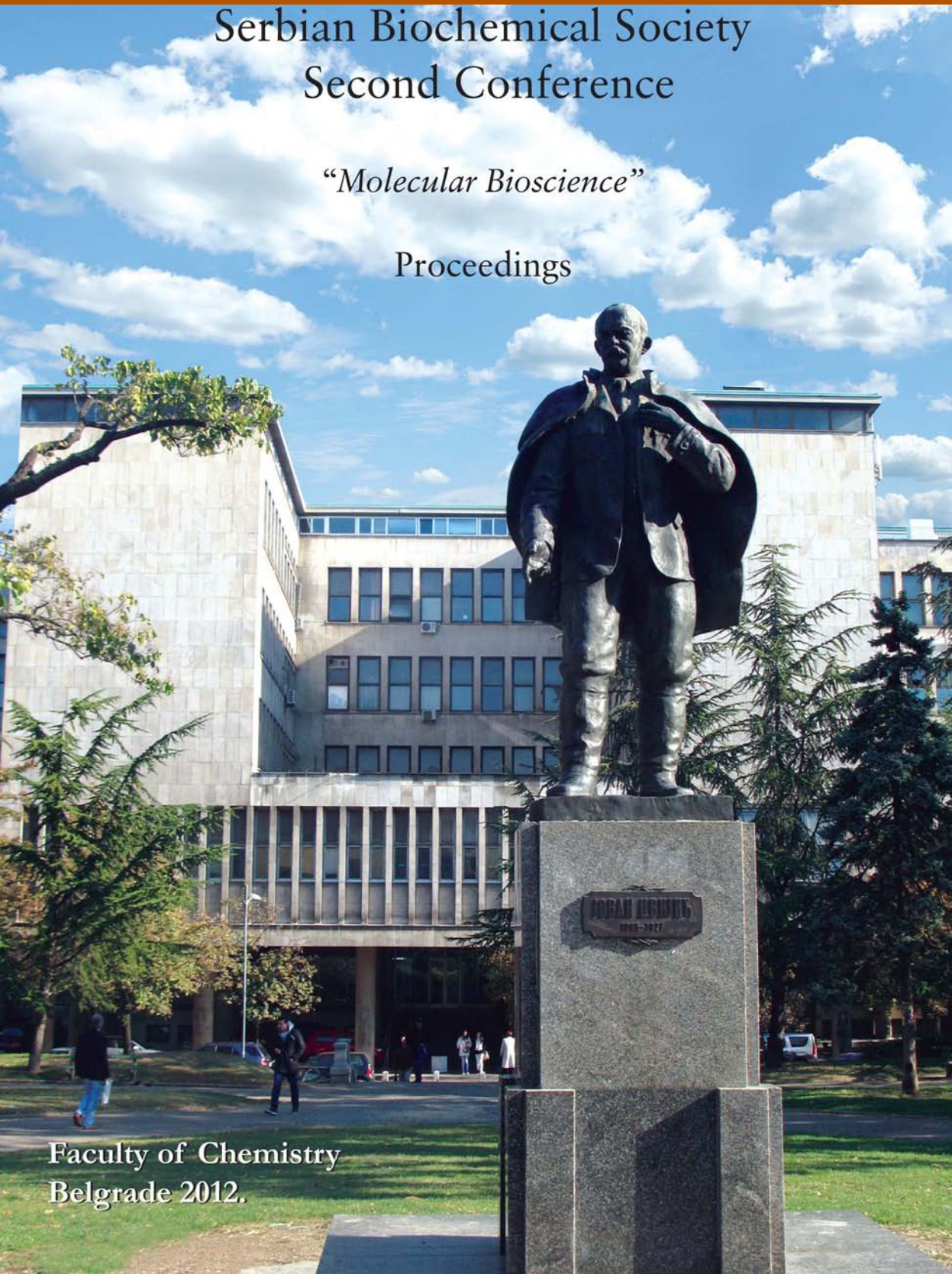
Serbian Biochemical Society
Second Conference

"Molecular Bioscience"

Proceedings



Faculty of Chemistry
Belgrade 2012.

Serbian Biochemical Society

Proceedings

Edited by: Mihajlo B. Spasić
Technical secretary: Jelena Nestorov
Cover and logo design: Aleksandra Nikolić - Kokić
Publisher: Faculty of Chemistry, Serbian Biochemical Society
Printed by: Colorgrafx, Belgrade

Serbian Biochemical Society Second Conference

University of Belgrade, Faculty of Chemistry.
9.11.2012. Belgrade, Serbia.

"*Molecular Bioscience*"

PROGRAM

10.00-10.10 Welcome messages from:

Prof. M. B. Spasi}
(President of the Serbian Biochemical Society)

Prof. B Jovan~i}evi}
(Dean of the Faculty of Chemistry)

10.10-10.55 *FEBS lecture*

Prof. Israel Pecht (FEBS Secretary-General)

Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel

The Type 1 Fce Receptor
A Double -faced Immunoreceptor

10.55-11.10 Discussion

11.10-11.30 Short break

11.30-11.50 Tanja] irkovi} Veli~kovi}, PhD

Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia.

Protein digestion, immunopathologies and health

11.50-12.10 Ivanka Karad`i}, PhD

Department of Chemistry, School of Medicine, University of Belgrade, Belgrade, Serbia.

Topology of proteasomal core particle of *Haloferax volcanii* by chemical cross-linking, mass spectrometry and bioinformatics

12.10-12.30 Edvard T Petri, PhD

Department of Biology and Ecology, University of Novi Sad, Novi Sad, Serbia.

Application of structural biochemistry to the study of mechanisms of ion channel activation

12.30-12.40 Discussion

12.40-13.00 Short break

13.00-13.20 *SCS lecture*

Nata{a Bo`i}, PhD

Centre for Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia.

Cold enzyme hydrolysis of starch

13.20-13.40 Niko S. Radulovi}, PhD

Department of Chemistry, Faculty of Science and Mathematics, University of Ni{, Serbia.

Harnessing the biological activity of natural products: Isolation, synthesis and screening of biological/pharmacological activity

13.40-14.00 Marina Mitrovi}, PhD

Department of Biochemistry, School of Medicine, University of Kragujevac, Kragujevac, Serbia.

Regulation of apoptosis in various experimental models of diseases

14.00-14.30 Break with refreshments provided.

14.30-14.50 Nevena Grdovi}, PhD

Institute for Biological Research “Sini{a Stankovi}” University of Belgrade, Belgrade, Serbia.

CXCL12 and PARP-1 are potential key molecules in the promotion of β-cell survival and diabetes attenuation

14.50-15.10 Aleksandra Stankovi}, PhD

Institute of Nuclear Sciences “Vin~a”, University of Belgrade, Belgrade, Serbia.

Genetic basis of inflammation in human diseases

15.10- Discussion and concluding remarks

Foreword

Dear Colleagues,

It is my great pleasure to wish you warm welcome to the Second Conference entitled “Molecular Bioscience” organized by the Serbian Biochemical Society.

Second Conference of the Serbian Biochemical Society indicate that wish from the foreword of the First Conference “that it is beginning of continual work for many years to come!” have start to be truth. We have invited Secretary General of FEBS to be lecturer and eight from Serbia to present their state of art in the field they work as invitation for further co-operation. Their presentations are published in Proceedings. I express my gratitude to the members of governing board of Serbian Biochemical Society who suggested lecturers and to all of them who accepted invitation.

Editor of the Proceedings
Prof. Mihajlo B.Spasić
President of the
Serbian Biochemical Society

Cold enzyme hydrolysis of starch

Nataša Božić*

* *Centre for Chemistry, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia.*
e-mail: nbozic@chem.bg.ac.rs

With efforts to reduce global reliance on fossil fuels and lower the greenhouse gas emission, an increasing search for renewably sourced materials, which can be used as feedstock for biofuel production, is ongoing in the past few decades. At the present, ethanol is the most common alternate fuel and is already produced on a fair scale, representing a sustainable substitute for gasoline in passenger cars. Basically, in the United States ethanol is produced by fermenting starch crops that have been converted into simple sugars, and the major feedstock for this fuel is corn. In Brazil ethanol is produced through the fermentation of sugar cane molasses. Various countries have been increasing their ethanol production as well, such as India (using sugar cane), Thailand (cassava), France (sugar beet), China (corn) and Canada (wheat), among others.
Improved molecular disassembly and depolymerization of grain starch to glucose are key to reducing energy use in the bioconversion of glucose to chemicals, ingredients, and fuels. In fuel ethanol production, these biorefining steps use 10-20% of the energy content of the fuel ethanol. The need to minimize energy use and to raise the net yield of energy can be met by replacing high-temperature, liquid-phase, enzymatic digestion with low temperature, solid-phase, enzymatic digestion. Also called cold hydrolysis, the approach is a step toward a "green" method for the production of fuel ethanol.

Introduction

Starch is the most important carbon and energy source among plant carbohydrates, and it is the second following cellulose in total biosynthesis[1]. Starch represents an inexpensive source for production of glucose, fructose and maltose syrups[2] and for obtaining the products of their fermentation; including food ingredients, biofuels, organic acids and other valuable compounds for industrial applications. Besides agricultural crops, starch is a significant component of domestic and commercial wastes and these could become useful resources to be converted into ethanol.

The disassembly and depolymerization of grain starch to glucose are the result of the hydrolysis of α-1,4- and α-1,6-linkages between glucose monomers. Acid hydrolysis was used for this from its discovery in 1813 at least until the 1970s. However, the dilute acid

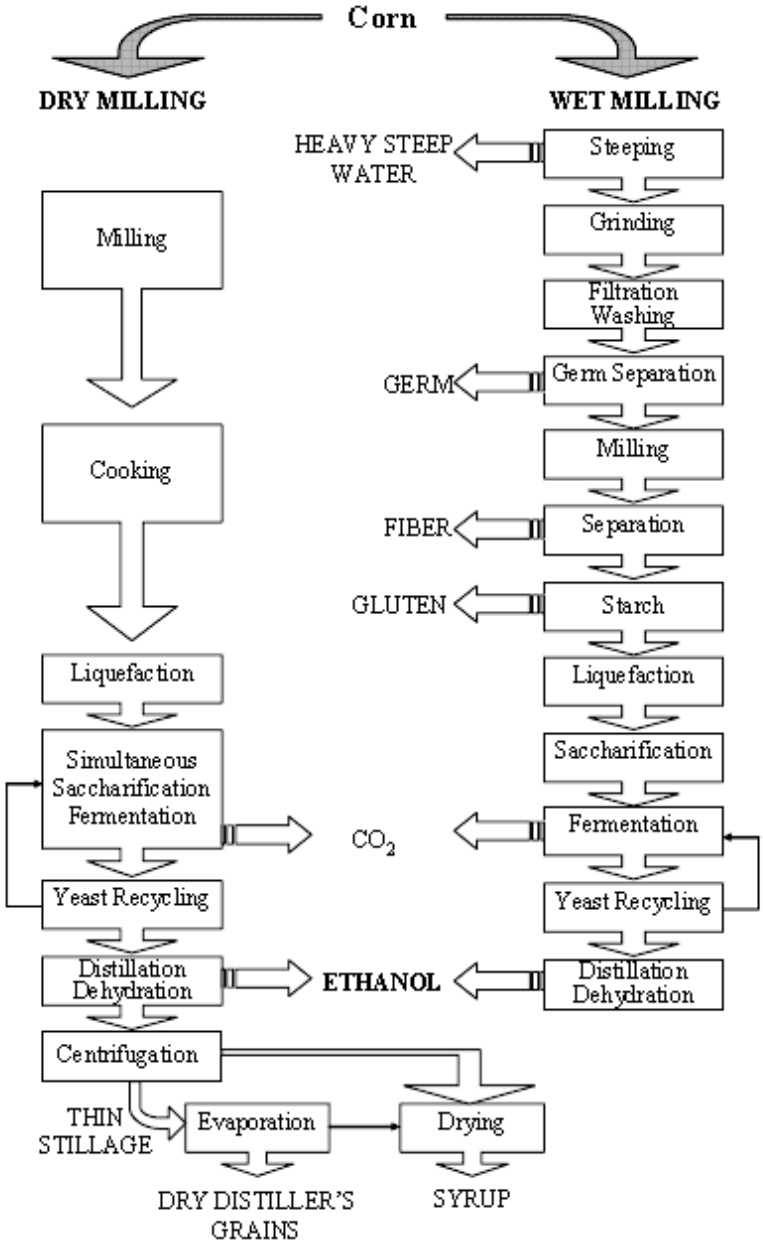


Figure 1.
Representation of dry milling and wet milling processes for bioethanol production[12]

and 120-150°C temperatures used in this process corroded equipment, formed undesirable byproducts, limited yield, and was costly[3-8]. High-temperature, liquid-phase enzymatic hydrolysis is now used for starch hydrolysis. One basic enzymatic hydrolysis configuration is a three-step sequence. In the first step, a 30% (by weight) slurry is cooked in the presence of α-amylase to 90-165 °C, cooled if necessary, held at 90 °C for 1-3 h, and then cooled further to 60 °C with the addition of glucoamylase. An energy-conserving alternative is to lower the starch-to glucose processing temperature below the onset of gelatinization which is for example, 54 °C for wheat, 60 °C for potato, or 65 °C for maize[6]. Regarding energy costs, effective utilization of natural resources, minimization of the formation of pollutants and viscosity (handling) problems, use of raw starch digesting enzymes that can perform direct hydrolysis of raw starch below gelatinization temperature is desirable[9]. The removal of the cooking stage also has the potential to increase the value of the co-products since valuable proteins would undergo less thermal stress[10].

Traditional production processes of ethanol from starch crops

Two different processes can be used to produce ethanol from starch crops: dry grind and wet milling, depicted in Figure 1. In dry grind, the feed material is ground mechanically and cooked in water to gelatinize the starch. Enzymes are then added to break down the starch to form glucose, which yeasts ferment to ethanol. In that case, a fixed amount of ethanol is produced, along with other feed products and carbon dioxide, and has almost no process flexibility. In wet milling, the insoluble protein, oil, fiber, and some solids are removed initially, remaining only the starch slurry fed to the ethanol production step. This process has the capability to produce various end products and considerable higher process flexibility, compared to the dry milling[11]. However, about 65% of the ethanol in the US is produced from dry grind corn processing plants[12], since initial investment in plant is 2 – 5 times cheaper.

Cold hydrolysis of starch

In addition to the most traditional processes for the production of ethanol from starchy materials, a nonconventional technology, named cold hydrolysis, has been investigated. Although the concept is not recent, since it was reported as a consequence of studies during the World War II[13,14], its application at large scale was demonstrated only recently[15,16]. The production of ethanol by cold hydrolysis of starch dispenses some of the steps of high energy demand in a plant, i.e., cooking and liquefaction. In this process, the raw (granular, non-cooked) starch is submitted to an initial hydrolysis step, in the presence of endoamylolytic and proteolytic preparations, so that it becomes more susceptible to saccharification. Unlike energy-demanding steps in traditional processes, the hydrolysis initiates at a temperature below that for the gelatinization of starch, for a few hours. The addition of proteases aims at to improve starch exposure by breaking down proteins associated to its polysaccharides[17,18]. The suspension, still rich in starch, goes to the fermenters, where more enzymes are added, mainly acid glucoamylases which are able to digest raw starch. The yeast is added to the vessel, so that the fermentation starts occurring simultaneously to the saccharification[19], Figure 2.

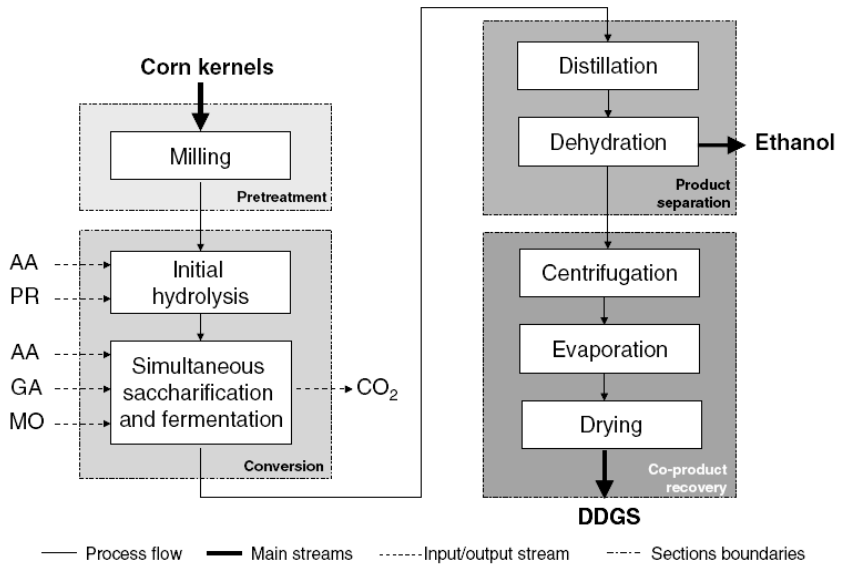


Figure 2. Simplified representation of cold hydrolysis process for production of ethanol from starch. AA α-amylase, PR protease, GA glucoamylase, MO microorganism, DDGS Distillers' Dried Grains with Solubles[20].

Besides the great energetic advantage of the cold hydrolysis process over the conventional technologies, the former also presents reduced water and chemicals consumption. The capital expenditure of a plant for conversion of raw starch is potentially lower, since the process is more integrated. The overall yield tends to be higher, due to the absence of Maillard reactions and reduced yeast inhibition. Since the sugars are gradually released, the cells tend to produce lower levels of coproducts, such as glycerol and higher alcohols and the osmotic stress is reduced[20]. Nevertheless, some drawbacks of the production of ethanol by cold hydrolysis includes: higher demand for enzymes (in both quantity and types of enzymes), since the hydrolysis of native starch presents some mass transfer limitations[21-23], which are present in a less extent or are nonexistent in the traditional processes; higher susceptibility for microbial contamination (by phytopathogens), which is avoided in the conventional technologies due to the high-temperature steps[24].

Starch structure

After its extraction from plants, starch occurs as a flour-like white powder insoluble in cold water. This powder consists of microscopic granules with diameters ranging from 2 to 100 μm, and with different size, shape, and chemical content depending on the botanic origin. Starch consists of mainly two glucosidic macromolecules: amylose and amylopectin. In

most common types of starch the weight percentages of amylose range between 72 and 82%, and the amylopectins range from 18 to 28%. However, some mutant types of starch have very high amylose content (up to 70% and more for amylomaize) and some very low amylose content (1% for waxy maize). Amylose is defined as a linear molecule of glucose units linked by (1-4) α-D-glycoside bonds, slightly branched by (1-6) α-linkages. Amylopectin is a highly branched polymer consisting of relatively short branches of α-D-(1-4) glycopyranose that are interlinked by α-D-(1-6)-glycosidic linkages approximately every 22 glucose units[25]. The multiplicity in branching lead Peat et al.[26] to describe the basic organization of the chains in terms of A, B and C chains. The single C chain per molecule, with a mean degree of polymerization (DP) above 60, carries other chains as branches and contains the terminal reducing end of the amylopectin macromolecule. The A chains are glycosidically linked to the rest of the molecule by their reducing group trough C6 of a glucose residue. The B chains are defined as bearing other chains as branches. They are linked to the rest of the molecule by their reducing group on one side and by a α-(1-6) linkage on the other, thus being the backbone of the grape-like macromolecule. From then, several models have been proposed, all referring to the cluster model.

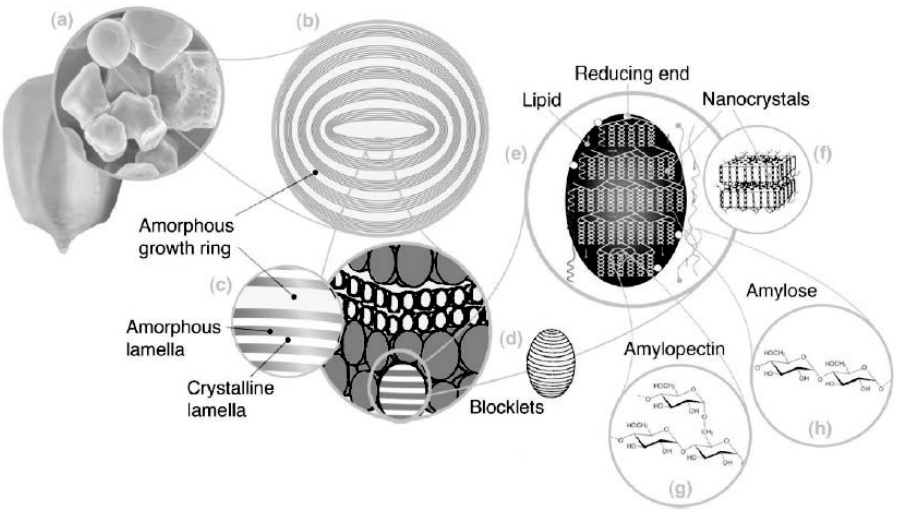


Figure 3. Starch multiscale structure: (a) starch granules from normal maize (30 μm), (b) amorphous and semicrystalline growth rings (120-500 nm), (c) amorphous and crystalline lamellae (9 nm), magnified details of the semicrystalline growth ring, (d) blocklets (20-50 nm) constituting a unit of the growth rings, (e) amylopectin double helixes forming the crystalline lamellae of the blocklets, (f) nanocrystals: other representation of the crystalline lamellae called starch nanocrystals when separated by acid hydrolysis, (g) amylopectin's molecular structure, and (h) amylose's molecular structure (0.1-1 nm)[27].

Minor components associated with starch granules are of three types: (i) cell-wall fragments, (ii) surface components, and (iii) internal components. The main constituents of surface components are proteins, enzymes, amino acids, and nucleic acids, whereas internal components are composed mainly of lipids. The proportion of these components depends on the botanical origin.

Starch structure has been under research for years, and because of its complexity, a universally accepted model is still lacking[27]. However, in this past decade a model seems predominant. It is a multiscale structure, shown in Figure 3, consisting of the (a) granule (2-100 μm) into which we find (b) growth rings (120-500 nm) composed of (d) blocklets (20-50 nm) made of (c) amorphous and crystalline lamellae (9 nm) containing (g) amylopectin and (h) amylose chains (0.1-1 nm). Starch granules consist of concentric alternating amorphous and semicrystalline growth rings.

α-Amylases

α-Amylases (E.C. 3.2.1.1.) are starch-degrading enzymes that catalyze the hydrolysis of internal α-1,4-*O*--glycosidic bonds in polysaccharides with the retention of α-anomeric configuration in the products. Most of the α-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) for their activity, structural integrity and stability. They belong to family 13 (GH-13) of the glycoside hydrolase group of enzymes[28]. Amylases are one of the most important industrial enzymes that have a wide variety of applications ranging from conversion of starch to sugar syrups, to the production of cyclodextrins for the pharmaceutical industry. These enzymes account for about 30% of the world's enzyme production[29]. The α-amylase family can roughly be divided into two groups: the starch hydrolyzing enzymes and the starch modifying, or transglycosylating enzymes. The enzymatic hydrolysis is preferred to acid hydrolysis in starch processing industry due to a number of advantages such as specificity of the reaction, stability of the generated products, lower energy requirements and elimination of neutralization steps[30]. Due to the increasing demand for these enzymes in various industries, there is enormous interest in developing enzymes with better properties such as raw starch degrading amylases suitable for industrial applications and their cost effective production techniques[31].

The α-amylase family, *i.e*. the clan GH-H of glycoside hydrolyses, is the largest family of glycoside hydrolases, transferases and isomerases comprising nearly 30 different enzyme specificities[32]. A large variety of enzymes are able to act on starch. These enzymes can be divided basically into four groups: endoamylases, exoamylases, debranching enzymes and transferases[29]:

1. endoamylases: cleave internal α-1,4 bonds resulting in α-anomeric products,
2. exoamylases: cleave α-1,4 or α-1,6 bonds of the external glucose residues resulting in α-or α-anomeric products,
3. debranching enzymes: hydrolyze α-1,6 bonds exclusively leaving long linear polysaccharides, and

4. transferases: cleave α-1,4 glycosidic bond of the donor molecule and transfer part of the donor to a glycosidic acceptor forming a new glycosidic bond.

α-Amylases are ubiquitous enzymes produced by plants, animals and microbes, where they play a dominant role in carbohydrate metabolism. Amylases from plant and microbial sources have been employed for centuries as food additives. Barley amylases have been used in the brewing industry. Fungal amylases have been widely used for the preparation of oriental foods. In spite of the wide distribution of amylases, microbial sources, namely fungal and bacterial amylases, are used for the industrial production due to advantages such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization[31].

Among bacteria, *Bacillus* sp. is widely used for thermostable α-amylase production to meet industrial needs. *B. subtilis, B. stearothermophilus, B. licheniformis* and *B. amyloliquefaciens* are known to be good producers of α-amylase and these have been widely used for commercial production of the enzyme for various applications. Similarly, filamentous fungi have been widely used for the production of amylases for centuries. As these moulds are known to be prolific producers of extracellular proteins, they are widely exploited for the production of different enzymes including α-amylase. Fungi belonging to the genus *Aspergillus* have been most commonly employed for the production of α-amylase.

Raw starch digesting amylase

Since many of the commercially available amylases do not withstand industrial reaction conditions, isolation and characterization of novel amylases with desirable properties is very important[33]. From that point of view screening of wild type strains of *Bacillus* sp. is very important. We have found several isolates with promising amylase characteristic[34]. It is important to emphasize that not all of the media used have induced expression of raw starch digesting amylase. Several raw starch digesting alpha amylases which can directly hydrolyze the raw starch in a single step at temperatures below the gelatinization temperature of starch has been reported[35]. Raw starch digesting amylases from *Bacillu*s sp. usually need prolonged time of incubation for efficient raw starch hydrolysis and are not able to digest all types of starch granules with same efficiency[36]. Often, better results were obtained with thermostable raw starch digesting amylases at temperatures between 60 and 70 °C[35]. Recently, amylase from *B. licheniformis* ATCC 9945a was purified and characterized[37]. The advantages of this amylase compared to previously reported ones are related to a high hydrolytic affinity of this enzyme towards different types of raw starch granules; cereals, tubers and roots. Enzyme appears to be a good candidate for the direct hydrolysis of diverse raw starches, using very low doses (0.07 U/mg of starch) and omitting energy intensive and expensive gelatinization step. Raw cereal starches are more completely and rapidly hydrolyzed than those from tubers or roots when digested by single, purified enzymes[38]. Moreover most raw starch digesting alpha amylase reported to date hardly digest potato starch[35,38]. Since corn, wheat and potato are the most important sources of starch in EU[1], enzymes that are capable of digesting all these types of raw starches efficiently are economically attractive.

Conclusion

Starch is a constituent of numerous agricultural feedstocks and a convenient substrate for bioethanol production. However, due to its polysaccharidic composition, it must be hydrolyzed exogenously to microbial cells, in order to be broken down into small sugars, e.g., glucose and maltose. Amylases are thus essential to enable efficient hydrolysis processes. Although processes for the production of bioethanol from starchy feedstocks have been used at large scale for decades, there is a continuous search for technological improvements leading to increases in yield as well as reductions in the costs associated to enzyme production and to the final bioethanol production process itself. Thus, technological challenges are being tackled in a number of fields, such as:

1. Plant biotechnology: The development of grains varieties containing genes for the expression of amylases[39], thus reducing enzyme dose during starch hydrolysis;
2. Microbial molecular biology: Genetic manipulation of strains to obtain strains expressing enzymes for starch hydrolysis[40-42]. Recently, we have produced extracellular recombinant amylase in *E. coli* using DsbA signal peptide sequence approach[43]. Recombinant α-amylase possessed the properties of the native enzyme. Furthermore the recombinant enzyme showed improved thermostability at 90°C and higher efficiency for digesting diverse raw starches comparing to the native enzyme, and comparative ability to hydrolyze raw corn and potato starches as a commercial α-amylase. The properties of the recombinant enzyme suggest the good potential of using this approach for production of fully active industrially important recombinant enzymes.
3. Microbiology: Understanding of metabolic mechanisms and adaptation of microbial cells for tolerance to higher concentrations of ethanol[44];
4. Enzyme technology: Formulation of synergistic enzyme pools for raw starch hydrolysis[45]; production of proteolytic enzymes for the pretreatment of grains, aiming at promoting higher exposure of starch to amylases[24, 46]; and use of protein engineering for the development of enzymes with improved action towards raw substrates[47];
5. Process engineering: Some trends comprise the integration of conversion steps, e.g., simultaneous liquefaction, saccharification and fermentation[47] or simultaneous fermentation and distillation; optimization of process control, by using dynamic strategies[45, 48]; very high gravity fermentation, which contributes to the reduction of capital costs and to the increase of plant throughput[49, 50]; and co-product valorization, through the post-processing and fractionation of DDGS for the separation of higher value-added components and improved use as animal feed.

Acknowledgements

This work was supported by the Serbian Ministry of Education and Science project grant number 172048 and ICGEB research project grant number CRP/YUG11-02.

References

1. Buchholz, K. & Seibel, J. (2008). Industrial carbohydrate biotransformations. *Carbohydr. Res.*, **343,** 1966-1979.
2. Roy, I. & Gupta, M.N. (2004). Hydrolysis of starch by a mixture of glucoamylase and pullulanase entrapped individually in calcium alginate beads. *Enzyme Microb. Technol*., **34,** 26–32.
3. Riley, G. (1850). Improved process in the manufacture of glucose. U.S. Patent 7,148.
4. Brown, J.M., Bemis, W.A., Monroe, F.A.Jr. (1948). .Dextrose syrup. U.S. Patent 2,438,033.
5. Wallerstein, L. & Gray, P. P. (1952). Production of dextrose. U.S. Patent 2,583,451.
6. Hartig, H.P. (1957)**.** Sugars derived from starch. *Food Technol. Aust.*, **9,** 73-83.
7. Honsch, W.M. (1957). Preliminary work for the manufacture of wheat starch glucose. *Starch/ Staerke* **9,** 45-47.
8. Rahman, S. (1995). Food Properties Handbook, CRC Press, Boca Raton.
9. Robertson, G. H., Wong, D.W.S., Lee, C.C., Wagschal, K., Smith, M.R., Orts, W.J. (2006). Native or raw starch digestion: a key step in energy efficient biorefining of grain. *J. Agric. Food. Chem.*, **54,** 353-365.
10. Bawa, N., *et al.,* (2010). Fermentation of glucose and starch particles using an inexpensive medium. *J. Chem. Technol. Biotechnol.,* **85,** 441–446.
11. Fernando, S., *et al.,* (2006). Biorefineries: current status, challenges and future direction. *Energy and Fuels,* **20,** 1727-1737.
12. das Neves, M.A., *et al.,* (2007). State of the Art and Future Trends of Bioethanol Production. *Dynamic Biochemistry, Process Biotechnology and Molecular Biology,* **1(1),** 1-14.
13. Balls, A.K., Schwimmer, S. (1944). Digestion of raw starch. *J. Biol. Chem.,* **156(1),** 203–210.
14. Williams, J. (2006). Break it down now. <http://www.ethanol producer.com/article.jsp?article_ id=319&q=&page=all>. Accessed 30 May 2011.
15. Swanson, T. (2004). Partnering in progress <http://www. ethanolproducer.com/article.jsp?article_ id=804&q=starch%20cold%20hydrolysis&page=all> Accessed 30 May 2011.
16. Galvez, A. (2005). Analyzing cold enzyme starch hydrolysis technology in new ethanol plant design. *Ethanol Prod. Mag.*, **11,** 58–60.
17. Wang, P., *et al.,* (2009). Effects of protease and urea on a granular starch hydrolyzing process for corn ethanol production. *Cereal Chem.,* **86(3),** 319–322.
18. Lehmann, U., & Robin, F. (2007). Slowly digestible starch—its structure and health implications: a review. *Trends Food Sci. Technol.,* **18,** 346–355.
19. Kopnieczny-Janda, G., Li, M., Shetty, J.K., Teunissen, P. (2008). Production of ethanol from barley and DDGS containing reduced beta-glucan and phytic acid. US Patent US2008/003344.
20. Castro, A.M., Castilho, L.R., Freire, D.M.G. (2011). An overview on advances of amylases production and their use in the production of bioethanol by conventional and non-conventional processes. *Biomass Conv. Bioref.,* **1,** 245–255.
21. Huston, A.L. (2008). Biotechnological aspects of cold-adapted enzymes. In: Margesin, R., Schinner, F., Marx, J.C., Gerday, C. (eds). Psychrophiles: from biodiversity to biotechnology. Springer Berlin Verlag, Heidelberg, pp. 347–364.
22. Vieira, B.C. Jr., v (2009). Kinetics of granular starch hydrolysis in corn dry-grind process. *Starch/ Starke,* **61,** 448–456.
23. Kumar, M., *et al.,* (2007). Biocatalytic conversion of granular starch to industrial chemicals. *ACS Symp. Ser.,* **972,** 32–45.

24. Schill, S.R. (2008). CUTC: Researchers continue to study corn utilization <http://www.ethanol-producer.com/article.jsp?article_id=4301&q=starch%20cold%20hydrolysis&category_id=35>. Accessed 30 May 2011.
25. Dufresne, A. (2007). Polymer nanocomposites from biological sources. Biopolymers Technology; Bertolini, A.C. (ed.), Cultura Academica, Sao Paulo, pp. 59-83.
26. Peat, S., Whelan, W.J., Thomas, G.J. (1952). Evidence of multiple branching in waxy maize starch. *J. Chem. Soc.*, 4546–4548.
27. Corre, D.L., Bras, J., Dufresne, A. (2010). Starch Nanoparticles: A Review. *Biomacromolecules,* **11,** 1139–1153.
28. Bordbar, A.K., Omidiyan, K., Hosseinzadeh, R. (2005). Study on interaction of α-amylase from *Bacillus subtilis* with cetyl trimethylammonium bromide. *Colloids Surf. B: Biointerfaces,* **40,** 67–71.
29. van der Maarel, M.J.E.C., *et al.,* (2002). Properties and applications of starch-converting enzymes of the α-amylase family. *J. Biotechnol*., **94,** 137–155.
30. Satyanarayana, T., Rao, J.L.U.M., Ezhilvannan, M. (2005). α-Amylases. In: Enzyme Technology, Pandey, A., Webb, C., Soccol, C.R., Larroche, C. (eds.), Asiatech Publishers Inc., New Delhi, India pp. 189–220.
31. Burhan, A., *et al.,* (2003). Enzymatic properties of a novel thermostable, thermophilic, alkaline and chelator resistant amylase from an alkaliphilic *Bacillus* sp. isolate ANT-6. *Process*. *Biochem*., **38,** 1397–1403.
32. Henrissat, B. (1991). A classification of glycosyl hydrolases based on amino acid sequence similiarities. *Biochem. J.,* **280,** 309–316.
33. Shafiei, M., Ziaee, A.-A., Amoozegar, M.A. (2010). Purification and biochemical characterization of a novel SDS and surfactant stable, raw starch digesting, and halophilic α-amylase from a moderately halophilic bacterium, *Nesterenkonia* sp. strain F, *Proc. Biochem.,* **45 ,** 694–699.
34. {okarda Slavi}, M., Bo`i}, N., Vuj~i}, Z. (2011). Screening a wild type strains of *Bacillus* sp. that produce α-amylases in solid state fermentation. *2nd FCUB ERA Workshop,* P21.
35. Goyal, N., Gupta, J.K., Soni, S.K. (2005). A novel raw starch digesting thermostable α-amylase from *Bacillus* sp. I-3 and its use in the direct hydrolysis of raw potato starch. *Enzyme Microb. Technol.,* **37,** 723–734.
36. Dettori, B.G., Priest, F.G., Stark, J.R., (1992). Hydrolysis of starch granules by the amylase from *Bacillus stearothermophilus* NCA 26, *Process Biochem.,* **27,** 17–21.
37. Bo`i}, N., *et al.,* (2011). Production and properties of the highly efficient raw starch digesting α-amylase from a *Bacillus licheniformis* ATCC 9945a. *Biochem. Eng. J.,* **53,** 203–209.
38. Qates, C.G. (1997). Towards an understanding of starch granule structure and hydrolysis. *Trends Food Sci. Technol.*, **8,** 375–382.
39. Sainz, M.B. (2009). Commercial cellulosic ethanol: the role of plant-expressed enzymes. *In Vitro Cell Dev. Biol. Plant,* **45,** 314–329.
40. Ghang, D.M., *et al.,* (2007). Efficient one-step starch utilization by industrial strains of *Saccharomyces cerevisiae* expressing the glucoamylase and α-amylase genes from *Debaromyces occidentalis*. *Biotechnol, Lett,,* **29,** 1203–1208.
41. Kim, J.H., *et al.,* (2010). Construction of a direct starch-fermenting industrial strain of *Saccharomyces cerevisiae* producing glucoamylase, α-amylase and debranching enzyme. *Biotechnol, Lett.,* **32,** 713–719.

42. Shigechi, H., *et al.,* (2004). Direct production of ethanol from raw corn starch via fermentation by use of a novel surface engineered yeast strain co-displaying glucoamylase and α-amylase. *Appl. Environ. Microbiol.*, **70(8),** 5037–5040.
43. Bo`i}, N. (2012). Hidroliza sirovog skroba - novi pravci. *Hemijski pregled,* **53(2),** 27-31.
44. Hu, X.K., *et al.,* (2007). Genetic dissection of ethanol tolerance in the budding yeast *Saccharomyces cerevisiae. Genetics,* **175,** 1479–1487.
45. Castro, A.M., *et al.,* (2011). Valorization of residual agroindustrial cakes by fungal production of multienzyme complexes and their use in cold hydrolysis of raw starch. *Waste Biomass Valor.,* **2,** 291–302.
46. Vidal, B.C. Jr., *et al.,* (2009). Protease pretreatment to improve ethanol fermentation in modified dry grind corn process. *Cereal Chem.,* **86(3),** 323–328.
47. Vikso-Nielsen, A., *et al.,* (2006). Development of new α-amylases for raw starch hydrolysis. *Biocatal. Biotrans.,* **24(1–2),** 121–127.
48. Murthy, G.S. (2007). Development of a controller for fermentation in the dry grind corn process. PhD Thesis, University of Illinois at Urbana-Champaign.
49. Devantier, R., Pedersen, S., Olsson, L. (2005). Characterization of very high gravity ethanol fermentation of corn mash. Effect of glucoamylase dosage, pre-saccharification and yeast strain. *Appl. Microbiol. Biotechnol*., **68,** 622–629.
50. Pereira, F.B., *et al.,* (2010). Optimization of low-cost medium for very high gravity ethanol fermentations by *Saccharomyces cerevisiae* using statistical experiments design. *Bioresour. Technol*., **101,** 7856–7863.

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

577.1(082)

SRPSKO biohemijsko društvo (Beograd).
Konferencija (2 ; 2012 ; Beograd)
[Proceedings] / Serbian Biochemical
Society Second Conference “Molecular
Bioscience”, 9.11.2012. Belgrade, Serbia. ;
[edited by Mihajlo B. Spasić]. - Belgrade :
Faculty of Chemistry : Serbian Biochemical
Society, 2012 (Belgrade : Colorgrafx). - 133
str. : graf. prikazi ; 24 cm

Tiraž 500. - Bibliografija uz svaki rad.

ISBN 978-86-7220-048-5 (FOC)

a) Биохемија - Зборници
COBISS.SR-ID 194357516