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Enhancing the activity of cellulase enzyme using ultrasonic irradiations

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ABSTRACT

The present work investigates the effect of low intensity ultrasonic irradiation on the cellulase activity. The effect on the kinetic and thermodynamic parameters as well as the molecular structure of cellulase enzyme was evaluated with the help of the chemical reaction kinetics model, Arrhenius equation, Eyring transition state theory, Michaelis–Menten equation, fluorescence spectroscopy and circular dichroism (CD) spectroscopy. It has been established that ultrasound had a positive effect on the activity of cellulase enzyme, though the selection of operating conditions played a crucial role in deciding the intensification. The maximum cellulase activity was observed at 17.33 W/cm^2 intensity and ultrasonic treatment time of 30 min, under which the enzyme activity was increased by about 25% over the untreated enzyme. After the ultrasonic treatment, thermodynamic parameters E_a, ΔH, ΔS and ΔG were reduced by 64.7%, 68%, 37.3% and 1.3%, respectively. In addition, fluorescence and CD spectra revealed that the ultrasonic treatment had increased the number of tryptophan on cellulase surface, and changed the molecular structure of cellulase enzyme favourably to provide more access to the active sites.

1. Introduction

In recent times power ultrasound has been shown to have significant application in food and biotechnology processes [1]. For several years, ultrasound has been used as a method for enzyme inactivation but recently it has been reported that ultrasound does not inactivate all enzymes especially under mild conditions [2]. The ultrasound generated by periodic mechanical motion of an ultrasonic probe transfers energy into the solution and causes alterations in pressure leading to the creation of small rapidly growing bubbles [3]. These bubbles are enlarged during the negative pressure cycle and after undergoing oscillations in size during multiple acoustic cycles, finally collapse violently which generates high pressures, temperatures and shear forces. Even though high ultrasonic intensity or extended sonication time can denature enzymes, it has been shown that use of ultrasonic treatment at appropriate frequencies and intensity levels can lead to enhanced enzyme activity [4]. Ultrasound also results in favourable conformational changes in protein molecules without altering the structural integrity of the enzymes [5].

The ability of ultrasound to increase the activity of enzymes and to reduce the mass transfer resistances in the process makes this treatment a potential option for the conversion of lignocellulosic biomass under mild conditions using cellulase for the production of bioethanol. Cellulase (endo-1,4-β-D-glucanase) refers to a group of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze the process of hydrolysis of cellulose. Cellulases are widely used in various fields such as pulp and paper industry, textile industry, bioethanol industry, wine and brewery industry, food processing industry, animal feed industry, agricultural industries, etc. [6]. Cellulase breaks down cellulose into smaller polysaccharides or completely into β-glucose units which can be further fermented to bioethanol. Considering the commercial importance of cellulase, it is essential to find an effective method to improve the activity of cellulase.

In spite of the fact that ultrasound shows a potential to modify the enzyme activity, very less publications have targeted to study the effect of power ultrasound on the performance of enzymes. Actually, majority of the papers published in this area evaluated the effectiveness of the ultrasound-aided processes, without focussing on the intensification of catalytic activity using ultrasound. Souza et al. [7] carried out a comprehensive study and examined the activity of a commercial amylase enzyme after ultrasonic treatment in an ultrasonic bath at 40 kHz. It has been reported that ultrasonic treatment can promote enzyme activity. Glucose oxidase enzyme activity under ultrasonic irradiation was evaluated by Guiseppi-Elie et al. [8]. The results proved that the sonicated enzyme at 23 kHz showed a altered composition with reduced α-helix and β-sheet fractions upon extended sonication compared with the

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unsonicated enzyme. Along with the changes in the secondary structure, small subsequent decrease in the enzymatic activity was observed indicating that time and intensity are the important parameters [8]. Effect of ultrasound on the activity of dextranase has been investigated by Bashari et al. [9]. The highest activity of dextranase was observed with ultrasound treatment at 25 kHz and 40 W for 15 min, under which the enzyme activity increased by 13.4% as compared to the untreated enzyme.

Enzymes are typically used at their optimal conditions, where they demonstrate highest activity, thus achieving maximum reaction rate. Therefore it is necessary to understand the influence of the ultrasound on the effectiveness of the enzyme functioning under ideal conditions. Thus there is a necessity of more research in this area to better understand the relationship of the 'sonication–enzyme action'. This would facilitate the development of effective processes in the field of sono-biotechnology. Nguyen and Le [10] have indicated that ultrasound intensity of 12 W/ml had a positive effect on the cellulase with 18% increase in its activity but, the mechanism of ultrasound action on cellulase has not been reported. Therefore, the major focus of this work was to investigate the effect of ultrasonic treatment on the activity of cellulase enzyme. In order to explore the change in enzyme activity, the effects of ultrasound on activity, thermodynamics as well as molecular structure of cellulase were investigated with the help of the Eyring transition state theory, Arrhenius equation, circular dichroism (CD) spectroscopy and fluorescence spectroscopy. A simple kinetic model, based on Michaelis–Menten equation, has also been introduced in order to investigate the variations in the kinetic parameters.

2. Materials and methods

2.1. Materials

Cellulase enzyme was obtained as a gift sample from Advanced Biotechnologies, Mumbai, India. The enzyme activity was 205,000 carboxymethyl cellulose unit per gram (CMCU/g). Carboxymethyl cellulose, citric acid, 3,5-dinitrosalicylic acid (DNSA), phenol, NaOH, and potassium sodium tartrate were procured from S.D. Fine Chemicals and all were of analytical grade. Bovine serum albumin was obtained from Sigma Aldrich.

2.2. Ultrasonic treatment of cellulase

The device used for ultrasonic treatment of enzyme was a probe sonicator obtained from Dakshin Ultrasonics, Mumbai. The ultrasonic irradiation at a frequency of 20 kHz was transferred through a cylindrical horn. The experimental setup is shown in Fig. 1. Cellulase powder was dissolved in citrate buffer of pH 4.8 and made up to the final concentration of 1.0 g/l. 200 ml cellulase sample solution was put into the 250 ml beaker, and the beaker was placed in a water bath maintained at temperature of 50 °C as it is the optimum temperature for cellulase enzyme. The effect of ultrasound at different ultrasonic time was investigated over the range 5 to 70 min, whereas the effect of ultrasound intensity was evaluated over the range 2.88 to 23.10 W/cm^2. The cellulase activities were also investigated at different temperatures over the range of 20 to 50 °C, under ultrasonic treatment time of 30 min and 17.33 W/cm^2 intensity.

2.3. Analysis

2.3.1. Assay of cellulase activity

The activity of cellulase enzyme was determined using DNSA method described by Miller [11]. 0.5 M CMC was used as the substrate for hydrolysis by cellulase. The absorbance was measured at 540 nm with Chemito Spectroscan UV 2700 Double beam UV–Vis spectrophotometer, which was used to calculate the concentration of glucose released from CMC. 1 CMCU is the amount of enzyme, which under standard conditions degrades CMC to reducing carbohydrates with a reduction power corresponding to 1 μmol glucose per minute.

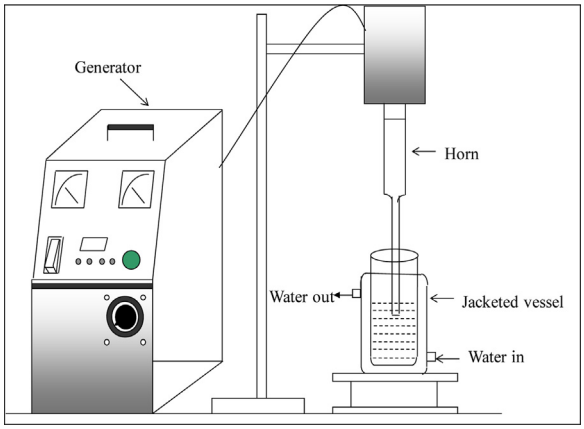

Fig. 1. Experimental setup for ultrasonic treatment of cellulase enzyme.

2.3.2. Soluble protein estimation

The total soluble protein content was determined by Bradford method, using bovine serum albumin as a standard [12]. To 0.25 ml of protein sample, 2.5 ml of Bradford reagent was added and the absorbance was immediately measured at 595 nm. For the calibration purpose, bovine serum albumin (BSA) was used.

2.4. Effect of ultrasound treatment on cellulase kinetics and thermodynamics

2.4.1. Ultrasonic treatment

For the determination of rate constants, 200 ml cellulase solution (1.0 g/l) was treated with ultrasound probe (20 kHz) at 17.33 W/cm^2 under different temperature (20, 30, 40, 50 °C) for 30 min. After that, 1.0 ml of ultrasound treated enzyme solution was added into the 3 ml of 0.5 M CMC solution. All the experiments were carried out at pH 4.8 in citrate buffer. For the determination of thermodynamic parameters, cellulase enzyme was treated with ultrasound probe (20 kHz) at 17.33 W/cm^2 for 30 min, and hydrolysis experiments were conducted at temperature of 20, 30, 40 and 50 °C, respectively.

2.4.2. Determination of kinetic parameters

The chemical kinetic model for cellulase enzyme used in the current work was based on the first-order kinetics as depicted in the following equation [13]:

$$\ln \frac{C}{C_0} = -kt \quad (1)$$

where C is the concentration of CMC at time $t = t$ (μg/ml), C_0 is the initial concentration of CMC, t is time, k is the total reaction rate constant involving the rate constants of ultrasonic action k_{us} and intrinsic activity k_c of cellulase (Eq. (3)). As it is difficult to measure the decrease in the CMC concentration, the reaction rate can be reflected by the increase in the amount of glucose released by CMC as follows [14]

$$\ln (V_\infty - V_t) = -kt + \ln V_\infty \quad (2)$$

where V_t is the concentration of glucose at time $t = t$ (μg/ml), V_∞ is the ultimate concentration of glucose (μg/ml), which is obtained from the hydrolysis experiment conducted under pH 4.8 at 50 °C

for 10 h. The k value can be determined experimentally from the slope by plotting $\ln(V_\infty - V_t)$ against t.

Compared with conventional enzymatic hydrolysis, the ultrasonic treated enzymatic hydrolysis reaction can be influenced by the ultrasound effect in addition to the thermal effect. Therefore, the k in ultrasonic treated enzymatic hydrolysis was expressed as:

$$k = k_c + k_{us} \quad (3)$$

where k_c is the reaction rate constant induced by thermal effect in conventional enzymatic hydrolysis, and k_{us} is the reaction rate constant induced by ultrasound effect in ultrasonic treated enzymatic hydrolysis.

2.4.3. Determination of thermodynamic parameters

The activation energy can be calculated from Arrhenius equation which is expressed as

$$k = Ae^{-E_a/RT} \quad (4)$$

where A is pre-exponential factor, E_a is the activation energy (J/mol) and R is the universal gas constant (8.314 J/mol K).

In the present study, Eyring transition state theory was used to obtain the thermodynamic parameters.

$$k = \frac{k_B T}{h} \exp\left(\frac{-\Delta G}{RT}\right) = \frac{k_B T}{h} \exp\left(\frac{-\Delta H}{RT} + \frac{\Delta S}{R}\right) \quad (5)$$

where T is the absolute temperature in K, k_B is Boltzman constant (1.38×10^{-23} J/K), h is Planck constant (6.6256×10^{-34} J/s). ΔG, ΔH and ΔS are the parameters of changes in free energy, enthalpy, and entropy for the process.

2.4.4. Determination of the kinetic parameters of the Michaelis–Menten equation

Michaelis–Menten constant (K_m) and maximum rate of reaction (V_{max}) values of untreated and ultrasound treated cellulases were determined by measuring the enzyme activities at various concentrations of CMC. K_m and V_{max} values were calculated according to Lineweaver–Burk plots.

2.5. Effect of ultrasonic treatment on the structure of cellulase enzyme

2.5.1. Intrinsic fluorescence analysis

Two samples were prepared to measure the intrinsic fluorescence. First sample was cellulase enzyme without ultrasonic treatment whereas the second sample was cellulase enzyme treated under optimized conditions of ultrasound intensity of 17.33 W/cm^2 for 30 min of irradiation time. Intrinsic fluorescence spectra of untreated (control) and ultrasound-treated sample in water were measured at room temperature (25 ± 1 °C) with fluorescence spectrophotometer (Jasco FP-6500 Spectrofluorometer) at 280 nm excitation wavelength (slit = 5 nm), 300–500 nm emission wavelength (slit = 5 nm) and 1200 nm/s of scanning speed.

2.5.2. Circular dichroism (CD)

CD spectra of untreated and ultrasound treated cellulase were recorded with a spectropolarimeter (Model MOS-450), using a quartz cuvette of 1 mm optical path length at room temperature (25 ± 1 °C). CD spectra were scanned in the far UV range (190 to 250 nm) with three replicates at 30 nm/min with 0.1 nm as bandwidth. The CD data were expressed in terms of mean residue ellipticity, $[\theta]$, in deg cm^2/dmol. The secondary structures of cellulase enzyme in the presence and absence of ultrasound irradiation were analyzed using an online server DICHROWEB [15].

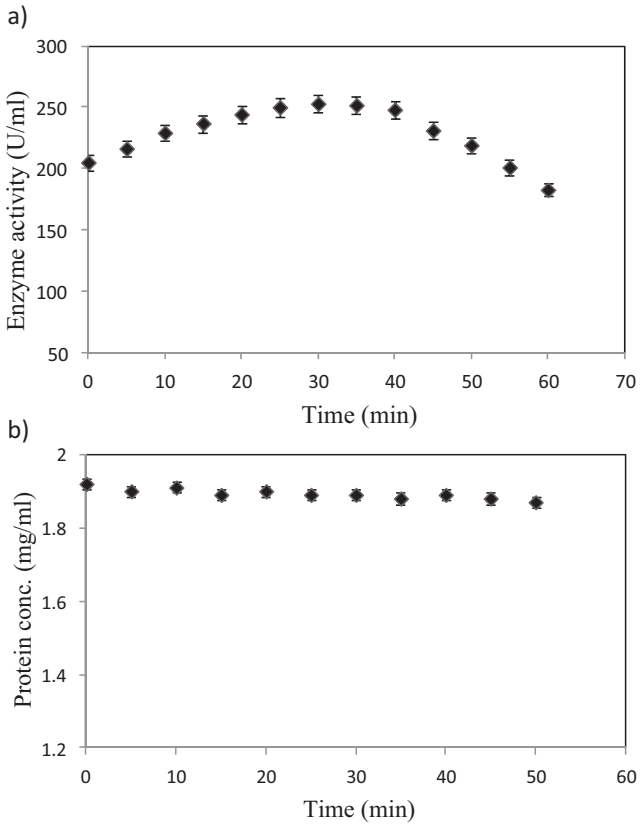

Fig. 2. (a) Effect of ultrasonic treatment on cellulase activity. (b) Effect of ultrasonic treatment on protein concentration.

3. Results and discussion

3.1. Effect of ultrasonic irradiation on activity of cellulase

The effect of ultrasonic treatment on cellulase activity has been shown in Fig. 2(a). The graph shows that an increase in ultrasonic treatment time significantly increased the cellulase activity till 30 min of irradiation time. When the treatment period was further increased to 50 min, cellulase activity was lower than that of control i.e. untreated enzyme. By using ultrasound, 23.4% increase in enzyme activity of cellulase was obtained at optimum treatment time of 30 min. Fig. 2(b) shows the effect of ultrasonic treatment on the protein content of cellulase enzyme. From Fig. 2(b), it can be clearly seen that there was no significant difference on the protein content after ultrasonic treatment. This result confirms that the change in enzyme activity was not due to the change of protein content in the preparation, including both enzyme and non-enzyme proteins.

Application of ultrasound generates the conditions of fluctuations in local velocity and pressure in adjacent fluid resulting in varied turbulence [16,17]. It can be stated that when ultrasonic treatment was conducted for an optimum time, most of enzyme molecules in the solution undergoes conformational change; which results in the enhancement in the cellulase activity [18]. Also the mass transfer resistances will be eliminated giving favourable results for the activity. It is important to note here that further increase in treatment time caused harmful effects, as continuous exposure to cavitating conditions for prolonged time led to degradation of the amino acid residues which contributes to the substrate binding domain or catalytic domain of the enzyme molecules resulting in decrease in enzyme stability [19]. Ertugay et al. [20] also reported that increased ultrasonic treatment time caused an

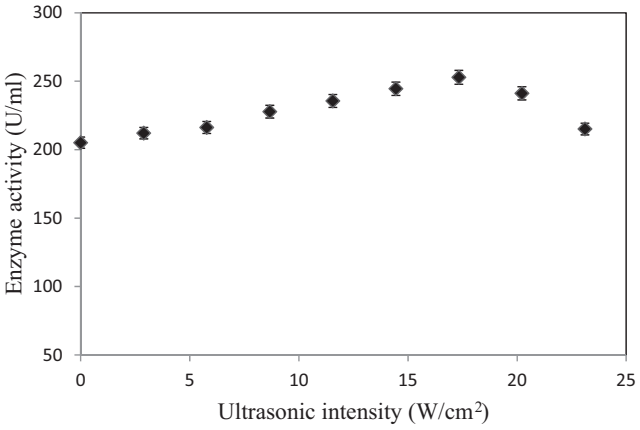

Fig. 3. Effect of ultrasonic intensity on cellulase activity.

inactivation effect, and resulted in a decrease in catalytic activity of lactoperoxidase in milk.

3.2. Effect of ultrasonic intensity on cellulase activity

Ultrasonic intensity is one of the important parameter which affects the catalytic activity of cellulase. The cellulase activity with varying ultrasonic intensity for optimum treatment time as 30 min has been shown in Fig. 3. The results demonstrated that, maximum activity of cellulase was observed for ultrasonic intensity of 17.33 W/cm^2. Cellulase activity increased by 23.4% over the untreated enzyme at 17.33 W/cm^2 intensity. When ultrasonic intensity exceeded 17.33 W/cm^2, the activity of cellulase decreased gradually with an increase in the ultrasonic intensity. Cavitation phenomenon occurring during ultrasonication was responsible for the observed behaviour of change in cellulase activity with varying intensity. Ultrasound can rupture the weak linkages like hydrogen bonds or Van der Waals interactions and bring conformational changes in protein structure [21]. Mild intensity and low frequency ultrasound irradiation in liquids causes the stable cavitation [22]. The forces induced by oscillation of stable cavitation bubbles changes the spatial conformation of enzyme, and thus enhances the activity of enzyme [23]. Szabo and Csiszar [24] observed 25% loss in the activity of cellulase at ultrasound intensity of 43.4 W/cm^2 whereas in the present study, cellulase activity was increased by 23.5% at ultrasound intensity of 17.33 W/cm^2. Thus it can be said that optimisation of the operating intensity is necessary so that beneficial effects are obtained for each of the enzymes used in sonicated system.

On the other hand, when ultrasound intensity exceeded 17.33 W/cm^2, decrease in cellulase activity was observed. The probable explanation for this is, high intensity ultrasound enhanced cavitation effects that caused significant shear in the liquid medium. Ultrasound irradiation, under these extreme conditions, could cause great damage to polypeptide chains, leading to inactivation of enzyme [25]. Additionally, extreme increase in localized pressure and temperature at higher intensity also leads to the generation of free hydroxyl and hydrogen radicals. These free radicals react with the enzyme causing its inactivation [26].

3.3. Effect of ultrasound on kinetic rate constants

In chemical kinetics, value of rate constant is an important parameter which is independent of the substrate concentration, and is mainly dependent on the temperature of reaction, medium and catalyst. The change in the cellulase activity occurred due to ultrasonic treatment will result in the change in the rate constants

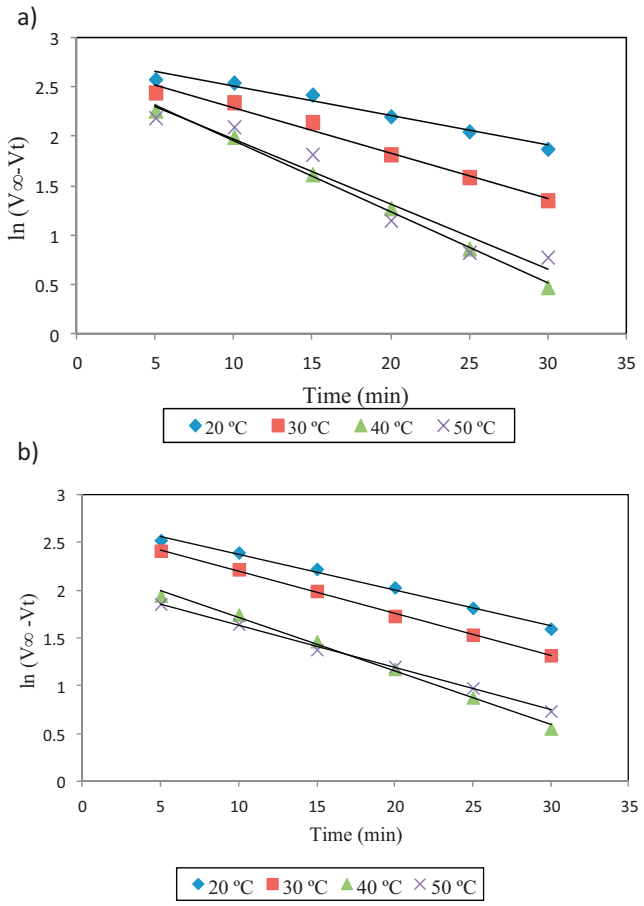

Fig. 4. (a) Relationship between $\ln(V_\infty - V_t)$ and reaction time for untreated cellulase. (b) Relationship between $\ln(V_\infty - V_t)$ and reaction time for ultrasound treated cellulase.

of hydrolysis of CMC to produce glucose. So in order to understand the effect of ultrasound irradiation on the rate of hydrolysis of CMC, experimental data have been fitted in the kinetic model. The ultimate hydrolysis extent in the absence of ultrasound was observed to be 31.8% of CMC.

Fig. 4(a) represents the plots of $\ln(V_\infty - V_t)$ versus time for the untreated cellulase whereas Fig. 4(b) shows the plots of $\ln(V_\infty - V_t)$ versus time for the ultrasound treated cellulase at 17.33 W/cm^2 for 30 min. It can be seen from Fig. 4(a) and (b) that, hydrolysis of CMC by untreated as well as ultrasound treated cellulase obeyed the first order kinetics. The rate constants k, k_c and k_{us} at different temperatures have been summarized in Table 1. It can be seen that rate constant k increased with increase in temperature from 20 to 50 °C. This can be attributed to the increase in collision frequency between the CMC i.e. substrate and cellulase enzyme at higher temperature [27].

3.4. Effect of ultrasound on the thermodynamic parameters

Activation energy is the minimum amount of energy required to convert a normal stable molecule into a reactive molecule and

Table 1
Reaction rate constants of untreated and ultrasound treated cellulase.

Temperature (K)	k_c (min^{-1})	k_{us} (min^{-1})	k (min^{-1})
293	0.0112	0.0432	0.0544
303	0.0212	0.0563	0.0775
313	0.0453	0.0687	0.114
323	0.083	0.0897	0.1727

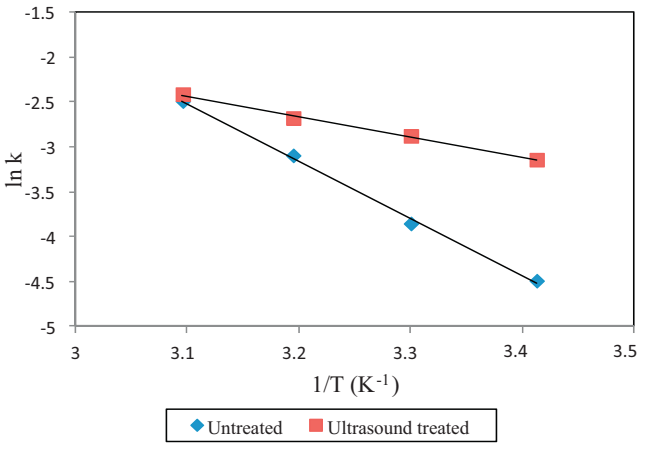

Fig. 5. Relationship between lnk and $1/T$.

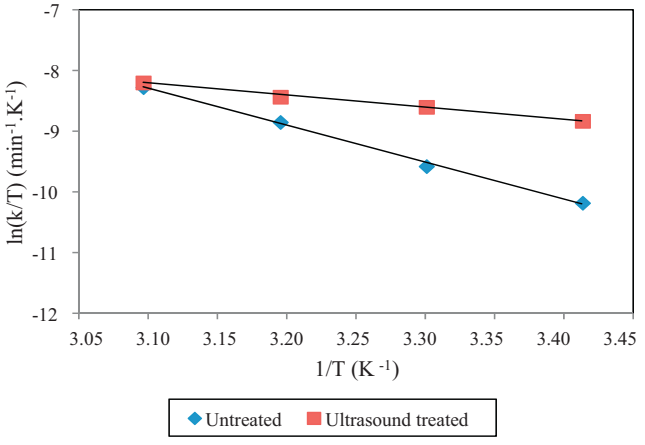

Fig. 6. Relationship between $ln(k/T)$ and $\left(1/T\right)$.

it reflects the rapidity of chemical reactions [28]. Arrhenius plots of ln k against reciprocal of absolute temperature (K^{-1}) have been shown in Fig. 5. Activation energy was determined from the slope of the Arrhenius plot. For the untreated and the ultrasound treated cellulase, the activation energy values were 53.3 and 18.8 kJ/mol, respectively, which shows that ultrasound decreases the energy barrier necessary for reaction remarkably. Ultrasonic treatment of cellulase reduced the activation energy by 65% than that of untreated cellulase. The greatly reduced E_a value by ultrasonic treatment indicates that the hydrolysis reaction catalyzed by cellulase could occur very easily.

Eyring plots of ln $\left(k/T\right)$ versus reciprocal absolute temperature, $1/T$ has been shown in Fig. 6. The plot resulted in good linear fits and ΔH was estimated from slopes of the curves and ΔS calculated from the intercepts. The values of ΔH, ΔS, and ΔG obtained from the data have been given in Table 2. It can be seen that ΔG decreased by 1.3% after ultrasonic treatment, which represents the increase in cellulase activity. After ultrasonic treatment, ΔH decreased by 68%, attributed possibly to the ultrasonically induced rupture of hydrogen bonds stabilizing the enzyme at ground state and the distraction of the internal hydrophobic core, both of which leads

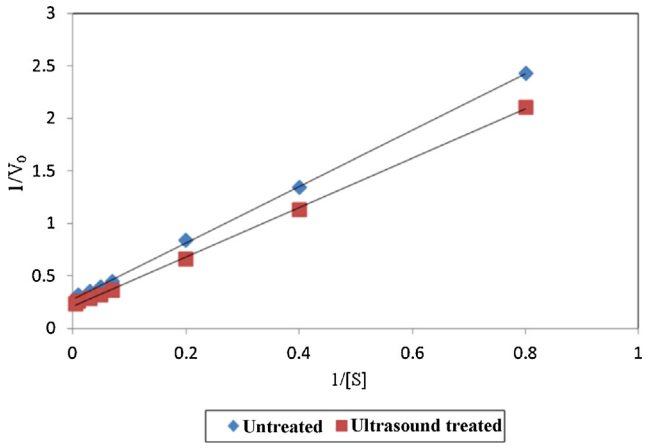

Fig. 7. Plots of the V_0 values obtained as a function of the [S] using Lineweaver–linearization.

to alteration in the protein structure. ΔS represent the variation in the extent of local disordering between transition state and the ground state. The 37.3% decrease in the ΔS can be attributed to the oxidative modification of amino acid residues and initiation of cross-linking and aggregation which leads to the increase in enzyme activity [14]. Qu et al. [29] have also reported the similar results for change in thermodynamic parameters of alcalase enzyme after ultrasonic irradiation, where ΔH, ΔS and ΔG were reduced by 74.1%, 34.3% and 1.4%, respectively. These values are in well agreement with the values obtained in the present work.

3.5. Effect of ultrasonic treatment on the kinetic parameters

By using a linear transformation of the Michaelis–Menten equation, the Lineweaver–Burk plot was made where the reciprocal of the initial reaction rate (V_0) was plotted against the reciprocal of the substrate concentration [S] as shown in Fig. 7. From Fig. 7, maximum rate of reaction, when the enzyme was saturated with substrate (V_{max}) and the Michaelis constant (K_m) were obtained. From the Lineweaver–Burk plot, the value of V_{max} can be obtained from the intercept and the value of K_m/V_{max} from the slope.

Results of the kinetic experiments have been summarized in Table 3. Studies of the ultrasonic effects on cellulase kinetics were carried out under the optimal ultrasonic conditions and compared to the control without the use of ultrasound. As can be seen in Table 3, the result showed that, V_{max} of cellulase increased whereas K_m decreased for the ultrasound-treated enzyme compared to the untreated enzyme. V_{max} reflect the limiting rate of the enzymatic reaction at substrate saturation, whereas K_m value indicates the affinity between the enzyme and the substrate. An increase in V_{max} indicates that a considerable movement of reactants to the active site of the enzyme and the reaction products to the medium were achieved under an ultrasonic field [23]. On the other hand, decrease in K_m under ultrasonic irradiation might be attributed to intense pressure, shear force and temperature which were generated as a result of the ultrasound cavitation. The exposure of active centre would make cellulase combine with the substrate easily and display better reaction ability [30]. Sulaiman et al. [31] studied the ultrasound assisted enzymatic hydrolysis of CMC. The

Table 2
Thermodynamic parameters of untreated and ultrasound treated cellulase.

Treatment	E_a (kJ/mol)	ΔH (kJ/mol)	ΔS (kJ/mol)	ΔG (kJ/mol)
Untreated	53.23	50.67	−109.37	84.90
Ultrasound treated	18.8	16.24	−142.72	83.67

Table 3
Kinetics parameters of untreated and ultrasound treated cellulase.

Treatment	K_m (μM)	V_{max} (μM/s)
Untreated	9.73	3.62
Ultrasound treated	8.31	4.76

Table 4
Contents of secondary structure of untreated and ultrasound treated cellulase.

Treatment	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Random coil (%)	Enzyme activity (U/ml)
Untreated	25.93	26.31	20.94	24.97	205.07
Ultrasound treated	22.72	24.58	22.86	32.36	252.87

optimum ultrasonic intensity for enhancing the hydrolysis of CMC was found to be 11.8 W/cm^2. Sonication at the power intensity of 11.8 W/cm^2 enhanced the V_{max} by 84% compared to untreated sample and reduced the value of K_m by 41% compared to control sample. These results are in well agreement with the present study where 17.33 W/cm^2 was found to be the optimum power intensity for the hydrolysis of CMC with an increase in V_{max} by 34% and decrease in K_m by 17% as compared to that of the untreated sample.

3.6. Effect of ultrasonic treatment on the structure of cellulase

In general, enzymes are monomeric globular proteins whose catalytic activity depends on the native configuration of their active sites. The irradiation with ultrasound might cause the changes in the secondary structure leading to better exposure of active sites of enzyme. Thus in order to get a better understanding about the effect of ultrasound irradiation on the activity of cellulase and the secondary structure and tertiary structure, the conformation changes in cellulase were evaluated by measuring the intrinsic fluorescence and CD spectra in the presence and absence of ultrasound.

Fluorescence spectroscopy is a useful method to analyze the structure alteration in proteins since the intrinsic fluorescence of aromatic amino acid residues is susceptible to the polarity of microenvironments along the transition [32]. The intrinsic fluorescence of enzyme is due to their amino acid groups; and mainly attributed to Trp, Tyr and Phe residues, particularly to Trp residue [33]. In the present work, changes in the enzyme conformation were investigated by Trp fluorescence spectrum (the maximum fluorescence emission wavelength is 348 nm). It can be seen from Fig. 8(a) that, the fluorescence intensity of the ultrasound treated cellulase decreased compared to that of untreated cellulase, which clearly confirmed that, the ultrasonic treatment increased the number of tryptophan on cellulase surface [34]. Additionally, the optimum fluorescence emission wavelength did not show a red or blue shift. Therefore, results confirmed that, ultrasound irradiation induced molecular unfolding of protein, destroyed hydrophobic interactions of protein molecules, caused more groups and regions inside the molecules to expose and therefore, decreased the fluorescence of cellulase [35].

CD is being increasingly being considered as a helpful technique for investigating the structure of proteins in the solution. This technique has been established to be easy and consistent for rapid evaluation of protein structure and monitoring conformational changes [36]. The results of the analysis of the CD spectra of untreated and ultrasound treated cellulase are shown in Fig. 8(b) and are summarized in Table 4. The content of α-helix, β-sheet and random coil of cellulase was calculated to understand the connection between enzyme activity and secondary structure. As can be seen in Table 4, the fraction of α-helix increased due to the ultrasound irradiation. Such an increase in the α-helical fractions in cellulase as a result of the ultrasonic treatment could be attributed to the pressure alterations and turbulence [37] and the induced structural transformations, which may perhaps affect the active site of the enzyme. Under optimum ultrasound condition, the contents of α-helix decreased by 12.4%, and random coil increased by 29.6%, respectively compared with that of untreated enzyme. These changes made cellulase show more uniformity and flexibility, which are useful for the enhancement of their activity and hence

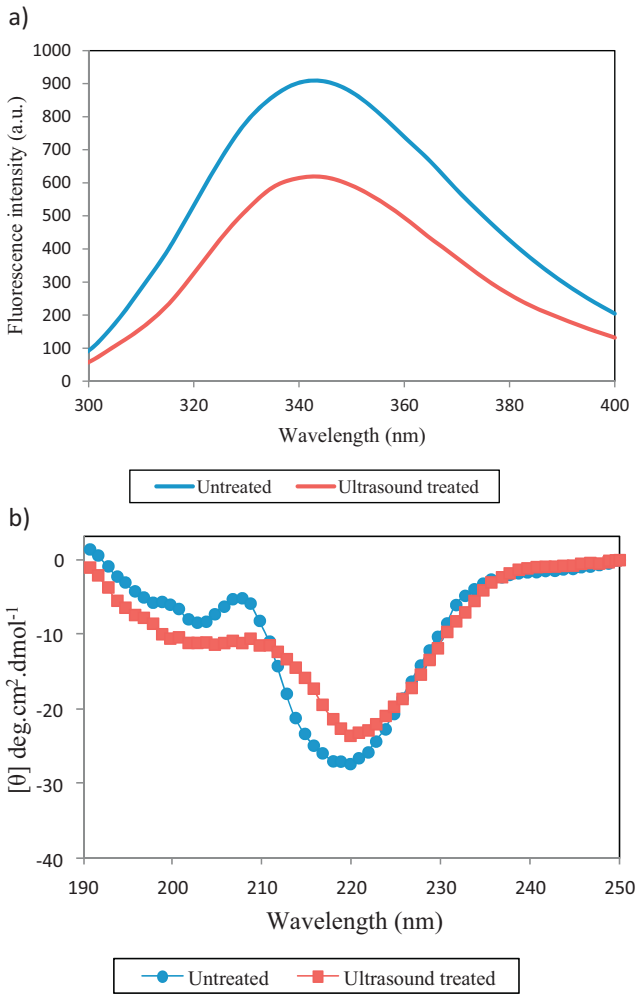

Fig. 8. (a) Intrinsic fluorescence spectra of untreated and ultrasound treated cellulase. (b) CD spectra of untreated and ultrasound treated cellulase.

in the catalytic efficiency. Wang et al. [38] have also reported similar results for change in composition in cellulase structure with 8.85% decrease in α-helix content and 29.5% increase in random coil. These results are in well agreement with the results obtained in the present work.

4. Conclusions

The present work has clearly established that, low intensity ultrasound has a positive effect on cellulase activity. The maximum activity of cellulase was observed when the enzyme was treated with ultrasound at 17.33 W/cm^2 intensity for 30 min, under which the enzyme activity increased by ~25%compared to the untreated sample. Significant reduction in thermodynamics parameters was observed after ultrasonic irradiation. E_a, ΔH, ΔS and ΔG were reduced by 64.7%, 68%, 37.3% and 1.3%, respectively. Results showed that, hydrolysis reaction under ultrasound irradiation obeyed Michaelis–Menten kinetics. Under the optimal

ultrasound conditions, the values of V_{max} and K_m were higher compared to untreated enzyme. Fluorescence spectra reflected that the ultrasonic treatment had increased the number of tryptophan on cellulase surface. The molecular structure of cellulase as established using CD spectra showed favourable changes in terms of composition of α-helix, β-sheets and random coil.

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