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APPENDICES

Appendix A Retention Time and Calibration Curve of Glucose, Acids, and Solvents by HPLC

The quantity of glucose, acids and solvents profile of fermentation process were determined by HPLC equipped with a refractive index detector (Series 200 LC/S/N291N5060508, Perkin Elmer) using an Aminex-HPX 87H column (300 mm x 78 mm, Bio-Rad Lab, USA) and a guard column (30 mm x 4.6 mm, Bio-Rad Lab, USA) under these following conditions: flow rate 0.60 ml/min, mobile phase 0.005 M of H₂SO₄ and column temperature was fixed at 60 °C. The retention times of glucose, acids and solvents shown in Table A1. For acids, there consists of butyric and acetic acid. For solvents are consists of acetone, butanol, and ethanol. In order to determine the quantity of glucose and products, calibration curve of each substance is necessary. Figure A1 shows the calibration curve of glucose, acids, and solvents.

Table A1 Retention time of substances

Substances	Retention time (min)
Glucose	9.24
Acetic acid	15.07
Butyric acid	21.09
Acetone	21.89
Butanol	37.19
Ethanol	22.39

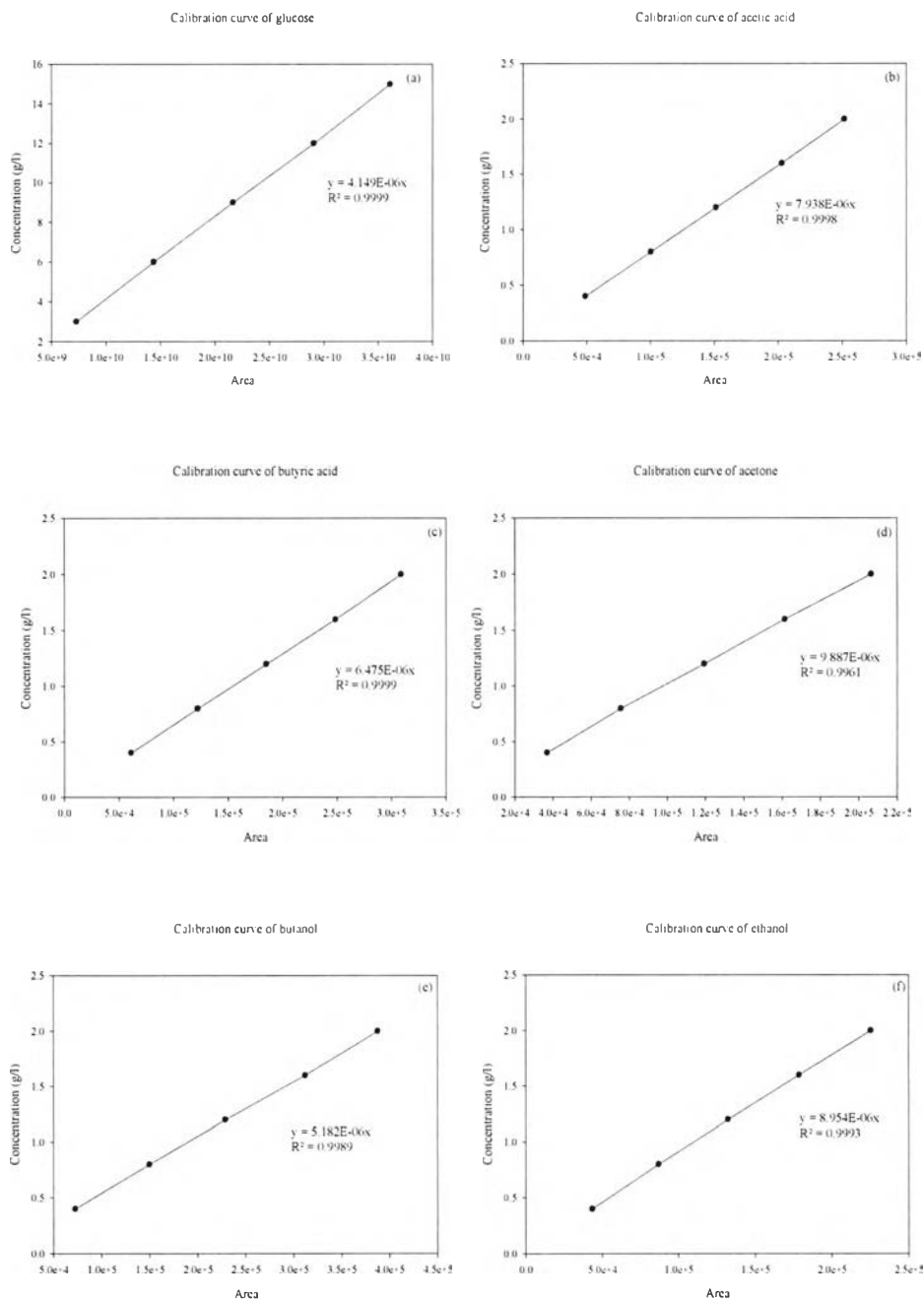


Figure A1 Calibration curve of glucose, acids, and solvents. Symbols; (a) calibration curve of glucose, (b) calibration curve of acetic acid, (c) calibration curve of butyric acid, (d) calibration curve of acetone, (e) calibration curve of butanol, and (f) calibration curve of ethanol.

Appendix B Retention Time and Calibration Curve of Acetone-Butanol-Ethanol by GC

For the fermentation step the samples products acetone-butanol-ethanol by *Clostridium beijerinckii* TISTR1461 at different times on stream were collected and detected by Gas Chromatography at the petroleum and petrochemical college, Chulalongkorn University. In order to determine the quantity of acetone-butanol-ethanol in product, the calibrational curve of acetone-butanol-ethanol is required. Figure B1 shows the calibration curve of standard acetone-butanol-ethanol under various concentrations in the range 0.5 g/l to 2 g/l using propanol as an internal standard.

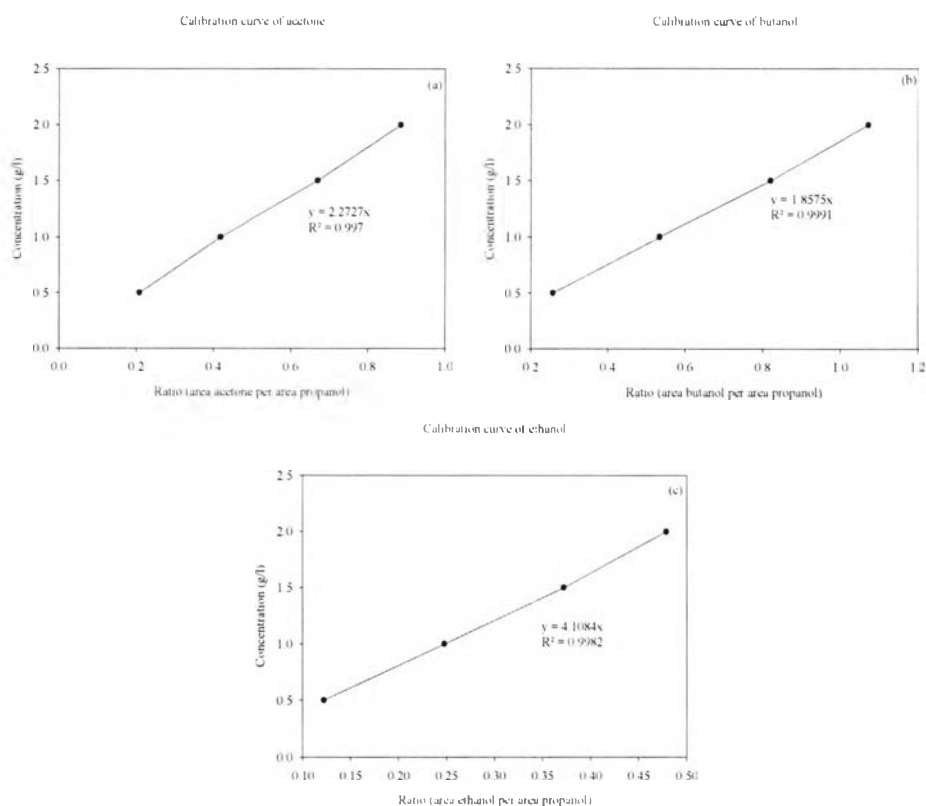


Figure B1 Calibration curve of acetone-butanol-ethanol. Symbols; (a) calibration curve of acetone, (b) calibration curve of butanol, and (c) calibration curve of ethanol.

Appendix C Immobilized Cells Fermentation on Untreated Zeolite

Figure C1 shows the pH profile of immobilized cells fermentation on untreated zeolite. The initial pH was about 7 at 0th hour and pH was significantly increased at 12th hour. After 12th hour it showed the dramatically decrease of pH profile and constant after 72 hour. The high pH value might be the effect from basicity of zeolite 13X leading to inappropriate initial pH to optimized the growth rates of *Clostridium* genus which should be in the range of 6.5-6.8 (Tashiro *et al.*, 2004; Schmidt and Weuster-Botz, 2012; Kumar *et al.*, 2013). This might be resulting to the low glucose consumption and low solvents production compare with free mobilized cells fermentation as shown in Figure C2 and C3, respectively. Hence, there has to had acid treatment process for zeolite in a preparation step to neutralize zeolite before using as a cell carrier.

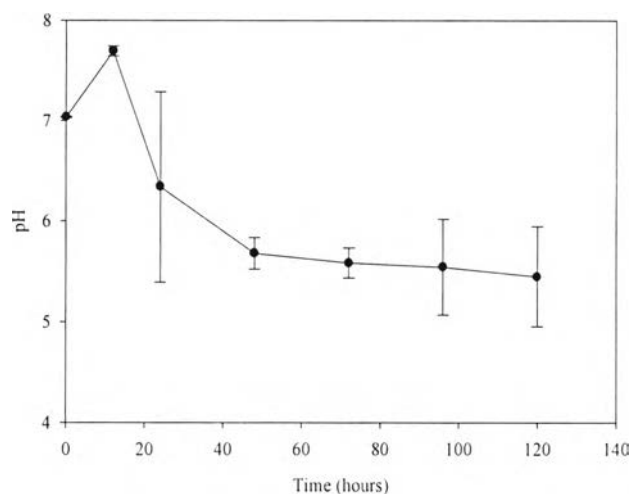


Figure C1 pH profile of immobilized cells fermentation on untreated zeolite.

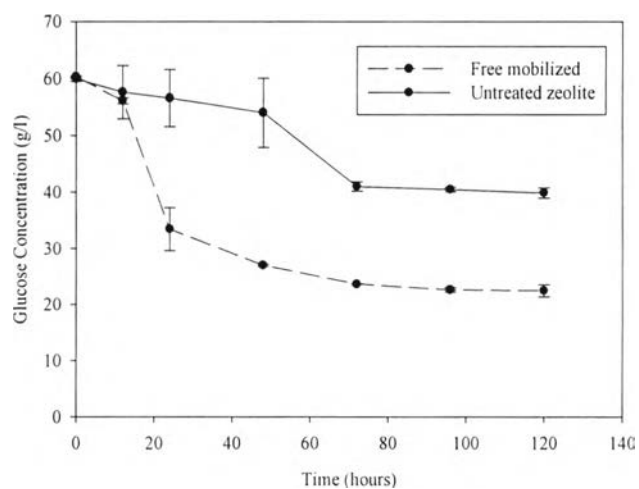


Figure C2 Glucose profile of immobilized cells fermentation on untreated zeolite.

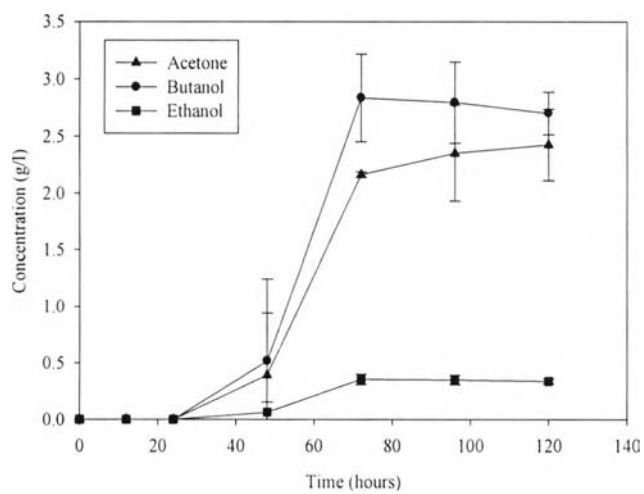


Figure C3 Solvents production of immobilized cells fermentation on untreated zeolite.

Appendix D Scanning Electron Microscope (SEM)

Figure D1-D4 shows the materials surface before cell immobilization of electron microscope at magnification x1000, x3000, and x5000 for brick, activated carbon, untreated zeolite and treated zeolite, respectively.

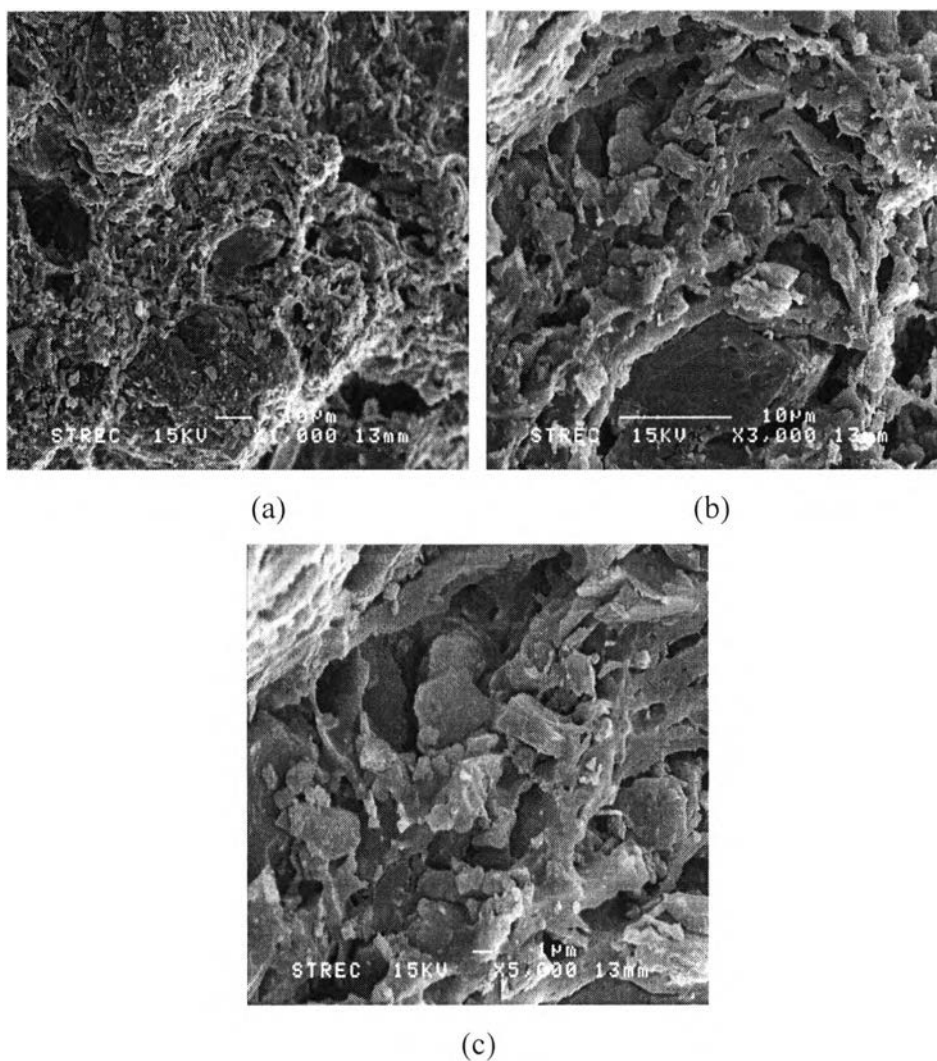


Figure D1 Scanning electron microscope images of brick before the immobilization; (a) magnification x1000, (b) magnification x3000, (c) magnification x5000

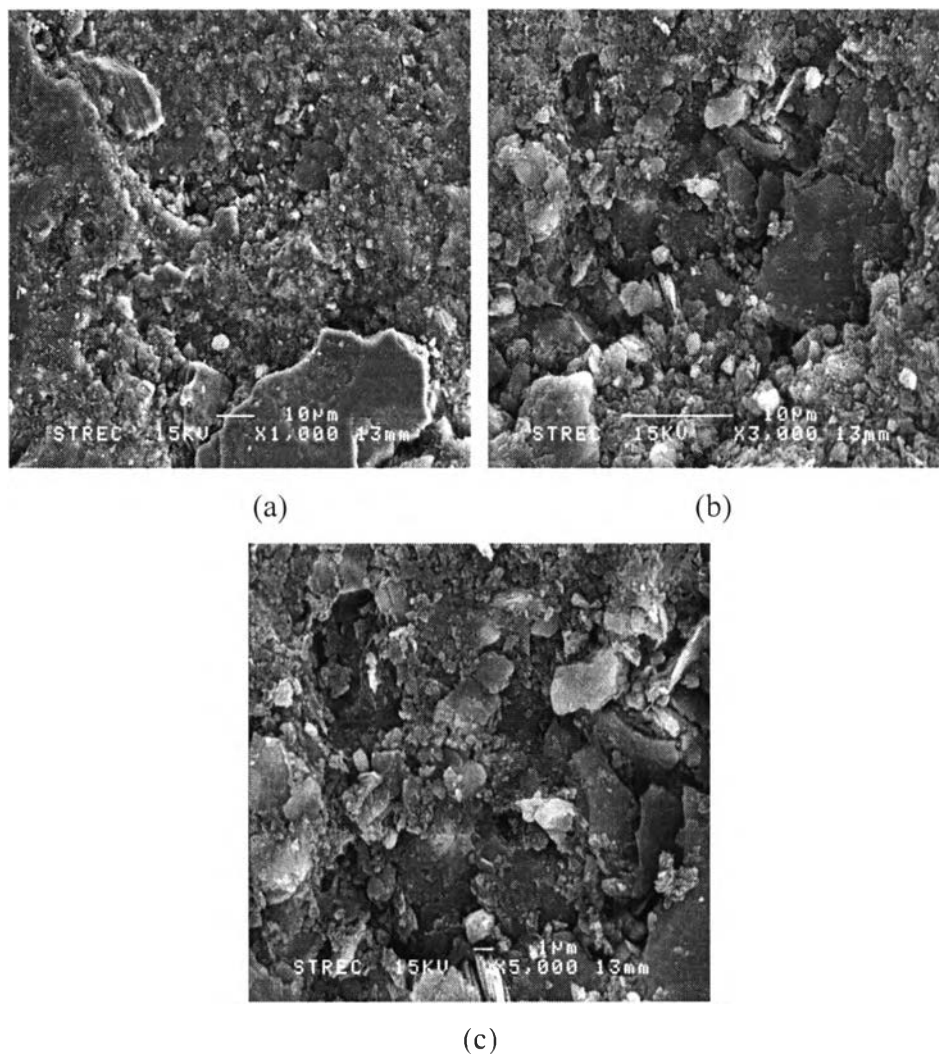


Figure D2 Scanning electron microscope images of activated carbon before the immobilization; (a) magnification x1000, (b) magnification x3000. (c) magnification x5000

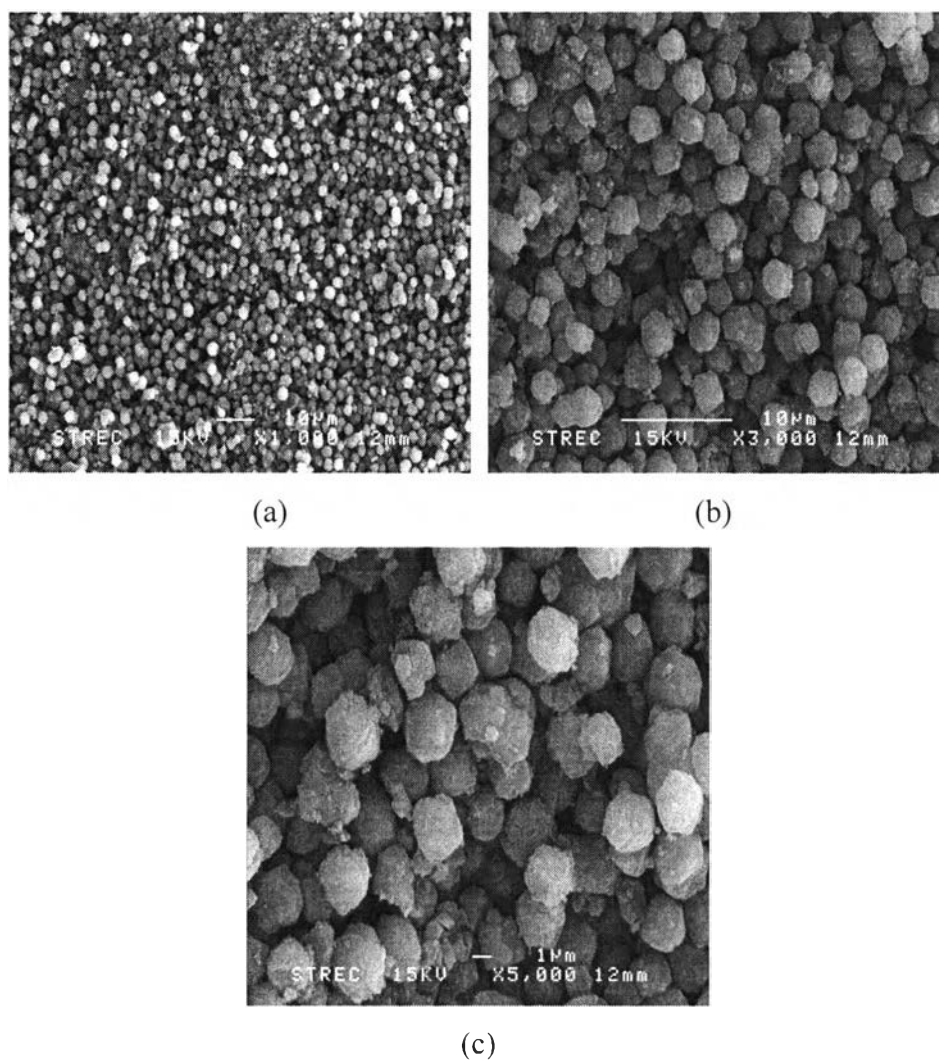


Figure D3 Scanning electron microscope images of untreated zeolite before the immobilization; (a) magnification x1000, (b) magnification x3000, (c) magnification x5000

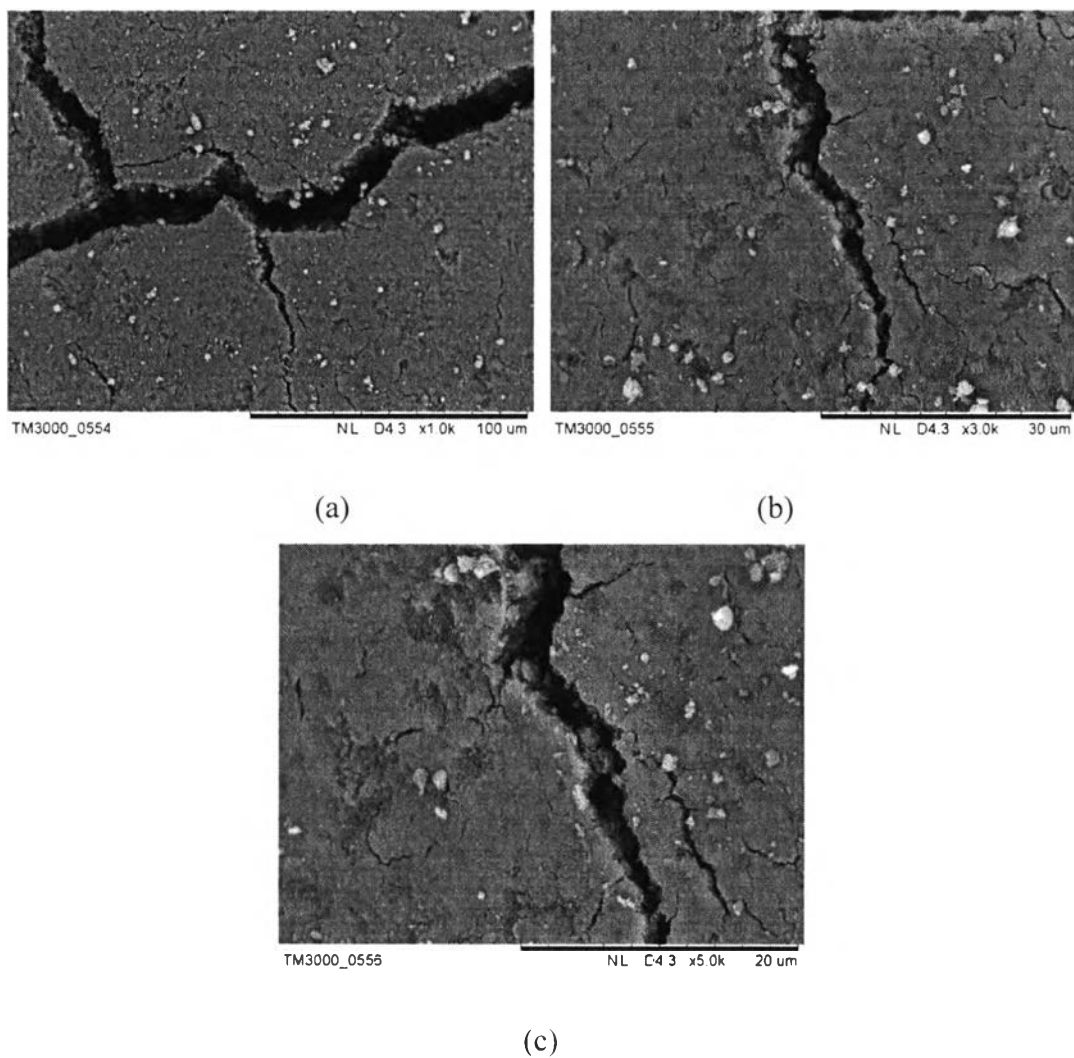


Figure D4 Scanning electron microscope images of treated zeolite before the immobilization; (a) magnification x1000, (b) magnification x3000, (c) magnification x5000

For Figure D5-D7 shows the materials surface with cell immobilization of electron microscope at different magnification for brick, activated carbon, untreated zeolite and treated zeolite, respectively.

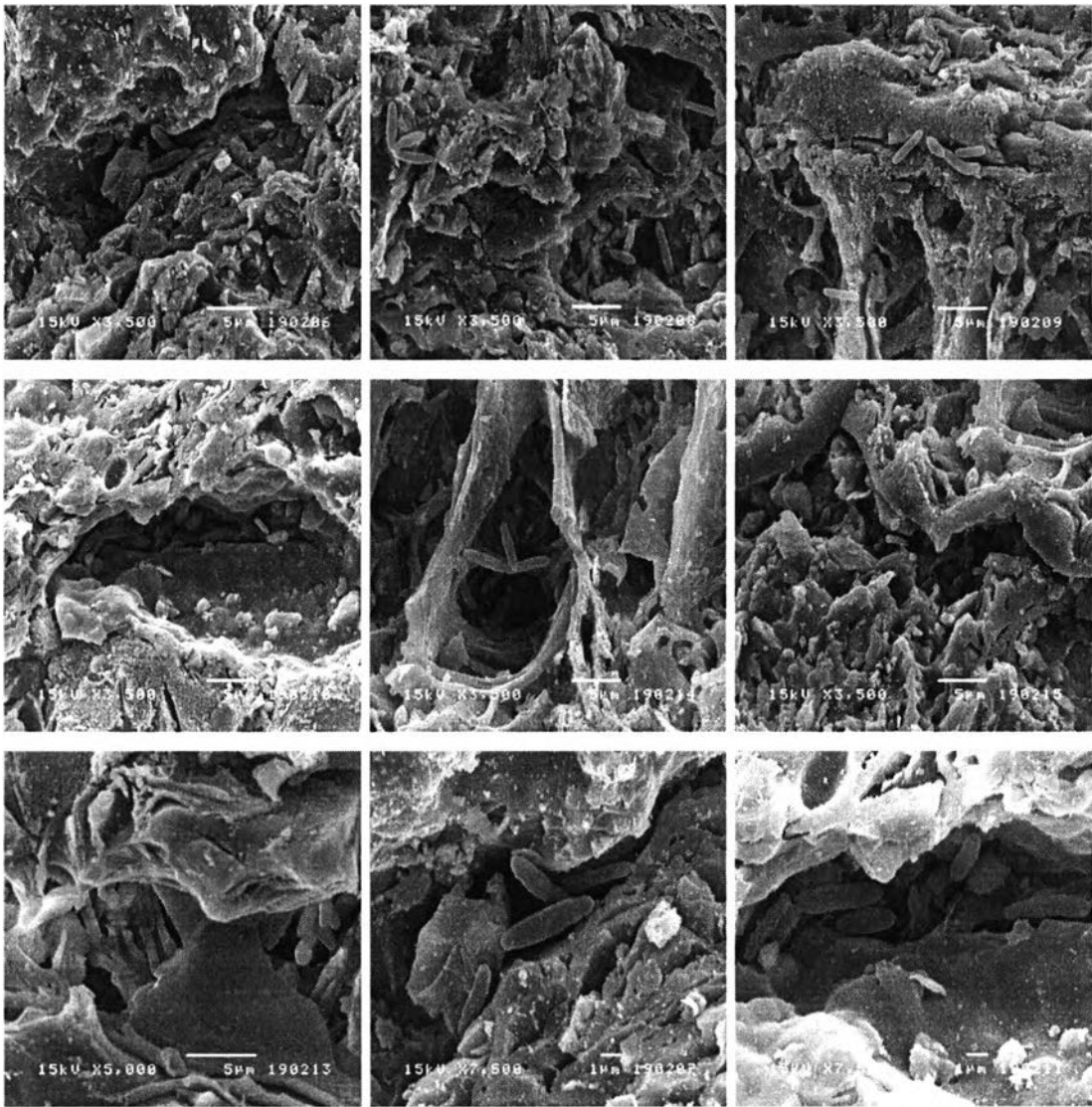


Figure D5 Scanning electron microscope images of brick after the immobilization at different magnification.

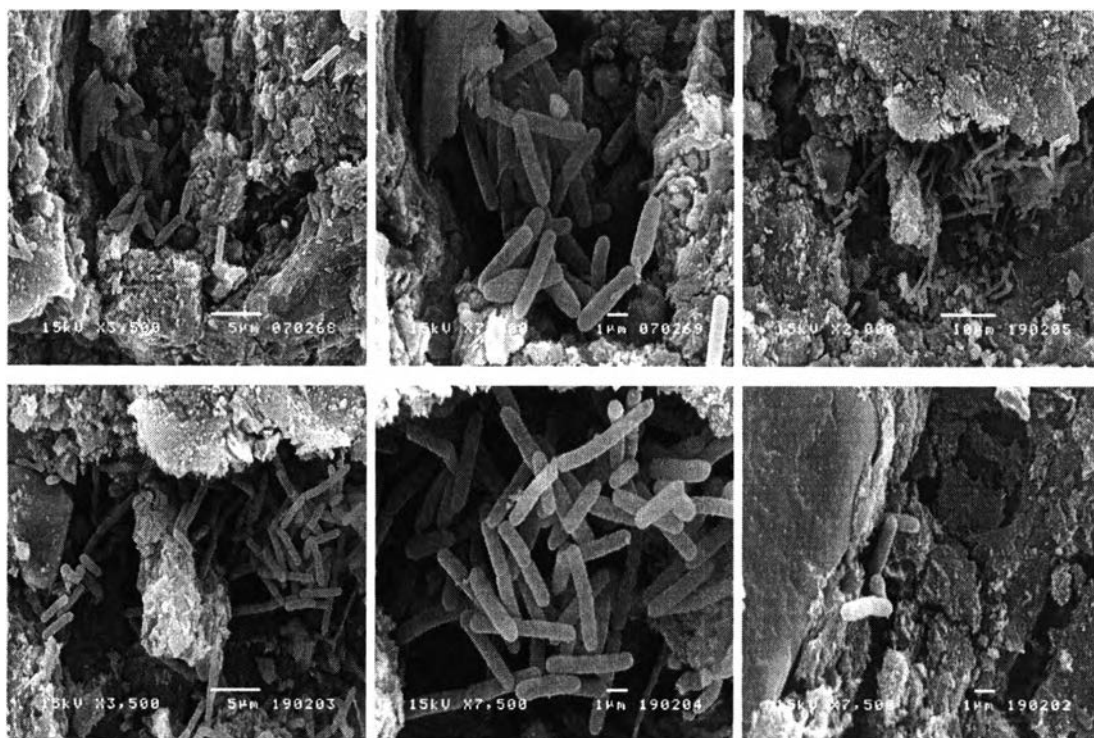


Figure D6 Scanning electron microscope images of activated carbon after the immobilization at different magnification.

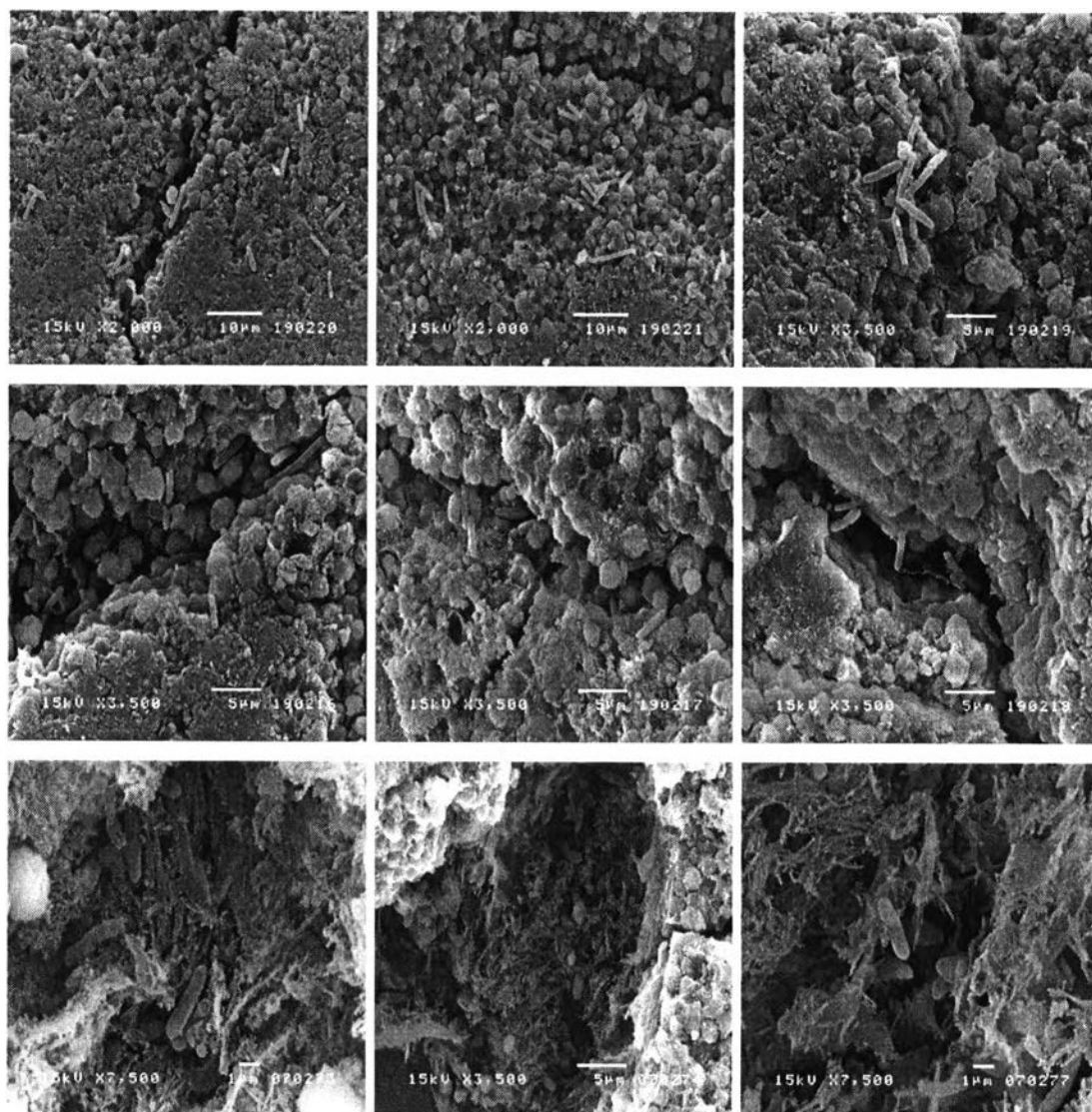


Figure D7 Scanning electron microscope images of zeolite after the immobilization at different magnification.

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