CALCIUM SALT ADDITION IN BIOETHANOL OBTAINING

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Abstract: *Current issue in bioethanol production concerns in increasing efficiency in alcohol, reducing energy consumption and water cooling. The paper presents a study about the influence of calcium lactate both on the percentage of alcohol obtained as on the energy consumption used in the technological process. The research has performed to non cooking mashes process by using a suitable enzyme mixture that allows lower heating temperature to 36 degrees, in terms of avoiding the appearance of unwanted fermentable processes. The monitoring of the fermenting process was controlled in the spectral infrared range by means of spectrometric sensors and a specialized software that through an electronic multiplexer allows a successive reading and with a high speed of the values for ethanol, carbon dioxide and oxygen concentrations. The chemical composition of the alcohol mixture obtained from the fermentation was determined by gaschromatography.*

Key words: *bioethanol, enzymes mixture, spectrometric detectors, calcium lactate, gaschromatography*

Introduction

Bioethanol is the most important alternative to the fossil fuels, alone or in a mixture with biodiesel made from virgin or used vegetable oils and animal fat [1]. Starchy and lignocellulosic materials are the most important biomass resources.

The use of corn as a source of fermentable sugars because of his high starch content is an important possibility of bioethanol obtaining [2]. Starchy biomass is hydrolyzed by enzymatic or acid procedure to release fermentable sugars. Acid hydrolysis has as results the formation of levulinic acid and formic acid which affects the yeast growth [3]. The enzymatic hydrolysis of starch materials is possible in a cooking process at a high amylolytic enzymes content to hydrolyse the starchy materials to glucose.

Method for converting granular starch into glucose without heat treatment for gelatinization and dextrinization presents some advantages as: reduce cost of converting starch to dextrin, low consumption of thermic energy and less equipment, a reduced initial sugar concentration so that the yeast has no osmotic stress, no undesirable Maillard reactions, an excellent growth and viability of yeast, less glycerine content [4], a low viscosity of mashes and a high yield in ethanol, a short time of non cooking process, less cooling water, no vapour added [5].

Energy consumption is reduced by approximately 50% reported to energy cost of cooking starch materials. Disadvantage is the possible contamination with Lactobacillus sp.of mash fermentation in bioethanol production with a decrease of production efficiency [6]. Many researches present attempts to improve ethanol yield by using a technological process with a law energy consumption, amylolytic enzymes with specificity for α-1,4-or 1,6-glucosidic linkages with a large capability of hydrolysing them to produce mono-or oligosaccharides [7], thermodynamics studies of industrial production of ethanol from corn and the possibilities of improving the yield [8]. A study about the influence of calcium lactate both on the percentage of alcohol obtained as on energy consumption used in the

technological process is the main goal of this paper.

Materials and methods

Sample handling

The experimental was carried on non cooked mashes from corn as raw material. The efficiency of using calcium lactate in a long process of non cooked mashes fermentation in 72 hours has been analyzed. Bioethanol was obtained in small bioreactors from Bluesens, 1 l volume, and parameters that pH, free carbon dioxide, free oxygen, bioethanol were monitored. Alcohol mixture was distilled by a rotaevaporator of 500 ml volume. Analyses of alcohol mixture was performed by a gaschromatograph Shimadzu, with flame ionization detector, copper chromatographic column of 30m length and 0.25 mm internal diameter, microsyringe 5µl, hydrogen generator, nitrogen cylinder, helium cylinder, computer, printer**.**

Materials

The composition of standard solution is: 20cm ^{3} ethanol, 2cm ^{3} methanol, 2cm ^{3} propyl alcohol, 2cm ^{3} isopropyl alcohol, 2cm ^{3} acetaldehyde, 2cm ^{3} ethyl acetate, reagents from Merck Company. The standard solution is obtained from the stock solution thined until a 1:5 ratio is achieved and contains ingredients in following concentration: acetaldehyde 3.12 mg/ ml, ethyl acetate 3.28 mg/ml, methanol 3.16 mg/ml, isopropyl alcohol 3.12 mg/ml, ethanol 31.6 mg/ml, propyl alcohol 3.2 mg/ml, amyl alcohol 3.3 mg/ml. Maize flour with 180 µm granulosity is used as starchy raw material and yeast for fermentation is a special one.

For industrial applications it is preferred an enzymatic hydrolysis by using a special enzymatic mixture. For the hydrolysis of corn starch in a non cooking process a combination of specific enzymes has been used The enzymes involved in hydrolysis include:α-amylase, glucoamylase, and protease.. The using of appropriate amounts of enzymes is very important. Larger amount of α-amylase may be detrimental to the production of dextrose because of a larger saccharides which may polymerize to unfermentable dextrose by transglucosidase.[7.] The enzymatic mixture used in corn non-cooked mashes is presented in the table no.1 and has the following composition [9]:

Table 1. Enzymatic mixture used in non cooked mashes**.**

Nr.crt	Enzyme	Micro – organism that creates it	Optimum pH	Optimum Temp (^{0}C)	Action time (min)	Enzimatic activity	Used doze (g/g s.u)
1.	**G-ZYME EXTRA** (α-amylase)	Bacillus stearothermophilus	5,4-5,8	55	120	14000 AAU/g	0,04
2.	**STARGEN™ 001** α-amylase, glucoamylase	Aspergillus kawaechi şi Aspergillus niger	4,0-4,5	32	60	456 GSHU/g	0,025
3.	**FERMGEN™**(protease)	Trichoderma reesei	3,0-4,5	35	15	1000 SAPU/g	0,02

FERMGEN ™ protease may be used with STARGEN ™ in enzymatic fermentative processes without mashes cooking. STARGEN ™ enzyme contains α-amylase

and glucoamylase which work together for the starch hydrolysis to glucose.

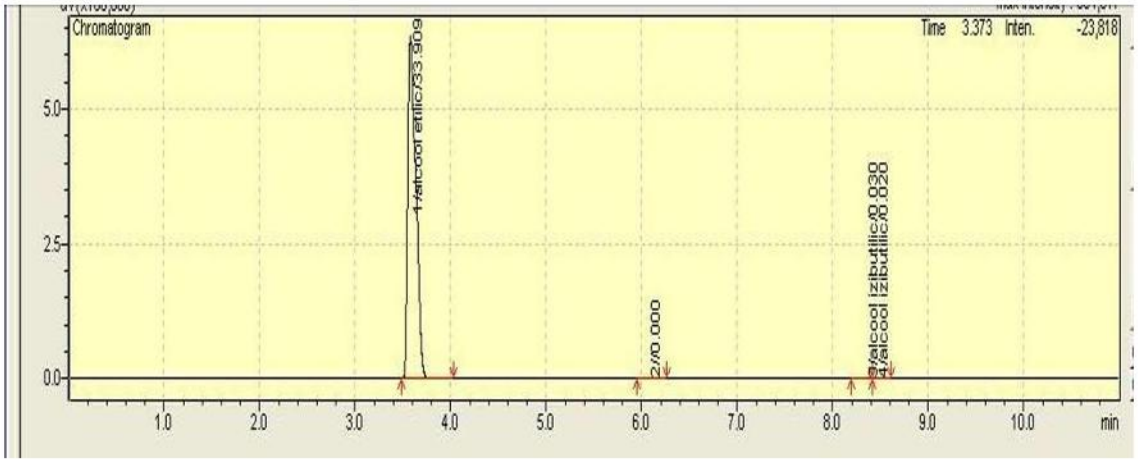
Experimental

Enzyme G-ZYME EXTRA has been added to melt samples for analysis (corn grist) and samples were maintained at a temperature of 55 ºC two hours. After cooling at 34 ºC enzyme STARGEN 001 is added to enhance enzymatic hydrolysis of starch. Samples were maintained at a temperature of 55 ºC for one hour. PH of saccharified mashes must be 5.3 to 5.7, because as the result of yeast activity during fermentation, pH decreases to 4.2 or 4.3. In most cases when the pH is below this limit value the mashes are infected with lactic acid bacteria. Then FERMGEN enzyme was added for a quickly hydrolysis of proteins to give more nutrients for the yeast fermentation stage. After a rest for 15 minutes mashes were fermented in special cells equipped with three sensors that continuously monitor gas content of CO_2, O_2 and ethanol, for 72 hours at room temperature. Cells fermentation content was distilled and samples were analyzed using a gas chromatograph to identify the components and to determine their concentrations. To increase efficiency and lower cost of ethanol the liquefaction temperature is decreased using a calcium salt. By adding calcium salt, in different quantities, of 1-3 grams per 100 g corn grist, thermostatic temperature decreased from 55 º C to 45 ° C and even 36 ° C. After two hours of controlled temperature maintenance the samples followed the same steps as were presented above, if the samples heated at 55 º C, without added calcium salts.

The fermentation monitoring was carried out by biosensors from Blue Sens Company, evolution of alcohol, CO_2, and consumed oxygen amounts can be analyzed on the fermentation curves recorded.

Groups of three mashes simultaneously fermentated, heated to 55, 45 and 36 degrees, were monitored. In each of mashes were added different amounts, 1, 2 and 3 grams of Puracal PP / FCC/100 g raw material, a very soluble calcium salt of natural lactic acid L (+), L-2-hydroxy-calcium propionate $(CH_3CHOHCOO)_2$ $5H_2O$, produced by fermentation. In the figure no. 1 it is shown the fermentation process of three mashes heated to 55 degrees with the addition of 1.2 3 g of Puracal.

Figure 1. Fermentation of non cooked mashes at 55 º C with various quantities of Puracal added (1,2,3 g /100 g raw material)



Results - Peak Table

Peak Table | Compound | Group | Calibration Curve

Peak#	Compound Name	Ret.Time	Area	Conc.	Units
1	alcool etilic	3.576	3729439.0	33.90910	mg/ml
2		6.063	3346.9	0.00000	
3	alcool izibutilic	8.349	4487.6	0.03039	mg/ml
4	alcool izibutilic	8.452	3001.8	0.02033	mg/ml

Figure 2. Chromatogram of mash heated to 55 degrees with the addition of 1 g Puracal / 100g raw material

In the same way, chromatograms were made for other samples and the results are presented in Table no.2

Table no.2 Ethanol content of heated mashes with Puracal addition

No.crt.	Temperature ^{0}C	Puracal added, g/100g raw material	pH of fermented mashes	Conc ethanol mg/ml
1	55	1	4.23	33.90910
		2	4.20	39.9820
		3	4.15	44.0487
2	45	1	4.20	29.85491
		2	4.17	30.11364
		3	4.10	54.70136
3	36	1	4.26	20.31805
		2	4.24	27.44803
		3	4.23	33.12690

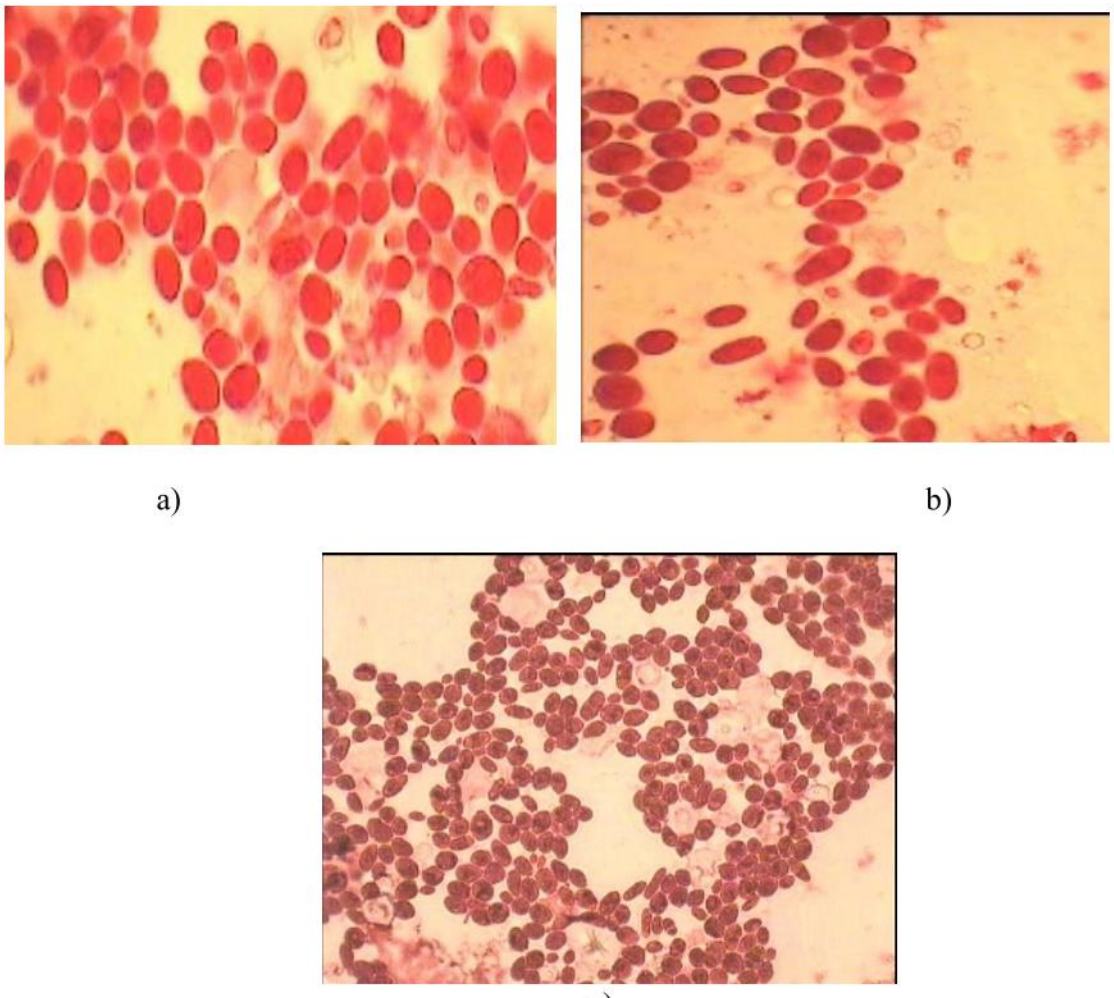
Each value represents the average of six determinations.

Grain mashes are a favorable medium for infectious microorganisms and the lowering temperature below 50 degrees it favors their development. Microbiological control is performed first to determine what kind of infectious bacteria (quality control), and secondly to detect and remove the source of infection.

Development of infectious microorganisms in mashes is accompanied by an increased acidity which is formed on account of sugar, so there is a decrease in pH below 4.2 and as a consequence, unsaccharified dextrins are remainin in the mash.

In a microscopic analysis, lactic bacteria distinguished by the shape of rods. In a distillery is accepted that in the final fermentation stage, mashes contain a maximum of 5% bacteria to yeast.

Various mashes heated at different temperatures , after 72 hours of fermentation were analised by microscop, figure no.3 and no lactic bacteria was found which can cause secondary fermentation.



a) b)

c)

Figure 3. Microbiological control of mashes at different temperatures: a) 36°C, b) 45° , c) 55 °C

Conclusions

Analyzing the results obtained, presented in Table 2 it is noted that the addition of Puracal increases the intake of ethanol obtained at all test temperatures. It notes that the largest amount of ethanol is obtained from mashes wich were heated at 45 degrees and with 3 grams of Puracal addition. This behaviour is explained by the role of calcium on amylase activity.

Amylase contains endogenous calcium linked over enzyme. Calcium added before liquefaction stabilizes very well the enzyme.

The calcium salt PURAC PP / FCC, calcium lactate, acts to stabilize amylase and to help the achievement of optimal pH for mash fermentation.

Saccharified mashes should have a pH of between 5.3 and 5.7 but as a result of yeast during fermentation, the pH decreases. pH of mashes in fermentation in normally drops to 4.2 to 4.3. In most cases when the pH is below this limit value, lactic acid bacteria infectes the mashes.

The analysis of pH values and the images from the microscope, figure no.3 show that there are no infection with lactic acid bacteria in mashes heated at 55, 45 or 36 degrees.

Taking into account the energy savings that are achieved by reducing the heating temperature for converting granular starch into glucose without heat treatment for gelatinization and dextrinization [9], from 55 to 36 degrees, and the economy of cooling water, it is recommended Puracal addition to improve yield in continuous process of ethanol obtaining.

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