Research Article

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# Effect of alternating magnetic field treatments on enzymatic parameters of cellulase

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## Abstract

**BACKGROUND: Cellulase is an enzyme of the glycosyl hydrolase family that catalyses the cleavage of $\beta$-1,4 glycosidic bonds in cellulose. In this study an alternating magnetic field was applied to evaluate cellulase activity using carboxymethyl cellulose (CMC) as substrate.**

**RESULTS: The maximum and minimum activities of cellulase occurred when magnetic fields of 2.2 and 4.2 mT respectively were applied for 20 min. Following these treatments, the enzymatic parameters $K_m$ and $V_m$ were determined based on fitting to the Michaelis–Menten equations. Generally, $K_m$ showed the opposite trend to $V_m$ under magnetic field treatments. Treatment of enzyme/substrate solutions at 4.2 mT inhibited enzyme activity whereas treatment at 2.2 mT promoted it.**

**CONCLUSION: It appears that treating enzyme/substrate solutions with different magnetic fields can inhibit or promote enzyme activity. Further research is needed to determine how the magnetic field influences the enzyme and substrate.**


**Keywords:** magnetic field; cellulase activity; Michaelis–Menten model; kinetics

## INTRODUCTION

Cellulose is the most abundant biological polysaccharide found in all plants and algae. It is widely contained in municipal solid wastes and agricultural energy resources, i.e. biomass resources. Development of an effective process for cellulose-containing biomass utilisation has been studied as one of the post-petrochemical methods that could help to solve the food supply problem caused by the rapid increase in population worldwide and the energy shortage due to a lack of fossil fuels.[1,2] It is important to utilise cellulose, since cellulase efficaciously hydrolyses cellulosic material. The efficiency of enzymatic hydrolysis of cellulosic materials depends on the cellulase activity.

Cellulase represents a group of enzymes that contribute to the degradation of cellulose to glucose. In most cellulolytic organisms, several cellulase components form a cellulase complex that synergistically hydrolyses cellulosic substrates. The nature of the cellulolytic enzyme system used determines the mode of action of cellulase, the activity of each enzyme component, the synergistic action among enzyme components and the inhibitory effect of reaction intermediates and products on the enzymatic action.[3]

It is well known that cellulolytic enzymes are inhibited by cellobiose and/or glucose. The pattern of this inhibition has been studied for a long time, with various points of view being expressed on the nature of the inhibition. For example, it has been reported that competitive inhibition is dominant,[4,5] that non-competitive inhibition is observed[6] or that combined inhibition is involved.[7] Holtzapple *et al*.[8] reported that all forms of enzyme species (free, adsorbed and complex) are subject to inhibition in the process of cellulose hydrolysis. They also stated that a competitive inhibition pattern can be exhibited only if a significant fraction of the enzyme exists as enzyme–substrate complexes.

All living organisms, including human beings, are continuously exposed to electromagnetic fields generated by environmental and domestic sources such as 50–60 Hz high-voltage transmission lines and various electronic appliances. A large number of attempts to explain biological effects of magnetic fields on the molecular level have been reported for prokaryotes and eukaryotes.[9–12] It has been shown that magnetic fields may affect biological functions of organisms via changes in cell growth, proliferation and viability[13–15] and cell morphotype,[13] DNA alteration in terms of point mutations,[16] inhibition of nocturnal levels of melatonin[17] and increase in calcium influx.[18]

There have been some reports concerning effects of electric or magnetic fields on enzyme activities. Serpersu and Tsong[19,20] reported activation of a $K^+$ pumping mode of (Na, K)-ATPase by an oscillating electric field of 20 V cm$^{-1}$ and 1 kHz. Liu *et al*.[21] observed activation of an $Na^+$ pumping mode with an oscillating electric field. Sakai *et al*.[22] suggested that the enzymatic reaction was apparently activated in an alternating magnetic field. Celik *et al*.[23] found superoxide dismutase activity *in vitro* or *in vivo* and catalase activity *in vivo* in soybean roots under a magnetic field. In previous studies, magnetic fields were initially explored to inhibit the activity of polyphenol oxidases (PPOs),[24,25] and the Michaelis–Menten

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model was found to be useful to describe effects of magnetic fields on PPO inhibition.[26,27] Since PPOs extracted from fruits or vegetables are crude enzymes, the results may be influenced by ingredients of the tested PPOs. Up to now, there is no report on effects of magnetic field pretreatments on the enzymatic kinetics of analytical-grade cellulase.

The objectives of this research were (1) to determine the optimum magnetic field and treatment time, (2) to obtain Michaelis–Menten model parameters of cellulase enzyme under different treatment methods and (3) to explore the reaction mechanism of cellulase enzyme based on the kinetic analysis.

## MATERIALS AND METHODS

### Materials

3,5-Dinitrosalicylic acid (DNS) cellulase was purchased from Sigma-Aldrich (Poole, UK). The product was standardised to a declared activity of 10 000 U $g^{-1}$. The pH was maintained at 4.8 with 0.2 mol $L^{-1}$ acetic acid/sodium acetate buffer. The concentration of enzyme used was 100 mg $mL^{-1}$. The carboxymethyl cellulose (CMC) employed as substrate was obtained from Wuxi Chemical Plant (Wuxi, China). Other solvents and chemicals were of analytical grade and used without further purification.

### Glucose standard curve

Seven concentrations (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 mg $mL^{-1}$) of 2 mL of glucose solution and 1.5 mL of DNS as colour reagent[3,28,29] were heated in a boiling water bath for 5 min and then cooled to room temperature with tap water. After shaking and stirring for 20 min and adding distilled water to give 20 mL samples, the absorption values of the samples at 540 nm were finally measured using a spectrophotometer (722S, Shanghai Precision and Scientific Instrument Co., Ltd, Shanghai, China), with an extinction coefficient of 98.7 L $mmol^{-1}$ $cm^{-1}$ and distilled water as control. The regression equation between glucose level ($M$, mg) and absorption value ($x$) was obtained as $M = 2.809x + 0.0288$ ($R^2 = 0.997$) and used to estimate the enzyme activity.

### Definition of activity of cellulase

Since one unit of enzyme was obtained with 1 μmol of glucose produced by hydrolysis of 1 g of enzyme powder for 1 min at pH 4.8 and 50 °C, the activity of cellulase ($A_c$, U $g^{-1}$ = μmol $min^{-1}$ $g^{-1}$) could be estimated as follows:

$$A_c\ (\mathrm{U\ g^{-1}}) = (M \times 5.555)/(V \times t) \quad (1)$$

where $M$ is the amount of glucose produced by hydrolysis (μmol), $V$ is the amount of cellulase (0.05 g) and $t$ is the reaction time (5 min).

### Magnetic field generator

The alternating magnetic field generator was designed at Yangtze University (Jingzhou, China) and comprised six sets of cylindrical coils powered by a transformer (Fig. 1). Each set included three coils for replicated samples. The samples were placed on the non-conductive stand in the centre of the coils. The rocking bed could be operated by a rotational controller but was not used in this study. The maximum effective current was 0–10 mA, corresponding to a magnetic field amplitude of 0–4.2 mT, with a frequency of 50 Hz. The sample temperature during pretreatments was maintained at 24–26 °C by a thermostat. A detailed description of the magnetic field unit can be found in Ref. 30.

### Magnetic field treatment and enzyme activity

To determine the maximum and minimum activities of cellulase enzyme under different magnetic fields, 18 samples of 0.5 mL of fully mixed cellulase solution at 10 g $kg^{-1}$ diluted in 0.2 mol $L^{-1}$ acetic acid/sodium acetate buffer with 1.5 mL of CMC solution at 10 mg $mL^{-1}$ were subjected to alternating magnetic field treatments. By adjusting the current three times in the six different sets of coils, 18 magnetic fields were used and maintained for 20 min at room temperature. After establishing the maximum enzyme activity, the corresponding magnetic field was used to determine the effect of treatment time on the enzyme activity of mixed samples. The enzyme activity was compared between control and magnetically treated samples at treatment times ranging from 0 to 60 min. Separate enzyme solution and CMC solution were also used to estimate the kinetic parameters.

After treatment, each sample was immediately placed in a 50 °C water bath to measure the reaction velocity over a time range from 0 to 9 min at 1 min intervals, which was plotted as a function of CMC concentration (0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.5 and 1 mg $mL^{-1}$).[31] Then enzyme solution or CMC solution was added to constitute enzyme and substrate systems for 30 min. Finally, the glucose concentration was determined by monitoring the absorbance at 540 nm. All experiments were carried out in triplicate.

### Kinetic modelling

According to the Lineweaver–Burk plot, i.e. $1/V = (K_m/V_m) \times 1/[S] + 1/V_m$, the reciprocal of the reaction velocity was first plotted against the reciprocal of the concentration of CMC solution. Then relationships and coefficients of determination were obtained by linear regression. Thereafter the regressed equations were converted to the equivalent Michaelis–Menten formula, i.e. $V = V_m \times [S]/(K_m + [S])$, which was used to calculate the rate of substrate turnover at saturation with enzyme ($V_m$) and the half-saturation constant ($K_m$). The criteria for model discrimination were the mean square error and normal distribution of the residuals.

### Statistical analysis

The mean and standard deviation of the kinetic parameters $K_m$ and $V_m$ were obtained over three replicates. The mean values were separated by $t$ tests at a significance level of $P = 0.05$.

## RESULTS AND DISCUSSION

### Determination of magnetic field and treatment time

Figure 2 shows the enzyme activity of mixed cellulose enzyme and CMC solution as a function of magnetic field for 20 min. The maximum and minimum activities of cellulase enzyme occurred at magnetic fields of 2.2 and 4.2 mT respectively. The maximum and minimum enzyme activities showed an approximately 25% increase and 8% decrease respectively compared with the control value at zero magnetic field.

Figure 3 shows the relationship between enzyme activity and treatment time at a magnetic field of 2.2 mT. It was observed that the maximum enzyme activity occurred at a treatment time of 20 min. Therefore magnetic fields of 2.2 and 4.2 mT with a treatment time of 20 min were selected as further experimental conditions.

Based on biophysics theory, the magnetic field is recognised as an important physical factor in biological effects. The two major elements of magnetic fields, i.e. threshold intensity and treatment time, were also found to be biological indicators in



  

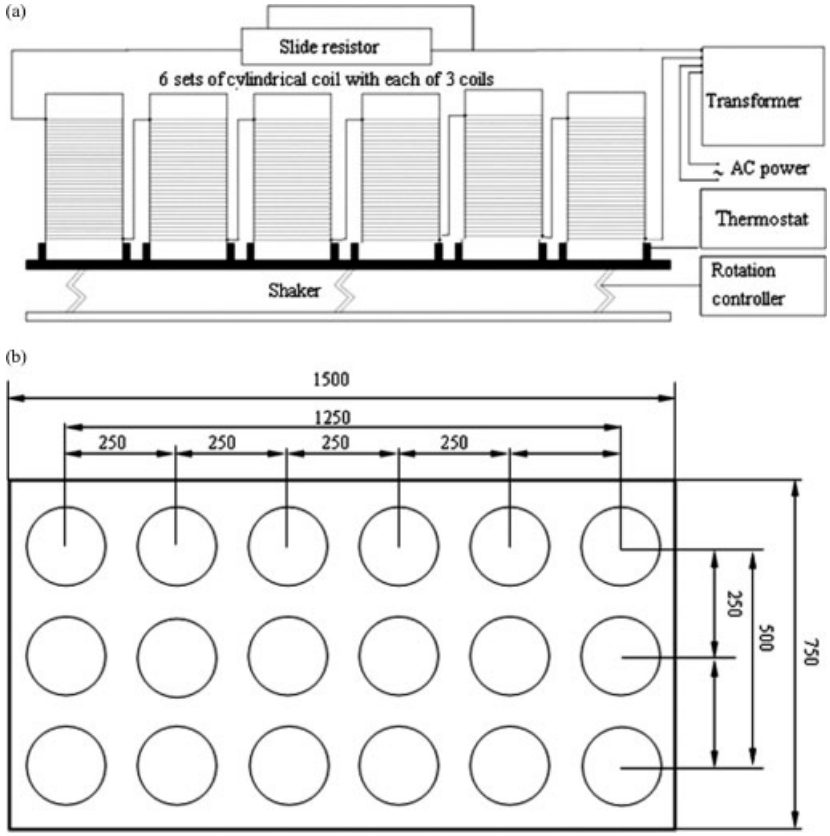


**Figure 1.** Magnetic field generator: (a) schematic of system; (b) vertical view of distance of each coil or glass bottle and table-board of rocking bed (all dimensions are in mm).[30].

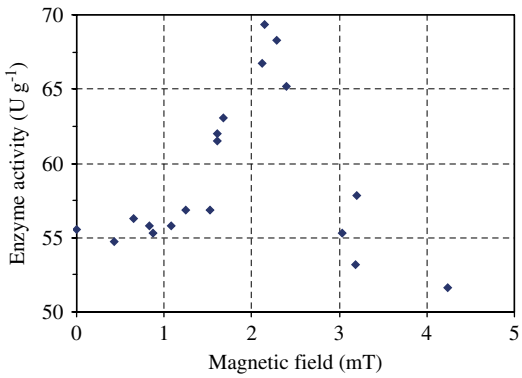


**Figure 2.** Enzyme activity of mixed cellulase enzyme and CMC solution as a function of magnetic field for 20 min.

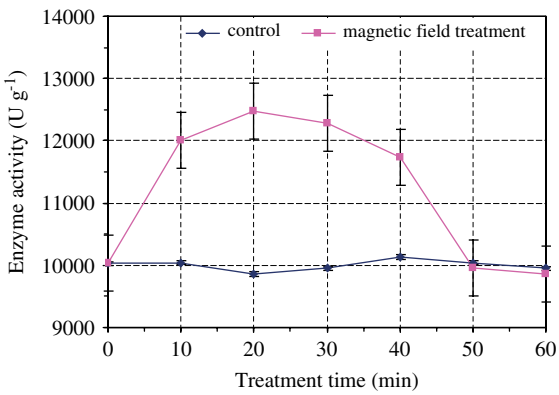


**Figure 3.** Relationship between enzyme activity and treatment time at magnetic field of 2.2 mT.

different materials.[32,33] Muraji *et al.*[34–36] reported the influence of an alternating magnetic field on the growth of the primary root of corn (*Zea mays*) seedlings. Strasak *et al.*[12] observed the effects of low-frequency magnetic fields at 2.7–10 mT and 50 Hz for 0–12 min on the viability and oxidoreductive activity of Gram-negative *Escherichia coli* bacteria, suggesting that bacterial growth is negatively affected by such magnetic fields. That is, the ability of bacteria to form colonies decreases with increasing magnetic field and treatment time.

The effects of magnetic fields on the activity of (Na, K)-ATPase have revealed several interesting properties of the electroactivation process.[19–21,37] Only the ouabain-sensitive ion-pumping modes of the enzyme are found to have electric field actions, while ion leakage and passive transport are not affected by electric field stimulation. These results imply that the electroactivation of the $Na^+$ and $K^+$ pumps follows mechanisms similar to those of ATP-dependent activation. Furthermore, the field-induced ion pumps have characteristic windows with respect to electric field frequency and amplitude.

### Kinetic parameters of Michaelis–Menten model

Figure 4 shows the initial enzymatic reaction velocity as a function of substrate concentration under seven treatment conditions. The reaction velocity increased linearly with increasing substrate



  

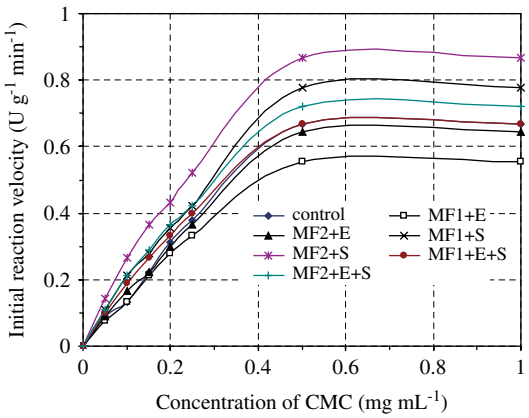


**Figure 4.** Effect of substrate concentration on initial enzymatic reaction velocity (see Table 1 for treatment methods).

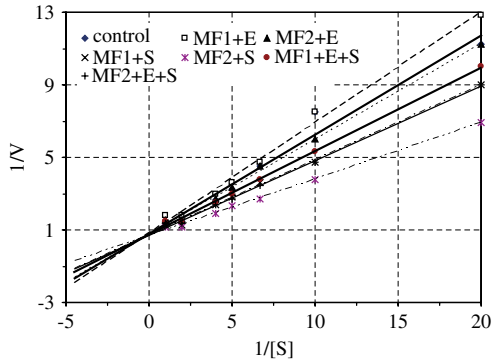


**Figure 5.** Relationship between reciprocal of reaction velocity ($1/V$) and reciprocal of concentration of CMC solution (1/[S]) (see Table 1 for treatment methods).

concentration until 0.5 mg mL$^{-1}$ for all treatment methods. The reaction proceeded fastest for the CMC solution treated by magnetic field at 2.2 mT (MF2 + S) and slowest for the cellulase enzyme solution treated by magnetic field at 4.2 mT (MF1 + E). At substrate concentrations above 0.5 mg mL$^{-1}$, all reaction velocities maintained a constant value.

The data in Fig. 4 were further processed via Lineweaver–Burk plots to obtain Michaelis–Menten equations (Fig. 5). The linear relationships with $R^2$ values of 0.972–0.997 (Table 1) between the reciprocals of reaction velocity and CMC concentration clearly indicated that the reaction mode could be explained by a simple Michaelis–Menten mechanism.

The linear equations were used to estimate $K_m$ and $V_m$ under the seven treatment conditions (Fig. 6). The results showed that the $K_m$ values obtained with a magnetic field of 4.2 mT were significantly larger than those obtained with a magnetic field of 2.2 mT ($P < 0.05$). The average $K_m$ values in cellulase enzyme solutions or mixed cellulase enzyme and CMC solutions treated by magnetic field at 4.2 mT were significantly larger than those in controls ($P < 0.05$). Thus the magnetic field of 4.2 mT could reduce the affinity of enzyme and substrate and thereby inhibit enzyme activity. This observed inhibition was similar to competitive inhibition.

However, the average $K_m$ values in all solutions treated by magnetic field at 2.2 mT were smaller than those in controls, but with significant differences ($P < 0.05$) only with CMC solutions

**Table 1.** Kinetic model parameters obtained with Michaelis–Menten equations under given treatment methods

| Treatment method[a] | Michaelis–Menten equation[b] | $R^2$ |
|---|---|---|
| Control | $V = 227.273 \times [S]/(0.6818 + [S])$ | 0.972 |
| MF1 + E | $V = 212.222 \times [S]/(0.7556 + [S])$ | 0.992 |
| MF2 + E | $V = 232.558 \times [S]/(0.6744 + [S])$ | 0.997 |
| MF1 + S | $V = 256.410 \times [S]/(0.5897 + [S])$ | 0.997 |
| MF2 + S | $V = 263.158 \times [S]/(0.4475 + [S])$ | 0.998 |
| MF1 + E + S | $V = 217.391 \times [S]/(0.7391 + [S])$ | 0.980 |
| MF2 + E + S | $V = 250.000 \times [S]/(0.5750 + [S])$ | 0.998 |

[a] Control, treated without magnetic field; MF1 + E, cellulase enzyme solution treated by magnetic field at 4.2 mT; MF2 + E, cellulase enzyme solution treated by magnetic field at 2.2 mT; MF1 + S, CMC solution treated by magnetic field at 4.2 mT; MF2 + S, CMC solution treated by magnetic field at 2.2 mT; MF1 + E + S, cellulase enzyme and CMC solution treated by magnetic field at 4.2 mT, respectively; MF2 + E + S, cellulase enzyme and CMC solution treated by magnetic field at 2.2 mT, respectively.
[b] $V$, reaction velocity (U g$^{-1}$ min$^{-1}$); [S], concentration of CMC solution (mg mL$^{-1}$).

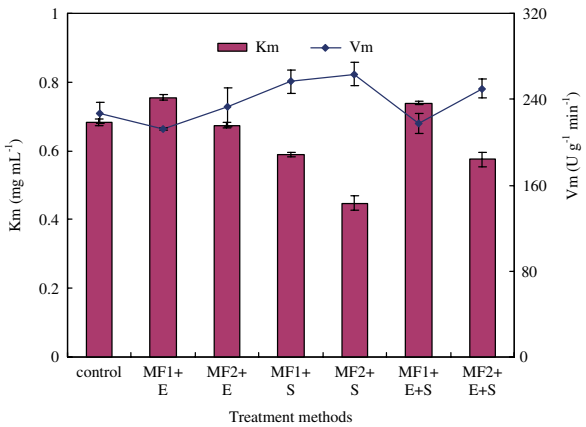


**Figure 6.** Effect of treatment methods (see Table 1) on concentration of CMC solution at half-maximum reaction velocity ($K_m$) and maximum reaction velocity ($V_m$).

(Fig. 6), suggesting that the magnetic field of 2.2 mT could increase the affinity of enzyme and substrate and thereby promote enzyme activity. The average $K_m$ values in CMC solutions treated with a magnetic field of 2.2 mT appeared to be the smallest among treatments. Generally, $V_m$ showed the opposite trend to $K_m$ under magnetic field treatments (Fig. 6).

Ghose and Das[4] reported that glucose inhibited the hydrolysis of cellulose by *Trichoderma viride* only slightly whereas cellobiose inhibited the reaction considerably even at very low concentration. They also concluded that the inhibition was competitive. This was also found by Gregg and Saddler,[5] who reported that end-product inhibition appeared to influence the initial rate of hydrolysis by increasing substrate recalcitrance, reducing the overall reaction rate and enhancing the hydrolysis reaction.

Gusakov and Sinitsyn[7] reported variations in inhibition pattern due to some key factors such as cellulase binding constant, enzyme concentration, available surface area of cellulose and $\beta$-glucosidase activity. In most cases the inhibition was either competitive or non-competitive. They also reported that the



  

enzyme/substrate ratio was a very important factor that could result in different product inhibition patterns.

Koneracka *et al*.[38] reported that bovine serum albumin could bind covalently to magnetic particles without losing its biological properties. They also found that magnetic particles immobilised with $\beta$-glucosidase responded quickly to the magnetic field and that 'on–off' control of the enzyme reaction was possible.

## CONCLUSIONS

Alternating magnetic fields at 50 Hz affected the activity of cellulase enzyme. The maximum and minimum activities of cellulase occurred when magnetic fields of 2.2 and 4.2 mT respectively were applied for 20 min. From kinetic analysis the enzymatic parameters $K_m$ and $V_m$ were obtained based on the Michaelis–Menten model. Magnetic field treatment of enzyme/substrate solutions at 4.2 mT inhibited enzyme activity whereas treatment at 2.2 mT promoted it. Further research is needed to determine how the magnetic field influences the affinity of enzyme and substrate and its possible effects on the structure of the substrate, the conformation of the enzyme and the valence state and position of metallic ions on active sites of the enzyme.

## ACKNOWLEDGEMENTS

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