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Bioethanol production from triticale by simultaneous saccharification and fermentation with magnesium or calcium ions addition

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HIGHLIGHTS

- The effect of magnesium or calcium ions in triticale mashes on bioethanol yield.
- When 160 mg/L of magnesium ions were added bioethanol content increased by 31.22%.
- When 160 mg/L of calcium ions were added bioethanol content increased by 21.04%.
- Magnesium ions had more significant effect on bioethanol yield than calcium ions.
- When magnesium ions are added there is no need to use commercial enzymes.

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ABSTRACT

The aim of this study was to determine the effect of magnesium or calcium ions content in triticale mashes on glucose and maltose content after liquefaction as well as on bioethanol yield after fermentation. Triticale variety Odyssey was used in this study. Liquefaction and saccharification in this study were performed without using any additional saccharifying enzymes, i.e. the triticale starch was hydrolyzed only by the enzymes present in triticale grain. Glucose and maltose content increased with the increase of magnesium and calcium ion content in mash. Glucose and maltose content increased by 30.16% and 9.58%, respectively, when 160 mg/L of magnesium ions were added, compared to the control sample. Glucose and maltose content increased by 69.31% and 61.66%, respectively, when 160 mg/L of calcium ions were added, compared to the control sample. According to the obtained results for glucose and maltose content increase during liquefaction, the supplementation of mashes with calcium ions had greater influence on the activity of triticale's amylases than the supplementation of mashes with magnesium ions. The present investigation shows that magnesium and calcium ions addition to triticale mashes improved bioethanol production during SSF processing. When 160 mg/L of magnesium ions were added bioethanol content increased by 31.22% compared to the control sample while when 160 mg/L of calcium ions were added bioethanol content increased by 21.04%. High percentage of the theoretical bioethanol yield (92.19%) was achieved after fermentation when 160 mg/L of magnesium ions were added to triticale mash. The obtained results show that the addition of magnesium and calcium ions in bioethanol production from triticale increase triticale's amylase activity as well as yeast enzyme activity. All this shows that when triticale with high amylolytic enzymes activity is used in bioethanol production with the addition of magnesium ions there is no need to use commercial enzymes in starch hydrolysis, which makes the use of triticale as a raw material for bioethanol production more economical.

1. Introduction

Bioethanol is regarded as a promising alternative energy source, which is both renewable and environmentally friendly. During bioethanol production, the composition of media affects the

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physiological state and, consequently, the fermentation performance of the microorganism employed [1]. Bioethanol is produced by fermentation of sugar, starch or cellulosic biomass and its utilization can significantly reduce fossil fuels use. It is expected to be one of the dominating renewable biofuels in the transportation sector within the twenty years to come [2]. The production of bioethanol is increasing over the years, and has reached the level of 85.2 billion litres in the year 2012 [3]. The governmental supports for the substitution of fossil fuels with bioethanol produced from biomass is predicted to result in global production of $125 \cdot 10^9$ L of bioethanol by 2020 [4]. The primary beneficial aspects of fermenting biomass-derived sugars to bioethanol as a fuel source is that it can be produced from renewable plant material that is able to photosynthetically re-fix CO_2 produced during bioethanol production and combustion [5]. Bioethanol production has remarkably increased because many countries look for reducing oil imports, boosting rural economies and improving the air quality [2]. One major problem with bioethanol production is the availability of raw materials for the production. There are several criteria for choosing raw materials for bioethanol production: price and yield of raw material, bioethanol yield, starch content, pest and diseases resistance, suitability for soil and weather conditions, harvesting transportation and storage options as well as the usability of by-products [6]. The availability of feedstocks for bioethanol production can vary considerably from season to season and depends on geographic locations [7]. However, feedstocks for bioethanol production must be sustainable and must not threaten biodiversity or food security [5].

Yeast strains of *Saccharomyces cerevisiae* have been extensively studied in recent years for fuel bioethanol production, in which yeast cells are exposed to various stresses such as high temperature, bioethanol inhibition, and osmotic pressure from product and substrate sugars and so on [8].

Triticale is a cereal crop adapted to less favorable soil conditions. It is suitable for low input farming because of lower demands on pesticides application [9]. Today, it has been reported that triticale is cultivated in more than 30 countries worldwide [10] on around 3.7 million ha in total, yielding more than 12 million tonnes a year [11]. Modern triticale varieties have been found to be very competitive as a feedstock for bioethanol production [12]. Triticale crops have a high yield potential as well as a high starch content, together with a low content of soluble polysaccharides and proteins, and is therefore considered to be ideal for bioethanol production [13]. There is high activity of triticale's own amylolytic enzymes, mainly α-amylase, and this is crucial in starch saccharification [14,15]. Considering the currently prevalent "cold" technique of saccharification, by means of commercial enzymes, the processing of triticale is economically beneficial as it enables the reduction of the commercial enzymes consumption [15]. In our previous research [16,17] it was shown that the addition of commercial enzymes was not necessary during liquefaction and saccharification step in bioethanol production from triticale variety Odyssey. Cereal α-amylases are known to be metalloenzymes. Is has been shown that these enzymes contain covalently bound calcium ions which act as an allosteric activator. Besides calcium ions, magnesium ions can also act as α-amylases activator. Studies on barley α-amylase show that these ions, especially calcium ion help in maintaining the three-dimensional structure of amylases [18].

The mineral metabolism of yeast is of interest to bioethanol producers looking to improve yields, increase fermentative capacity, and maintain consistency of product quality [19]. Metal ions especially divalent cations are necessary for the activation of several glycolytic enzymes and, in practical terms, if industrial media is deficient in them, the conversion of sugar to bioethanol may be suppressed leading to slow or incomplete fermentation process [20]. Magnesium is involved in many essential physiological and biochemical functions in yeast cells, including growth, cell division, enzyme activation, stimulation of synthesis of essential fatty acids, regulation of cellular ionic levels, and maintaining membrane integrity and permeability. Yeasts have a very high growth demand for magnesium ions, and magnesium accumulation by yeast correlates closely with the progress of fermentation [21,22]. Magnesium also plays roles in protecting yeast cells against environmental stresses during fermentation such as caused by bioethanol, high temperature, or high osmotic pressure [23]. The rate of uptake and utilization of metal ions by the yeast biomass depends both on the ion content in the medium, as well as on its bioavailability. It has been established that accumulation and release of metal ions during ethanolic fermentation is a dynamic process and that the intensity depends on the sugar and bioethanol content in the fermentation medium [24]. Calcium is not a requirement but may stimulate cell growth, protects yeast cell membrane structure and helps maintain membrane permeability under adverse conditions [22]. Calcium, being actively excluded from the yeast cell, acts mainly extracellularly for example, calcium is essential for amylase activity [25]. Metal ion deficiencies often occur in fermentation media, and studies on optimization of metal ions combinations are thus of great practical importance to improve bioethanol production [1,26].

The aim of this study was to determine the effect of magnesium or calcium ions content in triticale mashes on glucose and maltose content after liquefaction as well as on bioethanol yield after fermentation. Triticale variety Odyssey, from experimental fields, Rimski Šančevi location (Serbia) was used in this study. The effect of magnesium and calcium ions content in triticale mashes on glucose and maltose content after liquefaction and on bioethanol yield after fermentation was investigated by adding different amounts of $MgSO_4{\cdot}7H_2O$ or $CaCl_2$ solution in triticale mashes before liquefaction and saccharification. In this study the process was conducted without the addition of external amylolytic enzymes, and the liquefaction and saccharification of starch were performed only by enzymes present in triticale grain. The bioethanol yield and productivity were also assessed.

2. Materials and methods

2.1. Materials

Triticale variety Odyssey was obtained from Institute of Field and Vegetable Crops – Novi Sad (Serbia). Triticale was milled in a dry conical mill (Miag-Braunschweig, Germany) type: DOXY 71 b/4, mill motor power 0.22 kW, at 1375 r/min. The granulation of triticale meals was determined by sieving 100 g of milled sample for 3 min on the set of sieves with the following opening widths: 1000, 700, 500, 250 and 132 μm on Bühler MLU 300 sieve.

2.2. Yeast strain

Instant dry active baker's yeast *S. cerevisiae* provided from Alltech Fermin, Senta, Serbia was used as a producing microorganism. Prior to each experiment, the yeast was activated according to the following procedure: the yeast was measured and suspended in 0.1% sterile peptone water warmed up to 38 °C. The yeast cell count was determined in Neubauer's counting chamber. From the prepared yeast solution, the amount of inoculum needed to obtain $30–35 \cdot 10^6$ CFU/mL in the fermentation medium, was taken [27]. Indirect counting method, i.e. pour plate technique, was used to determine the number of viable cells. Serial dilutions of the samples were performed, and after the incubation time at 30 °C, colonies grown in Petri dishes were used to count the number of viable cells.