13rd International Electronic Conference on Synthetic Organic Chemistry (ECSOC-13), 1-30 Novermber 2009
http://www.mdpi.org/ecsoc-13 & http://www.usc.es/congresos/ecsoc/13/

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Microwave-assisted enzymatic hydrolysis of starch

Marcin Lukasiewicz[1], Magdalena Marciniak[1], Anna Osowiec[1]

[1]Department of Food Technology, University of Agricultural,
ul. Balicka 122 PL-30-149 Krakow, Poland

e-mail: rrlukasi@cyf-kr.edu.pl

Abstract Keywords Introduction Experimental Results and Discusion Literature

ABSTRACT

Enzymatic hydrolysis of potato starch by γ-amylase was investigated to reveal the potential coupling mechanism of MIECC. The MIECC effect on increasing initial reaction rate ~2.5 times was observed in case of low viscous reaction system i.e. low substrate concentration. Up to now amylases were known as microwave sensitive enzymes, that are strongly deactivated when placed in microwave filed. Conducted experiments testifies on γ-amylase specific activation done by microwaves.

KEYWORDS

enzym, hydrolysis, microwave, starch

INTRODUCTION

Microwave-assisted synthesis attracts nowadays a lot of attentions because of the shortening in the reaction time, which is very often followed by the improvement in the yield and selectivity [1]. The advantage of the application of microwaves in chemistry has already been shown in a number of publications [2]. However, these phenomenon are still beyond a clear explanation. Up to now some hypotheses have already been emphasized using dielectric and conducting mechanism of microwave dielectric heating as well as interphase polarization [3]. From the other hand Microwave Irradiation-Enzyme Coupling Catalysis (MIECC) has also been proven as an useful tool for many enzymatic transformation in both water and organic solutions [4]. Enzymes as biocatalyst are mainly a proteins that are very heat sensitive. According

to that the overheating of reaction mixture in this case by means of hot spot as well as whole system overheating may cause denaturation of proteins or even a changes in active center conformation. As a result a dramatic decrease of enzyme activity is observed [5]. It was also proven that in case of low power of high-frequency electromagnetic field the nonthermal activation of enzyme may be observed [6]. Most of known MIECC reaction was investigated in nonpolar solvents that may protected the enzyme from overheating [7]. Experiment in high polar i.e. water solutions are very rare [8]. From the other hand enzymatic hydrolysis of starch is a very important industry process that guide to the wide range of different products. Examination of amylolitic enzyme working at microwave conditions looks promising in both scientific and industrial interests. .

EXPERIMENTAL

Reaction setup

For all described experiments a commercial potato starch was used. Modification was done using glucoamylase from *Rhizopus sp.*(Fluka). All the processes was carried out at 40°C and at pH = 6,5. Temperature was controlled by means of fiber optic thermometer with accuracy of 0,1°C. Before hydrolysis starch pastes were obtained at 90°C. Conventional experiments (Δ) were carried out in water bath and the microwave ones in RM-800 microwave reactor (Plazmatronika, Poland) - Figure 1. In all cases the stirring of the reaction system were applied.

Figure 1: RM-800 microwave reactor (Plazmatronika, Poland)

In a typical experiment 6.48g of starch (40mmol) was dispersed in 240ml of deionized water. The suspension was heated up to 90°C in order to obtained a starch paste. After cooling down to 40°C the solution of enzyme was added - 15mg dissolved in 10mL of water (the enzyme activity was estimated as 400u/g) and the solution was fill up to the final volume of 250mL. The reaction was carried out in MW or Δ conditions. At specific time intervals the progress of starch degradation was checked according to DNS method (see below).

Degree of hydrolysis

Dextrose equivalent (DE - dextrose equivalent) was determined by standard procedure for reducing power with 3,5-dinitrosalicyl acid (DNS). In this method the

free carbonyl group are oxidized to carboxyl ones what follows the reduction of DNS to appropriate aldehyde [9]

RESULTS AND DISCUSION

At the very first stage of experiments the heating rate as a function of applied microwave field was measured (Figure 2). As may be seen the heating rate at low power level (up to 70mW/g) is almost constant. The very fast temperature increase may be observed in the range of 70 - 120mW/g. At extremely high power level the second plateau may be observed. The phenomenon may by interpreted by means of high enthalpy of water evaporation. According to that for further experiments the low level of MW power was used in order to avoid the enzyme denaturation and minimal of thermal effects of the process.

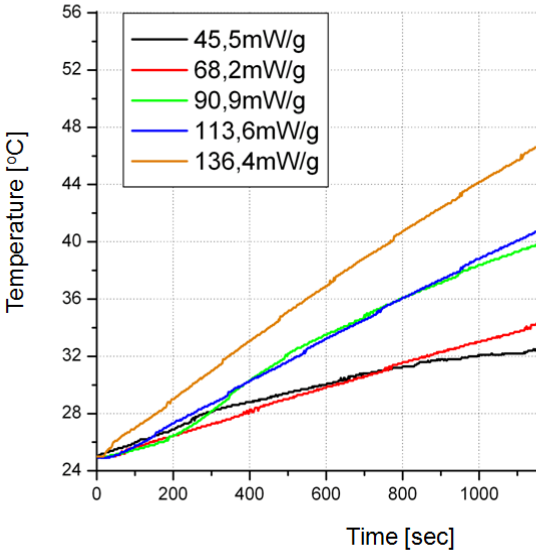

Figure 2: Temperature as function of heating time.

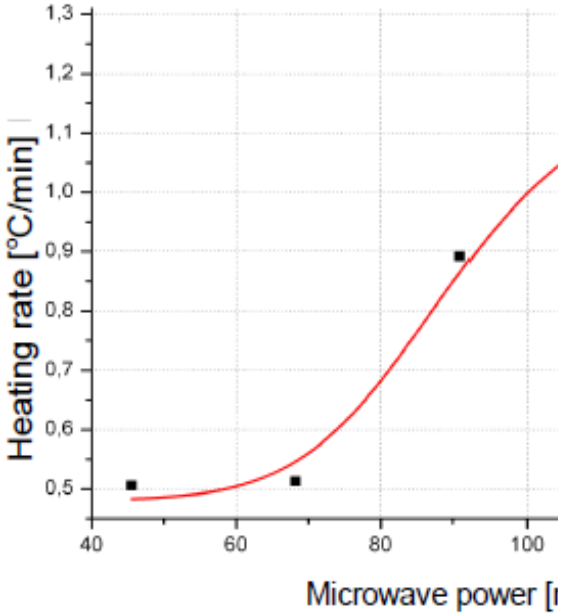

Figure 3: Heating rate as a function of MW power level.

The main experiments was carried out at low power level, at constant temperature but at different substrate (starch) concentration. The reason was well known phenomenon of viscosity of starch pastes that increase with carbohydrate concentration in enzyme. However hydrolysis of starch causes decreasing of viscosity, the high viscosity at the beginning of the process may cause heat exchange problem what influence on denaturation and activity of enzymes. The results of experiments at both conventional and microwave experiments drown as progression curves are presented at Figures 4-7.

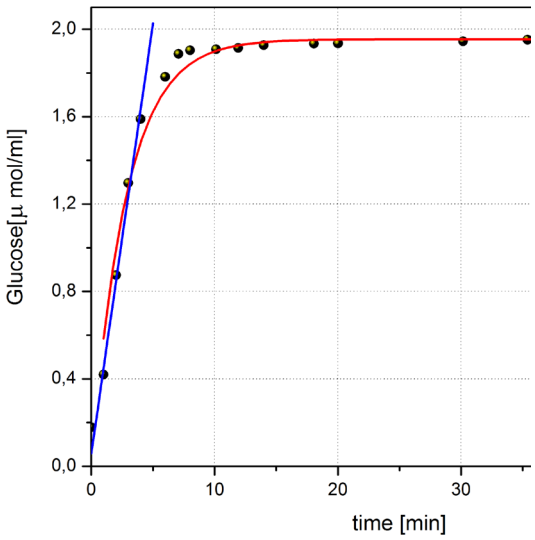

Figure 4: Conventional conditions $C_{0\text{-}AGU}$=0,160mmol/ml.

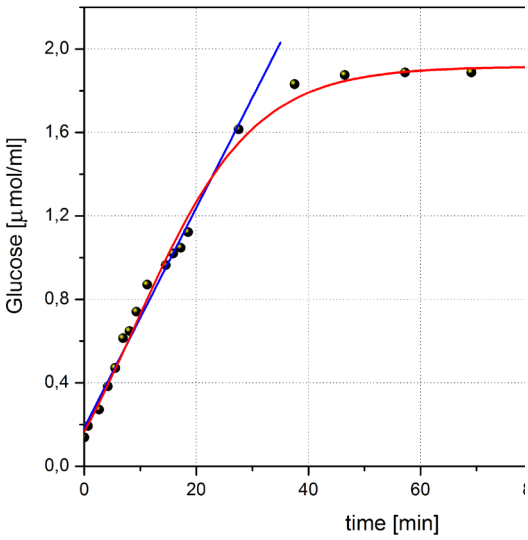

Figure 5: Conventional conditions $C_{0\text{-}AGU}$=0,101mmol/ml.

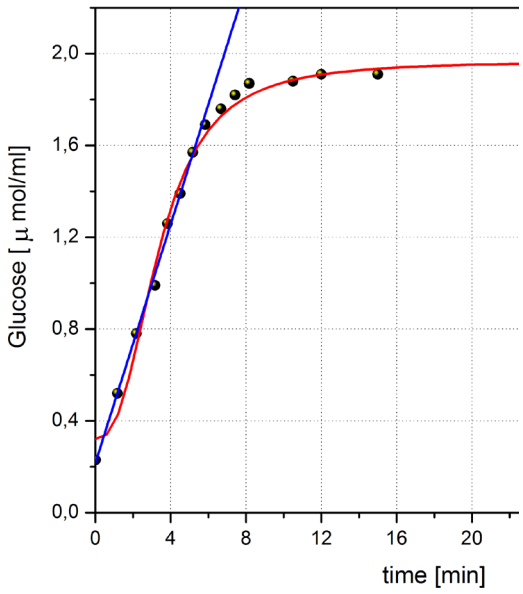

Figure 6: Microwave conditions $C_{0\text{-}AGU}$=0,160mmol/ml.

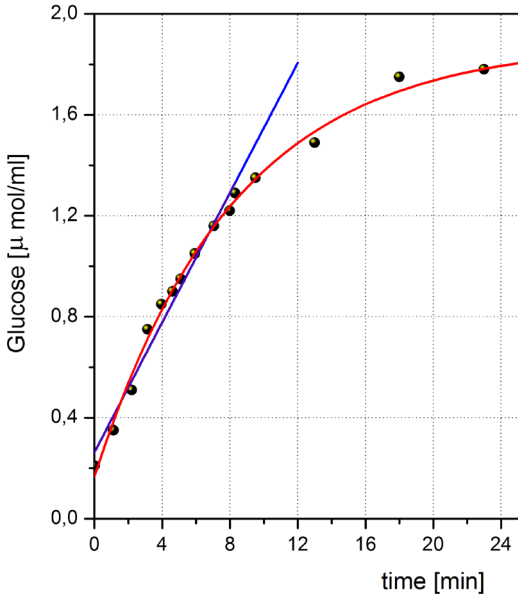

Figure 7: Microwave conditions $C_{0\text{-}AGU}$=0,160mmol/ml.

According to standard biochemical procedures for enzyme process kinetics it may be stated that all processes follows the rule of hyperbolic kinetics. It allows to determine so called initial rate that was collected in Table 1.

Table 1: Initial rates af enzymatic hydrolysis.

AGU [mmol/ml]	Conditions	Vo [mmol/min]	R^2
0.101	Δ	0.0528	0.998
0.101	MW	0.1285	0.975
0.160	Δ	0.3938	0.991
0.160	MW	0.2803	0.995

The obtained results and correctness of estimation may be proven by statistical analysis (R^2). Comparison of the results guides to the very interesting conclusions that are summarized at Figure 8. In high viscous environment i.e. higher starch concentration the inactivation of amylase at MW conditions may be observed. It testifies that overheating and hot spot are generating in the system with worst heat exchange. In opposite lower viscosity i.e. lower starch concentration gives completely different results. At low power level the additional activation of enzyme mau be observed. The electromagnetic field may in this case affect the conformation of active center to favor the cleavage of glicosidic bonds. Up to now there was no experimental proofs on activation of amylases by microwaves in water. At the present stage of research the nonthermal mechanism of activation may be proposed however further study are needed in this topic.

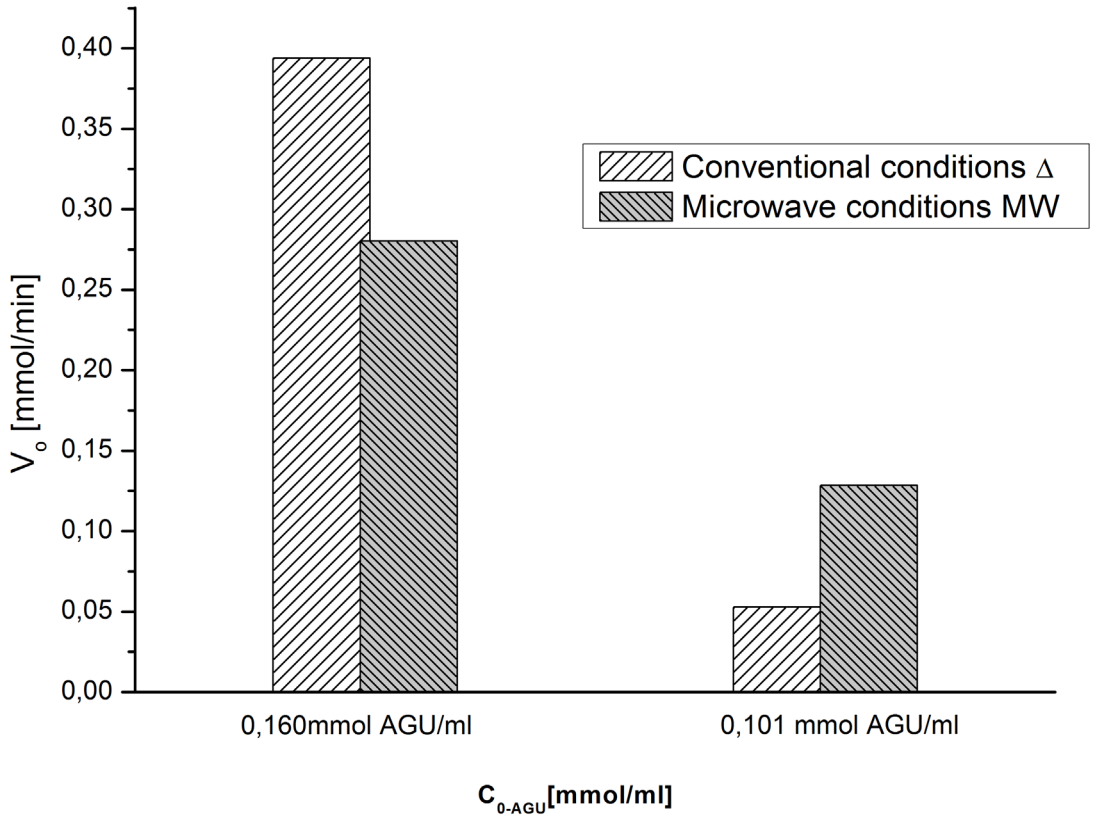

Figure 8: Comparison of conventional and microwave-assisted enzyme hydrolysis of starch.

Up to now amylases were known as microwave sensitive enzymes, that are strongly deactivated when placed in microwave filed. Presented experiments testifies on specific activation done by microwave. As a conclusion it is worth to point out some general statements:

(1) Enzymatic hydrolysis of starch using typical enzymes may successfully be carried out at microwave condition

(2) The effect of microwave irradiation strongly depends on:

Microwave power level - higher levels of MW may cause denaturation of the enzyme

Viscosity of the reaction system that is the function of starch concentration - in less concentrated pastes the diffusion of heat allowed to increase the reaction rate without denaturation of the enzyme

(3) The observed specific microwave effects may be treated as non-thermal, however there is a strong need to develop the research

(4) The MIECC effect on increasing initial reaction rate ~2.5 times was observed in

case of low viscous Microwave power [mW/g] reaction system i.e. low substrate concentration.

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