Kinetics of Xylanase Fermentation by Recombinant *Escherichia coli* DH5α in Shake Flask Culture

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**Abstract: Problem statement:** Interest in xylanase enzyme application has markedly increased in pulp and paper processing industries. The switch to xylanase-producing recombinant *Escherichia coli* DH5α pTP510 is seen here as an economic alternative towards higher productivity and easier downstream purification. Modeling of *E. coli* DH5α growth and enzyme secretion is thus desired for future optimization in fermentation process.

**Approach:** Kinetics of intracellular xylanase fermentation by a recombinant *E. coli* DH5α was studied in shake flask culture. The effect of different medium formulations (complex, minimal and defined), initial pH (6.5, 7.0, 7.4 and 8.0) and agitation speeds (150, 200 and 250 rpm) on cell growth and xylanase production were evaluated. Mathematical models based on Logistic and Luedeking-Piret equations had been proposed to describe the microbial growth and xylanase production.

**Results:** Highest xylanase production was obtained in defined medium. Based on medium formulation, the highest cell concentration (4.59 g L⁻¹) and xylanase production (2, 122.5 U mL⁻¹) was obtained when (NH₄)₂HPO₄ was used as the main nitrogen source, with an adjustment of the initial pH to 7.4 and agitation speed of 200 rpm. The maximum specific growth rate (µ_max), growth associated xylanase production coefficient (α) and non-growth associated xylanase production coefficient (β) was 0.41 h⁻¹, 474.26 U mg cell⁻¹ and 0 U mg cell⁻¹ h⁻¹, respectively.

**Conclusion:** Xylanase production was growth associated process and the enzyme secretion was greatly dependent on cell concentration and the specific growth rate of *E. coli* DH5α.

**Key words:** Recombinant *E. coli* DH5α, thermophilic xylanase, submerged fermentation, kinetics, mathematical model

**INTRODUCTION**

The polysaccharides of xylan typically associated with cellulose and lignins play an important structurally-supportive role in building up of plant cell walls. Degradation of hemicellulose component which mainly consists of xylan requires an efficient xylanolytic enzymatic systems; consisting several enzymes including endo-β-1,4-xylanase, β-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, acetyl xylan esterase, ferulic acid esterase and p-coumaric acid esterase. In recent years, there has been an increasing interest in xylanase enzyme fermentations regarding their wide applications in various industries, especially in paper and pulp industry.
of xylanase producer such as proteases and transglucosidases also affect the actual yield of the xylanase enzyme\[^{6,7}\]. In addition, the xylanases producing-fungi normally have slow growth rate thereby correspond to lower enzyme productivity which limits its application in commercial fermentation processes. Alternatively, enhanced enzyme production using recombinant strain such as *Escherichia coli* may be used to overcome these problems. Recombinant enzyme produced by *E. coli* generates higher productivity due to its faster growth rate compare to fungal producers.

Construction of the recombinant *E. coli* producing xylanases free of cellulolytic enzymes has been reported\[^{8}\]. However, very little attention has been paid to its process development which normally related to optimization, kinetic and modeling as well as fermentation modes. Proteins and enzymes production by recombinant strains could also be enhanced through optimization of medium and culture conditions\[^{9}\]. The mathematical model based on some equations can be used to describe the relationship of the experimental variables and explains the behavior of the system itself. The information gathered may be useful in optimization and designing of the fermentation process for improvement of the xylanase production.

The objective of the present study was to investigate the kinetics of cell growth and xylanase production by the recombinant *E. coli* DH5\(\alpha\) using different medium formulations and culture conditions. The information obtained from this kinetic study may be useful to obtain better understanding of the fermentation process for the improvement of intracellular xylanase production by *E. coli* DH5\(\alpha\).

**MATERIALS AND METHODS**

**Microorganism and cell maintenance:** *E. coli* DH5\(\alpha\), which has been constructed to secrete intracellular xylanolytic enzymes was obtained from Department of Chemistry, Airlangga University, Surabaya, Indonesia and employed throughout this study. The recombinant strain encodes genes for exo-xylanase (exo-xyn), \(\alpha\)-L-arabinofuranosidase (abfa) and \(\beta\)-xylosidase (xyl) from *Bacillus thermoleovorans* IT-08, isolated from Gunung Pancar Hot Spring, Bogor-West Java, Indonesia. The genes have been cloned into pTP510 and expressed in *E. coli* DH5\(\alpha\). The xylanase gene was targeted into *E. coli* DH5\(\alpha\) strain to obtain a single cell colony. For maintenance, the plate was subcultured at 1 week intervals and stored at 4\(^{\circ}\)C.

**Inoculums preparation:** A single cell colony picked from the stock culture was inoculated into 100 mL LB medium, with the addition of 100 \(\mu\)g mL\(^{-1}\) ampicillin in Erlemeyer flasks (250 mL). The inoculated flasks were incubated at 37\(^{\circ}\)C in rotary shaker (150 rev min\(^{-1}\)) until the Optical Density (OD) of the 8 h culture reached a value of around 0.8. This culture was used as standard inoculum for all fermentations.

**Medium and fermentation:** Initially, the feasibility of using three different media (LB, defined and minimal medium) on growth of *E. coli* DH5\(\alpha\) and xylanase production was investigated. LB medium consisted of (g L\(^{-1}\)) tryptone, 10.0; yeast extract, 5.0 and NaCl, 5.0.

Composition of defined medium is as follows (g L\(^{-1}\)): K\(_2\)HPO\(_4\), 13.2; KH\(_2\)PO\(_4\), 2.6; (NH\(_4\))SO\(_4\), 4.1; NaCl, 2.0; MgSO\(_4\).7H\(_2\)O, 0.5; FeCl\(_3\), 0.025 and trace element solution (TES), 3.2 mL L\(^{-1}\). TES constituted of (g L\(^{-1}\)): CaCl\(_2\).2H\(_2\)O, 1.44; AlCl\(_3\).6H\(_2\)O, 0.042; ZnSO\(_4\).7H\(_2\)O, 0.87; CoCl\(_2\).6H\(_2\)O, 0.16; CuSO\(_4\), 1.6; H\(_2\)BO\(_3\), 0.01; MnCl\(_2\).4H\(_2\)O, 1.42; NiCl\(_2\).6H\(_2\)O, 0.01 and Na\(_2\)MoO\(_4\).H\(_2\)O, 0.02. Minimal medium only consisted of (g L\(^{-1}\)): NH\(_4\)Cl, 1.0; KH\(_2\)PO\(_4\), 3.0; Na\(_2\)HPO\(_4\), 2H\(_2\)O, 6.0 and MgSO\(_4\), 1.0. In all fermentations, the media was supplemented with glucose and 100 \(\mu\)g mL\(^{-1}\) ampicillin.

For the subsequent experiments to investigate the effect of various nutrient sources on growth of *E. coli* DH5\(\alpha\) and xylanase production, the defined medium was employed. The medium with the preferred nitrogen sources (NH\(_4\)Cl, NH\(_4\)NO\(_3\), (NH\(_4\))\(_2\)HPO\(_4\) and NaNO\(_3\)) replacing (NH\(_4\))SO\(_4\) was used to investigate the effect of initial culture pH, ranging from pH 6.5-8, on growth of *E. coli* DH5\(\alpha\) and xylanase production. For the effect of agitation speed (150, 200 and 250 rpm), the optimal medium with an initial culture pH of 7.4 was used. All experiments were conducted in triplicates and results given are the average value.

**Analytical procedures:** During the fermentation, samples were withdrawn at 2 h interval for the analysis. The samples were centrifuged at 10,000 rpm, 4\(^{\circ}\)C, for 10 min (Centrifuge 5810R, Eppendorf, Germany). The cell pellets obtained were used for determination of cell growth and enzyme activity. The cells pellet were resuspended in 0.9% (w/v) NaCl and the OD was measured at 600 nm using spectrophotometer (Spectronic\(^{\circ}\)20, GENESYS, USA). The cell suspensions were then filtered through dry membrane
filter and then dried in an oven for 24 h at 90°C for measurement of Dry Cell Weight (DCW). Correlation between DCW and OD was estimated from several batch experiments. One OD unit was approximately equivalent to 0.6042 g L\(^{-1}\) of cells.

For the extraction of the intracellular xylanase, the cell pellet was washed with 0.9% (w/v) NaCl and resuspended in phosphate citrate buffer (pH 7). The cells suspension was subjected to ultrasonication using sonicator (XL-2020, Heat System, New York) operated at 20 kHz, 15 sec for cell disruption. The disrupted cell suspension was then incubated at 70°C for 1 h and then recentrifuged to obtain cell free extract or crude enzyme sample.

For xylanase activity assay, a mixture of 0.1 mL of substrate (1% w/v oat spelt xylan in citrate phosphate buffer, pH 7) and 0.1 mL of crude enzyme was incubated at 70°C in a shaking water bath for 1 h. The reaction was stopped by adding 0.6 mL of DNS (3, 5-dinitrosalicylic acid) reagent and the reducing sugar produced from the reaction was measured using modified DNS method as described elsewhere\(^{[10]}\). One unit of xylanase activity is the amount of enzyme in 1 mL sample solution producing 1 µg mL\(^{-1}\) of reducing sugar (xylose) in a minute incubation under the given experimental conditions. Residual glucose in the culture supernatant was analyzed using biochemistry analyzer (YSI 2700 Select Biochemistry Analyzer, YSI, Ohio).

**Kinetics and mathematical models:** The simplified batch fermentation kinetic models based on Logistic and Luedeking-Piret equations which have been described elsewhere\(^{[11]}\) were used to describe growth of *E. coli* DH5\(\alpha\) and xylanase production, respectively.

**Cell growth:**

\[
\frac{dX}{dt} = \mu_{\text{max}} \left( \frac{1 - X}{X_{\text{max}}} \right) X \\
(1)
\]

**Substrate consumption:**

\[
\frac{-dS}{dt} = \frac{1}{Y_{\text{x/s}}} \left( \frac{dX}{dt} \right) + m_s X \\
(2)
\]

**Xylanase production:**

\[
\frac{dP}{dt} = \alpha \left( \frac{dX}{dt} \right) + \beta X \\
(3)
\]

Where:

- \(X\) = The cell concentration (g L\(^{-1}\))
- \(X_{\text{max}}\) = The maximum cell concentration (g L\(^{-1}\))
- \(\mu_{\text{max}}\) = Maximum specific growth rate (h\(^{-1}\))
- \(S\) = The substrate concentration (g L\(^{-1}\))
- \(Y_{\text{x/s}}\) = Yield coefficient of cells on carbon substrate (g g\(^{-1}\))
- \(m_s\) = Maintenance coefficient (g g\(^{-1}\) h\(^{-1}\))
- \(\alpha\) = Growth associated xylanase production coefficient (U mg cell\(^{-1}\))
- \(\beta\) = Non-growth associated xylanase production coefficient (U mg cell\(^{-1}\) h\(^{-1}\))
- \(t\) = The fermentation time (h)

Equations 1-3 were fitted to the experimental data by non-linear regression through Marquadt algorithm using MATLAB software (matlab release 14.0, mathworks, USA). The model parameter values were evaluated by solving Eq. 1-3 with the computer program applied as a search method to minimize the sum of squares of the differences between predicted and calculated values. The predicted values were then used to simulate the profiles of cell, substrate and product formation during the fermentation process.

**Statistical analysis:** The statistical significance of the cells and xylanase produced during various treatments was then analyzed using the SPSS program (SPSS Version 15, SPSS Inc., USA). The Tukey’s Honestly Significant Difference (HSD) test multiple comparison method was used to compare the difference between treatment means.

**RESULTS**

**Effect of basal medium on growth of *E. coli* DH5\(\alpha\) and xylanase production:** Comparison of growth of *E. coli* DH5\(\alpha\), xylanase production and glucose consumption during batch fermentation using three different basal media is shown in Fig. 1. Higher growth rate was determined in LB where lag phase was not observed as compared to defined and minimal media (Fig. 1a). In fermentation using defined medium, growth reached a stationary phase after 24 h while for LB medium it was reached only after 14 h. However, the final cell concentration attained at the end of fermentations was not significantly different with different media used. The final cell concentration for defined, minimal and LB media was 2.47, 2.22 and 2.12 g L\(^{-1}\), respectively (Table 1). In all cases, xylanase production was increased concomitantly with growth, suggesting that the process is growth associated (Fig. 1c). It is interesting to note that the final xylanase activity obtained at the end of fermentations was significantly different with the different media used.
The highest xylanase activity (1526.5 U mL\(^{-1}\)) was obtained in defined medium, followed by minimal medium (1208.5 U mL\(^{-1}\)) and LB medium (1094.5 U mL\(^{-1}\)). Glucose was consumed for growth and complete utilization of glucose was observed at the end of the cultivation (Fig. 1b).

Figure 2 shows the time course of xylanase fermentation by the recombinant *E. coli*, which also includes the comparison between the calculated data according to Eq. 1-3 with the experimental data. The calculated data fitted well to the experimental data. The result suggests that the proposed models based on Logistic and Luedeking-Piret equations were sufficient to describe growth of *E. coli* DH5\(\alpha\) and xylanase production respectively. The kinetic parameter values for the fermentations employing three different basal media are shown in Table 1. The highest maximum specific growth rate (\(\mu_{\text{max}}\)) (0.59 h\(^{-1}\)) was obtained in LB medium, followed by minimal medium (0.54 h\(^{-1}\)) and defined medium (0.49 h\(^{-1}\)). Although defined medium gave the lowest \(\mu_{\text{max}}\), but this medium gave the highest growth-associated xylanase production coefficient (\(\alpha\)). The \(\alpha\) value for fermentation using defined medium (616.34 U mg cell\(^{-1}\)) was significantly higher than those obtained in minimal medium (436.06 U mg cell\(^{-1}\)) and LB medium (554.54 U mg cell\(^{-1}\)). In all fermentations, the value of non-growth associated xylanase production coefficient (\(\beta\)) was 0 U mg cell\(^{-1}\) h\(^{-1}\).
This result confirmed that the xylanase production by *E. coli* DH5α was growth associated process. It is also interesting to note the fermentation that produced the highest xylanase showed the highest maintenance coefficient (m). The m, value for defined medium (0.202 g substrate g cell⁻¹ h⁻¹) was about 2 and 4 times higher than those calculated for LB and minimal medium, respectively.

**Effect of nitrogen source on growth of *E. coli* DH5α and xylanase production:** Table 2 shows the kinetic parameter values for xylanase fermentation by *E. coli* DH5α using different types of nitrogen source. The kinetic parameter values were calculated from the modeling exercise using the data obtain from the time courses of the fermentation runs (data not shown). In all cases, the formulation was based on defined medium.

Both, cell growth and xylanase production were greatly influenced by the type of nitrogen source used. The highest final cell concentration (Xₘᵢₓ) (4.47) was obtained in fermentation using (NH₄)₂HPO₄ as nitrogen source, followed by (NH₄)₂SO₄ (3.02), NH₄Cl (2.99), NH₄NO₃ (2.64) and NaNO₃ (1.00 g L⁻¹). This is corresponded well to the final xylanase activity obtained at the end of fermentation, where the highest activity (1772 U mL⁻¹) was obtained in fermentation using (NH₄)₂HPO₄. The highest xylanase productivity (128.29 U mL⁻¹ h⁻¹) was also obtained in fermentation using (NH₄)₂HPO₄. The xylanase yield based on glucose consumed was not significantly different among the nitrogen sources tested. The lowest xylanase activity (696 U mL⁻¹) was obtained in fermentation using NaNO₃. However, the specific xylanase activity was the highest for this fermentation. The maximum specific growth rate (0.69 h⁻¹) was observed with NH₄Cl while the lowest observed with NH₄NO₃ (0.35 h⁻¹).

**Effect of initial culture pH on growth of *E. coli* DH5α and xylanase production:** Effect of initial culture pH on growth of *E. coli* DH5α and xylanase production is summarized in Table 3. The highest final cell concentration (Xₘᵢₓ) (4.92 g L⁻¹) was obtained at initial pH of 7.4 and corresponded to the highest xylanase production (1854.0 U mL⁻¹). Inhibition of growth and xylanase production was observed with a slight reduction in pH to 7: and greatly inhibited at pH 6.5. Final cell concentration and xylanase activity obtained at pH 6.5 were about 3 times lower than those obtained at pH 7.4. Only slight reduction in growth and xylanase production was observed at alkaline conditions (pH 8).

In terms of cell yield (Yₑ) and xylanase yield (Yₓₛ), the values were not significantly different at pH ranged from 7-8, but greatly reduced at pH 6.5. On the other hand, xylanase productivity (103.05 U mL⁻¹ h⁻¹) obtained at pH 7.4 was comparable to that obtained at pH 8, but significantly reduced at pH 6.5 and 7.
The XDH5 cell productivity (g L−1) and xylanase production was associated to growth of E. coli DH5α and xylanase production was proportionally increased with cell biomass. In other words, improvement of xylanase production was very much related to cell built-up, though some factors such as nutrients concentration, pH and dissolved oxygen level may enhance the ability of cell to express the xylanase genes. It is interesting to note that the specific growth rate also gave a profound effect on the final cell concentration and xylanase activity. This means that the specific production rate of xylanase was influenced by the specific growth rate. A moderate specific growth rate of E. coli DH5α (0.36 h−1) gave higher cell yield (0.37 g g−1) and xylanase yield (154.09 U mg−1) based on glucose consumed. It can be deduced that cultivation of cells at lower growth rate allowed them to allocate more cellular resources to express the xylanase gene.

The concentration of each nutrient in the defined medium such as glucose, ammonia, magnesium, phosphorus and zinc that inhibited growth and metabolite production shall be determined. The knowledge obtained from this study is very useful for further optimization of medium composition, as well as development of fed-batch culture, to enhance growth of E. coli DH5α and xylanase production. Specific nutrient could be controlled below inhibition levels in fed-batch culture to maintain high cell growth of E. coli[12]. For example, efficient production of truncated thermostable xylanase from Rhodothermus marinus in E. coli had been achieved in fed-batch culture employing complex medium[13]. Moreover, optimization of medium component using response surface methodology for phytase production by E. coli has also been reported[14].

The maximum specific growth rate obtained in fermentation using NH4Cl and NaNO3 as an individual nitrogen source was 0.69 h−1 and 0.52 h−1, respectively. High maximum specific growth rate of recombinant E. coli with a value of 0.67 h−1 and 0.44 h−1 was also obtained in fermentation using NH4Cl and NaNO3, respectively[15]. They also claimed that the ammonia-grown cultures of E. coli yielded a greater specific growth rate, cell yield and lipopolysaccharide production efficiency and a more hydrophilic surface than nitrate grown cultures. The use of (NH4)2HPO4 exhibited high cell concentration of E. coli (4.47 g L−1) and corresponded to the highest xylanase activity (1772 U mL−1). Most probably, the energy uptake by the cells was influenced by ionic strength of the substances and a well buffering system by (NH4)2HPO4. NH4NO3 and NaNO3 were found not suitable as the main nitrogen source for growth of E. coli DH5α and xylanase production due to significant reduction in productivity.

From the results of this study it can be suggested that initial culture pH 7.4 was the preferred pH for growth of E. coli DH5α and xylanase production.

**DISCUSSION**

Production of intracellular xylanase by E. coli DH5α was influenced by the medium composition and culture conditions. LB medium is commonly used for recombinant gene expression on laboratory scale. The effect of each nutrient component in LB medium on growth of the recombinant strain and metabolite production cannot be clearly identified. The use of defined medium permitted better understanding of nutrient requirement for growth and metabolism of the required product. Results from this study indicated that the type of nitrogen source was the important factor that determined growth rate of E. coli DH5α and xylanase production. Besides, the initial culture pH and oxygen supply were also another important factors that affect growth of E. coli DH5α and xylanase production. From the modeling study, it was found that the xylanase production cannot be clearly identified. The use of a, b and c defined medium permitted better understanding of nutrient requirement for growth and metabolism of the required product. Results from this study indicated that the type of nitrogen source was the important factor that determined growth rate of E. coli DH5α and xylanase production. From the modeling study, it was found that the xylanase production was associated to growth of E. coli DH5α and xylanase production was proportionally increased with cell biomass. In other words, improvement of xylanase production was very much related to cell built-up, though some factors such as nutrients concentration, pH and dissolved oxygen level may enhance the ability of cell to express the xylanase genes. It is interesting to note that the specific growth rate also gave a profound effect on the final cell concentration and xylanase activity. This means that the specific production rate of xylanase was influenced by the specific growth rate. A moderate specific growth rate of E. coli DH5α (0.36 h−1) gave higher cell yield (0.37 g g−1) and xylanase yield (154.09 U mg−1) based on glucose consumed. It can be deduced that cultivation of cells at lower growth rate allowed them to allocate more cellular resources to express the xylanase gene.

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The maximum specific growth rate obtained in fermentation using NH4Cl and NaNO3 as an individual nitrogen source was 0.69 h−1 and 0.52 h−1, respectively. High maximum specific growth rate of recombinant E. coli with a value of 0.67 h−1 and 0.44 h−1 was also obtained in fermentation using NH4Cl and NaNO3, respectively[15]. They also claimed that the ammonia-grown cultures of E. coli yielded a greater specific growth rate, cell yield and lipopolysaccharide production efficiency and a more hydrophilic surface than nitrate grown cultures. The use of (NH4)2HPO4 exhibited high cell concentration of E. coli (4.47 g L−1) and corresponded to the highest xylanase activity (1772 U mL−1). Most probably, the energy uptake by the cells was influenced by ionic strength of the substances and a well buffering system by (NH4)2HPO4. NH4NO3 and NaNO3 were found not suitable as the main nitrogen source for growth of E. coli DH5α and xylanase production due to significant reduction in productivity.
Further improvement of the production could be achieved by optimizing the (NH₄)₂HPO₄ concentration, to over-express the xylanase gene efficiently.

In most fermentations employing *E. coli*, the initial culture pH was adjusted to pH ranging from 7.0⁰ to 7.4⁰ for enhancement of growth. In this study, initial pH 7.4 was found optimal for the growth of *E. coli* DH5α and xylanase production. On the other hand, initial pH 6.5 strongly inhibited the growth of *E. coli* DH5α as well as xylanase production. Acidic condition was found not suitable for initial *E. coli* fermentation due to its adaptation phase. Duffy et al.⁰ also claimed that the initial pH 5.8 was not suitable for the growth of *E. coli*. During the fermentation with optimal initial pH, the pH decreased with fermentation time up to 12 h due to increased in proton electrochemical gradient (data not shown). Glucose consumption was the highest when the proton electrochemical gradient was the highest. After 14 h of fermentation, the culture pH started to increase due to H⁺ transport into the cells and depletion of glucose in the culture. The changes in proton electrochemical gradient resulted in differences in the metabolism. The highest specific growth rate of *E. coli* DH5α and xylanase production rate were occurred at pH 6.4, while glucose consumption rate was the highest at pH 5.8. This result indicates that manipulation of culture pH during the fermentation at different phases may enhance growth of *E. coli* DH5α and xylanase production, as reported for many fermentation processes.

*E. coli* is a facultative bacterium which can grow either in the presence of oxygen or absence of oxygen. Higher growth rate of *E. coli* DH5α and xylanase production was obtained in shake flask fermentation agitated at high speed (200-250 rpm), indicating that the oxygen transfer rate may influence the process. Previous researchers found that the mass transfer rate of nutrients and rate of oxygen supply to the cells were enhanced by small cell cluster with bigger interfacial areas, which in turn, improve the cultivation performance of *E. coli*. Oxygen supply to the shake flask culture was obtained by surface aeration, where the quantity of oxygen supplied was increased with increasing agitation speed. Improvement in xylanase production at higher agitation speeds (200 and 250 rpm) may be related to the level of dissolved oxygen in the culture. Vigorous agitation (250 rpm) may provide benefits for cell growth and protein production. This is in contrast to the findings from this study which indicated that moderate agitation (200 rpm) exhibit high xylanase activity and productivity. In shake flask culture incubated on rotary shaker, the agitation speed of 200 rpm was found optimal for growth of recombinant *E. coli*.

The xylanase production obtained in this study (2122.5 U mL⁻¹) was significantly higher than those reported in the literature. For example, the highest xylanase production obtained in submerged cultivation of *Aspergillus niger* B03 was 996.3 U mL⁻¹ and 592.7 U g/substrate by *Trichoderma longibrachiatum*. The highest activity of thermostable cellulase-free xylanase in shake-flask fermentations by *Bacillus subtilis* was 1056 U mL⁻¹. The advantages of intracellular as compared to extracellular xylanases include higher product yield and reduced degradation by proteolytic enzymes present in the culture. Further improvement of xylanase production by *E. coli* DH5α by refining the optimization of medium composition using Response Surface Methodology (RSM) is being carried out in our laboratory. In addition, the fed-batch culture technique, with various controlled parameters such as specific growth rate, nutrients level and the culture pH, is also being developed in our laboratory for efficient large scale fermentation process for xylanase production by the recombinant *E. coli* DH5α.

**CONCLUSION**

Results from this preliminary study showed that nutrient composition and optimum cultural conditions played an important role in growth of *E. coli* DH5α and intracellular xylanase production. Optimization of medium and culture conditions, as well as development of a suitable fermentation technique is required for enhancement of xylanase production by *E. coli* DH5α. From the kinetic and modeling study, it was found that the xylanase production by *E. coli* DH5α was growth associated process. Although the xylanase activity was increased concomitantly with increasing cell concentration in the culture, the growth associated xylanase production coefficient (α) was also depended on the maximum specific growth rate (μ_max) of the *E. coli* DH5α.

**REFERENCES**

1. Uffen, R.L., 1992. Xylan degradation: A glimpse at microbial diversity. J. Ind. Microbiol. Biotechnol., 19: 1-6. DOI: 10.1038/sj.jim.2900417
2. Haltrich, D., B. Nidetzky, K.D. Kulbe, W. Steiner and S. Zupancic, 1996. Production of fungal xylanases. Biore. Technol., 58: 137-161. DOI: 10.1016/S0960-8524(96)00094-6
3. Khasin, A., I. Alchanati and Y. Shoham, 1993. Purification and characterization of a thermostable xylanase from Bacillus steaerothermophilus T-6. Applied Environ. Microbiol., 59: 1725-1730. http://www.pubmedcentral.nih.gov/articlerender.fcgi?aid=182151

4. Sa-Pereira, P., M. Mesquita, J.C. Duarte, M.R.A. Barros and M. Costa-Ferreira, 2002. Rapid production of thermostable cellulose-free xylanase by a strain of Bacillus subtilis and its properties. Enz. Microbiol. Technol., 30: 924-933. DOI: 10.1016/S0141-0229(02)00034-0

5. Belancic, A., J. Scarpa, A. Peirano, R. Diaz, J. Steiner and J. Eyzayuirre, 1995. Penicillium purpurogenum produces several xylanases: Purification and properties of two of the enzymes. Biotechnology, 41: 71-79. DOI: 10.1016/1052-7110(94)00057-W

6. Hmrnova, M., E. Petrakova and P. Biely, 1991. Induction of cellulose and xylan degrading enzyme systems in Aspergillus terreus by homo and heterodisaccharides composed of glucose and xylose. Gen. Microbiol., 137: 541-547. DOI: 10.1016/0036-9114(91)90078-V

7. Penbroke, J.T., B. Sweeny and R.A. Whelan, 1992. Reduction in xylanase activity in 10 Pseudomonas laviengena Extracts is a Result of Protease Activity. In: Xylans and xylanases, Visser, J., G. Beldman, M.A.K. Someren and A.G.J. Voragen (Eds.). Elsevier, Amsterdam, ISBN: 0444894772, pp: 479-482.

8. Kulkarni, N., J. Chauthawale and M. Rao, 1995. Characterization of the recombinant xylanases in Escherichia coli from an alkalophilic thermophilic Bacillus sp. NCIM 59. Enz. Microb. Technol., 17: 972-976. DOI: 10.1016/0141-0229(94)00144-8

9. Kulkarni, N., A. Shendye and M. Rao, 1999. Molecular and biotechnological aspects of xylanases. FEMS. Microbiol. Rev., 23: 411-456. DOI: 10.1016/S0112-790X(99)00006-6

10. Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., 31: 426-428. DOI: 10.1021/ac60147a030

11. Weiss, R.M. and D.F. Ollis, 1980. Extracellular microbial polysaccharides I. Substrate, biomass and product kinetic equations for batch xanthan gum fermentation. Biotechnol. Bioeng., 22: 859-864. DOI: 10.1002/bit.260220410

12. Yee, L. and H.W. Blanch, 1992. Recombinant protein expression in high cell density fed-batch cultures of Escherichia coli. Nat. Biotechnol., 10: 1550-1556. DOI: 10.1038/nbt1292-1550

13. Karlsson, E.N., O. Holst and A. Tocajb, 1999. Efficient production of truncated thermostable xylanases from Rhodothermus marinus in Escherichia coli fed-batch cultures. Biosci. Bioeng., 87: 598-606. DOI: 10.1016/S1389-1723(99)01212-1

14. Sunitha, K., J.K. Lee and T.K. Oh, 1999. Optimization of medium components for phytase production by E. coli using response surface methodology. Bioprocess Eng., 21: 477-481. DOI: 10.1007/PL0009086

15. Chen, G. and K.A. Strevett, 2003. Impact of carbon and nitrogen conditions on E. coli surface thermodynamics. Colloids Surfaces B: Biointerfaces, 28: 135-146. DOI: 10.1016/S0927-765X(02)00143-1

16. Vidal, L., P. Ferrer, G. Alvaro, M.D. Benaiges and G. Caminal, 2005. Influence of induction and operation mode on recombinant rhamnulose 1-phosphate aldolase production by Escherichia coli using T5 promoter. Biotechnology, 118: 75-87. DOI: 10.1016/j.biotech.2005.02.012

17. Maldonado, L.M.T.P., V.E.B. Hernandez, E.M. Rivero, A.P. Barba de la Rosa, J.L.F. Flores, L.L.G.O. Acevedo and A.R. De Leon, 2007. Optimization of culture conditions for a synthetic gene expression in Escherichia coli using response surface methodology: The case of human interferon beta. Biomol. Eng., 24: 217-222. DOI: 10.1016/j.ibioeng.2006.10.001

18. Duffy, G., R.C. Whiting and J.J. Sheridan, 1999. The effect of a competitive microflora, pH and temperature on the growth kinetics of Escherichia coli O157:H7. Food Microbiol., 16: 299-307. DOI: 10.1006/fmic.1998.0242

19. Calik, P., G. Bilir, G. Calik and T.H. Ozdamar, 2002. Influence of pH conditions on metabolic regulations in serine alkaline protease production by Bacillus licheniformis. Enz. Microbiol. Technol., 31: 685-697. DOI: 10.1016/S0141-0229(02)00162-X

20. Coleman, M.E., M.L. Tamplin, J.G. Philips and B.S. Marmer, 2002. Influence of agitation, inoculum density, pH and strain on the growth parameters of Escherichia coli O157:H7-relevance to risk assessment. Int. Food Microbiol., 2589: 399-415. DOI: 10.1016/S0168-1605(02)00367-7

21. Beshay, U., H. El-Enshasy, I.M.K. Ismail, M. Hassan, W. Ewa and A.E.G. Sawsan, 2003. β-Glucanase production from genetically modified recombinant Escherichia coli: Effect of growth substrates and development of a culture medium in shake flasks and stirred tank bioreactor. Process Biochem., 39: 307-313. DOI: 10.1016/S0032-9592(03)00078-5
22. Sivakesava, S., Z.N. Xu, Y.H. Chen, J. Hackett, R.C. Huang, E. Lam, T.L. Lam, K.L. Siu, R.S.C. Wong and W.K.R. Wong, 1999. Production of excreted Human epidermal Growth Factor (hEGF) by an efficient recombinant Escherichia coli system. Process Biochem., 34: 893-900. DOI: 10.1016/S0032-9592(99)00013-8

23. Georgi, T.D., G.P. Ivan, S.S. Vaselin and M. Rositza, 2007. Optimization of nutrient medium containing agricultural wastes for xylanase production by Aspergillus niger B03 using optimal composite experimental design. Biore. Technol., 98: 2671-2678. DOI: 10.1016/j.biortech.2006.09.022

24. Azin, M., R. Moravej and D. Zareh, 2007. Production of xylanase by Trichoderma longibrachiatum on a mixture of wheat bran and wheat straw: Optimization of culture condition by Taguchi method. Enz. Microbial Technol., 40: 801-805. DOI: 10.1016/j.enzmietec.2006.06.013