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# Waste cellulose enzymatic hydrolysis kinetic

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***Abstract***

Lignocellulosic biomass can be used to produce ethanol, and single cell protein. There are two processes involved in the conversion: hydrolysis of cellulose in the cellulosic biomass to produce reducing sugars, and fermentation of the sugars to bioethanol or used by microrganism as a major source of carbon, under aerobic cultivation conditions, high yield coefficients of biomass.

***Keywords***: kinetic, cellulose, hydrolysis, bioethanol, single cell protein

## 1. Introduction

Lignocellulosic materials from agriculture, forest management and urban waste are the largest sources by hexose (C-6) and pentose (C-5), sugars with a potential for the production of biofuels, chemicals and other economic by-products. Directive was accepted that requests member states to establish legislation about utilization of fuels from renewable resources. Biofuels are considered to be most promising in the short-term as their market maturity.[13 Wiesenthal Tobias] The rate and extent of cellulose hydrolysis by cellulose enzymes is influenced by many substrate and enzyme factors, and operational conditions. (Champagne).The aims of this study were to optimise the enzymatic hydrolysis of lignocellulose materials.

## 2. Materials and method

Lignocellulosic materials used in this study are waste cellulosic materials, such as: office paper, newspaper and cardboard 1:1:1 (w/w).The used materials were pretreated as follows: milled at a vibratory ball milling, autoclaved of waste cellulosic materials at $120^0$C, in wet atmosphere, with $H_2SO_4$, 2% (w/w) concentration (1/7 w/v) by and a reaction time of 24 hours, washed with distillated water until neutral pH and dried to a 95% dry mateer.

The enzyme used in this study, Celulaza Onozuka R 10 (by Trichoderma viride), 1 U/mg, was a commercial broth obtained from SC. Nordic Invest SRL, Cluj-NapocaThe buffer used was 50 mM citrate, pH 4,8. The reducing sugar reagent was the DNS reagent containing 1416 mL of deionized water, 10,6 g of 3,5-dinitrosalicylic acid, 19.8 g of NaOH, 306 g of Rochelle salts (Na-K tartrate), 7,6 mL of phenol, and 8,3 g of sodium metabisulfite. Glucose was used as the reducing sugar stock standard and was diluted to produce a standard curve. All chemicals were obtained from "Elena Doamna" Food Industry College, Galati or from SC. Nordic Invest SRL, Cluj-Napoca. Glucose concentrations were analyzed using a dinitrosalicylic acid (DNS) essay. The essays were performed in duplicate. The hydrolysis essay procedure was performed in 250 ml flasks that contained different concentration of cellulosic material and cellulase, suspended in 100 ml final volume with acetate buffer, pH 4,8. Te sample flasks were incubated in a thermostat, at $45^0$C, for 24 hours. The enzymatic reaction was stopped by essays keeping, for 10 minutes, in boil water.

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The mixture was centrifuged at 5000 rpm for 10 minutes, to separate the hydrolyzate; the samples were analized for reducing sugar content in the supernatant by the dinitrosalicylic acid (DNS) method, used pure glucose to the standard curve. The achieved results are shown in the table bellow.

**Table 1:** Rate of waste cellulose enzymatic hydrolysis

| Substrat concentration, % | Substrat concentration, [S], mg/ml | Reducing sugar concentration, mg/ml | v, µM reducing sugar / min | 1 / v, min / µM reducing sugar | 1/ [S], ml/mg |
|---|---|---|---|---|---|
| 0,05 | 0,5 | 0,037 | 0,14 | 7,14 | 2 |
| 0,1 | 1 | 0,18 | 0,69 | 1,45 | 1 |
| 0,2 | 2 | 0,22 | 0,85 | 1,17 | 0,5 |
| 0,4 | 4 | 0,3 | 1,15 | 0,87 | 0,25 |
| 0,6 | 6 | 0,37 | 1,42 | 0,7 | 0,16 |
| 0,8 | 8 | 0,44 | 1,7 | 0,59 | 0,12 |
| 1 | 10 | 0,59 | 2,27 | 0,44 | 0,1 |
| 1,2 | 12 | 0,81 | 3,12 | 0,32 | 0,08 |
| 1,4 | 14 | 0,89 | 3,43 | 0,29 | 0,07 |
| 1,6 | 16 | 0,93 | 3,58 | 0,28 | 0,06 |
| 1,8 | 18 | 0,96 | 3,7 | 0,27 | 0,055 |
| 2 | 20 | 1 | 3,85 | 0,26 | 0,05 |
| 2,2 | 22 | 1 | 3,85 | 0,26 | 0,05 |
| 2,4 | 24 | 0,93 | 3,58 | 0,28 | 0,06 |
| 2,6 | 26 | 0,85 | 3,28 | 0,3 | 0,038 |

Polinoms of 1,2,3,4 grade were plotted through essays. The correlation and the average square deviation grew, so the polinom grade 3 best represents the graphic diagram.

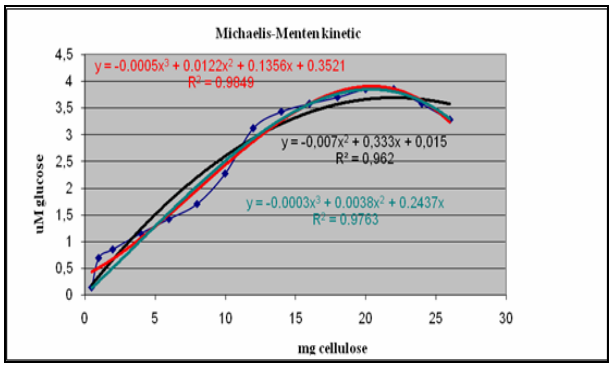


**Figure 1:** Michaelis-Menten kinetic of waste cellulose enzymatic hydrolysis

Were plotted the diagrams: $1/v = f(1/[S])$ and linear variation Lineweaver-Burk, V expresses in µM sugar reducer/minute, and[S] in mg/ml.. Where the linear variation meets the ordonate $K_m$ can be determined. So: $1/k_m = -5$, where $K_m$ is 0,2.

To calculate $K_m$ and $V_{max}$. kinetic parameters the Lineweaver-Burk variation was represented: $V_{max}$ = 3,85 µM reducing sugar, after Michaelis-Menten kinetic and after Lineweaver-Burk: $1/V_{max} = 0,7$, where $V_{max}$ = 1,43 µM reducing sugar, which represent the right value. A values range for [S] can be given to obtain $v_{max}$, between 20 and 22 mg/ml. At [S] bigger than 22 mg/ml, the enzymatic speed reaction lowers, due to substrat inhibition or the inhibition of reaction products (glucose, celobiose, etc.)

**Conclusions:** The enzymatic speed is maximum at substrat concentrations between 2-2,2%, respectively 3,58 µM sugar reducer / min, the obtained reducer sugar quantity is 1 mg/ml. 1 ml cellulase enzyme was used, 1% 1U/mg).

***Temperature optimisation***

For optimal temperature determination the procedure was: 0,2 g sample prepared as above was performed in 250 ml flasks with 2 ml cellulase 0,1%; at final volume by 50 with acetate buffer pH=4,8. Te sample flasks were incubated in a thermostat, at $45^0$C, for 24 hours, under agitation at a lab shaker at 150 rpm. The samples were kept frozen prior to enzymatic hydrolysis.

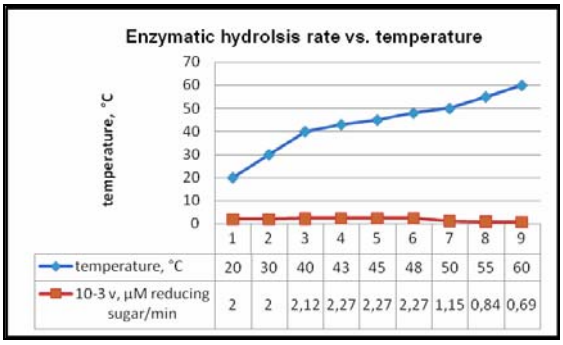


**Figure 3:** Enzymatic hydrolysis of waste cellulose vs. temperature

***Conclusions:*** maximum enzymatic reaction speed is obtained at temperatures between 43-48$^0$C; over or below, speed lowers. This is the optimal temperature interval for the enzyme in work Celulaza Onozuka R10. High temperatures make the enzyme inactive, hence proteic component abnormal.

***pH optimisation***

For optimal pH determination the procedure was: 0,2 g sample prepared as above was performed in 250 ml flasks with 2 ml cellulase 0,1%; at final volume by 50 ml with acetate buffer pH=4,8. The sample flasks were incubated in a thermostat, at 45$^0$C, for 24 hours, under agitation at a lab shaker at 150 rpm. The samples were kept frozen prior to enzymatic hydrolysis.

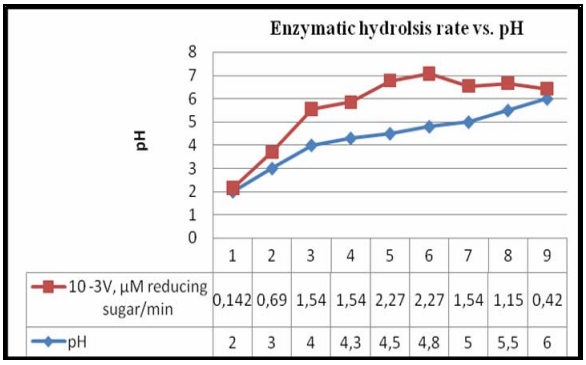


**Figure 4:** Enzymatic hydrolysis of waste cellulose vs. pH

***Conclusions:*** Enzymatic reaction speed grows proportionally with the reaction medium pH, with maximum values between pH: 4,5-4,8, at higher pH values, speed lowers. The optimal pH of cellulose enzymatic hydrolyse reaction ranges between pH: 4,5-4,8.

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