentation medium of raw juice obtained by enzymatic pressing exhibited importantly ($p<0.05$) lower ethanol yield (47.55%) theoretical yield (Table 3). Study by Balcerek and Pielech-Przybylska (2008) also showed high ethanol yield efficiency, 77 to 96% ethanol, but in the case of thick juice fermentation.

Taking into account low ethanol yield from enzymatic pressed juice, the next fermentation experiment was conducted on diluted 1:1 raw juice. Raw juice dilution caused the obtaining of importantly ($p<0.05$) higher ethanol yield of 85.39% theoretical yield, but still lower (of 10%) than that from non enzymatic treated juice (Table 3). Previous research described by Gumienna et al. (2009), as a result of the analysis concerning sugar beet media fermentation, also stated increase in ethanol yield together with the decrease of media density. Additional enzymatic treatment of the fermented media could cause extraction of some byproducts with inhibiting character according to the tested microorganisms. Dilution of such medium decreased the inhibiting effect and allowed increase of the ethanol yield efficiency.

On the basis of the obtained results, it was stated that raw sugar beet juice was a very good medium for ethanol production due to easy procedure of the fermentation process unlike sugar beet pulp. Although, sugar beet raw juice constitutes a source of easy available sugars for ethanol biosynthesis by yeast, the remains after pressing are left for further research to make the fermentation process more and more economical.

Bacterium *Z. mobilis* 3881, used for raw juice fermentation process, caused the obtaining of importantly lower ($p<0.05$) ethanol yield (Table 3). Raw juice (pressed without enzymatic treatment) was fermented with ethanol yield (65.55%), theoretical yield (Table 3). This ethanol efficiency with *Z. mobilis* was 30% lower as compared to *S. cerevisiae* fermentation for raw juice but comparable to ethanol yield obtained by *Z. mobilis* on sugar beet pulp medium (66.44 to 67.51% theoretical yield) (Table 2).

Taking into account low ethanol efficiency of not diluted raw juice pressed with enzymatic treatment, dilution 1:1 was applied for fermentation experiments with *Z. mobilis* 3881. The conducted research demonstrated that raw sugar beet juice pressed with enzymatic treatment was not suitable for ethanol fermentation with bacterium *Z. mobilis*, giving 3.88% theoretical ethanol yield (Table 3).

It can be observed that ethanol fermentation efficiency heavily depends on raw material used, in particular on fermentation sugars content and their availability. During raw materials processes to intermediate products, many physico-chemical changes are detected which can positively or negatively influence ethanol fermentation yield.

Research on sugar beet pulp and raw juices showed the most favourable conditions for sugar beet products ethanol fermentation process. Results indicate the use of distillery yeast *S. cerevisiae* in the form of Safdistil C-70

Table 4. Ethanol and byproducts content of sugar beet pulp raw distillates from ethanol fermentation (30°C, 72 h).

By product	*S. cerevisiae* (Safdistil C-70)		*S. cerevisiae* (Fermiol)		*Z. mobilis* 3881	
Dilution	1:1	1:0.75	1:1	1:0.75	1:1	1:0.75
Aldehydes						
g L^{-1} 100% spirit	0.786^{c}	1.600^{e}	0.430^{a}	0.554^{b}	1.948^{f}	1.584^{d}
Total compounds (%)	0.10	0.20	0.05	0.07	0.25	0.20
Esters						
g L^{-1} 100% spirit	0.031^{b}	0.089^{d}	0.046^{c}	0.048^{c}	0.109^{e}	0.010^{a}
Total compounds (%)	0.01	0.01	0.01	0.01	0.01	0.01
Higher alcohols						
g L^{-1} 100% spirit	2.882^{d}	3.903^{f}	2.724^{c}	3.285^{e}	1.944^{b}	0.785^{a}
Total compounds (%)	0.36	0.49	0.34	0.41	0.25	0.10
Methanol						
g L^{-1} 100% spirit	0.178^{a}	0.206^{c}	0.181^{a}	0.188^{b}	0.413^{e}	0.231^{d}
Total compounds (%)	0.02	0.03	0.02	0.02	0.05	0.03
Ethanol						
Total compounds (%)	99.51^{bcBCD}	99.27^{aA}	99.58^{cD}	99.49^{bBC}	99.44^{bB}	99.66^{dE}

The coefficient of variation was below 5% in all cases.
Means within rows with different small letters are significantly different ($p<0.05$).
Means within rows with different capital letters are significantly different ($p<0.05$), for sugar beet pulp and juices.

preparation both for sugar beet pulp and raw juices (pressed without and with enzymatic treatment) fermentation. Taking into account the density of fermentation media, in the case of sugar beet pulp and raw juice pressed with enzymatic treatment, dilution 1:1 of the media is necessary.

The quality of obtained raw distillates was determined using gas chromatography method. This method detected volatile compounds, except for ethanol, which constitutes the contaminants. The differences in distillates quality demonstrate that the final quantity and composition of fermentation byproducts depends on raw material and fermentation conditions. In the produced distillates, the following compounds were determined: higher alcohols, esters, aldehydes and methanol.

The research showed that the distillates from sugar beet juices fermentation were characterized with importantly ($p<0.05$) higher quantity of ethanol to distillates sugar beet pulp fermentation both in the case of yeasts *S. cerevisiae* (Safdistil C-70) and bacterium *Z. mobilis* 3881 utilization (Tables 4 and 5).

The highest ($p<0.05$) volatile byproducts content of 5.798 g L^{-1} 100% spirit was noticed in distillates obtained from sugar beet pulp yeasts fermentation with 1:0.75 diluted medium (Table 5) whereas the lowest content of volatile byproducts (0.546 g L^{-1} 100% spirit), was found in distillates from raw juice (obtained without enzymatic treatment) bacterium fermentation (Table 5).

Aldehydes content (Tables 4 and 5) in distillates obtained from sugar beet pulp and raw juice (obtained with enzymatic treatment) fermentation exceeded the quantity in Polish norms for agriculture spirit from molas-ses (0.3 g L^{-1} 100% spirit). In the case of distillates obtained from raw juice (pressed without enzymatic treat-ment) fermentation, aldehydes content, respectively, was lower ($p<0.05$) and compatible with the norms.

The requirement of raw spirits is very important when the spirit is intended for consumption. Taking into account the use of spirit for other needs e.g. bioethanol, higher content of volatile byproducts is not of great importance.

The highest group of contaminations, higher alcohols was obtained in distillates (Tables 4 and 5). The research shows the lowest content of higher alcohols in distillates from raw juice (obtained without enzymatic treatment) after bacterium fermentation and the highest for sugar beet pulp, diluted 1:0.75 yeasts (Safdistil C-70) fermentation ($p<0.05$). Polish norms do not regulate the content of higher alcohols for distillates from molasses.

Methyl alcohol (not also regulated for molasses distillate), as formed from pectins contained in sugar beet, was detected in the highest amount after bacterium fermentation of 1:1 diluted sugar beet pulp. It is noteworthy that methanol content in distillates obtained from juices after yeasts and bacterium fermentations was importantly lower ($p<0.05$) (0.028 to 0.168 g L^{-1} 100% spirit), taking into consideration low content of pectins remaining in the separated pulp (Tables 4 and 5).

Table 5. Ethanol and byproducts content of sugar beet raw juices distillates from ethanol fermentation (30°C, 72 h).

Raw juice obtained	*S. cerevisiae* (Safdistil C-70)		*Z. mobilis* 3881	
	Without enzymatic treatment	With enzymatic treatment	Without enzymatic treatment	With enzymatic treatment
Dilution	none	none	1:1	none
Aldehydes				
g L^{-1} 100% spirit	0.094^{a}	0.425^{b}	0.748^{c}	0.100^{a}
Total compounds (%)	0.01	0.05	0.10	0.01
Esters				
g L^{-1} 100% spirit	0.238^{c}	0.143^{b}	0.880^{d}	0.069^{a}
Total compounds (%)	0.03	0.02	0.11	0.01
Higher alcohols				
g L^{-1} 100% spirit	3.395^{d}	0.909^{b}	1.874^{c}	0.349^{a}
Total compounds (%)	0.43	0.12	0.24	0.04
Methanol				
g L^{-1} 100% spirit	0.035^{b}	0.093^{c}	0.168^{d}	0.028^{a}
Total compounds (%)	0.01	0.01	0.02	0.01
Ethanol				
Total compounds (%)	99.52^{aCD}	99.80^{bF}	99.53^{aCD}	99.93^{cG}

The coefficient of variation was below 5% in all cases.
Means within rows with different small letters are significantly different ($p<0.05$).
Means within rows with different capital letters are significantly different ($p<0.05$), for sugar beet pulp and juices.

Conclusions

The above results confirm that the efficient ethanol production from sugar beet pulp and raw juices is possible. As a result, it was stated that fermentation of sugar beet raw juice obtained by pressing without enzymatic treatment showed the highest ethanol yield among all applied sugar beet media. It was found that efficiency in sugar for ethanol bioconversion increased together with the medium dilution increase, both for sugar beet pulp and raw juice pressed with enzymatic treatment. Raw juice obtained without any support did not require any dilution for effective fermentation process. Distillery yeast *S. cerevisiae*, applied for sugar beet material ethanol fermentation, was more profitable microorganism than bacterium *Z. mobilis*.

The data obtained in this study showed effectiveness of bioethanol production from sugar beet and its intermediate products by *S. cerevisiae* yeast.

GC analysis showed that distillates obtained from raw juice fermentation contained lower quantity of volatile compounds as compared to distillates from sugar beet pulp.

The results presented show the possible use of sugar beet juice, without additional technical treatment for ethanol production, and the content of compounds accompanying sucrose favors the fermentation process with *S. cerevisiae*.

REFERENCES

Balcerek M, Pielech-Przybylska K (2008). Selection of yeast strains for fermentation of thick juice worts. Ferm., Fruits Vegetable Ind. 11:37-40

Cibis E, Krzywonos M, Miśkiewicz T (2006). Ethanol in the world-utilization trends, feedstocks and by-products. Przem. Chem. 85(8-9):1263-1267.

Dodić S, Popov S, Dodić J, Ranković J, Zavargo Z, Mucibabić RJ (2009). Bioethanol production from trick juice as intermediate of sugar beet processing. Biomass Bioenergy 33:822-827.

Dreszer K, Michałek R, Roszkowski A (2003). Renewable energy – possibility of its obtaining and utilization in agriculture. PTIR, Cracow – Lublin – Warsaw.

Grajek W, Gumienna M, Lasik M, Czarnecki Z (2008). Perspectives for ethanol production from starchy material. Chem. Industry. 87/11:1094-1101.

Gumienna M, Lasik M, Czarnecki Z, Szambelan K (2009). Applicability of unconventional energy raw materials in ethanol production. Acta Sci. Pol., Technol. Aliment. 8(4):17-24.

Henke S, Budnik Z, Hinkova A, Pour V (2006). Model of a sugar factory with bioethanol production in program sugars. J. Food Engin. 77:416-420.

Icoz E, Tugrul KM, Icoz E (2009). Research on ethanol production and use from sugar beet in Turkey. Biomass Bioenergy 33:1-7.

Kowalewska M, Broda M (2009). The status of the agricultural distilleries in Poland. Ferm., Fruits Vegetable Ind. 10:36-37.

Krełowska-Kułas M (1993). Analysis of nutrition products quality. PWE Warsaw.
Leiper K (2006). The fermentation of sugar beet syrup to produce bioethanol. J. Inst. Brew. Distilling 33:112-122.
Lin Y, Tanaka S (2006). Ethanol fermentation from biomass resources: current state and prospects. Appl. Microbiol. Biotechnol. 69:627-642.
Miller G (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31:426-428.
Ogbona JC, Mashima H, Tanaka H (2001). Scale up of fuel ethanol production from sugar beet juice using loofa sponge immobilized bioreactor. Biores. Technol. 76(6):1-8.
Patrascu E, Rapeanu G, Bonciu C, Hopulele T (2009). Bioethanol production from molasses by different strains of Saccharomyces cerevisiae. The Annals of the University Dunarea de Jos of Galati: 49-56. Paper presented at the International Symposium Euro-aliment 2009, 9th-10th of October 2009, Galati-Romania.
Ranković J, Dodić J, Dodić S, Popov S (2009). Bioethanol production from intermediate products of sugar beet processing with different types of Saccharomyces cerevisiae. Chem. Ind. Chem. Eng. Quarterly 15(1):13-16.
Sanchez OJ, Cardona CA (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. Biores. Technol. 99(13):5270-5295.
Stecka K (2006). Possibility of sugar beet utilization for bioethanol production. Village Tomorrow 8(9):13-14.