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APPENDICES

APPENDIX 1

Determination of chemical oxygen demand (COD)

COD was measured by Colorimetric determination using Reactor Digestion method (Hach COD reagent test kit, Hach Company, USA) as recommend by manufacturer.

Reagents

a. COD Digestion reagent vials (Hach COD reagent test kit; range 3 to 150 mg/L, 20 to 1500 mg/L) containing;

1. Potassium dichromate in sulfuric acid solution is a strong oxidizing agent.
2. Silver is a catalyst,
3. Mercury is used to complex Chloride interference

b. COD standard solution (Hach COD standard reagent)

Procedure

1. turn on the COD reactor and preheat the reactor to 150^{o}C.
2. add 2 ml of deionized water (blank) and samples to the COD digestion reagent vials.
3. tightly cap the vials and rinse them with deionized water. Then, wipe with a clean paper and invert gently each vial several times to mix completely. The sample vials will become very hot during mixing.
4. place the vial in the preheated COD reactor. Heat the sample at 150^{o}C for 2 hours.
5. turn off the COD reactor and wait about 20 minutes for the vials to cool to 120^{o}C or less. Invert gently each vial several times while still warm.
6. cool the vials to room temperature and place the vials in the test tube rack.
7. proceed to the colorimetric determination using Hach DR/2500 spectrophotometer (Hach Company, USA). Select the appropriate program for measuring the COD values. The programs are specified in Table11.1

Table A Method Performance

Program	Range in mg/L COD	COD Standard solution
430	3 to 150 mg/L	100 mg/L
435	20 to 1,500 mg/L	300 or 1,000 mg/L

Note: The colorimetric determination can measure COD value at the specific wavelengths which were specified in Table 11.2.

Table B The range-specific wavelengths

Range in mg/L COD	Wavelength
3 to 150 mg/L	420 nm
20 to 1,500 mg/L	620 nm

Determination of total nitrogen

Total Nitrogen was measured by Colorimetric determination using Persulfate digestion method (Test 'N Tube™ HR Total Nitrogen Reagent Set, Hach Company, USA) as recommend by manufacturer.

Reagents

a. Test 'N Tube™ HR Total Nitrogen Reagent Set containing: HR Total Nitrogen Hydroxide Digestion Reagent vials; range 10 to 150 mg/L, deionized water (free of all nitrogen-containing), Total Nitrogen Persulfate Reagent powder, Total Nitrogen (TN) Reagent A powder, TN Reagent B powder and TN Reagent C vials.

Procedure

1. turn on the COD reactor. Preheat to 103-106^{o}C.
2. add the contents of Total Nitrogen Persulfate Reagent powder to each of HR Total Nitrogen Hydroxide Digestion Reagent vials.
3. add 0.5 ml of deionized water included in the kit (blank) and sample to the vials. (Use only water that is free of all nitrogen-containing species as a substitute for the deionized water provided)
3. tightly cap the vials and shake vigorously for at least 30 seconds to mix.
4. place the vial in the preheated reactor and heat for 30 minutes.
5. remove the vials from the reactor and place in the test tube rack. Cool the vials to room temperature.
6. add the content of Total Nitrogen (TN) Reagent A powder to each vial.
7. cap the tubes and shake for 15 seconds. Then, stand the tubes for 3 minutes.
8. add TN Reagent B powder to each vial.
9. cap the tubes and shake for 15 seconds. Then, stand the tubes for 2 minutes.
10. add 2 ml of digested, treated samples (from item 9) to each TN Reagent C vial.
11. cap the vials and invert 10 times to mix. Then, stand the tubes for 5 minutes. The yellow color will intensify.
12. proceed to the colorimetric determination using Hach DR/2500 spectrophotometer (Hach Company, USA). Select the program 395N, Total HR TNT for measuring the total nitrogen values.

Note: The colorimetric determination can measure total nitrogen value at the specific wavelengths at 410 nm.

APPENDIX 2

The 16S rDNA sequences of bacteria

TGCTCTCGGG TGANGAGTGG CGGACGGGTG AGTAATGTCT GGGAAACTGC
CTGATGGAGG GGGATAACTA CTGGAAACGG TAGCTAATAC CGCATAACGT
CGCAAGACCA AAGAGGGGGA CCTTCGGGCC TCTTGCCATC AGATGTGCCC
AGATGGGATT AGCTAGTAGG TGGGGTAACG GCTCACCTAG GCGACGATCC
CTAGCTGGTC TGAGAGGATG ACCAGCCACA CTGGAACTGA GACACGGTCC
AGACTCCTAC GGGAGGCAGC AGTGGGGAAT ATTGCACAAT GGGCGCAAGC
CTGATGCAGC CATGCCGCGT GTATGAAGAA GGCCTTCGGG TTGTAAAGTA
CTTTCAGCGG GGAGGAAGGC GATNAGGTTA ATAACCTTGT CGATTGACGT
TACCCGCAGA AGAAGCACCG GCTAACTCCG TGCCAGCAGC CGCGGTAATA
CGGAGGGTGC AAGCGTTAAT CGGAATTACT GGGCGTAAGC GCACGCAGGC
GGTCTGTCAA GTCGGATGTG AAATCCCGGG CTCAACCTGG GAACTGCATN
CNAAACTGGC AGGCTGGANT CTTGTAAANG GGGNTAAANT CCNGGTGTAN
CGNTAAATGC NTAAAATCTG GAAGAATACC GGTGGCNAAG GGGCCCCCTG
GANAAAACTG ACCCTNAGTG CAAANCNTGG GGAACAANAG ATTAANANCC
CTGGNAAA

Figure A The partial 16S rDNA sequences of *Klebsiella oxytoca* (T1)

GGGACTTGCT CCCTGGGTGA NGAGCGGCGG ACGGGTGAGT AATGTCTGGG
AAACTGCCTG ATGGAGGGGG ATAACTACTG GAAACGGTAG CTAATACCGC
ATAACGTCGC AAGACCAAAG AGGGGGACCT TCGGGCCTCT TGCCATCAGA
TGTGCCCAGA TGGGATTAGC TAGTAGGTGG GGTAATGGCT CACCTAGGCG
ACGATCCCTA GCTGGTCTGA GAGGATGACC AGCCACACTG GAACTGAGAC
ACGGTCCAGA CTCCTACGGG AGGCAGCAGT GGGGAATATT GCACAATGGG
CGCAAGCCTG ATGCAGCCAT GCCGCGTGTG TGAAGAAGGC CTTCGGGTTG
TAAAGCACTT TCAGCGAGGA GGAAGGTGGT GAGCTTAATA CGNTCATCAA
TTGACGTTAC TCGCAGAAGA AGCACCGGCT AACTCCGTGC CAGCAGCCGC
GGTAATACGG AGGGTGCAAG CGTTAATCGG AATTACTGGG CGTAAAGCGC
ACGCCAGGCG GTTTGTTAAG TCANATGTGA AATCCCCGGN CTCAACCTGG
GAACTGCATT TTGAAACTGG CAAGCTANAG TCTCGTAAAG GGGGGTANAA
TTCCNGGTGT AACCGTGAAA TNCGTAAANA TCTGGANGAA TACCNGNTGG
NCAAAGGCGG CCCCCNTGGA CNAAAANNTN NCCTCAGGTN NNAAACCNNG
GGACCNNACN GNATTNATTC CCCTGGNNTN CCCNCCTTNA ANNNATGNCN
ANTTNNANGN TTGCCCCNTT NAGNCTNNT

Figure B The partial 16S rDNA sequences of *Serratia mercescens* (T2)

AGCAGCTTGC TGCTTCGCTG AACGAGTGGC GGACGGGTGA GTAATGTCTG
GGAAACTGCC CGATGGAGGG GGATAACTAC TGGAAACGGT AGCTAATACC
GCATAATGTC GCAAGACCAA AGAGGGGGAC CTTCGGGCCT CTTGCCATCG
GATGTGCCCA GATGGGATTA GCTTGTTGGT GAGGTAACGG CTCACCAAGG
CGACGATCCC TAGCTGGTCT GAGAGGATGA CCAGCCACAC TGGAACTGAG
ACACGGTCCA GACTCCTACG GGAGGCAGCA GTGGGGAATA TTGCACAATG
GGCGCAAGCC TGATGCAGCC ATGCCGCGTG TATGAAGAAG GCCTTCGGGT
TGTAAAGTAC TTTCAGCGGG GAGGAAGGTG TTGTGGTTAA TAACCGCAAC
AATTGACGTT ACCCGCANAA NAAGCACCGG CTAACTCCGT GCCAGCAGCC
GCGGTAATAC GGAGGGTGCA AGCGTTAATC GGAATTACTG GGCGTAAAGC
GCACGCAGNC GGTCTGTCAA GTCGNATGTG AAATCCCCGG GCTCAACCTG
GGAACTGCAT NCAAACTNGG CANGCTTGAN TCTCNTAAAA GGGGGNTAAA
ATTCCNGNTT TACNGNTNAA TTGCCTAAAA ATNTTGGAGN AAAACCNGTG
GNAAAGGCGC CCCCCTTGAA AAAAANTGAC CCTCANGTGC NAAACNTGGG
GAANAAAAAG ATNNANAANC CCCGGNTAAA A

Figure C The partial 16S rDNA sequences of *Citrobacter farmeri* (T3)

TTGCTCCTTG GGTGANGAGT GGCGGACGGG TGAGTAATGT CTGGGAAACT
GCCCGATGGA GGGGGATAAC TACTGGAAAC GGTAGCTAAT ACCGCATAAC
GTCGCAAGAC CAAAGAGGGG GACCTTCGGG CCTCTTGCCA TCGGATGTGC
CCAGATGGGA TTAGCTAGTA GGTGGGGTAA CGGCTCACCT AGGCGACGAT
CCCTAGCTGG TCTGAGAGGA TGACCAGCCA CACTGGAACT GAGACACGGT
CCAGACTCCT ACGGGAGGCA GCAGTGGGGA ATATTGCACA ATGGGCGCAA
GCCTGATGCA GCCATGCCGC GTGTATGAAG AAGGCCTTCG GGTTGTAAAG
TACTTTCAGC GAGGAGGAAG GNGTTGTGGT TAATAACCGC ANCGATTGAC
GTTACTCGCA GAAGAAGCAC CGGCTAACTC CGTGCCAGCA GCCGCGGTAA
TACGGAGGGT GCAAGCGTTA ATCGGAATTA CTGGGCGTAA AGCGCACGCA
GGCGGTCTGT CAAGTCNGAT GTGAAATCCC CGGGCTCAAC CTGGGAACTG
CATCCGAAAC GGCAGGCTAG AGTCTTGTAN AGGGGGGTAA ANTCCAGGTG
TAGCGGTGAA ATGCGTAAAN ATCTGGAAGA ATACCGGTGG CNAANGCGNC
CCCCTGGANA AAACTGACNC TCANGTGCNA AAGCGTGGGG AACAACAGNN
TNAAANNNCC CCNGGNAAAA

Figure D The partial 16S rDNA sequences of Unknown bacterium (T4)

APPENDIX 3

Experimental Techniques for $k_L a$ determination

The $K_L a$ is measure of quantity of oxygen that can actually be transferred into a volume element, and it characterizes the efficiency of oxygen transfer.

Theory:

$$\frac{dC_{AL}}{dt} = k_L a\left(C^*_{AL} - C_{AL}\right)$$

Integrating....

$$\int_{C_{AL_1}}^{C_{AL2}} \frac{1}{\left(C^*_{AL} - C_{AL}\right)} dC_{AL} = k_L a \int_0^t dt$$

$$\ln\left(\frac{C^*_{AL} - C_{AL1}}{C^*_{AL} - C_{AL2}}\right) = k_L at$$

Therefore a plot of $\ln\left(\frac{C^*_{AL} - C_{AL1}}{C^*_{AL} - C_{AL2}}\right)$ versus **t** should result in a straight line of slope $K_L a$.

C_{AL} = Dissolved oxygen concentration in the broth (mMol O_2 L^{-1})
C^* = Saturated oxygen concentration in the broth (mMol O_2 L^{-1})
$K_L a$ = Oxygen transfer coefficient (h^{-1})
t = time (h)

KLa is measured by Static Gassing out method (Wise, 1951)

Procedure

1. In the absence of respiring organism (no O_2 consumption). Sparge vessel contents with N_2 to displace O_2. Monitor variation in dissolved oxygen concentration (DO) using a DO probe. Allow DO to fall to 0% saturation, then turn off N_2 flow

2. Sparge vessel contents with air at a known flow rate. Monitor and record variation of DO concentration with respect to time.

3. Plot of $\ln\left(\frac{C^*_{AL} - C_{AL1}}{C^*_{AL} - C_{AL2}}\right)$ versus **t** should result in a straight line of slope k_La

Oxygen trasfer in melanoidins-containing wastewater medium

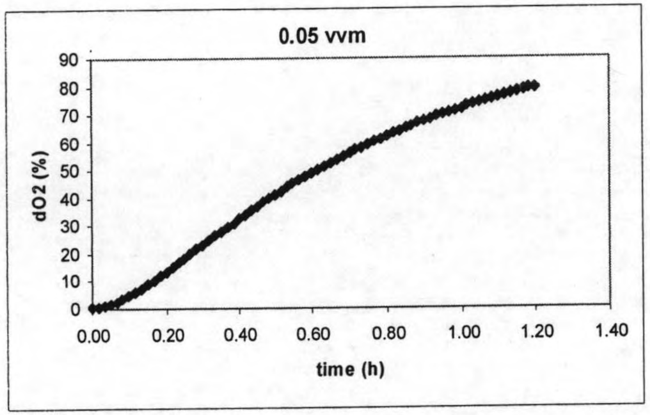

Figure E DO concentration with respect to time at 0.05 vvm

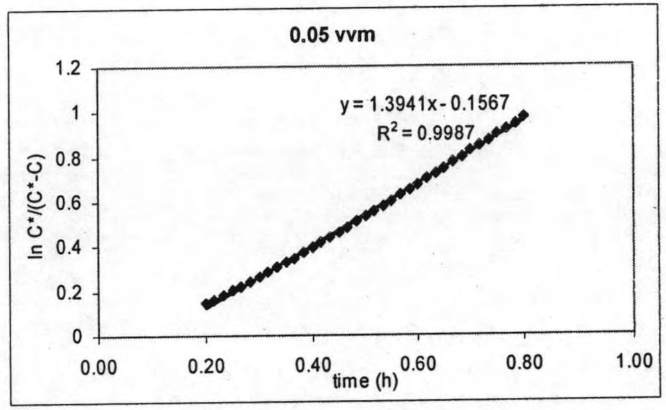

Figure F ln C*/(C*-C) value with respect to time at 0.05 vvm

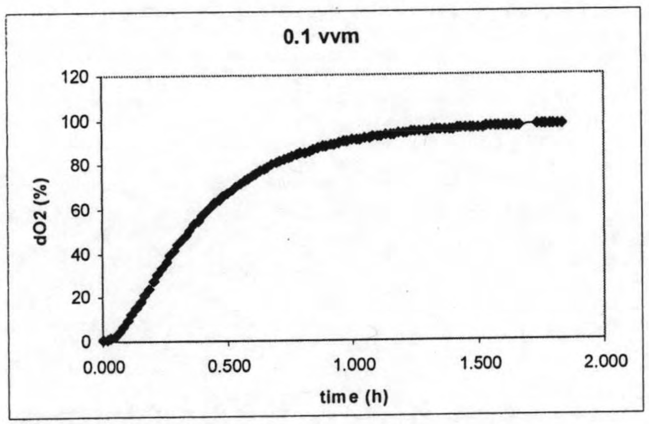

Figure G DO concentration with respect to time at 0.1 vvm

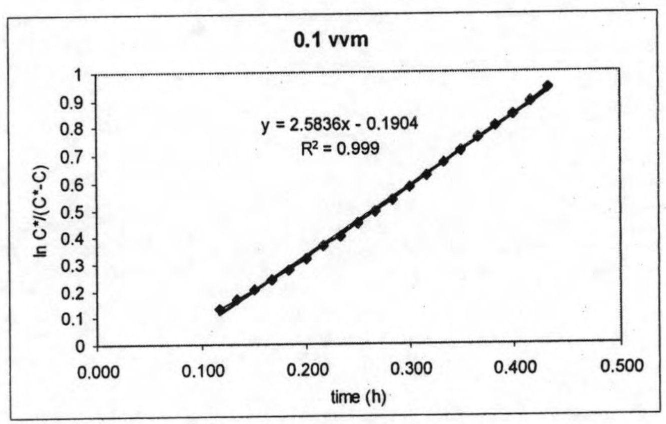

Figure H ln C*/(C*-C) value with respect to time at 0.1 vvm

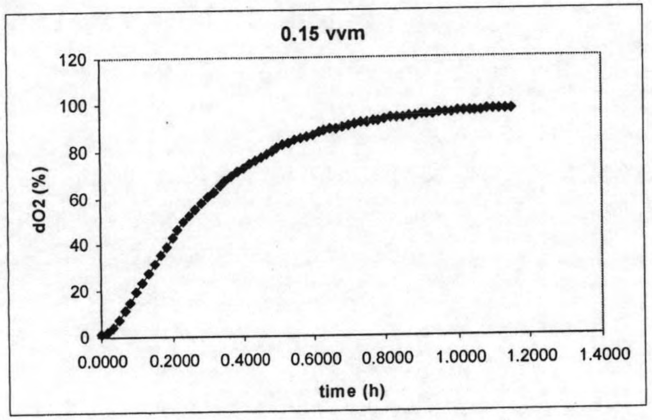

Figure I DO concentration with respect to time at 0.15 vvm

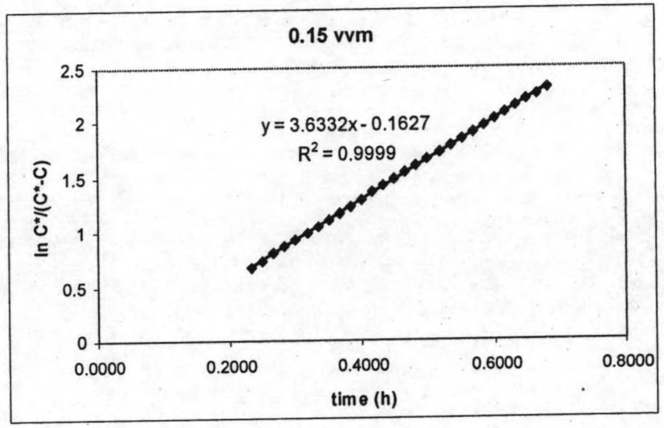

Figure J ln C*/(C*-C) value with respect to time at 0.15 vvm

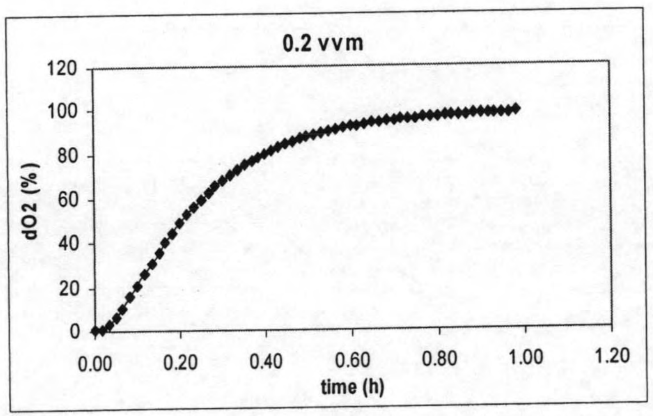

Figure K DO concentration with respect to time at 0.2 vvm

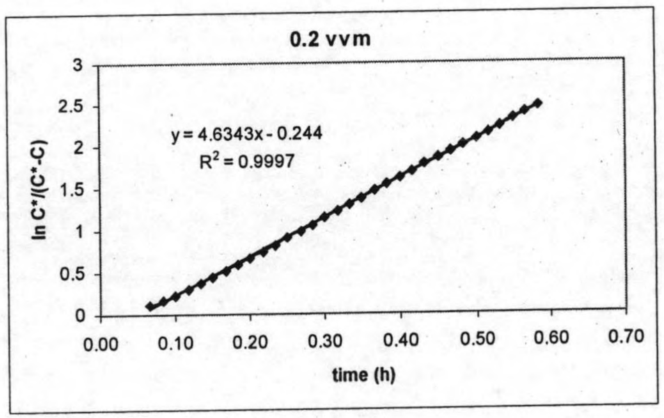

Figure L ln C*/(C*-C) value with respect to time at 0.2 vvm

Appendix 4

Method for testing membrane permeability

The test of membrane permeability was conducted under controlled laboratory conditions with the temperature of 25°C. The membrane module was a stainless-steel monochannel microfiltration module, a mineral M14 Carbosep® membrane. This membrane module was equipped separately with a bioreactor tank having a working volume of 1.6L. The reactor was filled with double distilled water (DDW). A peristaltic pump (Master Flex I/P) was used to supply the operating pressure and DDW circulation through the membrane module. A water level was used to control the influent pump and to keep the water level in the bioreactor constant.

The membrane permeability was determined by measuring the variation of permeate flux at different transmembrane pressure (TMP), which adjusted by stepwise increasing pump rotational speed from 50 to 750 rpm. At different pump speed, permeate flux through the membrane were recorded. During the experiment, the withdrawn water was returned to the tank to keep the water level in the tank constant. A plot of permeate flux (J) versus transmembrane pressure (represent by pump speed) should result in a straight line of slope permeability. Therefore, if the slope of the permeability curve is changed, then permeability of a given membrane is changed. Figure M shows the example of water permeability of the mineral membrane before and after operation.

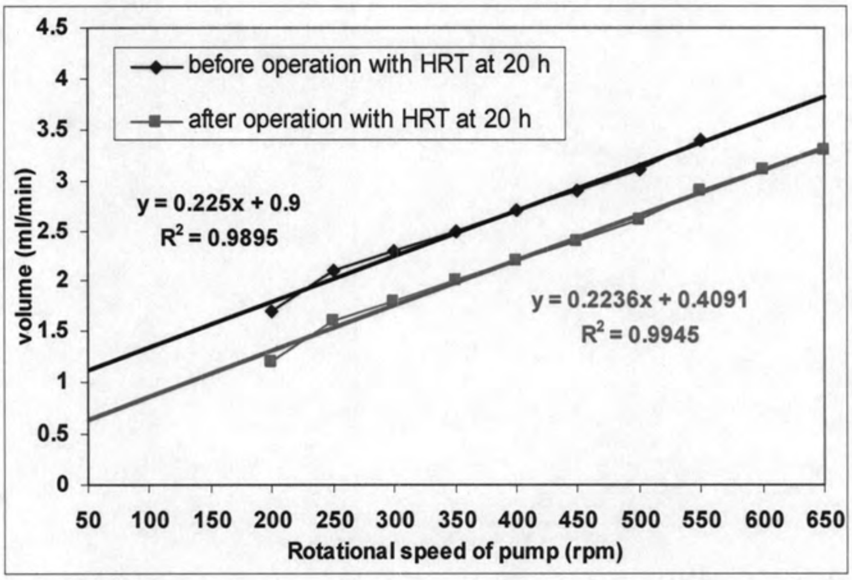

Figure M Membrane permeability of the mineral membrane before and after operation

Theory:

The relationship between the permeate flux and transmembrane pressure is given in the following equation:

$$J = \frac{\Delta P}{\mu R_t} \qquad \text{Eq. (1)}$$

where	J	=	Equilibrium permeate flux (L/m^2.h)
	ΔP	=	Transmembrane pressure, TMP (kPa)
	μ	=	Viscosity of the permeate (kN.s /m^2)
	Rt	=	Total membrane resistance

$$R_t = R_m + R_f = R_m + R_c + R_p \qquad \text{Eq. (2)}$$

R_m = Intrinsic membrane resistance,

R_c = Cake resistance,

R_f = Fouling resistance and

R_p = Resistance due to pore plugging and irreversible fouling.

Permeate flux and TMP data were used to calculate resistances by Eq. (1); filtration of pure water with a new membrane before operation gives the R_m, and R_t was calculated from the final flux and TMP values at the end of the operation.

$R_m + R_p$ is measured after removing the cake layer by washing the membrane with water after the operation followed by filtration with pure water. From these values each of R_t, R_m, R_c, R_p and R_f can be obtained using Eq. (2).

BIOGRAPHY

Miss Suhuttaya Jiranuntipon was born on July 5, 1976 at Bangkok, Thailand. She has graduated and holds a B.Sc. in Microbiology from Department of Microbiology, Faculty of Science, Chulalongkorn University in 1996. She has graduated and holds a M.Sc. in Industrial Microbiology from Department of Microbiology, Faculty of Science, Chulalongkorn University in 2001. She has applied to study Ph.D. in Biotechnology at the Program in Biotechnology, Faculty of Science, Chulalongkorn University in 2004. The Royal Golden Jubilee Program of the Thailand Research Fund and Embassy of France in Thailand financially supported her Ph.D. program.