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OPTIMIZATION OF ENZYMATIC HYDROLYSIS OF WASTE BREAD BEFORE FERMENTATION

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Abstract

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Finding of optimal hydrolysis conditions is important for increasing the yield of saccharides. The higher yield of saccharides is usable for increase of the following fermentation effectivity. In this study optimal conditions (pH and temperature) for amylolytic enzymes were searched. As raw material was used waste bread. Two analytical methods for analysis were used. Efficiency and process of hydrolysis was analysed spectrophotometrically by Somogyi-Nelson method. Final yields of glucose were analysed by HPLC.
As raw material was used waste bread from local cafe. Waste bread was pretreated by grinding into small particles. Hydrolysis was performed in 100 mL of 15 % (w/v) waste bread particles in the form of water suspension. Waste bread was hydrolysed by two commercial enzymes. For the liquefaction was used α-amylase (BAN 240 L). The saccharification was performed by glucoamylase (AMG 300 L). Optimal conditions for α-amylase (pH 6; 80 °C) were found. The yield of total sugars was 67.08 g·L^{-1} (calculated to maltose). As optimal conditions for glucoamylase (pH 4.2; 60 °C) were found. Amount of glucose was 70.28 g·L^{1}. The time of waste bread liquefaction was 180 minutes. The time of saccharification was 90 minutes. The results were presented at the conference CECE Junior 2014.

Keywords: ethanol, waste bread, enzymatic hydrolysis, amylases, α-amylase, glucoamylase, pH, temperature

INTRODUCTION

Saccharides and other nutrients contained in food waste are suitable for fermentation. Microorganisms could convert it into profitable products such as biofuels, useful chemicals or monomers for production of bioplastics. (LEUNG *et al*., 2012)

Searching of new materials for ethanol production is necessary, due to increasing prices of raw material (corn, cereals, potatoes, etc.). Food waste seems to be one of the suitable inexpensive fermentation feed used in distilleries (EBRAHIMI *et al*., 2008). Different types of food are globally wasted. The main categories of food waste are meat, fruit and vegetable, and bakery products. In the last category is the most abundant wasted bread (KOSSEVA, M. and WEBB, C., 2013). It follows that waste bread is suitable for ethanol production (EBRAHIMI *et al*., 2008).

Food waste often consists of polysaccharides which should be hydrolysed before fermentation. Pretreating of substrate as waste bread is necessary before fermentation. One of typical pretreatment method is enzymatic hydrolysis. Finding the optimal conditions of hydrolysis related to obtaining a higher yield of fermentable saccharides. More efficient hydrolysis is important to obtain a higher yield of ethanol. (PIETRZAK, W. and KAWA-RYGIELSKA, J., 2014)

The objective of this study was to optimise enzymatic hydrolysis of waste bread before ethanol fermentation. Main parameters of optimization were pH and temperature of enzymatic hydrolysis.

MATERIALS AND METHODS

Raw materials

For experiment was used wheat-rye bread (after shelf life). It was obtained from local cafe. Raw material has not been attacked by mold. Bread was crushed by mixer and chopper. The moisture of bread was 11.34 % (w/w). Content of starch was 62.4 % (w/w).

Enzymes

Two commercially available hydrolytic enzymes were used, α-amylase (BAN 240 L; Novozymes A/S, Bagsværd, Denmark) and glucoamylase (AMG 300 L; Novozymes A/S, Bagsværd, Denmark).

Liquefaction and saccharification

Experiments were performed in 250 mL Erlenmeyer flasks. 100 mL of 15 % (w/v) homogenized waste bread in water suspension was prepared for experiment. Size of bread particles was less than 1,25 mm. For liquefaction was added 4 % (w/v) of α-amylase. Liquefaction was proceeded in thermostat (Memmert UM 500) with 3 different values of pH (5; 6; 7) and under 3 different temperatures (70 °C; 80 °C; 90 °C). The pH of the suspension was adjusted by 1 % (w/v) solution of H_2SO_4 and 1 % (w/v) solution of NaOH. The liquefaction was ended by freezing the suspension. Time of liquefaction was 240 minutes.

The saccharification was performed in liquefied suspension by addition of 4 % (w/v) of glucoamylase. Then the Erlenmeyer flasks were placed into tempered shaker (Heidolph UNIMAX 1010, Heidolph 1000). There were applied 3 different temperatures (55 °C; 60 °C; 65 °C) and 3 different values of pH (3.2; 4.2; 5.2). Shaking was set to 130 min^{1}. Enzyme activity was ended by heating of the suspension at 80 °C for 5 minutes. Time of saccharification was 105 minutes. All experiments were performed in triplicate.

Analytical methods

The water content of waste bread was measured by drying 5.0 ± 0.1 g of sample at 105 °C in oven (ČSN ISO 712). Starch content in investigated raw material was measured by Ewers polarimetric method (ČSN 56051216).

Particular yields of the liquefaction and the saccharification were analysed spectrophotometrically by SomogyiNelson method. This method was used for determination the amount of total sugars. The yields of the liquefaction were calculated to maltose. The yields of the saccharification were calculated to glucose.

Efficiency of enzymatic hydrolysis and the concentration of glucose was analysed by HPLC. The glucose content was determined using Polymer IEX H form 8 μm (Watrex, CZ) at 60 °C with refractive index detector (RIDK 102 Ecom, CZ). For analysis on HPLC as mobile phase $5 \cdot 10^{3}$ M solution of H_2SO_4 was used. The separation was performed at a flow rate set to 1 $mL \cdot min^{-1}$ and remained constant throughout the measurement. Time of lactic acid analysis was 15 minutes.

Statistical evaluation of the results

All measured values were determined three times. These three measured values were averaged by function AVERAGE. For calculation of value of the confidence interval was used function CONFIDENCE in Microsoft office Excel 2010 (Microsoft, USA). Statistical significance level $\alpha = 0.05$ was used.

RESULTS

In this work hydrolysis of 15 % (w/v) suspension of waste bread and distilled water was optimised and characterised. According to the analysis was found that optimal conditions for liquefaction of waste bread were at 80 °C and pH 6. Under these conditions was the highest yield of reducing sugars. The yield of reducing saccharides was 67.08 $g \cdot L^{-1}$. These conditions were used before all saccharification experiments by glucoamylase.

It was found that optimal conditions for saccharification were at 60 °C and pH 4.2. Under these conditions was the highest yield of reducing sugars. The yield of reducing saccharides was 70.75 $g \cdot L^{-1}$. Final yields of total sugars were calculated from the difference of initial and final concentrations. Final yield of glucose was 70.28 $g \cdot L^{-1}$ (by HPLC analysis). The yield of glucose was also calculated from the difference of initial and final concentrations.

Particular concentrations of total sugars formed during the liquefaction are summarized in Tab. I. Particular concentrations of total sugars formed during the saccharification are summarized in Tab. II. In the Tab. III initial and final yields of glucose during the saccharification analysed by HPLC are shown. Graphical comparison of particular concentrations of glucose obtained during the saccharification are shown at Fig. 1.

DISCUSSION

There are few works deals with the use of waste bread as raw material for fermentation (KAWA-RYGIELSKA, 2012; KOSSEVA and WEBB, 2013). The use of food waste as raw material for fermentation requires knowledge of their chemical composition. It is also necessary to know the changes occurring in the material during production and storage (KAWA-RYGIELSKA, 2012).

The main ingredient of the bread is starch. The starch is used as raw material for fermentation for example in distilling (MONTESINOS, T. and NAVARRO, J.. 2000). The waste bread could be considered as suitable raw material for production of different fermentation products (EBRAHIMI, F.

I: *The concentration values [g·L^{-1}] of total sugars during the liquefaction by α-amylase*

temperature [°C]	time [min]	concentration of total sugars [g · l^{-1}]		
		pH 5	pH 6	pH 7
70	0	13.06 ± 0.05	9.9 ± 0.4	12.2 ± 0.7
	30	31.58 ± 0.17	24.24 ± 0.14	33.24 ± 0.06
	60	45.4 ± 1.6	32.95 ± 0.12	43.16 ± 0.06
	90	55.3 ± 1.0	44.6 ± 0.3	49 ± 3
	120	60.4 ± 0.9	49.9 ± 0.2	54.8 ± 0.3
	150	64 ± 4	57.3 ± 0.7	55.37 ± 0.13
	180	66 ± 2	67 ± 3	62.9 ± 0.3
	210	71.45 ± 0.13	70.8 ± 0.5	63.3 ± 0.4
	240	71.4 ± 1.6	71.7 ± 0.4	63.1 ± 0.4
80	0	9.0 ± 0.6	7.6 ± 0.5	7.3 ± 0.5
	30	21.6 ± 1.1	18.2 ± 1.3	19.78 ± 0.08
	60	27.8 ± 0.3	24.9 ± 1.1	25 ± 2
	90	35.8 ± 0.7	45.0 ± 0.3	34 ± 2
	120	43.2 ± 1.5	57.60 ± 0.19	40 ± 4
	150	53 ± 2	70 ± 3	51.4 ± 0.2
	180	65 ± 3	78 ± 2	55.4 ± 0.3
	210	62.8 ± 1.3	75.4 ± 0.6	57.0 ± 0.4
	240	65.7 ± 1.4	74.7 ± 0.4	55.7 ± 0.4
90	0	7.32 ± 0.15	11.84 ± 0.19	12.11 ± 0.02
	30	35.69 ± 0.14	35 ± 2	36.8 ± 0.9
	60	40.24 ± 0.09	48.63 ± 0.09	49 ± 3
	90	44.3 ± 0.9	47.35 ± 0.10	57.2 ± 1.3
	120	47 ± 3	51.25 ± 0.09	70.7 ± 0.4
	150	51.3 ± 1.3	56.94 ± 0.03	71.2 ± 0.4
	180	51 ± 2	62.7 ± 0.4	67.3 ± 1.5
	210	55.05 ± 0.13	63.4 ± 0.3	71 ± 4
	240	52 ± 3	62.72 ± 0.06	65 ± 3

et al. 2008). Suitability of bread as raw material for fermentation could be reduced by some changes. During production and storage are forms some changes of bread ingredients. These are formation of lipid-gluten network (SINGH, 2005), Maillard reactions and retrogradation of starch (RIBOTTA, P. and LE BAIL, A., 2007).

In this work ideal conditions for enzymatic hydrolysis of wheatrye bread by two commercial enzymes (BAN 240 and AMG 300L) were found. These commercial enzymes are suitable for starch hydrolysis in the industry. Their thermostability is useful in terms of increasing the efficiency of hydrolysis and reduction of the risk of microbial contamination. Finding the optimal conditions of hydrolysis is important for the following use of the hydrolysed material. Efficient hydrolysis leads to a higher yield of fermentation.

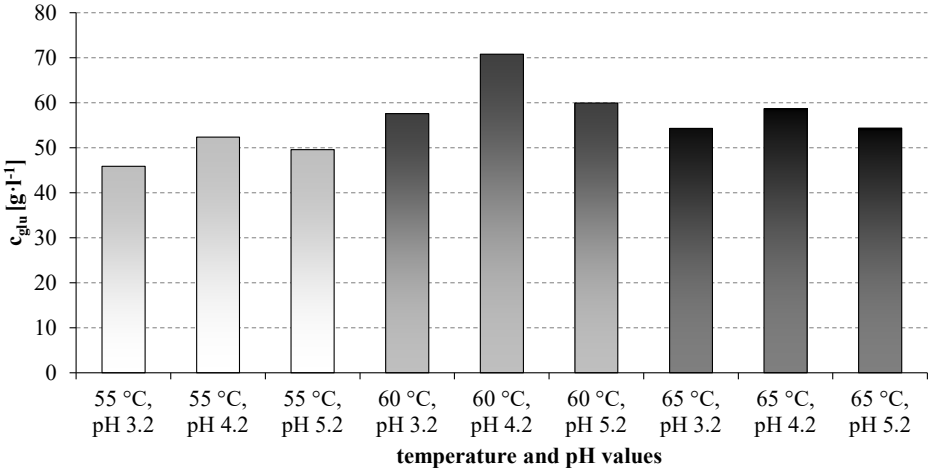
The aim of this work was study the other possibility of bakery waste hydrolysis. Bakery waste hydrolysates could be used for the other utilization to valuable products. Problem with using the bakery waste as common raw material is in pretreatment before hydrolysis and fermentation. Different kinds of pretreatment could be expensive and increase price of finally products. Moreover, the problem is storage of waste bakery (KAWA-RYGIELSKA *et al.*, 2007). It is often spoilage by microorganisms. Contamination of raw material by mold cause large economic losses (NEEDHAM *et al.*, 2005). However these problems and utilization of waste bread for production of biotechnological products requires further study.

II: *The concentration values [g·L^{-1}] of total sugars during the saccharification by glucoamylase*

temperature [°C]	time [min]	concentration of total sugars [g · l^{-1}]		
		pH 3.2	pH 4.2	pH 5.2
55	0	32 ± 2	24.78 ± 0.10	24.58 ± 0.07
	15	48.6 ± 0.2	47.47 ± 0.07	42.0 ± 0.4
	30	58.1 ± 0.2	56.73 ± 0.19	48.80 ± 0.06
	45	64.1 ± 0.4	61 ± 4	62.6 ± 1.0
	60	67.5 ± 0.3	65.57 ± 0.14	66.67 ± 0.18
	75	74.36 ± 0.14	68.0 ± 0.2	73 ± 3
	90	76.8 ± 0.6	76.8 ± 0.4	73.6 ± 0.5
	105	77.84 ± 0.14	77.12 ± 0.07	74.16 ± 0.11
60	0	18.9 ± 0.8	14.7 ± 0.7	15.51 ± 0.14
	15	42.3 ± 1.9	32.4 ± 1.0	41 ± 2
	30	47.35 ± 0.12	41.28 ± 0.07	48.59 ± 0.07
	45	58.4 ± 0.2	61.0 ± 0.5	62.4 ± 0.5
	60	61 ± 2	71.7 ± 0.4	66.4 ± 1.3
	75	70 ± 3	75.5 ± 1.3	71.36 ± 0.04
	90	79.0 ± 1.8	84.5 ± 0.8	74 ± 3
	105	76.5 ± 0.7	85.5 ± 0.5	75.4 ± 0.5
65	0	19.5 ± 1.9	20.50 ± 0.11	22.8 ± 1.7
	15	40.58 ± 0.12	39 ± 3	44.1 ± 0.2
	30	57 ± 3	54.8 ± 0.3	55.29 ± 0.15
	45	63 ± 2	62.0 ± 0.6	57.1 ± 0.8
	60	64 ± 6	66.47 ± 0.04	67.2 ± 0.5
	75	67 ± 8	73.9 ± 0.8	72.0 ± 0.2
	90	72.46 ± 0.18	78.2 ± 0.2	76.01 ± 0.04
	105	73.8 ± 0.5	79.14 ± 0.14	77.12 ± 0.07

III: *The concentration values [g·L^{-1}] of glucose during the saccharification by glucoamylase (HPLC)*

temperature [°C]	time [min]	concentration of glucose [g · l^{-1}]		
		pH 3.2	pH 4.2	pH 5.2
55	0	64.6 ± 0.4	57.4 ± 0.3	53.4 ± 0.4
	105	105.4 ± 0.8	102.6 ± 0.7	101.6 ± 0.3
60	0	42.9 ± 0.2	35.59 ± 0.07	43.9 ± 0.7
	105	101.8 ± 0.8	105.9 ± 1.8	98.6 ± 0.6
65	0	47.9 ± 0.3	45.1 ± 0.7	58.2 ± 0.3
	105	101.4 ± 0.9	101.0 ± 0.4	101 ± 3



1: *Graphical comparison of yields of glucose obtained during saccharification due to temperature and pH*

CONCLUSION

In this article the most suitable conditions for enzymatic hydrolysis of waste bread were finding. For experiments the waste dry bread was disintegrated into particles less than 1.25 mm. Thereafter were created several suspensions of bread and distilled water with different concentrations. It was found that 15 % (w/v) is the most suitable concentration of waste bread for hydrolysis.

After finding a suitable concentration of suspension for hydrolysis, enzymes were applied. In this work were used commercial enzymes BAN 240L (α-amylase from *Bacillus amyloliquefaciens*) and AMG 300L (glucoamylase from *Aspergillus niger*) by Novozymes.

Several enzymatic hydrolysis of waste bread were done. The α-amylase (BAN 240L) was used for the liquefaction of waste bread and glucoamylase (AMG 300L) for the saccharification. For both enzyme preparates was found optimal pH and temperature.

Optimal conditions for the liquefaction (pH 6; 80 °C) were found. The final concentration of total sugars was 67.08 g·L^{-1}. The time of liquefaction was about 180 minutes. After liquefaction was applied glucoamylase for saccharification. Optimal conditions for saccharification (pH 4.2; 60 °C) were found. The final concentration of total sugars was 70.75 g·L^{-1}. The time of saccharification was about 90 minutes. The final concentration of glucose analysed by HPLC was 70.28 g·L^{-1}.

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