Romanian Biotechnological Letters *Vol. 14, No. 5, 2009, pp. 4673-4680*

ORIGINAL PAPER

Researches regarding bioethanol from renewable sources such as sweetpotato, as an ecotoxic alternative to fossil fuels

Received for publication, May 15, 2009
Accepted, September 25, 2009

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Abstract

Sweetpotato (Ipomoea batatas) represents an important food source, but in the same time, a notable source of bioethanol, due to the large quantities of glucide polymers that can be transformed with high randaments in fermentable glucides. The glucides represent 80-90 % of the dry matter in the sweetpotato tuberized roots, from which: 2-5 % cellulose, 3-4 % hemicellulose, 70 % starch, 2.5-3 % pectic substances (pectic acids, galactans and arabans connected by glycosidic links). The content in monoglucides and oligoglucides is about 10 times lower compared to that of starch. Consequently, the hydrolysis of starch, hemicellulose and cellulose, can be performed by using enzymatic mixtures, with specificity for the mentioned substrates. In the present paper a comparison between some enzymatic products and their mixtures is achieved, as regards the efficiency of hydrolyzing sweetpotato polyglucides (a variety with orange flesh) to reducing sugars. The following enzymatic products were used: Amylex 3 T (α-amylase), Laminex 440 (glucanase, pentosanase), Diazyme X4 si Diazyme X5 (amyloglucosidases), MethaPlus (β-glucanase, xylanase, cellulase), Veron M4 (α-amylase) and BG α-malt (α-amylase). Especially active, proved to be the mixture of Diazyme X4 and Laminex 440 (glucose 78.71 % on DM bases), Diazyme X4 (glucose 75. 81 % on DM basis), followed by the mixture Amylex 3T+Laminex 440 (glucose 64.92 % on DM basis) and MethaPlus (glucose 59.70 % on DM basis). The other enzymes expressed more modest efficiency in transforming polyglucides in reducing sugars. The content increase in reducing sugars, compared to the control, varied between 32.69-328.23 %, according to the enzyme used for hydrolysis. After the enzymatic hydrolysis, the glucide extract was fermented by using *Saccharomyces* species, then distilled and rectified, in order to be purified to obtain bioethanol. The yeast vitality was increased by adding some fungal proteases. The bioethanol anhydrization was efficiently performed by using molecular sieves.

Keywords: *sweetpotato, enzymatic hydrolysis, reducing sugars, fermentation, bioethanol*

Introduction

The increased dependence on fossil fuel, in meeting the energy demands of the modern society, results in elevated CO_2 levels in the atmosphere, linked to global warming. As fossil energy supplies will no longer be adequate and affordable in the near future, the attention is driven to industrial biotechnology, able to develop biofuels from renewable energy sources [**8**].

Different types of plant biomass sources are already assessed for their potential to produce biofuels. Among these, some starch crops, like sweetpotato, represent attractive candidates to be used as renewable biomass sources to produce ethanol, as an unconventional fuel. Considered of being one of the most competitive vegetables, due to the high production/ha and to the exquisite nutritive qualities, the sweetpotato cultivation is more and more extended in the temperate zones. Apart from its value as food for humans and animals,

sweetpotato biomass is highly appreciated as an important raw material for the starch and alcohol industry [**3, 4**].

The important quantities of glucide polymers and the high efficiency of their transformation in fermentable glucides, recommend sweetpotato as a notable source to produce bioethanol. The glucides represent 80-90 % of the total dry substance. Of the total polyglucides, 2-5 % is represented by cellulose, 3-4 % by hemicellulose, 70 % by starch and 2.5-3 % by pectins. The mono- and oligoglucides are present in concentrations of 10 times lower compared to the starch ones, the reducing glucides are present as traces, while the dextrines are missing [**5**]. Conclusively, sweetpotato starch, cellulose and hemicellulose hydrolysis can be performed by using enzyme mixtures, having specificity for these substrates [**2, 6, 7**].

One of the main goals of this research is the testing of the efficiency of some enzymes and enzymatic mixtures, used for the hydrolysis of polyglucides from the sweetpotato storage roots, to reducing sugars. Another goal is referring to the fermentation of the reducing sugars to bioethanol, followed by its purification and anhydrization.

Materials and Methods

Conditioned sweetpotato storage roots, belonging to a variety with orange flesh (pre-dried and crumbled), were used as the starting material. The first step performed was the laboratory analysis concerning the % moisture content of the substrate, at 60 and 105^0 C (The Practical Reference Method, SR ISO 712/1999).

After that, the polyglucide substrate was hydrolyzed using some commercial fungal or bacterial enzymatic preparates mixtures (the volumes of enzymatic preparates/g sweetpotato dry matter, was calculated according to the technical specifications), from different sources (DANISCO, GENENCOR): Amylex 3 T (α-amylase), Laminex 440 (glucanase, pentosanase), Diazyme X4 (amyloglucosidase), Diazyme X5 (amyloglucosidase), MethaPlus (β-glucanase, xylanase, cellulase), Veron M4 (α-amylase) and BG α-malt (α-amylase). The most of the enzymatic preparates contained water, glycerol, sodium sulfate as stabilizer, sodium chloride, potassium sorbate or sodium benzoate as preservatives. The enzymatic preparates were ready buffered to the activity optimum pH.

Enzymatic hydrolysis was performed at 60^0 C, during 20 h period, on a rotary shaker at 200 rpm, at pH=5,8 (the temperature and pH were adequate for the heat proof enzymes we used, according to the technical specifications). The efficiency of hydrolysis was estimated by quantifying the amount of reducing sugars. The concentration of reducing sugars % were recorded as glucose content, by reading the absorption at 640 nm, using 3,5-dinitrosalicilic acid (Peterson and Porath modified method) [**1**]. Assuming the variability among the characteristics of the sweetpotato, we also calculated some statistics of seven different samples, regarding the % of reducing sugars after hydrolysis with Amylex 3T.

Following enzymatic hydrolysis, the glucide juice was fermented with some *Saccharomyces* species (Ethanol Red dry alcohol yeast from LESAFFRE), inoculated at a rate of 5 %. Anaerobic fermentation was performed for 24 and 48 hours, at 28^0C, without shaking. Yeast vitality was enhanced by adding some fungal proteases from *Aspergillus niger*, namely Diazyme FP and Alphalase FP2. The fermented juice was distilled. The rectification of the distillate was also achieved, in order to purify the bioethanol. Total anhydrization was efficiently performed on a 3 A^0 molecular sieve (15 % molecular sieve to the whole mixture).

Results and Discussions

Following the sweetpotato root biomass conditioning, the following parameters were evaluated: relative dry matter, absolute dry matter and total dry matter, being registered the following values: relative DM % = 20.395; absolute DM % = 86. 48; total DM % = 17.63. The reducing sugars values obtained after the enzymatic hydrolyses are given in percents, calculated on dry matter basis. The content in reducing sugars, expressed as g of glucose %, was calculated after a regression equation corresponding to the etalon curve. Also, the efficiency increase of the reducing sugars was recorded, compared to the control (Table 1).

Table 1. Reducing sugars obtained by enzymatic hydrolysis of sweetpotato root biomass

No.	Enzymes added to sweetpotato samples	g glucose in % DM	Increasing percent compared to control %
	Control (no enzyme added)	18.38	-
1	Amylex 3 T	56.53	207.56
2	Amylex 3T +Laminex 440	64.92	251.57
3	Diazyme X4	75.81	312.45
4	Diazyme X4 + Laminex440	78.71	328.23
5	Laminex 440	33.92	84.54
6	MethaPlus	59.70	224.80
7	Veron M4	45.92	149.83
8	BG α-malt	45.56	147.87

The mixture Diazyme X4 + Laminex 440 proved to be especially efficient (78.71 %), due to the fact that being a combination of an amyloglucosidase and a glucanase, it is hydrolyzing both the 1,4- and 1,6-alfa glucosidic links from starch, as well as the cellulosic glucans. The hydrolysis performed by using only Diazyme X4 was efficient as well (75.81 %). If the product Laminex 440 (glucanase, pentosanase) is used alone, the concentration of the reducing sugars decreases to 33.92 %. On the other hand, the mixture of Laminex 440 with an α-amylase such as Amylex 3T, improves the hydrolysis result up to 64.92 % reducing sugars. In fact, Amylex 3T alone is able to hydrolyse the starch in a proportion of 56.53 % (average of three repeats of the same sample), being very close to the values obtained with MethaPlus (59.70 %).

The Amylex 3T enzyme, an α-amylase, is frequently used in bioethanol industry, due to its effect of starch liquefaction. Though Veron M4 and BG α-malt are also α-amylases, their hydrolytic activity is somehow limited (45.92 %, respectively 45.56 %) as regards the starch from sweetpotato, being preferred in the bread industry (to hydrolyze starch from wheat).

The percentages of the increase in reducing sugars, compared to the control, following the enzymatic activities, were situated between 84.54 % and 328.23 %, thus proving their very good efficiency in hydrolyzing the polyglucides from the sweetpotato biomass (Fig. 1).

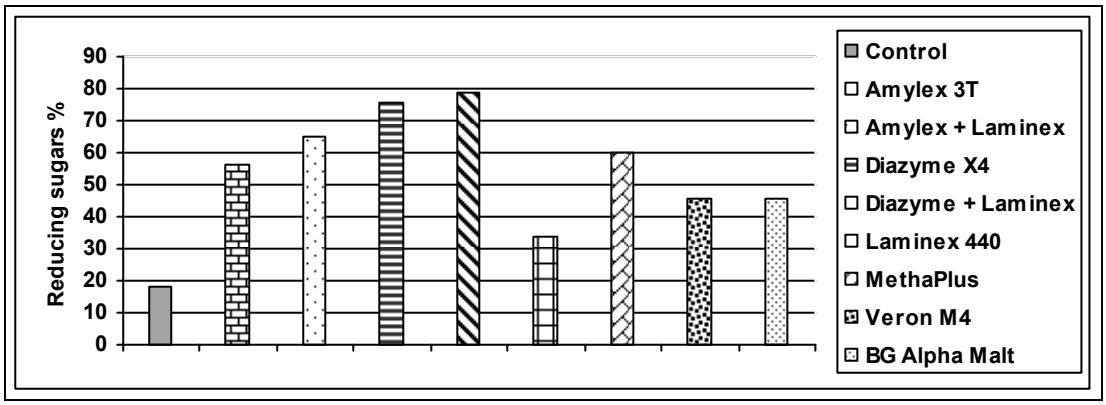

Figure 1. The percents of reducing sugars increase, compared to the control.

Follow-up, we experimented the fermentation of reducing sugars, resulted from sweetpotato starch by hydrolysis. The hydrolysis was performed with only one enzymatic preparate, namely Amylex 3T, because this preparate is frequently used in bioethanol industry. By using the Amylex 3T α-amylase (n=7) for hydrolyzing the sweetpotato starch, an average and a standard deviation of 44.674 ± 8.485, with a variance of 72.004 and a variability coefficient of 18.993 %, for the reducing sugars % (calculated on dry matter basis), were obtained. The variability is relatively increased, this implying strictly controlled conditions of the process technological parameters, especially at industrial scale. The pH values after the enzymatic hydrolysis, in the case of the samples hydrolyzed with Amylex 3T (n=7), have been of 5.664 ± 0.253, with a variance of 0.055 and a variability coefficient of 4.467 %. Having in view these values, we appreciate that the pH did not varied much, remaining relatively constant during the hydrolysis.

Further, the hydrolyzed juice (obtained with Amylex 3T enzyme) was fermented with Ethanol Red alcoholigen yeast, for 24 and 48 hours. The values of the reducing sugars, after 24 and respectively 48 hours of fermentation, alongside the control values (glucides after the enzymatic hydrolysis), are presented in the Table 2.

Table 2. Reducing sugars after 24 and 48 hours of fermentation (n=7)

Parameter	X±s	s	CV %
% Reducing sugars after hydrolysis (a)	44.674 ± 8.485	72.004	18.993
% Reducing sugars after 24 h of fermentation (b)	13.843 ± 6.249	39.056	45.142
% Reducing sugars after 48 h of fermentation (c)	7.404 ± 1.955	3.824	26.405

After 24 hours of fermentation, the reducing sugars display a very high variability coefficient (45.142 %), which decreases after 48 hours almost to one half (26.405 %). The significance of reducing sugars decrease following fermentation F test and t test is presented in Table 3.

Table 3. The significance of the differences between reducing sugars, after hydrolysis and after 24 or 48 h of fermentation.

Parameter	Average	Average	F	t
% Reducing sugars, difference (a)-(b)	44.674	13.843	0.542 ns.	7.740 ***
% Reducing sugars, difference (b)-(c)	13.843	7.404	10.213 *	2.601 *

*ns - insignificant, * significantly different, ** distinctly significant different , *** very significantly different*

It is obvious that after 24 hours of fermentation, the reducing sugars concentration decreased very significantly and continued to decrease significantly up to 48 hours.

The pH values also registered alterations, as long as the reducing glucides concentration decreased and ethanol accumulated (Table 4).

Table 4. The pH values after 24 and 48 h of fermentation (n=7)

Parameter	X ± s	s	CV %
pH values after hydrolysis % (d)	5.664 ± 0.253	0.055	4.467
pH values after 24 h of fermentation % (e)	3.914 ± 0.497	0.247	12.698
pH values after 48 h of fermentation % (f)	3.335 ± 0.602	0.363	18.051

The variability coefficient increased over the limit, especially during the last 24 hours out the 48 hours of fermentation (18.051 %), but is high enough during the first 24 hours, fact that can be explained by different speeds of the enzymatic reaction in sweetpotato samples, submitted to fermentation. The significance of pH values decrease, following fermentation (F test and t test), is presented in Table 5.

Table 5. The significance of the differences in pH values after hydrolysis and after 24 or 48 h of fermentation

Parameter	Average	Average	F	t
pH values difference (d)-(e)	5.664	3.914	1.024 ns.	8.425***
pH values difference (e)-(f)	3.914	3.335	0.680 ns	1.961 ns

It is noticeable that the pH decrease, after 24 h of fermentation, is very significant (8.425***), while the pH decrease after 48 h is not significantly different from the pH values after 24 h of fermentation (1.961 ns).

In this situation, a problem could be raised, that of the proportionality dependence between the decrease in reducing sugars and pH decrease during hydrolysis and fermentation. The reducing sugars – pH regression after hydrolysis, is presented in Fig. 2.

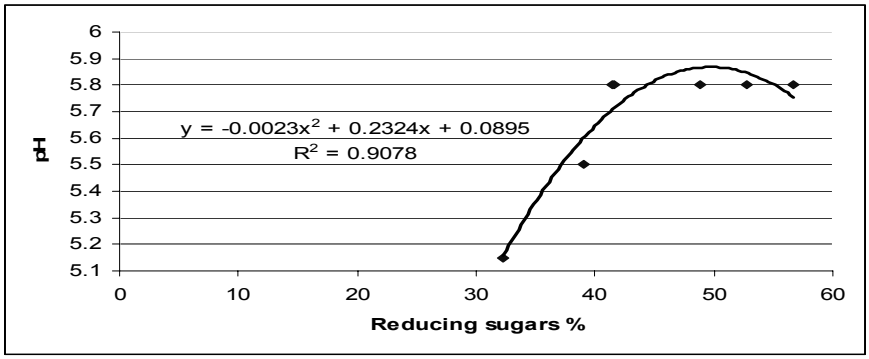

Figure 2. The dependence between reducing sugars – pH, in the samples hydrolyzed with Amylex 3T

A very good dependence between reducing sugars - pH is evident, expressed by a polynominal curve, having a determination coefficient R^2= 0.9087.

In Fig. 3 the dependence reducing sugars - pH, after 24 h of fermentation, is showed by a polynominal curve with a determination coefficient R^2= 0.5771.

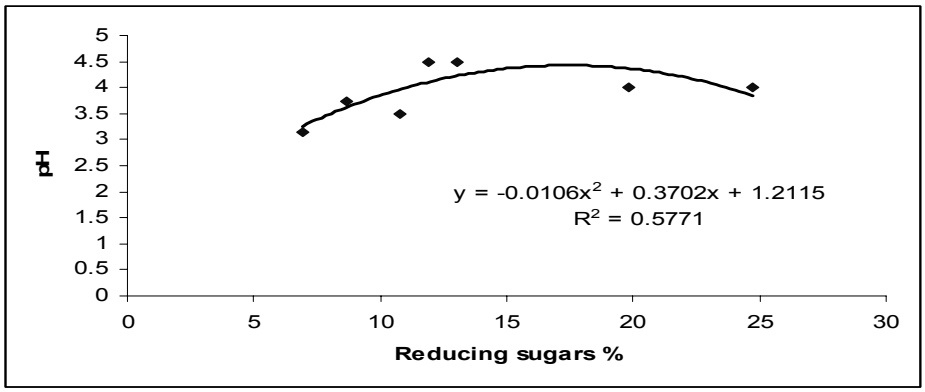

Figure 3. Dependence between reducing sugars – pH, after 24 h of fermentation

The determination coefficient was reduced almost to a half, compared to the determination coefficient of the first curve, though we may appreciate that having in view the reciprocal influence in a proportion of 57 %, between the reducing sugars and pH, there is still a notable interdependence between them.

The regression reducing sugars – pH, after 48 h of fermentation (Fig.4), is represented as a polynominal curve with a very reduced determination coefficient (R^2= 0.0039). Practically, at this level, dependence between reducing sugars and pH is not evident anymore.

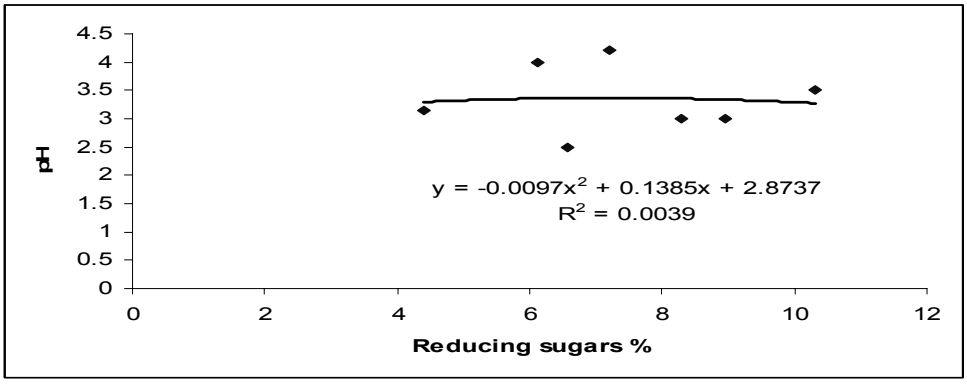

Figure 4. Correlation between reducing sugars – pH after 48 h of fermentation

In order to optimize the technological process of fermentation, it is recommended to have in view the fact that the medium acidification, expressed on one hand by pH decrease and on the other hand by the deployment of reducing sugars concentration, can be statistically foreseen only up to 24 hours of fermentation, because after this period the proportionality between the parameters is not maintained.

The fermentation was favored, with the decrease in reducing sugars, up to 15 % after 48 hours, by adding some fungal proteases such as Diazyme FP and Alphalase FP2, separately or in mixtures, together with the yeast Ethanol Red. It is known that the bioethanol accumulation in the medium slows down the fermentation processes. After 48 hours of fermentation, the distillation at 78.15^0 C of the ethanol-water azeotrop was performed. The distillation was followed by a concomitant rectification and anhydrization of the azeotrop.

The anhydrization was achieved by introducing a molecular sieve (Zeolit), with pore size of 3 A^0, into the distillation still, during the rectification process (15 % of the whole mixture). These procedures increased the alcohol concentration from 95.5 la 99.99 %. In the same time, the heavy metals detected in the azeotrop, at levels of 80.38 ppb Pb and 0.955 ppb Cd, were not present in the rectified bioethanol.

Conclusions

♦ The most active, proved to be the mixture of Dyazime X4 and Laminex 440 (78.71 % glucose on dry matter basis), Dyazime X4 (75.81 % glucose), followed by the mixture of Amylex 3T + Laminex 440 (64.92 % glucose on dry matter basis) and MethaPlus (64.92 % glucose on dry matter basis);

♦ The increase in reducing sugars, comparative to control, was between 32.69 - 328.23 %, reported to the enzymatic mixtures used;

♦ The variability coefficients of the reducing sugars, after hydrolyses and after 24 and 48 hours of fermentation, register high values, which implies the achievement of a strict control of the biotechnological processes parameters, as well as of an increased homogeneity of the raw material;

♦ The reducing sugars concentration decreased very significantly after 24 h of fermentation (t=7.740***) and significantly from 24 to 48 h of fermentation (2.601*);

♦ The pH value decreases very significantly after 24 h of fermentation (8.425***), but does not present significant differences at 48 h comparative to 24 h of fermentation (1.961 ns);

♦ The regressions reducing sugars–pH, presented high R^2 determination coefficients after hydrolysis and after 24 h of fermentation, but not after 48 h of fermentation. Consequently, the medium acidification can be statistically foreseen, based on the reducing sugars concentration (and inversely), only up to 24 h of fermentation, after this period the proportionality between the parameters is not maintained;

♦ The addition of fungal proteases, along with yeast, amplified the fermentation process, decreasing the concentration of the reducing sugars with up to 15 % of the value registered in control (without proteases);

♦ The bioethanol rectification produces the increase of the alcohol concentration and clears away various impurities (heavy metals);

♦ The anhydrization on molecular sieves, directly introduced in the distillation still, is both efficient and feasible, with no separate equipment necessary for this step (columns, transfer on sieve as vapors etc.).

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