The potential for sustainable bioethanol production in Serbia: available biomass and new production approaches

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Bioethanol has become one of the most promising biofuels today in response to uncertain fuel supplies and efforts to reduce greenhouse gas emissions. The biofuels are easily available from common biomass sources, biodegradable and contribute to sustainability. In Serbia, the industrial production of bioethanol still relies on conventional energy crops containing starch and sugar such as corn, wheat and triticale, which are the most suitable and available agricultural raw materials. The preview of bioethanol production possibilities and available feedstocks in Serbia are presented in this study. Several production approaches based on crop selection, process integration and waste utilization were also considered in order to increase production efficiency and to avoid the competition of the feedstock utilization for food and energy. Utilization of corn, wheat and triticale (plant resistant to severe climate and soil conditions) were investigated for bioethanol production as well as utilization of damaged crops (e.g. wheat) that are not appropriate for food consumption. Also, utilization of the stillage for the production of lactic acid could also improve the bioethanol production. The economy of bioethanol production was analyzed in order to decrease the production costs and make this biofuel competitive with fossil fuels. The analysis has compared the cost of bioethanol produced from three crops that can be cultivated in Serbia: corn, wheat, and triticale, and the triticale has shown as the most favourable.

Keywords: bioethanol; biofuel; feedstocks; corn; triticale; wheat; stillage

1. Introduction

The economic development of modern societies is crucially dependent on energy. The way energy is produced, supplied, and consumed strongly affects the local and global environment and is therefore a key issue in sustainable development. With the increase of human population and industrial prosperity, global energy consumption also has increased gradually. The worldwide energy consumption increased 17-fold in last century. The International Energy Agency, in its report on world energy consumption, projected that energy consumption will more than double by 2030, primarily because of growth in the developing countries [1-3].

The world's economy today highly depends on fossil energy sources (coal, oil, natural gas) which are used to produce fuels, electricity, chemicals, and other goods. The utilization of these conventional fossil energy sources in the long run is not sustainable [4]. In the 21st century, the greatest challenges for the society are to meet the growing energy demand and to provide raw material for the industry in a sustainable way. Non-renewable energy sources such as fossil fuels are limited and have a considerable negative environment impact [5]. Bioenergy from renewable resources is already today an alternative to fossil fuels; however, to meet the increasing need for bioenergy new and abundant raw materials have to be considered. All petroleum-based fuels can be replaced by renewable fuels produced from biomass such as bioethanol, biodiesel, biohydrogen etc. In this perspective, global production of renewable energy from the biomass of energy crops has been increasing rapidly, particularly after 2000.

Bioethanol produced from renewable biomass is believed to be one of the dominating renewable biofuels. Bioethanol is a liquid biofuel which can be produced from several different biomass feedstocks and conversion technologies. It is produced by fermentation of simple sugars present in biomass or the sugars obtained by prior chemical or enzymatic treatment of the biomass. The bioethanol fermentation is performed by microorganisms, traditionally by yeasts (*Saccharomyces* strains), although some types of engineered bacteria such as *Zymomonas mobilis*, *Escherichia coli* or *Klebsiella oxytoca* [6, 7] could also be used. After the fermentation, the bioethanol is being separated from the fermentation broth, conventionally by means of distillation and rectification or by using more efficient separation technologies such as pervaporation, membrane filtration or molecular sieves [8, 9].

Bioethanol has the potential to be a sustainable fuel, as well as a fuel oxygenate that can replace gasoline, in transport sector which is considered as one of the largest energy consumers as well as environmental pollutant [10]. According to International Energy Agency statistics [11], the transport sector accounts for about 60% of the world's total oil consumption. It is responsible for about one fifth of CO_2 emission on a global scale [12].

The production of bioethanol is increasing over the years, and has reached the level of 85.2 billion litres during the year 2012 [13]. An increase in bioethanol production up to more than 125 billion litres until 2020 has been predicted assuming the production support by governmental policies and exemptions [14]. Currently, nearly all bioethanol is produced from corn in the United States, or from sugar cane in Brazil, but any country with a significant agronomic-based economy can use current technology for bioethanol fermentation. Even though bioethanol production for decades mainly depended on energy crops containing starch and sugar (corn, sugar cane etc.), new technologies for converting lignocellulosic biomass into bioethanol are under development today and have not yet been demonstrated commercially on a larger scale [5]. The fact that the existing fossil fuel infrastructure, eventually with minor modification, can be used for bioethanol distribution and utilization puts this biofuel in front of other energy alternatives [11].

The preview of bioethanol production possibilities and available feedstocks in Serbia are presented in this study. Several production approaches based on crop selection, process improvement and waste utilization were also considered in order to increase production efficiency and to avoid the competition of the feedstock utilization for food and energy. Utilization of corn, wheat and triticale (plant resistant to severe climate and soil conditions) were investigated for bioethanol production as well as utilization of damaged crops (e.g. wheat) that are not appropriate for food consumption. Also, utilization of thin stillage for the production of lactic acid could improve the bioethanol production. Our analysis has compared the cost of bioethanol produced from three crops that can be cultivated in Serbia: corn, wheat, and triticale, and the triticale has shown as the most favourable.

2. Bioethanol production in Serbia

Over the past 10–15 years in the EU, heat and electricity production from biomass increased with 2% and 9% per year, respectively, between 1990 and 2000 and biofuel production increased about eight-fold in the same period [15]. The EU is today the third producer of fuel-bioethanol in the world behind the United States and Brazil, however its production is much lower than the first two (by a factor of about 10). Bioethanol production in EU was 4.97 billion litres in 2012, which is an 11% increase over 2011 [16]. However, although the amount of biofuels produced in the EU is growing; the quantities in general remain small compared to the total volume of mineral-based transport fuel sold.

One of the significant regulations which impact the EU biofuels market is the European Directive 2009/28/EC on the promotion of renewable energy which aims at achieving by 2020 a 20% share of energy from renewable sources in the EU's final consumption of energy and a 10% share of energy from renewable sources in each member state's transport energy consumption. To achieve that effect, EU members follow the Directive implementing with various political, fiscal and technical measures and incentives. Each EU Member State should adopt a national renewable energy action plan setting out its national targets for the share of energy from renewable sources consumed in transport, electricity, heating and cooling in 2020 [17, 18]. Serbia and other Balkan countries, which are interested in joining the EU, has accepted the obligation to follow EU policies and programs, including those that obliges them to introduce the production and use of fuels from renewable energy sources. Better coordination between institutions and academic and private sector is a very important step in order to realize cost-effective bioethanol production in Serbia. By implementation of the Kyoto Protocol, Serbia sends to international community and the EU a clear signal of readiness to implement the concept of sustainable development and monitor of global socio-economic trends, which will improve the investment climate and increase the confidence of potential foreign investors.

However, bioethanol production in Serbia is now at low level, even lower than the production scale at the very end of twentieth century, and it is not enough to fulfil the country's ethanol needs just for beverages, medical and pharmaceutical purposes. This is a main reason why in Serbia, there is still not an organized production and utilization of bioethanol or other biofuels for gasoline substitution. Our recent analysis revealed that Serbia will need to build new bioethanol plants in order to produce enough bioethanol for use as a fuel and thus to follow the aims of the European directive 2009/28/EC. Based on the given replacement of 20% of gasoline by bioethanol by 2020 according to European directive 2009/28/EC, it has been reported [19] that it will be needed to produce 233,000 t of bioethanol until 2026. If it is assumed that the needs of the ethanol industry and pharmacy stay the same (52,000 t), total demand for bioethanol would be 285,000 tons, which is 12 times the installed capacity in Serbia. It is now clear that in Serbia, it is necessary to introduce new plants for the bioethanol production - large capacity plants that besides bioethanol include the production of animal feed and carbon dioxide; or introduce a network of small plants for the production of raw bioethanol (65-70% vol.) which would be further separated from mash using different techniques within the petroleum industry.

Today in Serbia, the bioethanol production is performed in seven plants ("Panon"-new and old plant in Crvenka; "Kadaks" in Crvenka; "Vrenje" in Belgrade; "Lukas" in Bajmok; "Reahem" in Srbobran and "Alpis-SLC" in Kovin) with total production capacity of 23,225 t/year. Raw materials used in these plants are molasses and cereals. In these plants, 96% vol. bioethanol is produced, which is used mainly for alcoholic drinks and for medical and pharmaceutical purposes. Bioethanol production in Serbia from fermented materials in the period 2004-2011 is presented in Figure 1.

As shown in Fig. 1, bioethanol production in Serbia was 8.69 million litres in 2011, which is only 30% of annual production capacity in Serbia. This indicates that the capacity utilization of bioethanol plants is rather small. The biggest bioethanol production of 19.17 million litres was achieved in 2007 [20, 21].

It is important to point out that despite current low level of industrial production of bioethanol, Serbia has main prerequisites to develop and improve this production since it possess a large feedstock potential, tradition and also educated human potential.

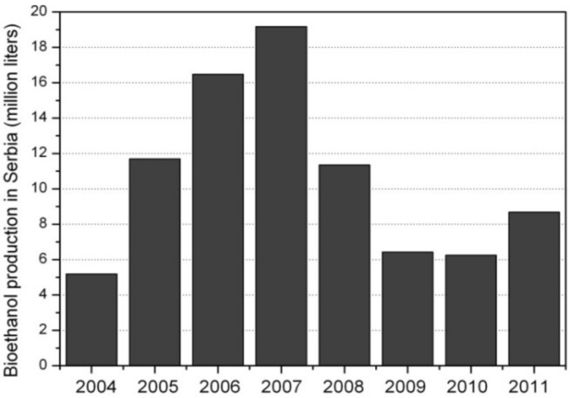

Fig. 1 Bioethanol production in Serbia in the period 2004-2011 [20, 21].

3. Feedstock potential for bioethanol production in Serbia

Bioethanol can be produced from a wide range of feedstocks, which can be classified into three categories: sugar-materials (e.g. sugarcane, sugar beet, sweet sorghum, molasses and fruits), starch materials (e.g. corn, wheat, triticale, rice, potatoes, cassava, Jerusalem artichoke, sweet potatoes and barley) and lignocellulosic materials (e.g. wood, straw and grasses). Sugars can be converted into bioethanol directly, while starch must firstly be hydrolyzed to fermentable sugars by the action of enzymes. Cellulose must likewise be converted into sugars, generally by the action of mineral acids. Fuel bioethanol has been produced commercially using starch and sugar feedstocks. Technologies for the production of ethanol from lignocellulosic biomass have been developed and tested at various scales up to semi-commercial demonstration plants. The productivity, energy input, and costs, as well as environmental impact vary for different feedstocks [22, 23]. Currently, there is a growing interest worldwide to find out new, abundant and economically more favourable feedstocks such as agricultural wastes; however, crops such as corn, wheat and sugar cane are still dominant at the industrial level [24-26].

The largest producers of bioethanol, Brazil and USA, produce the bioethanol from sugar cane and corn, respectively. In Europe, the feedstocks used for bioethanol are predominately cereals-wheat and corn (57%), sugar beet and molasses (32%), waste from the wine industry (10%) and other feedstocks (1%) [27]. In general, feedstock availability is directly related to land availability which seems to be an important and critical factor, affecting the feedstock cost. The availability of feedstocks for bioethanol production can vary considerably from season to season and depends on geographic locations. Locally available agricultural biomass will be used for the bioethanol production. Unlike food crops that require high production standards for uniformity, appearance, and safety, energy crops used for bioethanol production mainly need only to produce biomass, thus may be grown on marginal lands with little input and protection from pests. For a given bioethanol production line, the comparison and choice of the feedstock includes several issues: 1) chemical composition of the biomass, 2) cultivation practices, 3) availability of land and land use practices, 4) use of resources, 5) energy balance, 6) emission of greenhouse gases, acidifying gases and ozone depletion gases, 7) absorption of minerals to water and soil, 8) injection of pesticides, 9) soil erosion, 10) contribution to biodiversity and landscape value losses, 11) farm-gate price of the biomass, 12) logistic cost (transport and storage of the biomass), 13) direct economic value of the feedstock taking into account the co-products, 14) creation or maintain of employment and 15) water requirements and water availability [22, 28].

Annual biomass energy potential in Serbia is around 3.2 Mtoe (about 134,000 TJ – terajoules). Of these, about 1.7 Mtoe (about 71,000 TJ) comes from agricultural biomass, while the energy potential of biomass from forestry and wood industries approximately 1.5 Mtoe (about 63,000 TJ) [20]. Serbia, especially province Vojvodina, is very suitable for the production of bioethanol for the following reasons: a high-quality soil; an excellent raw materials for bioethanol production and the possibility of production of alternative raw materials (such as grain sorghum, corn and sorghum, Jerusalem artichokes); a tradition in the production of alcohol; a tradition in the production of seed varieties and hybrids, an existing professional staff etc. The basic raw materials for the production of bioethanol are agricultural raw materials, and they are connected with the structure of agricultural production in Serbia.

Serbia is a country with a developed agricultural production. It has about 5,092 million ha of agricultural land (0.68 ha per capita), of which 4,218 million ha is arable land (0.56 ha per capita) [20]. According to available arable land per capita, it is above the European average. In the structure of the realized value of the agricultural production 70% comes from the crop field production, and 30 % from the livestock production. In 2012, the production of wheat and corn was 1.63 and 7.2 million ton, respectively [21]. It is estimated that the agriculture and the industry based on agriculture (such as food and feed industry) participate in the gross domestic product (GDP) in the amount close to 40 % [29]. Current production of bioethanol in Serbia is based on molasses (50%) and cereals (50%) [30]. In Serbia, conventional energy crops such as starch-based raw materials are the most suitable and available agricultural raw material which can be used for industrial bioethanol production. However, a growing demand for the corn and wheat on global market are currently increasing their price and make these feedstocks less appropriate. For these reasons, possibilities of using cheaper substrates such as damaged crops and for example, triticale are being investigated [30, 31]. Agricultural production in Serbia in the period 2006-2010 is presented in Table 1 [20].

Table 1 Production of cereals, sugar beet and potato (in thousand tonne) in Serbia in the period 2006-2010 [20]

Agricultural crops	2006	2007	2008	2009	2010
Wheat	2,007	2,864	2,095	2,068	1,630
Corn	6,017	3,905	6,158	6,396	7,207
Barley	311	276	344	303	244
Oat	90	84	96	74	68
Rye	16	15	14	13	10
Sugar beet	3,189	3,206	2,299	2,798	3,325
Potato	646	740	844	898	887

As shown in Table 1, the largest production achieved is corn production of over 7 million ton, which completely satisfied domestic needs of corn for food (estimated domestic needs for corn are only 4-4.5 million ton) [20]. It remains approx. 2 million ton which can be directed to the production of bioethanol instead of its export, and thus decrease significant quantities of imported oil.

Generally, in Serbia there are significant surpluses of corn, wheat and sugar beet. In 2011 surpluses of these raw materials were 2.35 million ton [20]. The surplus of wheat only was 311,000 ton which is enough for the production of 85.4 thousand ton of bioethanol [32]. Although the production of bioethanol on corn is economically more favourable than on wheat, the advantage is that wheat kernels contain autoamylolytic enzymes able to degrade starch contained in the grains and thus could enable an easier pretreatment of the raw material and decrease the consumption of technical enzymes [30, 33].

Even higher autoamylolytical quotient is noticed for triticale (mean value for varieties Oganj, Jutro, Odisej, and NST 21/06 was 97.93%), which is a hybrid of wheat and rye [30]. It is an appropriate plant for Serbian climate which can grow on a land of quite low quality [30, 31]. Triticale shows a number of advantages for the grower. The main distinguishing features are as follows: higher grain yield even in unfavorable conditions, higher test weight, resistance to soil-climatic conditions, tolerance to dryness, tolerance to more acid soils and a lower requirement of nutrient substances. Also, it does not need as much fertilizer when compared to types and varieties providing the same yields [34].

Molasses, as a by-product of sugar production from sugar beet, is also very important feedstock for bioethanol production in Serbia. In 2011, the export of sugar beet molasses was 59,655 ton which could be a great potential for production of bioethanol of 10,880 t (specific conversion rate molasses to bioethanol is 230-310 l/t) [20, 21, 35].

When considering potential biomass for bioethanol production, a special attention should be paid on biomass which could be produced on marginal land. It is estimated that there are about 100 thousands hectares of low quality lands in Serbia which are not appropriate for conventional agricultural cultures, but could be used to cultivate alternative feedstock for bioethanol production such as sorghum, Jerusalem artichoke or triticale, and which could produce 3 million tons of bioethanol per year [21]. Another issue that should be explored is the utilization of wasted crops, damaged cereals or that of lower quality which do not meet the food requirements. According to one global analysis [36], there are about 73.9 Tg of dry wasted crops in the world that could potentially produce 49.1 GL of bioethanol annually. It should be emphasized that the varieties and hybrids of domestic seed selection are greatly appreciated in a foreign market.

Production of first generation bioethanol in Serbia based on available feedstocks taking advantage of surplus of crops and use of marginal land, is presented in Table 2. As shown in Table 2, there is great potential for bioethanol production in Serbia of $1{,}135{,}784 \cdot 10^3$ liters, ie. 896,134 ton in 2010. If it is calculated in lge (liter of gasoline equivalent) or toe (tons of oil equivalent) units it is 726,902 10^3 lge ie. 573,526 toe. Estimation of the amount of bioethanol that could be produced in Serbia in the future (from 2015 to 2030) was performed according to the methodology used by the International Energy Agency - IEA [37]. The agency in its regional and global estimates of energy potentials used data recommended from FAO that assume global annual crop growth of about 1.3% and about the same global growth of agricultural biomass (waste). For Serbia, this analysis assumed slightly lower amount of crop growth of 0.9%.

Similar analysis has been performed to estimate the country potential for the production of second generation of bioethanol and is presented in Table 3. For this purpose a recomended conversion factor of lignocellulosic wastes to ethanol of 214 lge/t_{dm} was taken in consideration [37]. The analysis was performed for two possible scenarios, the first for the possible utilization of 10% of the lignocellulosic biomass for the production of bioethanol and the second for the utilization of 25% of the biomass for the production of bioethanol. As shown in Table 3, a significant amount of $739{,}270 \cdot 10^3$ lge that corresponds to 583,480 toe of bioethanol could be produced on lignocellulose in Serbia by 2030.

This amount could be even enlarged by planting of fast growing energy crops such as *Miscanthus* x *giganteus,* willow etc. [38].

Table 2 Production of first generation bioethanol in Serbia in 2010 and prediction for period 2015-2030.

Feedstock		Yield,t/ha	Amount, t	Bioethanol, 10^3 l	Bioethanol [e], t
Molasses [a]		-	100,000	31,675	25,000
Corn [b]		5,5	2,000,000	700,000	552,300
Wheat [b]		3,5	500,000	170,000	134,130
Sugar beet which is used for production of surplus of sugar [b,c]		50	1,460,000	116,800	92,155
Feedstocks on marginal land 100,000 ha	Triticale	3,4	340,000	112,200	88,525
	Sorghum	3,6	360,000	122,400	96,573
	Triticale/Sorghum [d]	-	-	117,300	92,549
Total bioethanol production, year	2010			1,135,784	896,134
	2015			1,188,030	937,356
	2020			1,242,679	980,474
	2025			1,299,842	1,025,576
	2030			1,359,635	1,072,752

[a] statistic for 2010 [20]
[b] surpluses of corn, wheat and sugar according to data of Chamber of Commerce and Industry of Serbia [29]
[c] taking into account the current structure of agricultural production it is calculated how much ton of sugar beet that is used for production of surplus of sugar of 200,000 ton, can be redirected for bioethanol production (statistically 7.3 t of sugar beet is used for production of 1 kg of sugar)
[d]- it is estimated that triticale and sorghum are cultivated on marginal land in the ratio 50:50
[e]- 1t~$1.267 \cdot 10^3$ l; density of alcohol is ρ=0.789 g/cm^3

Table 3 Potential for second generation bioethanol production in Serbia in 2010 and prediction for period 2015-2030.

Feedstock		Bioethanol production if 10% of the biomass is utilized, 10^3 lge	Bioethanol production if 25% of the biomass is utilized, 10^3 lge
Biomass from agricultural residues [a]		225,583	563,958
Biomass from forestry [b]		21,439	53,597
Year	2010	247,022	617,555
	2015	258,385	645,962
	2020	270,271	675,678
	2025	282,703	706,758
	2030	295,708	739,270

[a] calculated on the basis of annual productions of agricultural crops and recommended ratios of crop to residue by IEA [37]
[b] calculated on the basis of forest residues of ~ 3.3 million m^3 [39]

4. Bioethanol production from corn, wheat and triticale

4.1 Selection of corn hybrids for bioethanol production

Today, there is a great interest in the development and cultivation of corn hybrids that can obtain higher bioethanol yields. In that manner, corn hybrids with higher content of fermentable sugars are developed. In America, two great companies "Pioneer" and "Monsanto" make efforts to identify and develop new corn hybrids, examine the impact of the environment on their growth, and the impact of corn varieties on the composition of useful by-products. Both companies have commercial corn varieties specifically cultivated for use in bioethanol production with obtained ethanol yield up to 4% higher than with conventional varieties (for the annual production of bioethanol of 150 million litres it means annual profit increase of 1-2 million US dollar). Further research in the world should result in modification of properties of starch and other complex carbohydrates by methods of genetic engineering.

Six domestic corn hybrids (ZP-434, ZP-633, ZP-611k, ZP-74b, ZP-704wx and ZP-Rumenka) of different genetic background produced in Corn Research Institute “Zemun Polje”, Belgrade, Serbia were tested for bioethanol production at the Faculty of Technology and Metallurgy, Belgrade, Serbia [40]. Dry matter of kernels varied between 866.4 and 914.0 g/kg. Hybrid ZP 74b had the highest starch content of 749.4 g/kg on dry matter basis (DM) and ZP Rumenka the lowest, only 653.80 g/kg DM. Protein content ranged from 91.2 to 132.5 g/kg DM. Oil content ranged from 53.6 to 70.8 and ash from 12.1 to 15.8 g/kg DM. Highest cellulose content was determined in hybrid ZP 74b. Fermentation efficiency of the studied hybrids represented as a percentage of theoretical bioethanol yields after 48 h of fermentation is presented in Fig. 2. The highest bioethanol fermentation efficiency of 94.5% was obtained on ZP 434 hybrid. This hybrid showed best fermentative characteristics since the maximum ethanol concentration of 96.40±0.70 g/l, the bioethanol yield of 0.535±0.004 g/g, the volumetric productivity of 2.01±0.01 g/l·h and the glucose concentration of 3.22±0.30 g/l were achieved after 48 h of fermentation [40, 41].

Beside the fermentation efficiency, an important issue in the crop evaluation for bioethanol production is land requirement, e.g. bioethanol yield which can be produced per land area. Among tested hybrids, ZP 434 gave the highest yield of bioethanol per area of 5783.4 t/ha or 7330 l/ha which is rather higher than average bioethanol yield per area of 6600 l/ha reported in the literature [22, 40].

It was found that one of the most important factors that could influence bioethanol yield on corn was the amount of hard (vitreous) and soft (floury) endosperm in kernel. As reported by Wang et al. [42] soft endosperm resulted in higher final bioethanol concentrations compared to ground corn and hard endosperm. Larger amount of soft fraction of the endosperm enables easier decomposition of the starch granules during enzymatic hydrolysis, leading to a higher bioethanol yield. In accordance with these facts, among six tested hybrids the lowest amount of hard fraction (566.9±10.1 g/kg DM basis) and therefore the highest amount of soft endosperm fraction (433.1±6.7 g/kg DM basis) was determined in hybrid ZP 433. This makes it a very good candidate for the industrial utilization for bioetahnol production [40, 41].

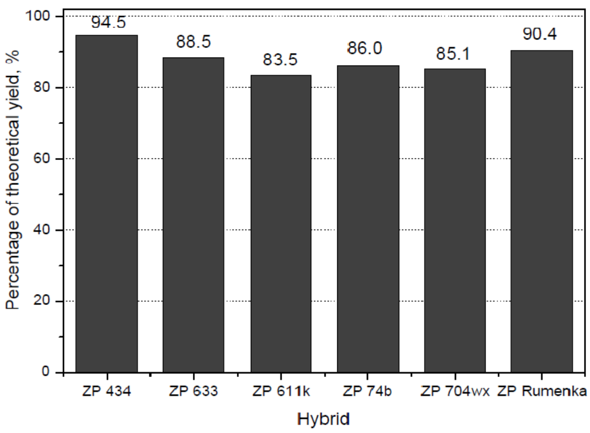

Fig. 2 Percentage of the theoretical bioethanol yield after 48 h of fermentation by six different corn hybrids.

4.2 Utilization of damaged or wasted wheat for bioethanol production

One of the possibilities to improve the economy of bioethanol production is to use damaged cereals or that of lower quality which do not meet the food requirements. Pejin et al. [43] explored the possibilities of using a domestic wheat variety Kantata for the bioethanol production. This wheat variety, obtained from the localities Kovin, Zrenjanin, Pančevo and Vrbas, was unsuitable in bread production. However, bioethanol yields higher or close to 40% (g bioethanol/100g of dry matter) were achieved by using this wheat variety in bioethanol production, as presented in Table 4. The obtained bioethanol yield varied with the enzymatic liquefaction temperature (70-90 °C) and duration (30 min and 1 h). The highest yield of 43.4% was achieved when the liquefaction was conducted at 80 °C for 30 min for wheat from Vrbas locality and at 85 °C for 1 h for wheat from Pančevo locality. Liqufaction at 90 °C did not increase bioethanol yield. Also, liquefaction at 90 °C is not justified because of high-energy consumption.

Table 4 Bioethanol yield obtained from wheat variety Kantata from various localities and at different enzymatic liquefaction temperature and duration.

Temperature, °C	Bioethanol yield, g bioethanol/100g of dry matter							
	Kovin		Zrenjanin		Pančevo		Vrbas	
	30 min	1 h	30 min	1h	30 min	1 h	30 min	1h
70	38.8	40.9	41.9	40.6	39.5	39.4	39.5	41.2
80	41.9	41.4	40.0	40.8	41.2	41.2	43.4	41.0
85	41.2	42.1	41.2	40.6	39.9	43.4	41.2	40.3
90	40.6	41.4	40.2	40.6	41.1	41.5	40.8	40.5

4.3 Utilization of microwave and ultrasound pretreatments in bioethanol production

The application of ultrasound or microwaves in the field of biorenewables is a relatively new concept and has a potential as a pretreatment method to increase the conversion of starch materials to glucose as well as overall bioethanol yield [44-46]. Besides being considered as a crucial step in the biological conversion to bioethanol, biomass pretreatment represents one of the main economic costs in the process [47]. Many recent studies have shown that ultrasound or microwaves pretreatments influence the process of swelling and gelatinization of corn starch granules and thus could be very efficient in destroying the corn starch crystalline arrangement and obtaining a soft gel. These phenomena could also enhance the enzyme susceptibility needed for the efficient hydrolysis, which may later on improve the outcome of bioethanol fermentation. The important parameters that should be optimized when applying these treatments are temperature, power, the length of treatment and its dynamics. Since these treatments may be energy consuming, the obtained benefits should be considered and compared with the increased production costs [44-49].

The changes in physical structure of control (without pretreatment) and pretreated samples of corn meal suspensions before liquefaction, were imaged by scanning electron microscope (SEM), as presented in Fig. 3. The SEM images show that both ultrasound and microwaves affected the decomposition of starch granules even before the liquefaction started which consequently enhancing enzyme hydrolysis. The conventional heating observed in the control sample caused less change in structure [50].

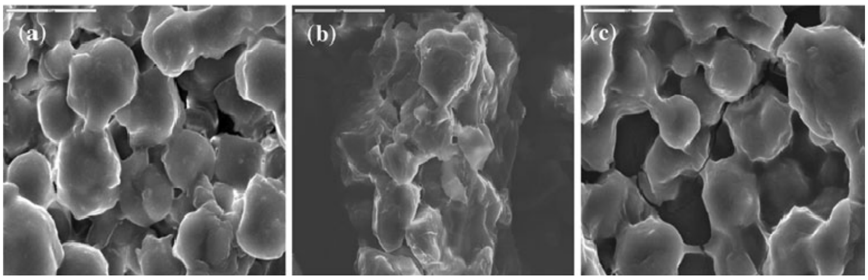

Fig. 3 SEM images of samples of corn meal suspensions: a) control sample (without pretreatment) before liquefaction, b) ultrasound pretreated sample before liquefaction, c) microwave pretreated sample before liquefaction. The length of the scale bar is equivalent to 20 µm (magnification 2000×).

Table 5 compares the effects of the microwave and ultrasound pretreatment achieved after 32 h of the SSF (simultaneous saccharification and fermentation) process of corn meal hydrolyzates by *Saccharoymces cerevisiae* var. *ellipsoideus* under optimized conditions previously determined in the study of Mojovic et al. [51]. The results indicate that the ultrasound and microwave pretreatments increased maximum bioethanol concentration by 11.15% and 13.40% (compared to the control sample), respectively, and consequently increased the bioethanol yield as well as other process parameters. Bioethanol concentration of 9.87% (w/w), bioethanol yield of 0.52 g/g, percentage of theoretical bioethanol yield of 90.80% and volumetric productivity of 3.08 g/(l·h) were achieved after 32 h of SSF process on corn meal with prior microwave treatment (Table 5). The improvement in bioethanol production obtained in pretreated samples could be attributed primarily to the effect of ultrasound and microwaves on disintegration of corn starch granules, acceleration of starch hydrolysis, and enhanced release of fermentable sugars and thereby increased bioethanol productivity [44, 52-55]. It should be noted that a critical assessment of the costs and benefit analysis is needed before an industrial application which should take into account the initial capital investment and operation cost of ultrasound and microwave pretreatments as well as the benefits achieved by these treatments.

Table 5 Values of the significant parameters obtained after 32 h of the SSF process of corn meal hydrolyzates by *Saccharomyces cerevisiae* var. *ellipsoideus* with and without the ultrasound and microwave pretreatments. Both pretreatments were performed within 5 min before addition of the liquefying enzyme. Presented data are expressed as the mean value and standard deviation from three independent experiments.

Process parameter	SSF process without pretreatment (control)	SSF process with ultrasound pretreatment	SSF process with microwave pretreatment
Biothanol concentration, % w/w	8.70±0.09	9.67±0.11	9.87±0.10
Biothanol yield $Y_{P/S}$,g bioethanol/g starch	0.450±0.005	0.500±0.006	0.510±0.005
Percentage of the theoretical biothanol yield[a], %	80.04±0.83	88.96±1.01	90.80±0.92
Volumetric productivity P, g/l·h	2.72±0.03	3.02±0.03	3.08±0.03
Utilized glucose [b],%	77.33±0.44	85.22±0.56	88.40±0.48

[a] Percentage of the theoretical bioethanol yield was calculated as the ratio between actual bioethanol yield and theoretical bioethanol yield, assuming all starch was converted to glucose and then to bioethanol.
[b] Utilized glucose (%) was calculated as the ratio of the consumed mass of glucose to initial mass of glucose.

Pejin et al. [32] investigated the possibilities of increasing fermentable sugars and bioethanol yields by applying an ultrasound pretreatment in bioethanol production by simultaneous saccharification and fermentation of triticale meal with *Saccharomyces cerevisiae* yeast in a batch system. In this study the process was conducted without the addition of external amylolytic enzymes, and the liquefaction and saccharification of starch was performed only by enzymes natively present in triticale grain. The ability of ultrasound pretreatment to disintegrate triticale starch granules and therefore improve the release of fermentable sugars was studied at different sonication temperatures. The ultrasound pretreatment was performed under the following conditions: 5 min, at 40, 50 or 60°C. Glucose and maltose contents in triticale meal suspensions increased with ultrasonic treatment compared with the untreated control sample, at all temperatures investigated. Increase in temperature of ultrasound pretreatment caused glucose and maltose content to increase. Glucose content at 40°C was 12.30% of dry matter higher in the ultrasound-pretreated sample than in the control sample, while at 60°C glucose content was 15.71% of dry matter higher in the ultrasound-pretreated sample than in the control sample. Maltose content at 40°C increased by 46.67% of dry matter in the ultrasound-pretreated sample compared with the control sample, while at 60°C maltose content increased by 52.57% of dry matter. During the SSF process the bioethanol contents obtained in ultrasound pretreated samples were higher than in the control sample, especially in the sample ultrasound pretreated at 60°C. In the sample ultrasound pretreated at 60ºC, a maximum bioethanol content of 9.55% (w/v) was achieved after 48 h of the SSF process. In this case, the increase in bioethanol content was 21.99% compared with the control sample after 48 h, and 10.57% after 72 h.

Bioethanol contents and the percentage of the theoretical bioethanol yield obtained after the fermentation of samples of triticale meal suspensions without and with an ultrasound treatment at 40, 50, or 60°C are given in Table 6. Bioethanol content increased by ultrasonic pretreatment compared to the untreated control sample, especially with the temperature increase. Temperature increase from 40 to 60°C caused bioethanol content increase by 15.02%. Application of ultrasound at 40°C increased bioethanol content by 8.16% compared to the control sample, while at 60°C bioethanol content increased by 10.89% compared to the control sample. The highest bioethanol contents were achieved in the ultrasound treated samples at 60°C (28.61 g/100g of dry matter). The results show that the temperature of ultrasound treatment significantly affects bioethanol yield and the percentage of theoretical bioethanol yield. This increase in bioethanol yield with the application of ultrasound is very important in bioethanol technology.

Table 6 Effect of temperature of ultrasound pretreatment on the bioethanol content (g/100g of dry matter) and the percentage of the theoretical bioethanol yield obtained after the fermentation of samples of triticale meal suspensions. The ultrasound pretreatment was performed at 40, 50, or 60°C, and with frequency of 40 kHz. Experimental conditions for fermentation: 30°C, 72 hours. Presented data are expressed as the mean value and standard deviation from three independent experiments.

Temperature, °C	Pretreatment conditions	Bioethanol content, g/100g of dry matter	Percentage of the theoretical bioethanol yield,%
40	Without ultrasound	22.43±0.21	66.29±0.62
	With ultrasound	24.26±0.23	71.68±0.67
50	Without ultrasound	24.10±0.17	71.23±0.51
	With ultrasound	26.08±0.19	77.08±0.56
60	Without ultrasound	25.80±0.18	76.23±0.53
	With ultrasound	28.61±0.24	84.56±0.70

4.4 Utilization of stillage as a by-product from bioetahnol production on corn

Stillage is one of the major by-products of bioethanol production, besides carbon dioxide. An average stillage amount produced in the bioethanol process is approximately 13 hl per hl of bioethanol [56]. There are many possibilities for valorization of the stillage from bioethanol processing. Some of them are the stillage recirculation and reuse [57-59], production of soil fertilizers [57], the production of various types of animal feed [60-62] and anaerobic fermentations for the production of lactic acid or butanol [63-65]. Some economical analysis have shown that the price of the by-products from bioethanol processing can reach up to 30 % of the price of the primary product and thus significantly improve the production economy [40].

In the majority of industrial facilities in Serbia, the bioethanol by-products have not been utilized posing therefore a serious environmental problem. The complex composition of stillage causes high BOD_5 values which range from 15–340 g/l [63]. Physical, chemical and nutritive characteristics of stillage are highly variable and dependent on the raw materials and various aspects of the bioethanol production process [66]. It has around 7-10 % of dry matter that originates from the grains used as a raw material. Although the most of the carbohydrates and sugars from the crops are utilized by yeast for the production of bioethanol, CO_2 and other volatile compounds, one small part between 2-3% remains in the stillage as unusable. Besides non-converted substances of the raw materials used, the stillage also contains all products of yeast fermentation such as the complex of B vitamins and growth supporting compounds [67].

One of the interesting, but quite unexplored possibilities of utilization of the liquid (thin) stillage is for the production of lactic acid [64, 68, 69]. Thin stillage is a liquid part of the fermentation mash remaining after the distillation of the bioethanol. The stillage, as an inexpensive feedstock, can be a valuable source of nutrients for growth of lactic acid bacteria. Fermentative sugar content is not high because of the previous alcoholic fermentation; however, the stillage is a good source of nitrogen, vitamins and minerals which originate both from the feedstock and residual yeast. This kind of stillage utilization is particularly supported by the world growing demand for lactic acid due to its versatile and increasing utilization in chemical, food, pharmaceutical, cosmetic and polymer industries [67]. Serbia itself imports lactic acid and this approach would be of great interest from the economical point of view.

Table 7 compares nine tested lactic acid bacteria regarding significant parameters achieved during lactic acid fermentation on corn thin stillage such as: the yield on the substrate (Y), volumetric productivity (P), substrate conversion and growth characteristics (number of cells). As shown in Table 7, the highest yield of 0.76 g/g and the productivity of 0.24 g/l·h were obtained with the strain *Lb. paracasei ssp. paracasei* NRRL B- 4564. After 72 h, this strain produced 17.37 g/l of lactic acid and consumed 72.90% of the sugars present in the stillage. The results showed that the strain *Lb. casei ssp. casei* NRRL B- 441 was also good candidate for lactic acid production on corn stillage, although it achieved to some extent lower yield, productivity and the substrate conversion. It is also interesting to note that the bacterial growth and lactic acid production were not in strict proportion e.g. these two strains which exhibited the best lactic acid productivity did not show the highest viable cell number at the end of fermentation. This could be partially due to different initial numbers of cells, but also due to differences in lactic acid production abilities and growth characteristics.

Table 7 Comparison of different strains of *Lactobacillus* sp. according to significant parameters obtained at the end of lactic acid fermentation (72 hours)

Species of *Lactobacillus*	Y, g/g	P,g/l·h	Substrate conversion,%	Number of cells, logN
Lb. paracasei ssp. paracasei NRRL B- 4564	0.76	0.24	72.90	8.15
Lb. casei ssp. casei NRRL B- 441	0.61	0.20	60.93	7,44
Lb. pentosus NRRL B- 227	0.60	0.19	49.21	8.90
Lb. rhamnosus ATCC 7469	0.55	0.17	61.91	7.79
Lb. acidophilus ATCC 4356	0.47	0.15	38.96	8.22
Lb. plantarum PL-4	0.41	0.13	24.78	8.26
Lb. helveticus ATCC 15009	0.33	0.10	35.05	4.90
Lb. fermentum NRRL B-75624	0.27	0.09	48.86	6.19
Lb. fermentum PL-1	0.24	0.08	38.24	8.69

Although the yields of lactic acid produced on the liquid stillage are lower than the yields reported on common substrates such as cheese whey, barley starch, beet molasses, sugar cane etc. [70-72], they are of great value considering the fact that the production is realised on the agro-industrial waste substrate with a serious negative environmental impact. Unsolved problem of the stillage disposal and domination of a starch based bioethanol production in Serbia made its utilization for lactic acid and/or probiotic biomass production a favourable approach. However, one of the major problems of the lactic acid production on liquid stillage could be a variability of its chemical composition, which should be regularly controlled.

Pejin et al. investigated the possibility of thin stillage recirculation in the mashing process during the production of bioethanol from corn [73]. In order to investigate the efficiency of the thin stillage recirculation to the mashing phase, different amounts of stillage were added. In this work, six cycles of mashing were performed in such a way that the stillage obtained after the first cycle was used in the second mashing and the stillage after the second cycle was used in the third mashing, etc. Various process parameters such as fermentation rate, bioethanol yield, percentage of the theoretical bioethanol yield, and the content of solids in stillage after distillation were evaluated. It was shown that as the amount of recirculated stillage increased (from 10 to 30%), higher bioethanol yields and starch utilization efficiencies were observed. The bioethanol yield was increased from 97% to more than 100% (Fig. 4). Yields higher than 100% could be explained by the fact that stillage enriched the slurry with surplus of products of carbohydrate (organic acids), amino acids, vitamins and yeast cells (phosphates) degradation. Yeast cells can utilize organic and amino acids as C-sources and N-sources.

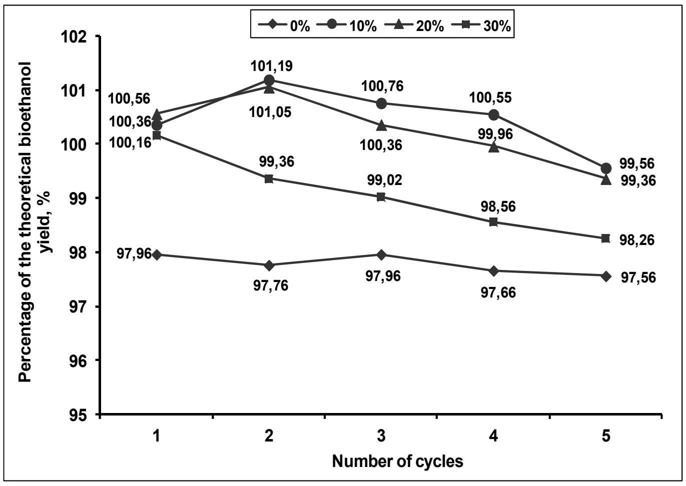

Fig. 4 Percentage of the theoretical bioethanol yield in fermentations with different quantity of recycled stillage

Analyzing the bioethanol yields after the third cycle, it could be concluded that stillage recirculation did not adversely affect the bioethanol yields. The yields after the fourth and fifth cycles were higher than the average (>95%). Recirculation of 20 and 30% of stillage in the sixth cycle lowered the bioethanol yields as compared to the previous cycles, but higher yields than the average were maintained. So far, it could be concluded that even the addition of 30% of recirculated stillage to the mashing phase did not negatively affect the bioethanol production. Gumienna et al. [59] showed that the 75% addition of thin stillage to the mashing process instead of process water and 40 recirculation cycles did not negatively affect the bioethanol production from triticale.

The dry matter content in the slurry after the fermentation also increased with the increasing amount of the recirculated stillage. The dry matter remaining after filtration of the slurry could be used as a cattle feed because of its high total protein content. As the amount of recirculated stillage increased (from 10 to 30%) higher protein content was achieved (1.5% increase). This study suggests that the recirculation of thin stillage should be the first and inexpensive step which should be undertaken in the bioethanol production process in order to decrease water consumption, and also the amount of the generated wastewater (e.g. thin stillage). In addition, the recirculation can result in higher bioethanol yield and thus improved production economy.

4.5 Some economical evaluations

The economy of bioethanol production was analyzed in order to decrease the production costs and make this biofuel competitive with fossil fuels in the study of Denčić et al. [74]. In this study cost of biotehanol production from three crops that can be cultivated in Serbia: corn, wheat, and triticale, was compared. The total costs of triticale production in Serbia are by 26% lower compared to those of wheat and corn. The first stage in bioethanol production is milling of the raw material. The energy consumed in the milling of triticale and wheat was by 32% lower compared to that of corn, which is in concordance with the findings of Offer and Haldenwanger [75]. Next phase in the production of bioethanol is the thermal pretreatment of the milled material. The corresponding costs consist of the costs of purchasing the appropriate enzymes for degradation of the starch contained in the wheat and corn samples and costs with the energy consumed in the thermal degradation of all the investigated raw materials. The triticale varieties did not require the use of enzymes since their kernel contains higher amounts of amylolytic enzymes [34, 30]. Thermal degradation in the process of bioethanol production is realized by heating the mixture of milled raw material with water to the given temperature. The thermal pretreatment temperature depends on the nature of the raw material, and it was 60°C for triticale, 65°C for wheat, and 95°C for corn [76]. The amount of energy needed for thermal pretreatment of triticale was by 44.43% lower compared to corn. The results of the economic analysis showed that the expenses of thermal pretreatment in bioethanol production from triticale were the lowest, somewhat higher from wheat, and the highest from corn. On a cost per litre of bioethanol produced basis triticale was calculated to be the most efficient crop [74, 77].

5. Conclusions

Energy systems today need to be renewable, sustainable, efficient and cost-effective, convenient and safe. One of the solutions is the use of biofuels such as bioethanol. The economy of bioethanol production should be analyzed and improved in order to decrease the production costs and make this biofuel competitive with fossil fuels. In this view, the choice of suitable and abundant raw material and appropriate production approach are of great importance since the feedstock cost represents a major part of the production cost. Utilization of waste crops or by-products from bioethanol production for the production of animal feed and lactic acid can significantly improve the economy of bioethanol production.

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