Bioethanol fermentation from untreated and pretreated lignocellulosic wheat straw using fungi *Fusarium oxysporum*

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A comparison has been made among untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw for bioethanol production by simultaneous saccharification and fermentation (SSF) process in a continuous stirred batch bioreactor (CSBR) using fungi *Fusarium oxysporum*. The optimum parameters used for the bioethanol fermentation are: time 48 h, pH 6, temperature 50, stirring speed 35, and wheat straw loading 35 g/L. Maximum yield of ethanol is found to be 0.756, 0.796 and 0.810 g/g of wheat straw under optimum conditions for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw. The sp. fungal growth rate is found to be 5.26, 5.40, and 5.88 s\(^{-1}\) and maximum sp. fungal growth rate is 10.52, 10.80 and 11.76 s\(^{-1}\) using the Monod model for the fermentation of untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw under optimum conditions respectively. The fungal growth kinetic parameters are 33.6, 33.9 and 34.7 g/L respectively for fermentation of the three samples under the optimum conditions. The sp. carboxy methyl cellulase (*CMCase*) activity are 1185, 1455 and 1545 min\(^{-1}\) and maximum sp. *CMCase* activity are 2370, 2910 and 1545 min\(^{-1}\) using Michaelis-Menten enzyme kinetic model for the fermentation of three samples respectively under optimum conditions. The *CMCase* enzyme kinetic parameters are 34.3, 34.5 and 34.7 g/L for the three samples respectively under optimum fermentation conditions. Fungi *Aspergillus oryzae* show the better conversion of ethanol than fungi *Fusarium oxysporum*. The fermentation process follows the first order rate equation in CSBR.

**Keywords:** Biofuel, Ethanol, Fermentation, Saccharification, Wheat straw

The use of ethanol as a source of energy would be more than just complementing for solar, wind, and other intermittent renewable energy sources in future. Ethanol has already been introduced on a large scale in many countries, and it is expected to be one of the dominating renewable biofuels in the transport sector. Ethanol can be blended with petrol and used as neat ethanol. Compared to single gasoline, ethanol has a higher octane number (96-113) that reduces the need for toxic octane enhancing additives. It is also a provider of oxygen, which helps to reduce the emission of carbon monoxide, nitrogen oxides, non-combusted hydrocarbon, and volatile organic compounds after combustion. In addition, ethanol is about 15% more efficient than gasoline in optimized spark-ignition engines. Furthermore, the production of ethanol by fermentation offers a more favorable trade balance, enhanced energy security, and a major new crop for a depressed agricultural economy\(^{1,2}\). Ethanol also reduces smog formation because of its low volatility and photochemical reactivity, its combustion products are low, and only low levels of smog producing compounds are formed by its combustion. When ethanol fuel is produced from lignocellulosic materials such as wood, herbaceous plants, agricultural residues and forestry wastes, its use as a transportation fuel reduces dependence on imported petroleum, decreases the balance of trade deficit, improves urban air quality, and contributes no net carbon dioxide to the atmosphere\(^{3-8}\).

The increased concern for the security of the oil supply and the negative impact of fossil fuels on the environment, particularly greenhouse gas emissions, has put pressure to find renewable fuel alternatives. The most commonly used renewable fuel is ethanol produced from sugar or grain (starch); however, this raw material base will not be sufficient to meet demand. Consequently, in future the large-scale need of ethanol will most certainly have to be based on

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production from lignocellulosic materials. A more possible alternative is the use of ecofriendly lignocellulosic agro-wastes, industrial cellulosic wastewaters, and effluents to satisfy the ecological balances and pollution abatement and simultaneous fuel production. There are a variety of biotechnological processes and microbial mechanisms for biological fuel (ethanol) production from lignocellulosic and cellulosic materials. Microorganisms are required to produce ethanol from lignocellulosic hydrolysates with a high yield from all sugars present using an economically feasible process. Different fermentation organisms among bacteria, yeasts, and fungi (natural as well as recombinant) have been reviewed with emphasis on their performance in lignocellulosic hydrolysates. The simultaneous saccharification and fermentation (SSF) process is a favored option for conversion of the lignocellulosic ecofriendly biomass into ethanol using different fungus because it provides enhanced rates, yields and concentrations of ethanol. There are mainly two processes involved in the conversion (i) hydrolysis of cellulose in the lignocellulosic biomass to produce reducing sugars (hexoses as well as pentoses) and (ii) fermentation of the reducing sugars to ethanol. In recent years, progress has been made in developing more effective pretreatment of lignocellulosic biomass and hydrolysis processes leading to higher yield of sugars and biofuel from agro-residues.

The present study was undertaken for aerobic ethanol production from untreated and pretreated (lime and dilute alkaline peroxide) lignocellulosic agro-residue (wheat straw) in a continuous stirred batch bioreactor (CSBR) using fungi Fusarium oxysporum (MTCC 1755). Attempts were also made to optimize process parameters like fermentation time, pH, temperature, stirring speed and wheat straw (untreated and pretreated) loading for maximum ethanol production. The sp. fungal growth rate (μ) and maximum sp. fungal growth rate (μ_max) of Fusarium oxysporum was determined by Monod growth model. Carboxymethyl cellulase (CMCase) enzyme activity was also assayed for untreated and pretreated wheat straw loading and plotted using Michaelis-Menten enzyme kinetic model. The sp. CMCase activity (ν) and maximum sp. CMCase activity (ν_max) of Fusarium oxysporum were determined using Michaelis-Menten enzyme kinetic model. The fungal growth kinetic parameters (K_s) and cellulose enzyme kinetic parameters (K_m) were determined for untreated and pretreated wheat straw respectively. The first order rate constant (k) was compared for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw as substrate in CSBR.

**Experimental Procedure**

**Collection of fungi and culture preparation**

The freeze-dried (lyophilized) fungi Fusarium oxysporum (MTCC 1755) was collected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India, and then stored in a freezer at – 4°C. The slant culture was aseptically prepared in growth medium of potato-sucrose-agar mixture [preparation steps: scrubbed and diced potato (200.0 g) was boiled in 1.0 L of water for 1 h; the pulp was squeezed through cheese cloth; agar (20.0 g) and sucrose (20.0 g) were added to it and boiled until it was dissolved; then it was transferred to measuring flask (1.0 L) and made up the volume with distilled water; The pH was adjusted at 6.5; and finally it was sterilized at 15 psi for 15 min in an autoclave]. The slant culture was kept for a period of 7 days in an incubator at 25°C for sufficient sporulation, spore crops were then harvested by washing the fully-grown slants with sterile distilled water and the content was transferred to suspension culture media in Erlemeyer flasks (250 mL). It was again kept in an incubator at 25°C for 7 days for proper fungal growth. The suspension fungal culture was filtered and the filtrate was further used for the present studies. The following constituents were used as suspension culture media: KH2PO4 4.0 g, MgSO4.7H2O 5.0 g, CaCl2 1.0 g, MnSO4.7H2O 0.05 g, FeSO4.7H2O 0.10 g, CaCl2.6H2O 0.10 g, AlK2(SO4)2.12H2O 0.01 g, and Na2MoO4.2H2O 0.01 g.

**Collection and analysis of wheat straw**

Wheat straw (Triticum durum) was collected from local agricultural activity and stored in laboratory under atmospheric pressure and room temperature. Samples were cut into usable sizes, dried in shadow and stored for studies. Samples were grinded (25 mesh size) in a grinder. The raw material wheat straw (25 mesh) was initially analyzed for physicochemical properties (lignin 19.85% (w/w), total
cellulose 47.72% (w/w) with degree of polymerization (DP) of 835, hemicelluloses 19.68% (w/w) and silica 2.81% (w/w) respectively).

**Pretreatment of wheat straw**

The cellulose content of wheat straw (substrate) is one of the most important factors for ethanol production from lignocellulosic agro-residues. To increase the cellulose content of substrate and ethanol yield in SSF process, a pretreatment of wheat straw with dilute alkaline peroxide (7.50% v/v H$_2$O$_2$, pH 11.5, temperature 35 °C, time 6 h) and with lime (100 mg/g wheat straw, temperature 121°C, time 6 h) was done. After pretreatment, the cellulose content in wheat straw increased to 65.25% (w/w) for diluted alkaline peroxide and 68.79% (w/w) for lime treatment respectively.

**SSF Method**

The SSF was carried out in CSBR (a conical flask of volume 3 L, with motor fitted stirrer inserted into it for proper fungal growth and well mixing of the fungi with wheat straw) containing untreated wheat straw (25 g/L). Suspension fungal culture (1 L) as inoculum was added to it. Suspension culture media (1 L) was added to the bioreactor. The initial pH (4.5) of content was maintained by using 0.1 N H$_2$SO$_4$ and/or 1 M CaCO$_3$ slurry. The mouth of the bioreactor was corked with sterilized non-absorbent cotton for aeration. The initial temperature of the contents was maintained at 35°C by means of heating coil fitted with off-on temperature controller. The temperature of bioreactor was measured by a thermocouple. The initial stirring speed was maintained at 20 rpm. The batch operations were also repeated for lime and alkaline peroxide pretreated wheat straw. Fermented ethanol was filtered and determined by gas chromatographic method (GC).

**Effect of fermentation conditions**

The SSS method was performed at a regular interval of fermentation time (12, 24, 36, 48, and 60 h), for various pH (5, 5.5, 6.0 and 6.5) and for different temperatures (40, 45, 50 and 55°C) respectively. The method was also repeated for various stirring speed (20, 25, 30, 35 and 40 rpm) and for various wheat straw loading (25, 30, 35, 40 and 45 g/L) respectively. The yield of ethanol was measured for different variable parameters while other conditions were kept constant at optimum level (time 48 h, pH 6, temperature 50°C and stirring speed 35 rpm).

**Luckey drop method for fungal growth estimation**

Exactly 0.1 mL of the fermented mixture was put using a calibrated medicinal dropper onto a glass slide and was seen under microscope. Number of fungal population was calculated and following calculation was performed, counting must be quick to avoid drying of the sample:

Number of fungi per mL = (No. of fungi counted in all fields × Area of cover slip, mm$^2$)/(Area of one macroscopic field, mm$^2$ × No. of field counted × volume of sample in the cover slip).

**Estimation of bioethanol by gas chromatograph**

The gas chromatography (Chrompack Model CP 9001), equipped with a flame ionization detector and a CP-9010 automatic liquid sampler was used. Data handling was done with the Maestro chromatography data system. The injection port of the chromatograph was installed with a hand-made glass liner (length 8 cm, o.d. 6 mm, i.d. 3 mm). This liner, which acts as a precolumn to prevent contamination of the gas chromatographic column with nonvolatile material from fermentation process, was stoppered with a dimethylchlorosilane-treated glass wool plug and partly filled with small glass beads with a diameter of 1 mm. The fermented ethanol was injected by means of a 50 µL Hamilton syringe (Model 1705, Chrompack) with a removable needle (needle gauge 22 S), penetrating the glass beads by at least 1.5 cm. Injection by <1.5 cm beneath the surface of the glass beads mostly resulted in a broad tailing peak for ethanol. The plunger of the syringe had a teflon tip to provide an inert leak-tight seal. For routine analyses, 2 µL injections were performed. The liner was replaced within seconds by a new one after some 50 injections (2 µL of ethanol).

The operating conditions were as follows: column 2 m ×2 mm i.d, glass packed with 10% SP 1200/1% H$_4$PO$_4$ on 80/100 Chromosorb WAW, column temperature 120 °C, injection port temperature 200°C, detector temperature 180°C, detector output attenuation 27, carrier gas (N$_2$) 20 mL/min, H$_2$ 30 mL/min and air 300 mL/min. Freshly packed columns were conditioned overnight at 190 °C with a flow of N$_2$ carrier gas, before being connected to the detector. A few 1 µL injections of 10% formic acid were made to clear the column from unknown impurities. When using a new liner, two 2 µL injections of distilled water were made to clear the new glass beads inside the liner of some unknown impurities. The time to replace the liner, to stabilize
the system, and to decontaminate the new liner took ~3 min. To 1 mL samples of water, fermented ethanol of the conc 1, 2, 4, 6, 8, and 10 µL was added to this stock calibrator, resulting in solutions of 0.5, 1, 2, 3, 4, and 5 g/L respectively. An aqueous stock calibrator of ethanol was prepared with a concentration of 500 g/L. This solution was stored at 4°C. This aqueous calibrator was used for daily calibration\textsuperscript{26,27}.

**Assay of carboxymethyl cellulase (CMCase) activity**

Fermented solution was filtered and taken (1.0 mL) in a clean dry test tube containing 4.0 mL of 0.05 M acetic acid at pH 5.0. It was incubated\textsuperscript{26} with shaking for 2 h at 37°C. Tubes were removed to an ice bath and allowed sediment to settle followed by clarification by centrifugation. It was stored in an ice bath. 3.0 mL glucose reagent (ATP 0.77 µmol/mL, hexokinase 1.5 units/mL, NAD 0.91 µmol/mL, glucose-6-phosphate dehydrogenase 1.9 units/mL and Tris-HCl buffer of pH 7.6 ±0.2, 0.1 M) was placed in a cavetti and incubated in spectrophotometer set at 340 nm slide and 25°C to achieve temperature equilibration. Absorbance was measured for the mixture at 340 nm ($A_{340}$) for 1 min using UV-Vis Spectrophotometer (M/S Perkin-Elmer, Lambda Bio-40). $A_{340}$ of the glucose reagent in the cuvette was also recorded. The supernatant (CMCase 0.1 mL) was added to each reaction tube and recorded increase in $A_{340}$ until no further change occurred in 1 min. Final $A_{340}$ was also recorded. It was compared with blank (without CMCase ) reading. CMCase activity was calculated using the following relationship:

$$\text{CMCase activity, Units/mL min} = \left(\frac{\Delta A_{340} \text{ Sample} - \Delta A_{340} \text{ Blank}}{6.22 \times 0.1 \times 2 \times 0.01 \times \text{ mL enzyme in mixture}}\right) \times 3.1 \times 180.5$$

where $\Delta A_{340} = A_{340 \text{ Final}} - A_{340 \text{ Initial}}$

**Results and Discussion**

**Effect of fermentation time**

The yield of ethanol is found to be proportional to fermentation time. It increases with the increase in time up to 48 h and then declines (Fig. 1). Maximum yield of ethanol is 0.37, 0.427 and 0.454 g/g of wheat straw at 48 h of fermentation time for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw respectively. The maximum ethanol production is 0.192, 0.222 and 0.236 g/g h for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at optimum time. After 48 h of time, the yield of ethanol decreases by

**Effect of pH**

The increase in yield of ethanol is observed with the increase in pH upto 6.0 and then it declines (Fig. 2). Maximum yield of ethanol is found to be 0.494, 0.572 and 0.634 g/g of wheat straw at pH 6.0 for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at 48 h fermentation time respectively. Operating pH of 6.0 is therefore taken as optimum for the ethanol fermentation of the three samples with fungi *Fusarium oxysporum* in CSBR. The maximum ethanol production is 0.257, 0.297 and 0.330 g/g h for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at optimum pH respectively. With the increase in pH (>6.0), the yield of ethanol sharply decreases (Fig. 2). When pH differs from the optimal value, the SSF process of wheat straw (pretreated and untreated) using fungi *Fusarium oxysporum* in CSBR (Fig. 1). Fermentation time of 48 h is therefore taken as optimum time for ethanol fermentation of pretreated and untreated wheat straw with fungi *Fusarium oxysporum*.
maintenance energy requirement of the fungi increases, which causes death rate to increase\textsuperscript{28}, leading to decrease in ethanol fermentation.

**Effect of temperature**

With the increase in temperature, the yield of ethanol increases up to 50ºC and then it decreases (Fig. 3). Maximum yield of ethanol is found to be 0.630, 0.664 and 0.706 g/g of wheat straw at 50ºC for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at 48 h of fermentation respectively. Operating temperature of 50ºC is therefore taken as optimum temp. for study. The maximum ethanol production is 0.372, 0.390 and 0.402 g/g h for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at optimum temperature respectively.

With the increase in temperature beyond 50ºC, yield of ethanol declines. Temperatures below the optimum (<50ºC) depress the rate of metabolism of fungal cells. The higher the optimal temperature (>50ºC), the lesser is the growth rate and finally thermal death occurs. At high temperature (>50ºC), death rate exceeds the growth rate, which causes a net decrease in the concentration of viable fungal populations with lower generation of ethanol. It is, therefore, not surprising that a multitude of physical and chemical parameters may cause perturbations\textsuperscript{29} in protein–geometry and structure of CMCase enzymes particularly.

**Effect of stirring speed**

With the increase in stirring speed, the yield of ethanol increases up to 35 rpm and then it decreases (Fig. 4). Maximum yield of ethanol is found to be 0.716, 0.750 and 0.772 g/g of wheat straw at 35 rpm for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at 48 h of fermentation respectively. Stirring speed of 35 rpm is therefore taken as optimum speed for ethanol fermentation of the three samples with fungi *Fusarium oxysporum* in a continuous stirred batch bioreactor. The maximum ethanol production is 0.372, 0.390 and 0.402 g/g h for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at optimum speed respectively. With the increase in stirring speed (>35 rpm), the yield of ethanol is declined. Increase in stirring speed can disturb the elaborate shape\textsuperscript{28} of enzyme CMCase of *Fusarium oxysporum* to such a degree that the denaturation of the protein occurs which deactivates the enzymes. Therefore, the yield of ethanol is decreased with the increase in stirring speed beyond the optimum value (>35 rpm).

**Effect of wheat straw loading**

With the increase in loading of pretreated and untreated wheat straw, the yield of ethanol increases up to 35 g/L and then decreases in both the samples (Fig. 5). Maximum yield of ethanol is 0.756, 0.796 and 0.810 g/g of wheat straw (Fig. 5) at 35 g/L loading of for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at 48 h of fermentation respectively.
fermentation respectively. Loading of 35 g/L is therefore taken as optimum value for ethanol fermentation of pretreated and untreated wheat straw using fungi *Fusarium oxysporum* in CSBR. The maximum ethanol production is 0.393, 0.414 and 0.422 g/g h for the three samples respectively at optimum loading. Low yield of ethanol with the increase in wheat straw loading can be attributed to product and substrate (wheat straw) inhibition. Some enzymes are produced from fungi, whereas others are influenced by substrate. The repression and depression processes allow *Fusarium oxysporum* to regulate their CMCase content in direct response to the environment. Inhibitory compounds limit efficient utilization of the hydrolysates for lower ethanol production by fermentation.

Analysis of Monod growth model

The sp. growth rate (μ= no. of cells/mL. s) of fungi *Fusarium oxysporum* for untreated and pretreated wheat straw at different loadings using the optimum bioethanol fermentation parameters in bioreactor was calculated from respective growth data and then plotted (Fig. 6) against substrate wheat straw loading to analyze Monod model, as shown below:

$$
\mu = \mu_{\text{max}} \left( \frac{S}{K_s + S} \right)
$$

where $K_s$ is the kinetic parameter at which the sp. growth rate ($\mu$) is half of maximum growth rate ($\mu_{\text{max}}$), i.e. $\mu = \mu_{\text{max}}/2$, at $K_s = S$ (upto linear portion of the curve). The model indicates a division between the lower concentration range where $\mu$ is strongly (linearly) dependent on $S$, and the higher concentration range where $\mu$ becomes independent of $S$ (curve portion of Fig. 6); here, $S$ is the loading of the substrate wheat straw.

With the increase in pretreated and untreated wheat straw loading, the sp. growth rate of *Fusarium oxysporum* is first increased, and then it decreases due to substrate and product inhibition effect. For Monod model, the sp. growth rate ($\mu$) of fungi is found to be 5.26, 5.40 and 5.88 s⁻¹ and maximum sp. growth rate ($\mu_{\text{max}}$) is 10.52, 10.80 and 11.76 s⁻¹ for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw respectively. The kinetic parameter ($K_s$) is 33.6, 33.9 and 34.7 g/L for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw respectively (Fig. 6).

An important case of decrease of fungal growth is that of product ethanol concentration and substrate. The utilization pattern of substrate wheat straw is significantly influenced by adaptation characteristics of fungal culture. Adaptation of fungi significantly affects the sp. growth rate, length of lag - phase and overall fermentation of bioethanol. Though the fermentation media contains many numbers of sugars (hydrolysis products of wheat straw like hexoses and pentoses) and culture media also contains other carbon sources, fungi does not show diauxic behaviour, whereas in the presence of two or more carbon source, fungi first utilize preferential one (wheat straw) till exhausted, and then utilize second or other carbon source. They do not utilize both carbon sources at a time. The diauxic inhibition has an effect on sp. growth rate as well as ethanol fermentation with *Fusarium oxysporum*.

Analysis of Michaelis-Menten enzyme kinetic model

The enzyme-substrate (CMCase-wheat straw) interaction varies from one enzyme-substrate complex to another. The CMCase activity of fungi *Fusarium oxysporum* for ethanol fermentation of untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw at different wheat straw loading under optimum fermentation conditions is shown in Fig. 7. For analysis of Michaelis-Menten enzyme kinetic model, sp. CMCase activity ($v = \text{Units/mL. min}$) is plotted against pretreated and untreated wheat straw loadings by using Michaelis-Menten enzyme kinetic model, as given below:

$$
v = v_{\text{max}} \left( \frac{S}{K_m + S} \right)
$$

where $K_m$ is the intrinsic kinetic parameter; and $S$, the substrate concentration, at which the sp. enzyme activity
activity \((\nu)\) is half of maximum sp. enzyme activity \((\nu_{\text{max}})\) i.e. \(\nu = \nu_{\text{max}}/2\), at \(K_m = S\) The model indicates a division between the lower concentration range where \(\nu\) is strongly (linearly) dependent on \(S\), and the higher concentration range where \(\nu\) becomes independent of \(S\). \(\nu_{\text{max}}\) is solely a function of rate parameters and is expected to change with temperature or pH.

With the increase in pretreated and untreated wheat straw loading, the sp. CMCase activity \((\nu)\) of *Fusarium oxysporum* first increases, and then decreases (Fig. 7) due to substrate and product inhibition effect. For Michaelis-Menten enzyme kinetic model, the sp. CMCase activity \((\nu)\) is 1185, 1455 and 1545 min\(^{-1}\) and maximum sp. CMCase activity \((\nu_{\text{max}})\) is 2370, 2910 and 1545 min\(^{-1}\) for ethanol fermentation of untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw respectively. The kinetic parameters \(K_m\) \((K_m = S)\) are 34.3, 34.5 and 34.7 g/L for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw respectively.

The kinetics of aerobic bioethanol fermentation of untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw in CSBR were compared using fungi *Fusarium oxysporum* (MTCC 1755) and fungi *Aspergillus oryzae* (MTCC 1847). The first order rate equation is shown as:

\[
\ln (1-X) = kt \quad \ldots (3)
\]

where \(X\) is the wheat straw conversion at time \(t\).

Straight lines are obtained by plotting fermentation time \((t)\) against \([- \ln (1-X)]\). The rate constant \((k)\) is measured from slopes of the straight lines. The rate constant \((k)\) is found to be 0.012, 0.013 and 0.014 h\(^{-1}\) for fermentation of untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw in CSBR using fungi *Fusarium oxysporum* respectively. The rate constant \((k)\) is 0.014, 0.016 and 0.019 of the three samples using fungi *Aspergillus oryzae*. Ethanol fermentation from wheat straw in SSF process shows good agreement with the first order rate kinetics. Fungi *Aspergillus oryzae* shows the better conversion of ethanol from wheat straw than fungi *Fusarium oxysporum*.

**Conclusion**

Fermentation of bioethanol from pretreated and untreated wheat straw with fungi *Fusarium oxysporum* in CSBR is found to be an effective biofuel production process. The optimum parameters for bioethanol fermentation are: time 48 h, pH 6.0, temperature 50ºC, stirring speed 35 rpm and wheat straw loading 35 g/L. Maximum yield of ethanol in SSF process is found to be 0.756, 0.796 and 0.810 g/g of wheat straw at optimum parameters for untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw respectively. The sp. fungal growth rate \((\mu)\) is 5.26, 5.40 and 5.88 s\(^{-1}\) and maximum sp. fungal growth rate \((\mu_{\text{max}})\) is 10.52, 10.80 and 11.76 s\(^{-1}\) using Monod model for fermentation of untreated, lime pretreated and dilute alkaline peroxide pretreated wheat straw samples respectively. The Monod kinetic parameters \((K_s)\) are 33.6, 33.9 and 34.7 g/L for the fermentation of three wheat straw samples at optimum fermentation conditions respectively. The sp. CMCase activity \((\nu)\) is 1185, 1455 and 1545 min\(^{-1}\) and maximum sp. CMCase activity \((\nu_{\text{max}})\) is 2370, 2910 and 1545 min\(^{-1}\) using Michaelis-Menten enzyme kinetic model for the fermentation of the three wheat straw samples respectively. The enzyme kinetic parameters \((K_m)\) are 34.3, 34.5 and 34.7 g/L for ethanol fermentation of the three wheat straw samples at optimum fermentation conditions respectively. The first order rate constants \((k)\) are found to be 0.012, 0.013 and 0.014 h\(^{-1}\) for ethanol fermentation of the three wheat straw samples in CSBR using fungi *Fusarium oxysporum* respectively. The rate constants \((k)\) are 0.014, 0.016 and 0.019 h\(^{-1}\) for ethanol fermentation of the three wheat straw samples to ethanol using fungi *Aspergillus oryzae* in CSBR respectively. Fungi *Aspergillus oryzae* show the better conversion of ethanol from wheat straw than fungi *Fusarium oxysporum*. 

![Fig. 7—Michaelis – Menten kinetic model for sp. Enzyme activity](image_url)
References