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APPENDICES

Appendix A Autoclave

Batch reactor system used to study the effects of added acid on pretreatment. Autoclave (Parr reactor), which can operate in high temperature and high pressure, was created in order to use with heater stirrer because of the homogeneous system. The reactor was created from Stainless steel type 316 in order to withstand with acid solution. Autoclave was shown in Figure A1.

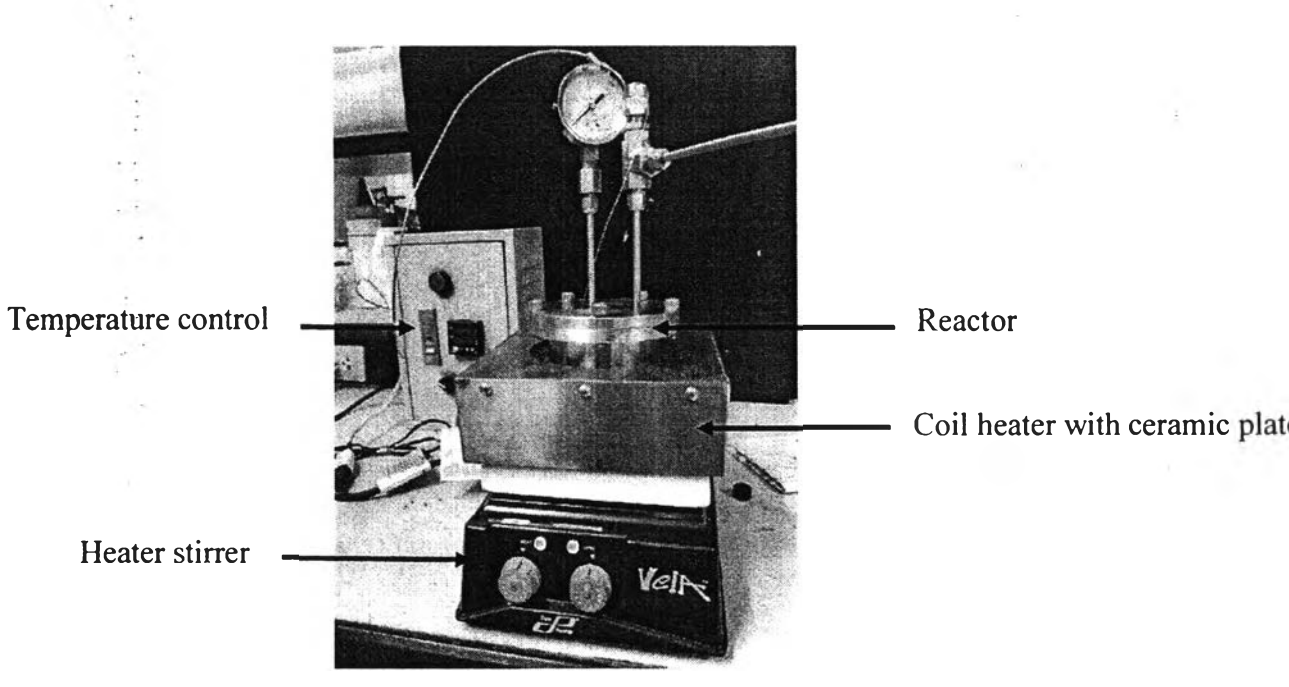

Figure A1 Autoclave.

Autoclave consisted of temperature control, coil heater with ceramic plate, heater stirrer, Reactor, pressure gauge, and pressure release value. About heating rate of autoclave, the researcher explored the heating rate of autoclave system by plotting the graph between temperature from room temperature to temperature setting point (100 °C, 120 °C, 140 °C, and 160 °C) and time (1 min interval). The result is shown showed in Figure A2.

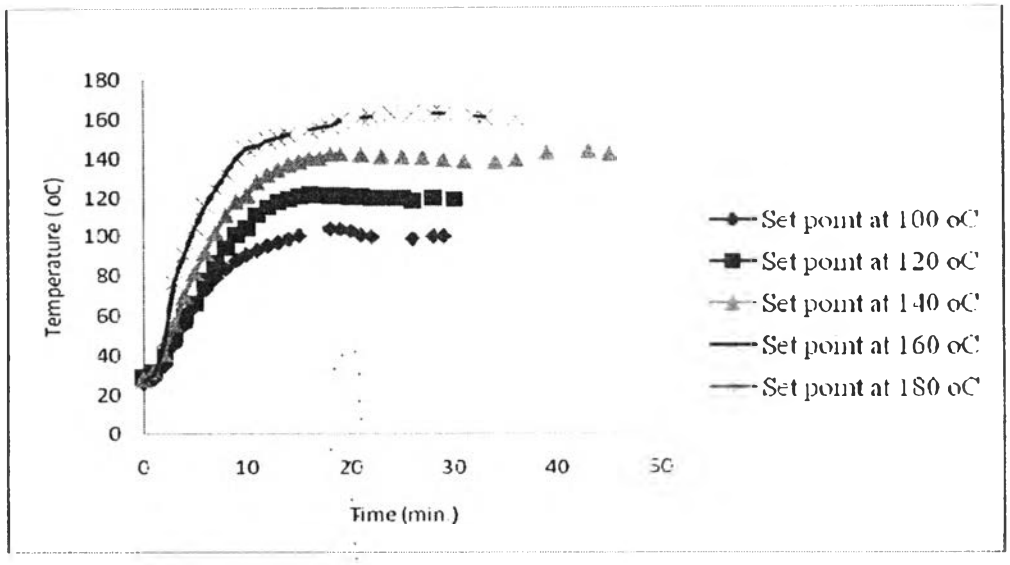

Figure A2 Heating rate of autoclave system.

Figure A2 showed that the system had approximately used 15 minutes to enter steady stage (Temperature setting point). Therefore, heating of system approximately equaled 5 °C/min.

Appendix B Activity of Enzyme

The enzymatic activity was determined according to method developed by T.K. Ghose. First, Glucose standard curve was plotted between amounts of glucose (micromole/ml) and value of absorbance of UV–VIS Spectrometer. The calibration curve is shown in B1.

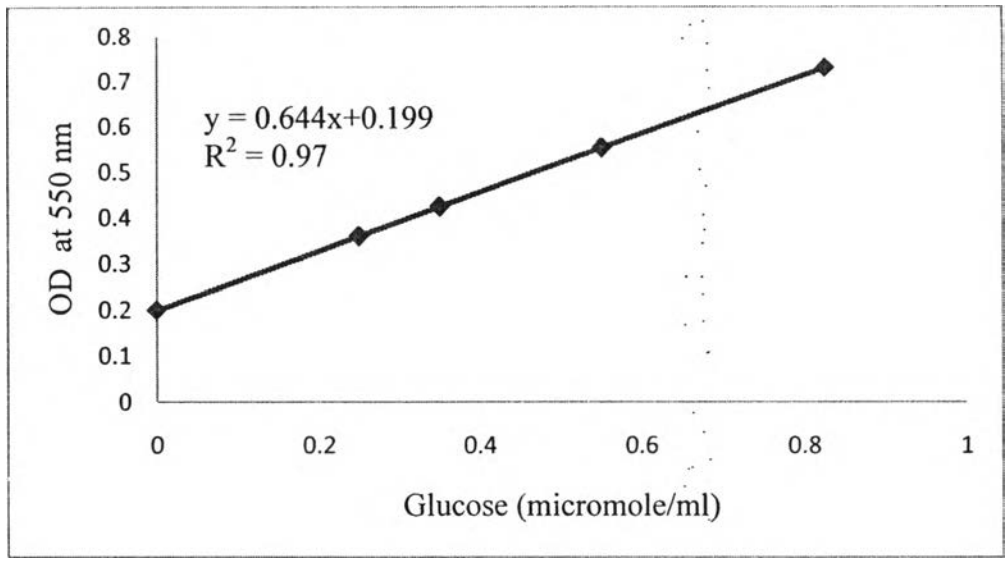

Figure B1 Glucose standard curve.

After plotting Glucose standard curve, cellulase (from Novozyme) and ACCELLERASE@1500 (from Genencor) was investigated activity of enzyme. The result is shown in Table B1.

Table B1 Activity of Enzyme

Type of Enzyme	Activity (unit/ml)
Cellulase	2889.27
ACCELLERASE@1500	2482.45

Table B1 showed that cellulase from Novozyme has activity higher than ACCELLERASE@1500 from Genencor because this T.K. Ghose's method can detect only cellulase. Therefore, it is possible that cellulase in ACCELLERASE@1500 might be less than cellulase from Novozyme.

Appendix C Standard Curve of HPLC

The fermentable sugars were identified and quantified by both Aminex column HPX–87H. The column was maintained at 65 °C and 20 µl of each sample and eluted by 0.005 M sulfuric acid at the flow rate 0.6ml/min and Lichrospher NH_2 250*4mm column in HPLC–ELSD), and organic acid were identified and quantified by both Aminex column HPX–87H. The column was maintained at 25 °C and 10 µl of each sample and eluted by 84% of ACN: 16% of water at the flow rate 1.6 ml/min. Analytical grade glucose, xylose, mannose, galactose, and cellubiose were used as authentic standards. The result of standards was shown in Table C1 and Figure C1.

Table C1 Retention time of each fermentable sugar

Standard	Aminex column (min)	Lichrospher column (min)
Glucose	8.638	9.633
Xylose	9.216	5.466
Mannose	9.114	8.516
Galactose	9.137	10.466
Cellubiose	7.143	N/A
Arabinose	9.939	6.433
Rhamnose	N/A	4.350
Furfural	N/A	N/A

N/A; Not Available

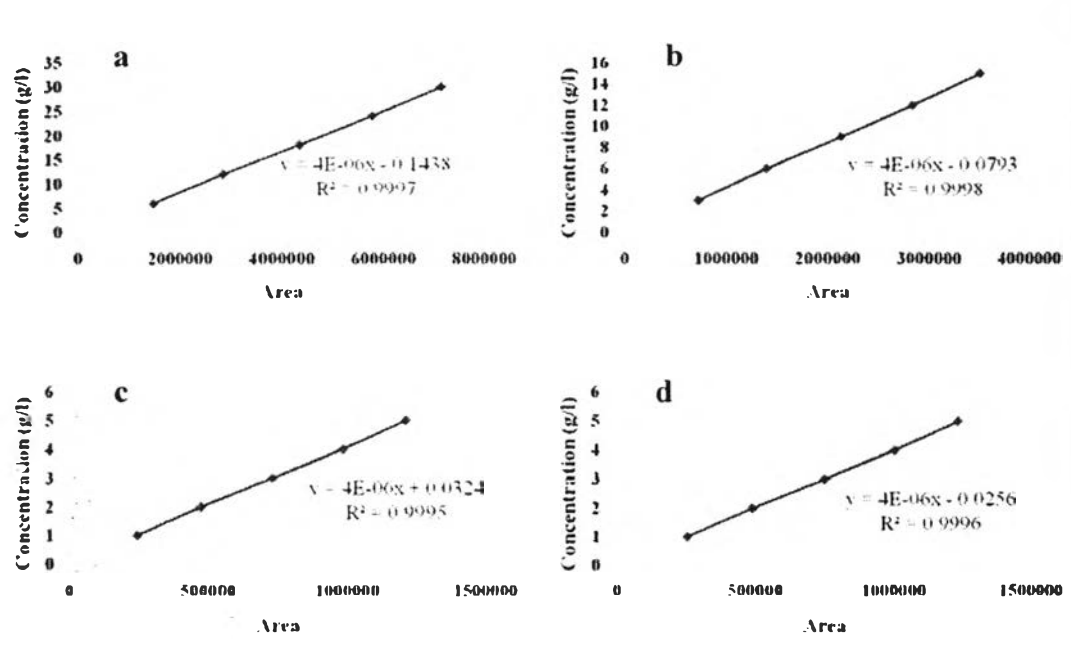

Figure C1 Standard curve of sugar for (a) standard glucose (b) standard xylose (c) standard cellubiose and (d) standard arabinose.

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