POSSIBILITY OF USING OF TREATED BEET SHREDS FROM PROCESS OF BIOETHANOL PRODUCTION FOR ANIMAL FEED MOGUĆNOST PRIMENE TRETIRANIH REPINIH REZANACA IZ PROCESA PROIZVODNJE BIOETANOLA KAO HRANE ZA STOKU

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ABSTRACT

Bioethanol can be produced from various feedstocks, including lignocellulosic materials, such as sugar beet shreds. In the case of sugar beet shreds, at first, it is necessary to extract pectin, which itself can be used for different purposes. As a result of that the cellulose becomes more accessible for enzymes degradation to simple sugars, and obtained sugars could be fermented to ethanol. The aim of this study was to evaluate the chemical composition of the raw beet shreds, beet shreds without pectin and solid residue after enzymatic hydrolysis in order to identify whether these materials can be used as animal feed. On the base of the obtained results for dry matter, ash, total Kjeldahl nitrogen, total, soluble and insoluble fibers, it can be concluded that all these materials can be used as animal feed. This would considerably contribute to profitability of bioethanol production from sugar beet shreds.

Key words: sugar beet shreds, pretreatment, chemical composition.

REZIME

Bioetanol se može proizvoditi iz različitih sirovina, između ostalog i lignoceluloznih materijala u koje spadaju repini rezanci. Kada se koriste repini rezanci potrebno je prvo izdvojiti pektin, koji se i sam može koristiti za različite svrhe. Kao rezultat ovoga celuloza postaje lakše dostupna za enzimsku degradaciju do prostih šećera, a dobijeni šećeri se mogu fermentisati do etanola. Cilj rada je bio da se ispita hemijski sastav sirovih repinih rezanaca iz kojih je uklonjen pektin i čvrstog ostatka nakon enzimske hidrolize da bi se sagledalo da li se i ovi materijali mogu koristiti kao stočna hrana. Na bazi dobijenih rezultata za suvu materiju, pepeo, ukupni azot po Kjeldahl-u, ukupna, rastvorljiva i nerastvorljiva vlakna se može zaključiti da se svi ovi materijali mogu koristiti kao stočna hrana. To će značajno doprineti profitabilnosti proizvodnje bioetanola iz repinih rezanaca.

Ključne reči: repini rezanci, prethodni tretman, hemijski sastav.

INTRODUCTION

Sugar beet shreds generated from beet sugar processing, both dry or wet, are commonly used as an animal feed. More effective utilization of sugar beet shreds can improve the economic viability of beet sugar production. Beet shreds are untapped source of pectin, cell wall polysaccharides that can be converted into higher-value biobased products. On the other hand, the increasing necessity for energy leads to the development of new energy sources (Dakić et al., 2009; Dželetović and Mihailović 2011). One possible solution is the production of bioethanol and its use as a gasoline substitute. Bioethanol can be produced from various feedstocks, including lignocellulosic materials (second generation bioethanol), such as sugar beet shreds (Gnansounou, 2010). The main advantages of lignocellulosic substrates for bioethanol production, in comparison with sugar and starch substrates, are low price, absence of competition with food and feed, and lack of direct and indirect land-use impact. The main disadvantage of lignocellulosic substrates is pretreatment which is needed prior bioethanol production, and which is relatively complicated and expensive. Pretreatment involves the separation of cellulose and its degradation to simple, fermentative sugars. After that, obtained sugars are fermented to ethanol under the action of yeasts. Pretreatment step demands large quantities of energy, water, chemicals and enzymes, and produces large quantities of wastewater that should be purified before discharging back to the nature, and some solid waste. Because of that, the bioethanol production from lignocellulosic biomass is still not economically viable using existing technologies in the context of current petroleum price. Promising option to meet this challenge, among others, is valorisation and reuse of by-products and wastes obtained from that process (Gnansounou and Dauriat, 2010).

In the case of sugar beet shreds, it is necessary to extract pectin, and as a result of that the cellulose becomes more accessible. Then the solid residue after depectination is treated with enzymes to degrade cellulose to simple sugars. Enzymatic hydrolysis is currently preferred because it uses mild reaction condition and has less effect on by-products. The solid residue after enzymatic hydrolysis occurs as waste shreds. Another advantage of sugar beet shreds as a source for bioethanol production, is that the delignification step is not necessary (*Ivetić et al., 2012*), what leads to increasing economy of the entire process. The aim of this study was to evaluate the chemical composition of the raw beet shreds, beet shreds without pectin and solid residue after enzymatic hydrolysis in order to identify whether these materials can be used as animal feed. This would considerably contribute to profitability of bioethanol production from sugar beet shreds.

MATERIAL AND METHOD

Sugar beet shreds were kind gift from A.D. Šajkaška, Helenic Sugars, Serbia. Dry raw beet shreds were milled on Miag laboratory cone mill and sieved by Bühler laboratory sifter (gyratory in a horizontal plane), model MLU-300 (Uzwil, Switzerland). The fraction of particles from 224 to 400 µm was used in the experiments. Beet shreds are treated by predefined procedure (*Ivetić et al., 2012*). In order to remove pectic substances sugar beet shreds were suspended in HCl solution, at pH 1.5 and 85°C according to *Sun and Hughes* (1998). After cooling down, the suspension was filtered through laboratory filter paper Macherey-Nagel MN 651/120 and filter cake was washed in order to remove residual HCl. After pectin extraction, one part of shreds was dried and analysed, and other part was treated enzymatically. Enzymatic hydrolysis was carried out by commercial cellulases (Celluclast, Novozyme) in 0.1 M acetate buffer, with enzyme concentration of 0.67 FPU/g, at pH 4.8 and 40°C during 72 hours. Solid residue was than separated, dried and analysed. In the samples the dry matter, ash, total Kjeldahl nitrogen were determined according the *MEBAK* (1997), as well as total (*AOAC*, 1986), soluble (*AOAC*, 1996) and insoluble fibers (*AOAC*, 1994).

RESULTS AND DISCUSSION

After depectination the mass of shreds was about two times less than the initial mass of raw shreds. The mass loss during the enzymatic hydrolysis was approximately equal. Therefore, in the process of bioethanol production, from 1 t of raw shreds, besides ethanol, 0.25 t of waste shreds, pectin solution and stillage can be obtained (*Ivetić et al., 2011; Ivetić et al., 2012*). By-products from processes of bioethanol production from other lignocellulosic materials are somewhat different (*Gnansounou and Dauriat, 2010*). After the process of depectination, about 0.11 m³ solution containing pectin was obtained per 1 kg of beet shreds, and additionally, about 0.08 m³ of wastewater from rinsing. Method of extraction, treatment and use of pectin is the subject of other investigations (*Chakraborty and Ray, 2011; Willats et al., 2006*).

The results of analysis of raw sugar beet shreds, shreds after depectination and after enzymatic hydrolysis were presented in Table 1.

Table 1. Characteristics of raw and treated sugar beet shreds

	Raw shreds	Shreds after depectination	Shreds after hydrolysis
Dry matter - DM (%)	91.9	97.3	95.9
Ash (%)	4.1	0.90	0.33
Organic dry matter -	87.8	96.4	95.5
ODM (%)			
Percentage of ODM in	95.5	99.1	99.7
DM (%)			
Total Kjeldahl nitrogen	1.62	1.94	3.14
(% _{DM} *)			
Proteins (% _{DM})	10.1	12.1	19.6
Total fibers - TF (% _{DM})	75.0	82.7	73.7
Insoluble fibers - IF	57.8	80.5	72.2
(% _{DM})			
Soluble fibers - SF (% _{DM})	17.1	2.2	1.63
Percentage of SF in TF	22.8	2.66	2.21
(%)			

* percentage is calculated on the base of dry matter (DM)

Both untreated (raw) and treated shreds have dry matter over 90%. Untreated beet shreds have 4.1% of ash, and the residues after both treatments contain four times lower contents of ash. Accordingly, the percentage of ODM in DM, in treated shreds is almost 100%. This organic matter contains proteins and fibers, and small amount of other components. Raw shreds contain about 10% proteins, shreds after depectination 12%, and residue after enzymatic hydrolysis almost 20% proteins, which is two times more than in raw shreds. While the sugar beet shreds are used primarily as a source of fiber, it is very beneficial that the protein content in enzymatically treated shreds is higher than in raw shreds.

According to *AOAC* Official Method 985.29 (*1986*) the total fiber includes cellulose, hemicellulose, lignin and pectin. The raw shreds contain 69% of total fibers, with a percentage of soluble fibers about 23%. Soluble fibers in raw shreds include mainly pectin. After depectination, soluble fibers were removed

from shreds and consequently, only insoluble fibers remained. According to our results (Table 1) depectinated shreds contain 80% of fibers, with percentage of soluble fibers only 2%. As a result of pectin removal, the celulose became available to cellulolytic enzymes. One part of cellulose was hydrolyzed to sugars, and other part remained in shreds as insoluble fibers, along with hemicellulose and lignin. Waste beet shreds after enzymatic hydrolysis contain 70% of total fibers and the proportion of soluble fibers in the treated shreds is almost negligible. The presence of only insoluble fibers in waste shreds is not suitable in terms of their use as animal feed. Feeds containing high fiber content are important components of the diet of ruminant farm animals. It is known, that high-starch feed has some disadvantages for ruminants (González et al., 2012). One approach of reducing the use of cereals in ruminant rations involves their replacement by noncereal by-products such as sugar beet pulp. The results of Mandebvu and Galbraith (1999) indicated that replacement of barley by molassed sugar beet pulp had no effect on food intake, growth rate or feed conversion efficiency. In opposite, González-Alvarado et al. (2010) established that sugar beet pulp improves feed to gain ratio of broilers only from 1 to 10 days of age, but after that the effects disappeared. Sugar beet pulp has high pectin content and pectins are characterized by their high swelling capacity. A wetter and bulkier digesta, as occurs when sugar beet pulp is included in the diet, causes physical distension of the gastrointestinal tract which might affect the physiological mechanisms that regulate feed intake. In this respect, the depectinated or enzymatically treated sugar beet shreds could be a good solution. Anyway, the inclusion of insoluble fiber sources in the diet improve digestive health of both ruminant and non-ruminant animals (Montagne et al., 2003; Hedemann et al., 2006). Since the effect depends on the physicochemical characteristics of the dietary fibers, their level of incorporation in the diet, the duration of ingestion, the animal species and age, and so on, it can be assumed that in the appropriate fodder mix, both the residue of sugar beet shreds after extraction of pectin and the residue after enzymatic hydrolysis could be used. In addition, chemicals used in this pretreatment procedures are non-hazardous and safe, and consequently it should not be any obstacles for the use of the obtained residues as animal feed.

CONCLUSION

The results of analysis of raw and treated sugar beet shreds have showed that the residues after depectination and hydrolysis contain lower contents of ash, 30% and 100% more protein as compared to untreated shreds, respectively, and almost the same percentage of fibers, but the percentage of soluble fibers in the treated shreds is almost negligible. On the base of the obtained results it can be concluded that solid residue from treatment of sugar beet shreds for bioethanol production, can be used as animal feed.

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