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APPENDICES

Appendix A SEM images of untreated and pretreated celluloses

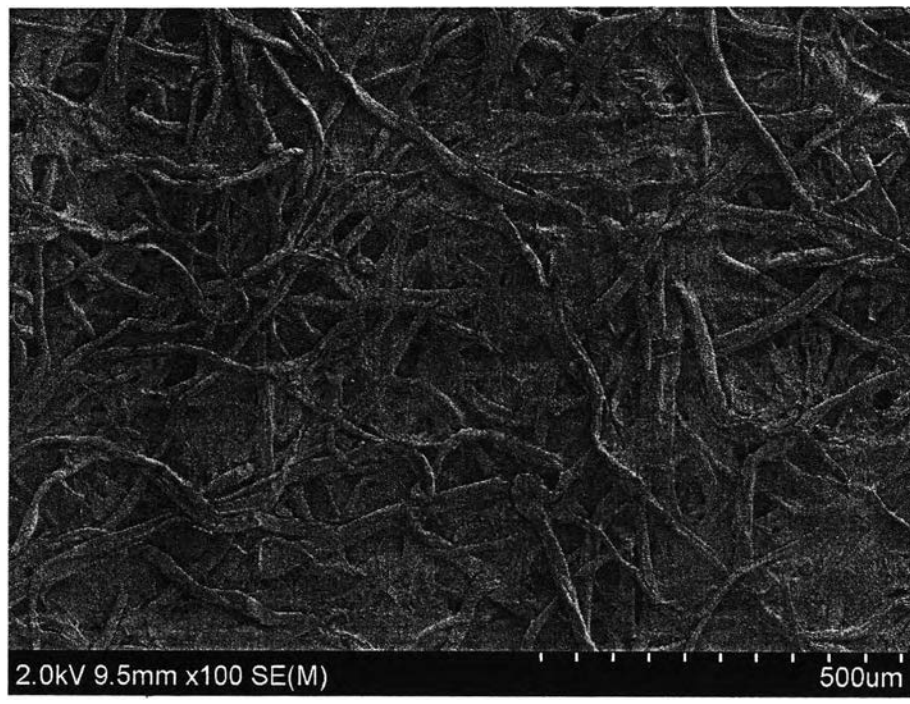

Figure A1 Untreated cellulose Whatman No.1 (low magnification).

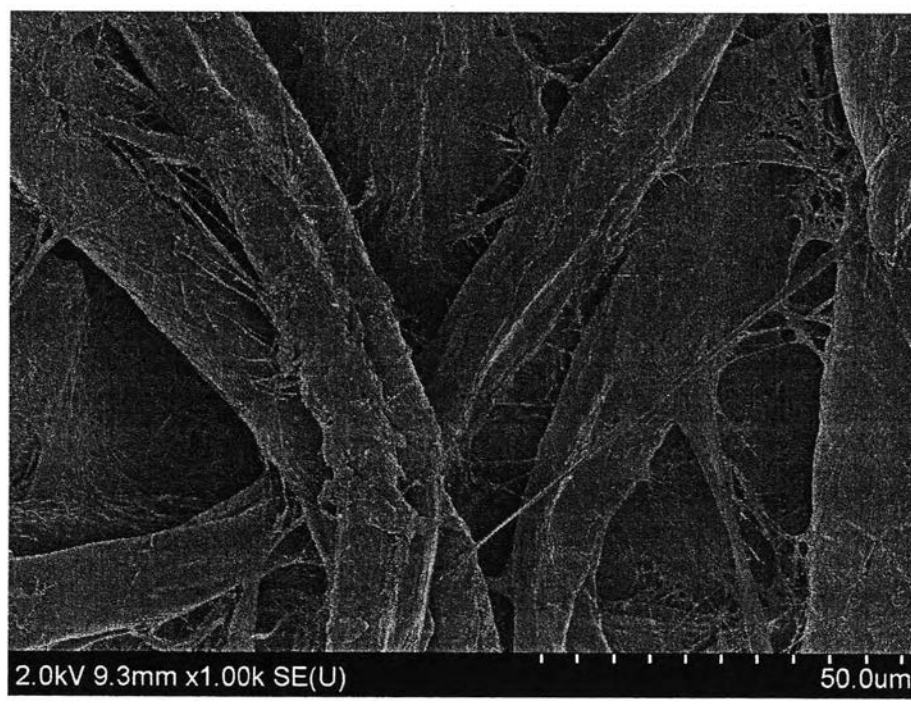

Figure A2 Untreated cellulose (high magnification).

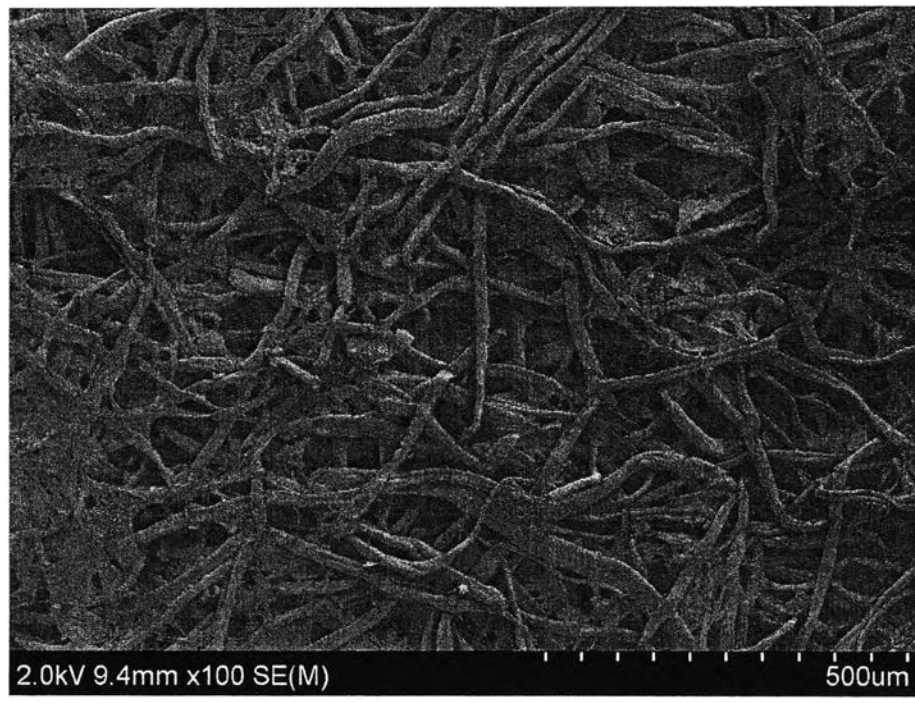

Figure A3 Pretreated cellulose Whatman No.1 (low magnification).

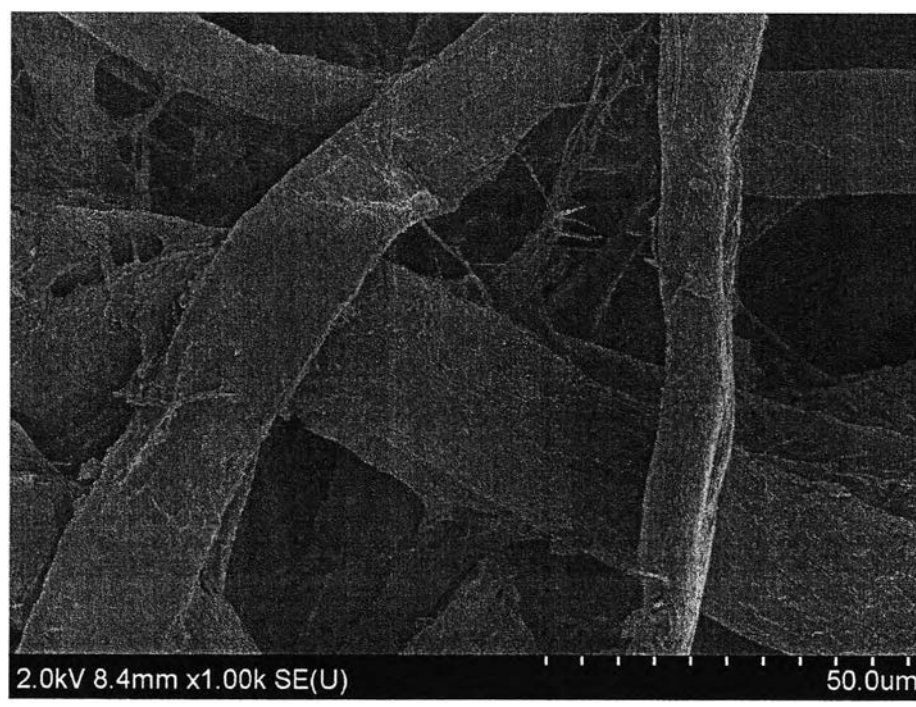

Figure A4 Pretreated cellulose Whatman No.1 (high magnification).

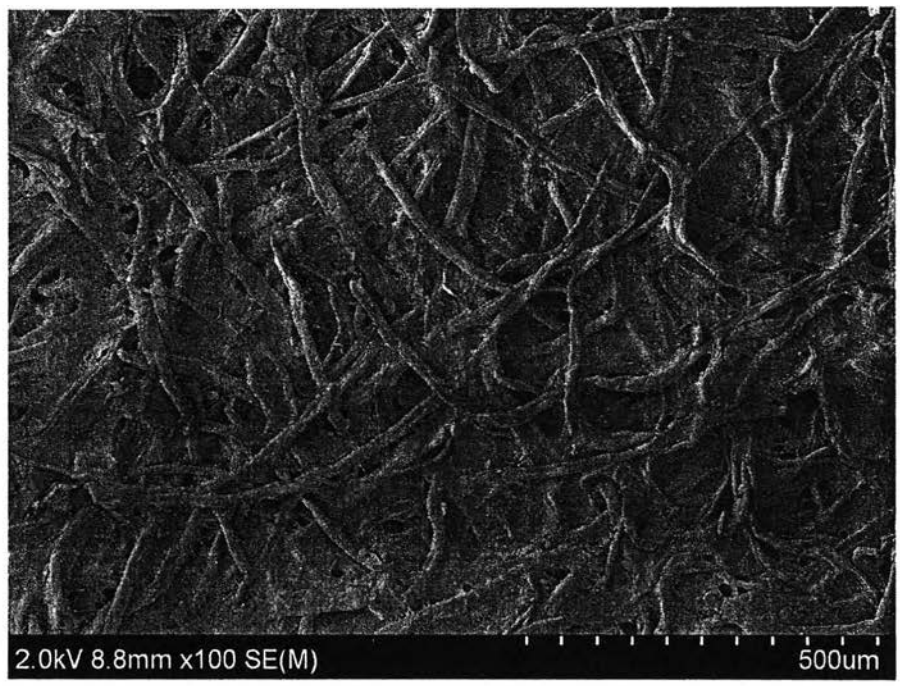

Figure A5 Untreated cellulose Whatman No.2 (low magnification).

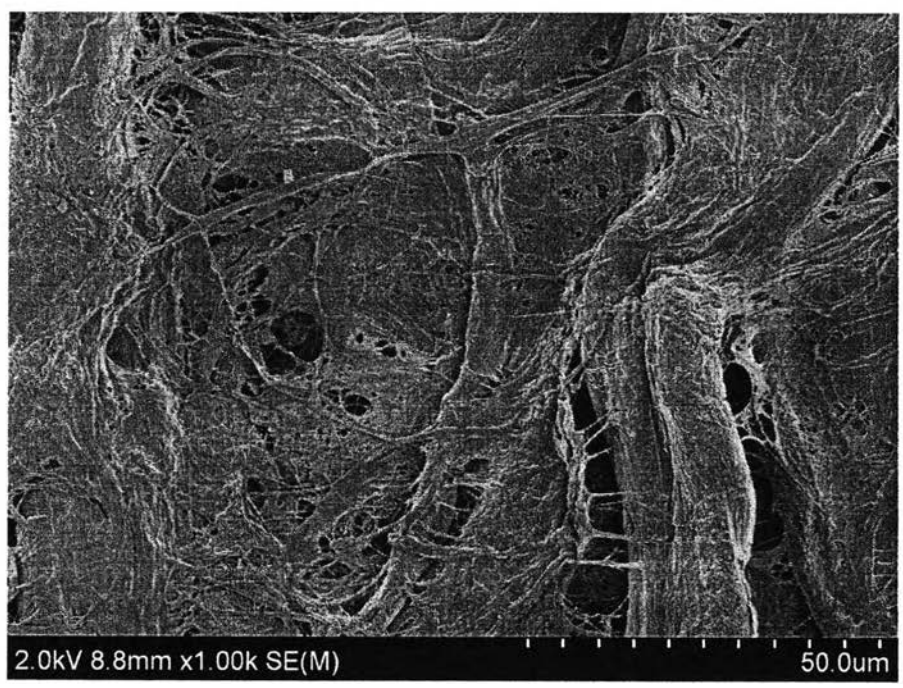

Figure A6 Untreated cellulose Whatman No.2 (high magnification).

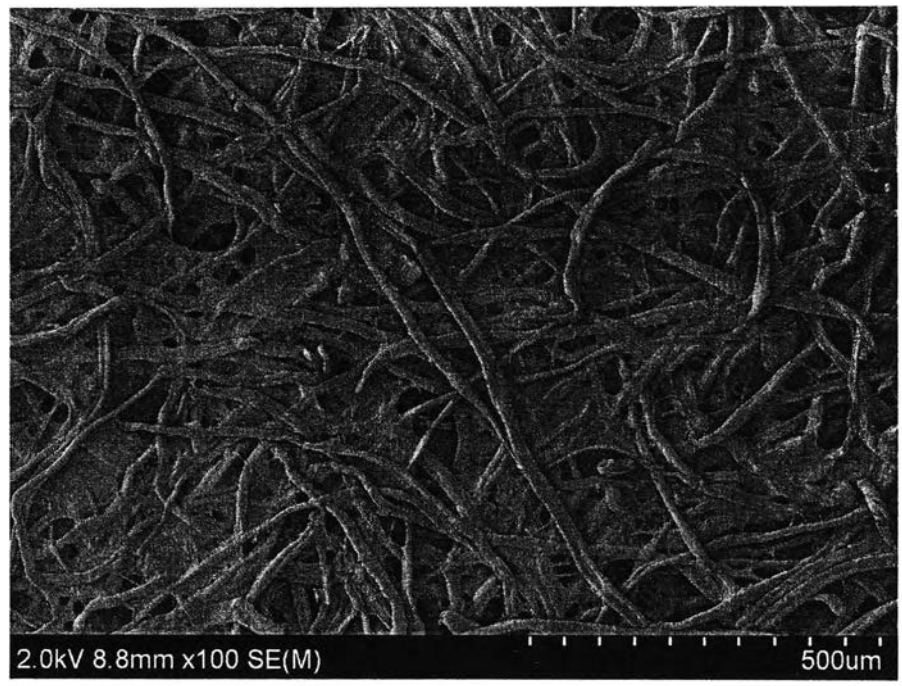

Figure A7 Untreated cellulose Whatman No.4 (low magnification).

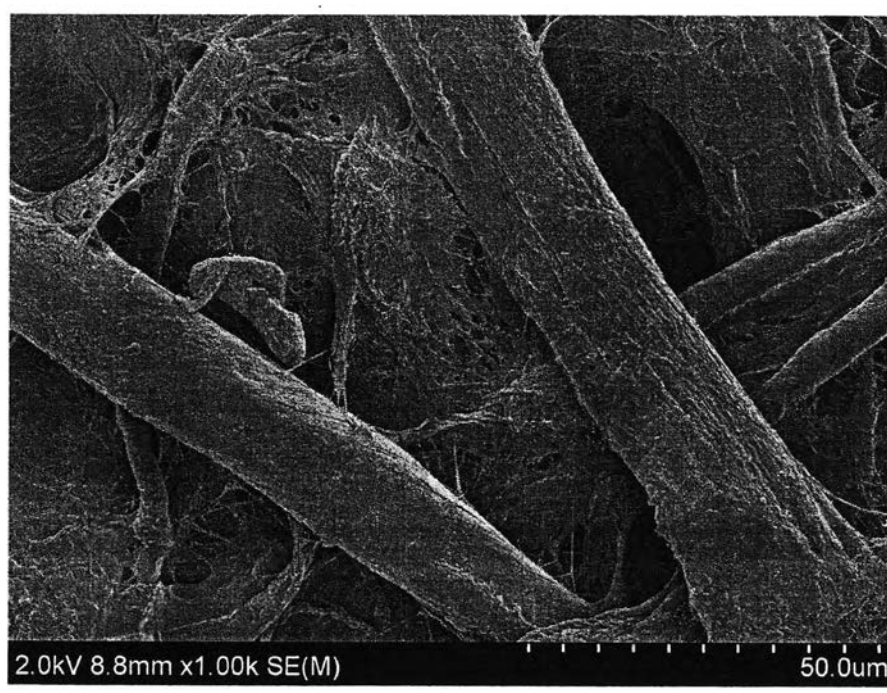

Figure A8 Untreated cellulose Whatman No.4 (high magnification).

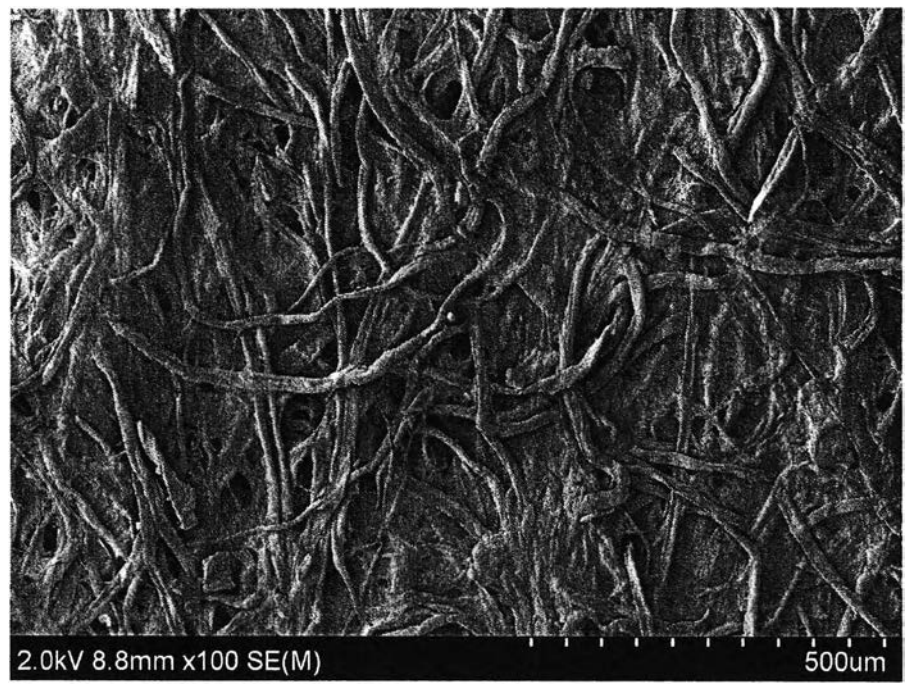

Figure A9 Untreated cellulose Whatman No.5 (low magnification).

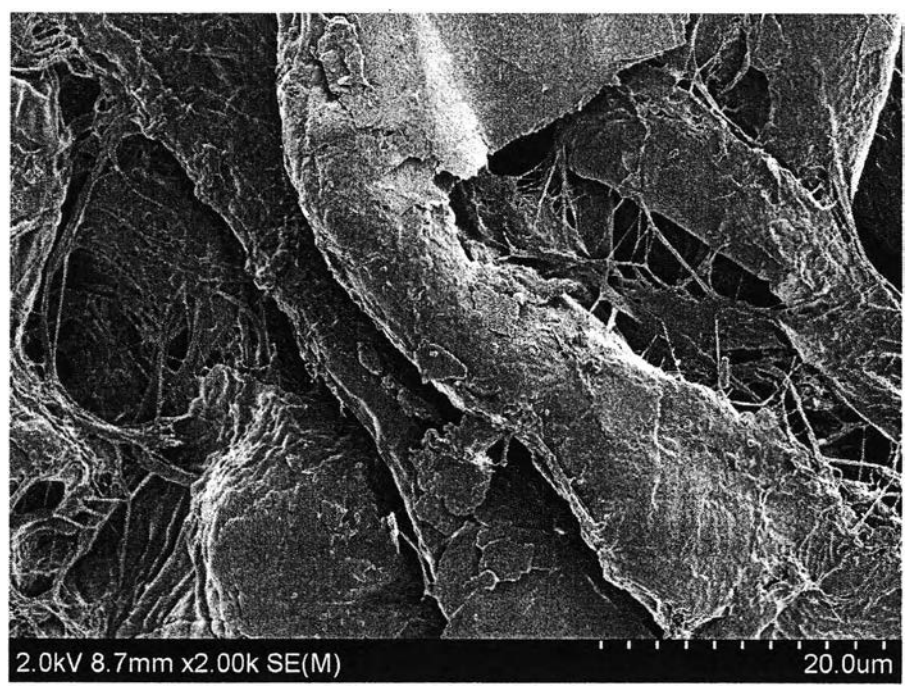

Figure A10 Untreated cellulose Whatman No.5 (high magnification).

Appendix B Area-volume of Whatman Filter Paper

Whatman No.	Crystallinity intensity	Surface area (m^2/g)	Pore volume (cc/g)	Pore size (nm)
1	1498	0.710	$7.870x10^{-3}$	44.3
2	1573	1.56	$1.034x10^{-2}$	26.5
4	1223	1.443	$8.129x10^{-3}$	22.5
5	2015	1.250	$7.283x10^{-3}$	23.3
Pretreated No.1	153	1.200	$6.802x10^{-3}$	22.7

Appendix C Media for Microorganisms

1. 65 modified DSMZ broth medium 2

Approximate Formula* Per Liter

Carboxymethyl Cellulose (CMC)	5.0	g
Yeast extract	4.0	g
Malt extract	10.0	g

Dissolve and adjust pH to 7.2

Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes

2. 65 modified DSMZ agar medium 2

Approximate Formula* Per Liter

Carboxymethyl cellulose (CMC)	5.0	g
Yeast extract	4.0	g
Malt extract	10.0	g
Agar	12.0	g

Dissolve and adjust pH to 7.2

Autoclave at 121°C and pressure at 15 pounds/square inch for 15 minutes

Appendix D Reagent Preparations

1. 0.85%(w/v) NaCl in 1000 mL

Sodium chloride (NaCl)	8.5	g
Distilled water	1000	mL

2. Hydrochloric acid 1 N in 100 mL

Hydrochloric acid (HCl conc.)	8.29	mL
Distilled water	91.71	mL

3. Sodium hydroxide 1 N in 100 mL

Sodium hydroxide (NaOH)	4.0	g
Distilled water	100	mL

4. Sodium hydroxide 0.1 N in 1000 mL

Sodium hydroxide (NaOH)	4.0	g
Distilled water	1000	mL

Appendix E Measurement of glucose concentration

Glucose concentration was determined using glucose (HK) assay kit.

Components of 1 mL glucose (HK) assay reagent:

1.5 mM NAD

1 mM ATP

1 unit/mL of hexokinase

1 unit/mL glucose-6-phosphate dehydrogenase with sodium benzoate and potassium sorbate as preservatives

Procedure

1. Pipette the following solution into the appropriate marked test tubes.

Tube	Glucose Assay Reagent (mL)	Sample Volume (μL)	Volume of Deionized Water (mL)
Sample Blank	-	100	1.0
Reagent Blank	1.0	-	0.1
Test	1.0	100	-

2. Mix all solutions homogeneously and incubate for 15 minutes at 18-35°C.

3. Measure the absorbance at 340 nm versus deionized water.

Calculation

The contribution of the absorbances of the sample and the glucose assay reagent must be taken into account for the total blank.

$$A_{Total} = A_{Sample\ Blank} + A_{Reagent\ Blank}$$

$$\text{mg glucose/mL} = \frac{(\Delta A)(TV)(\text{Glucose molecular weight})(F)}{(\varepsilon)(d)(SV)(\text{Conversion factor for } \mu g \text{ to mg})}$$

$$\text{mg glucose/mL} = \frac{(\Delta A)(TV)(180.2)(F)}{(6.22)(1)(SV)(1{,}000)}$$

$$\text{mg glucose/mL} = \frac{(\Delta A)(TV)(F)(0.029)}{(SV)}$$

$\Delta A = A_{Test} - A_{Total}$

TV = Total assay volume (mL)

SV = Sample volume (mL)

Glucose MW = 180.2 g/mol

F = Dilution factor from sample preparation

ε = Millimolar extinction coeffient for NADH at 340 nm millimolar^{-1}cm^{-1} or equivalently (mL/μmoles)(1/cm)

d = Light path (cm) = 1 cm

1,000 = Conversion factor for μg to mg

Appendix F Bacteria Concentration

Bacteria concentration was determined using total nitrogen test kit.

1. The bacteria concentration from enzymatic hydrolysis

During enzymatic hydrolysis, bacteria growth was monitored by withdrawing samples from the hydrolysis reactor periodically. Solid that obtained from centrifuging of the sample, contained of cellulose and bacteria. Method that can calculate weight of bacteria and cellulose is shown in equation F1.

$$\text{wt. Solid} = \text{wt. Cellulose} + \text{wt. Bacteria} \qquad \text{(F1)}$$

Then, a concentration of bacteria was determined by the total nitrogen test kit.

$$\text{wt. Bacteria} = \frac{\text{g Nitrogen contained in sample}}{\text{(g Nitrogen / 1 g Bacteria)}} \qquad \text{(F2)}$$

1.1 The amount of nitrogen in bacteria

The amount of nitrogen in each strain was determined in triplicates by using the total nitrogen test kit. Figure F1 shows procedure for determination

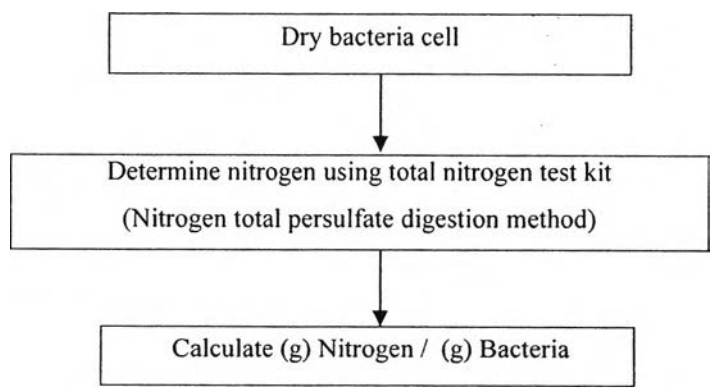

Figure F1 Diagram for determination the amount of nitrogen in bacteria.

Procedure

Nitrogen total persulfate digestion method is conducted in order to check amount of nitrogen which directly related to amount of bacteria during hydrolysis.

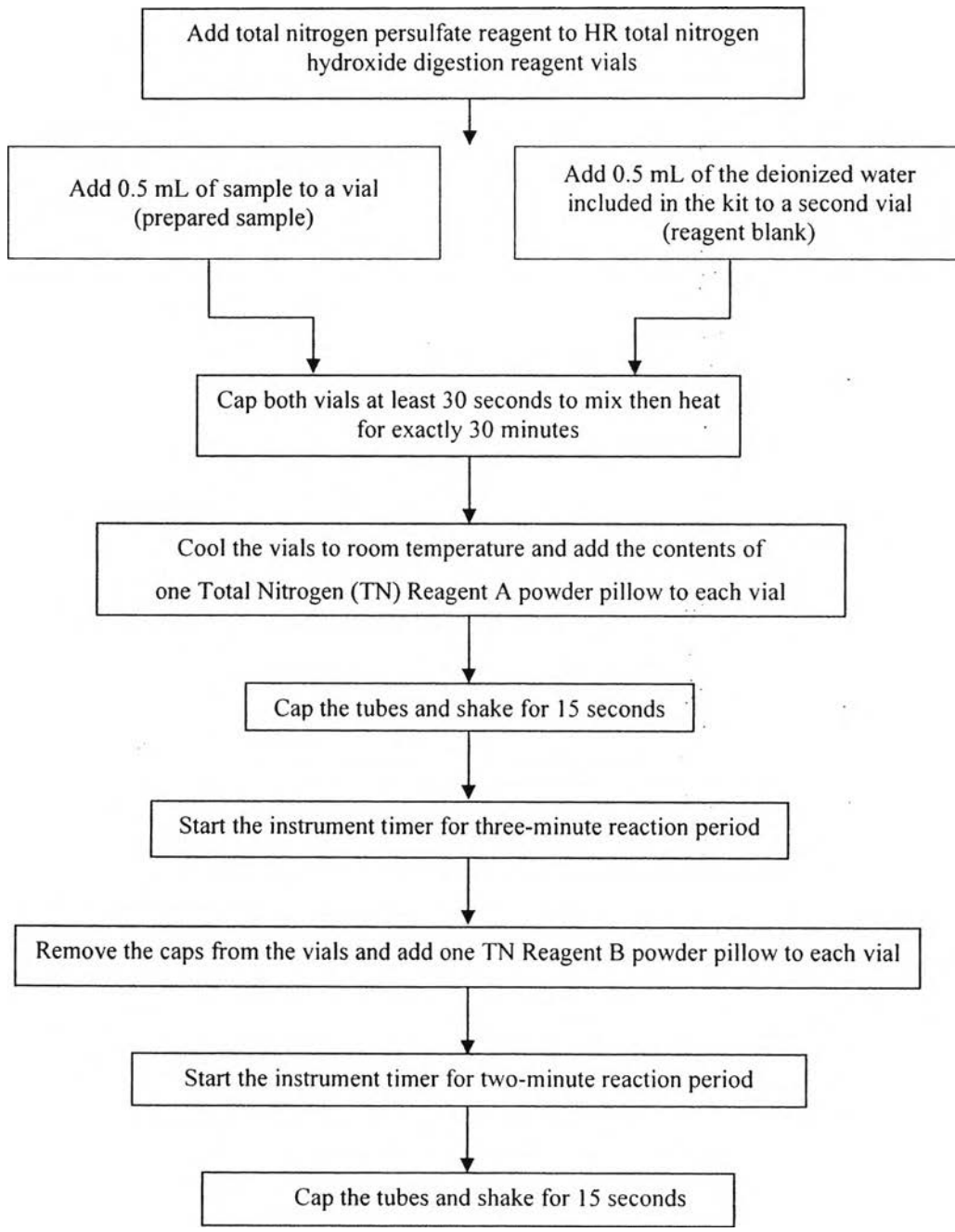

Figure F2 Procedure for analyzing amount of nitrogen.

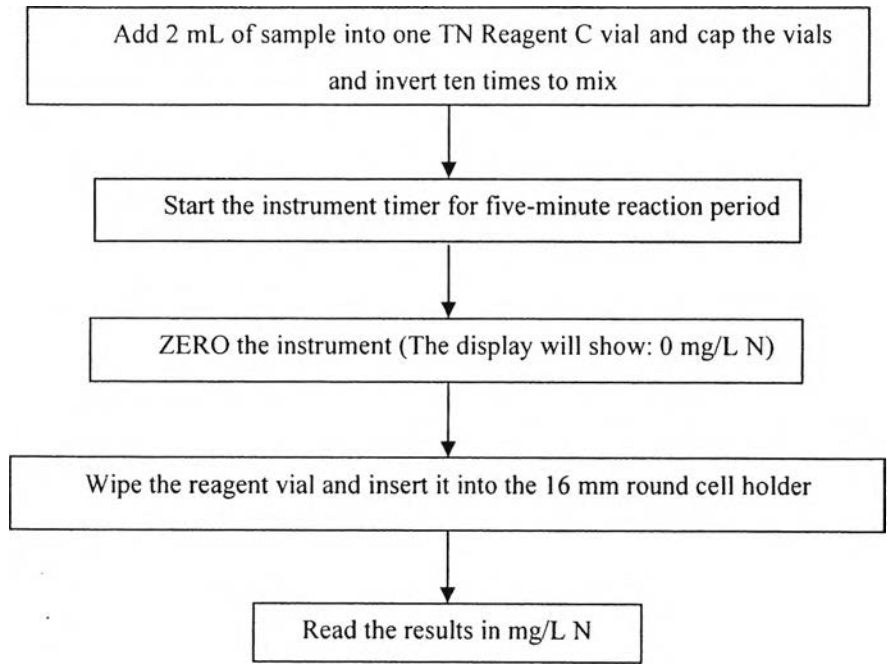

Figure F2 Procedure for analyzing amount of nitrogen (countinued).

Appendix G Experiment data of enzymatic hydrolysis

Table G1 Glucose, bacteria, and cellulose concentration of enzymatic hydrolysis of untreated cellulose using strain A 002

Time (h)	0	1	2	3	4	5	6	9	12	24
Glucose (g/L)	0.10	0.19	0.20	0.21	0.21	0.22	0.23	0.25	0.10	0.01
Bacteria (g/L)	0.38	0.70	0.80	0.90	1.00	1.20	1.20	1.50	1.70	2.10
Cellulose (g/L)	1.00	0.12	0.15	0.13	0.19	0.13	0.18	0.23	0.15	0.13
Yield	0.00	0.22	0.24	0.24	0.26	0.26	0.28	0.33	0.12	0.01

Table G2 Glucose, bacteria, and cellulose concentration of enzymatic hydrolysis of pretreated cellulose using strain A 002

Time (h)	0	1	2	3	4	5	6	9	12	24
Glucose (g/L)	0.10	0.20	0.22	0.23	0.25	0.24	0.24	0.29	0.24	0.03
Bacteria (g/L)	0.36	0.90	1.00	1.20	1.30	1.30	1.40	1.60	1.50	2.20
Cellulose (g/L)	1.00	0.83	0.78	0.80	0.76	0.72	0.73	0.67	0.66	0.67
Yield	0.00	1.18	1.00	1.16	1.06	0.86	0.91	0.88	0.72	0.08

Table G3 Glucose, bacteria, and cellulose concentration of enzymatic hydrolysis of untreated cellulose using strain F 018

Time (h)	0	1	2	4	5	6	9	12	24
Glucose (g/L)	0.10	0.46	0.53	0.50	0.57	0.54	0.42	0.14	0.07
Bacteria (g/L)	0.46	2.00	1.90	1.00	1.50	1.40	5.60	5.70	4.70
Cellulose (g/L)	1.00	0.10	0.11	0.12	0.11	0.13	0.12	0.10	0.12
Yield	0.00	0.52	0.60	0.57	0.65	0.63	0.48	0.16	0.08

Table G4 Glucose, bacteria, and cellulose concentration of enzymatic hydrolysis of pretreated cellulose using strain F 018

Time (h)	0	1	2	4	5	6	9	12	24
Glucose (g/L)	0.10	0.49	0.55	0.59	0.56	0.56	0.58	0.32	0.02
Bacteria (g/L)	0.46	1.40	1.80	0.90	0.70	0.60	1.00	1.40	2.90
Cellulose (g/L)	1.00	0.50	0.42	0.38	0.40	0.39	0.38	0.39	0.39
Yield	0.00	0.98	0.95	0.96	0.93	0.92	0.93	0.52	0.04

Table G5 Glucose, bacteria, and cellulose concentration of enzymatic hydrolysis of untreated cellulose using strain M 015

Time (h)	0	1	2	3	4	5	6	9	12	24
Glucose (g/L)	0.10	0.12	0.13	0.17	0.15	0.16	0.18	0.19	0.08	0.00
Bacteria (g/L)	0.47	1.25	1.80	1.70	1.90	2.10	2.00	2.90	3.30	3.8
Cellulose (g/L)	1.00	0.12	0.18	0.23	0.11	0.14	0.17	0.18	0.17	0.15
Yield	0.00	0.14	0.16	0.22	0.17	0.19	0.22	0.23	0.10	0.00

Table G6 Glucose, bacteria, and cellulose concentration of enzymatic hydrolysis of pretreated cellulose using strain M 015

Time (h)	0	1	2	3	4	5	6	9	12	24
Glucose (g/L)	0.10	0.14	0.17	0.17	0.17	0.18	0.22	0.24	0.18	0.10
Bacteria (g/L)	0.47	1.00	2.00	2.00	2.30	2.50	2.30	2.80	3.10	3.50
Cellulose (g/L)	1.00	0.88	0.83	0.82	0.80	0.78	0.76	0.74	0.75	0.74
Yield	0.00	1.19	0.97	0.92	0.87	0.84	0.93	0.91	0.73	0.37

Table G7 Glucose, bacteria concentration of enzymatic hydrolysis of untreated cellulose using strain M 015 (0.22g of dry weight bacteria /L at the initial concentration)

Time (h)	0	1	2	3	4	5	6	9	12	24
Glucose (g/L)	0.10	0.11	0.11	0.14	0.16	0.17	0.16	0.17	0.12	0.02
Bacteria (g/L)	0.20	0.30	0.40	0.70	0.90	0.60	1.10	1.90	2.00	3.30

Table G8 Glucose, bacteria concentration of enzymatic hydrolysis of pretreated cellulose using strain M 015 (0.22g of dry weight bacteria /L at the initial concentration)

Time (h)	0	1	2	3	4	5	6	9	12	24
Glucose (g/L)	0.10	0.12	0.14	0.17	0.18	0.17	0.18	0.22	0.21	0.04
Bacteria (g/L)	0.23	0.50	0.90	1.00	0.90	0.80	0.90	1.10	1.60	2.00

Table G9 Glucose, bacteria concentration of enzymatic hydrolysis of untreated cellulose using strain A 002 + M 015

Time (h)	0	1	2	3	4	5	6	9	12	24
Glucose (g/L)	0.10	0.12	0.17	0.19	0.16	0.15	0.17	0.18	0.11	0.00
Bacteria (g/L)	0.45	1.40	0.80	0.50	0.70	2.00	1.00	1.50	0.80	3.10

Table G10 Glucose, bacteria concentration of enzymatic hydrolysis of pretreated cellulose using strain A 002 + M 015

Time (h)	0	1	2	3	4	5	6	9	12	24
Glucose (g/L)	0.10	0.15	0.18	0.20	0.21	0.20	0.19	0.13	0.11	0.02
Bacteria (g/L)	0.47	2.20	2.50	2.00	2.30	1.90	1.50	2.50	2.10	4.20

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Proceeding:

1. Worasamutprakarn, C., Rangsunvigit, P., Chavadej, S., Sreethawong, T., and Rengpipat., S. (2010, April 22) *Conversion of Cellulose to Glucose by Microbes Isolated from Higher Termits* Proceeding of The 1st National Reseach Symposium on Petroleum, Petrochemicals, and Advanced materials and The 16th PPC Symposium on Petroleum, Petrochemicals, and Polymers, Bangkok, Thailand.