# APPENDIX

# APPENDIX A

## Reagent

**1. 0.7 M Sodium Chloride (NaCl)**

| | | |
|---|---|---|
| NaCl | 4.10 | g |
| Distilled water ($dH_2O$) | 100 | ml |

Dissolve NaCl 4.10 g in $dH_2O$ and adjust the volume to 100 ml.

**2. 10% Cetyl trimethylammonium bromide (CTAB)**

| | | |
|---|---|---|
| CTAB | 10 | g |
| 0.7 M NaCl | 100 | ml |

Dissolve CTAB 10 g in 0.7 M NaCl and adjust the volume to 100 ml.

**3. 240 mM Potassium phosphate buffer (pH 8.0)**

| | | |
|---|---|---|
| Monopotassium phosphate ($KH_2PO_4$) | 3.27 | g |
| Dipotassium phosphate ($K_2HPO_4$) | 4.18 | g |
| $dH_2O$ | 100 | ml |

Dissolve $KH_2PO_4$ 3.27 g and $K_2HPO_4$ 4.18 g in $dH_2O$. Adjust the volume to 100 ml.

**4. CTAB extraction buffer**

| | | |
|---|---|---|
| 10% CTAB | 50 | ml |
| 240 mM Potassium phosphate buffer | 50 | ml |

Combine the solutions and mix thoroughly. Autoclave at 121 °C for 15 min.

**5. Chloroform : Isoamyl alcohol (24 : 1)**

| | | |
|---|---|---|
| Chloroform | 96 | ml |
| Isoamyl alcohol | 4 | ml |

Combine the solutions and mix thoroughly. Store in brown glass bottle at 4 °C.

**6. Phenol : Chloroform : Isoamyl alcohol (25 : 24 : 1)**

| | | |
|---|---|---|
| Phenol | 50 | ml |
| Chloroform | 48 | ml |
| Isoamyl alcohol | 2 | ml |

Combine chloroform and isoamyl alcohol in a glass measuring cylinder to make 24 : 1, then combine with phenol and mix gently. Store in brown glass bottle at 4 °C.

**7. 3M Sodium acetate (NaOAc) (pH 5.2)**

| | | |
|---|---|---|
| NAOAc. $3H_2O$ | 40.83 | g |
| $dH_2O$ | 100 | ml |

Dissolve NAOAc. $3H_2O$ 40.83 g in $dH_2O$. Adjust pH using acetic acid to 7.4. and bring volume to 100 ml. Aautoclave at 121 °C for 15 min.

**8. 70% Ethanol**

| | | |
|---|---|---|
| 100% Ethanol | 70 | ml |
| $dH_2O$ | 30 | ml |

Combine the solutions and mix thoroughly. Store at -20 °C.

**9. 1 M Tris-HCl (pH 7.4)**

| | | |
|---|---|---|
| Tris-base | 12.11 | g |
| $dH_2O$ | 100 | ml |

Dissolve Tris-base 12.11 g in $dH_2O$. Adjust pH using HCl to 7.4 and bring volume to 100 ml.

**10. 100 mM Tris-HCl (pH 7.4)**

| | | |
|---|---|---|
| 1 M Tris-HCl | 10 | ml |
| $dH_2O$ | 90 | ml |

Combine the solutions and mix thoroughly. Autoclave at 121 °C for 15 min.

**11. 0.5 M Ethylenediaminetetraacetic acid (EDTA) (pH 8.0)**

| | | |
|---|---|---|
| EDTA.2Na | 18.16 | g |
| $dH_2O$ | 100 | ml |

Dissolve EDTA.2Na 18.16 g in $dH_2O$. Adjust pH to 8.0 and bring volume to 100 ml.

**12. 10 mM EDTA (pH 8.0)**

| | | |
|---|---|---|
| 0.5 M EDTA | 2 | ml |
| $dH_2O$ | 98 | ml |

Combine the solutions and mix thoroughly. Autoclave at 121 °C for 15 min.

**13. 10X Tris-EDTA buffer (TE buffer)**

| | | |
|---|---|---|
| 100 mM Tris-HCl | 10 | ml |
| 10 mM EDTA | 2 | ml |
| $dH_2O$ | 88 | ml |

Combine the solutions and mix thoroughly.

**14. 1X TE buffer**

| | | |
|---|---|---|
| 10X TE buffer | 10 | ml |
| $dH_2O$ | 90 | ml |

Combine the solutions and mix thoroughly. Autoclave at 121 °C for 15 min.

**15. 100 mg/ml RNase**

| | | |
|---|---|---|
| RNase | 0.1 | g |
| $dH_2O$ | 1 | ml |

Dissolve RNase 0.1 g in $dH_2O$ and store in -20 °C.

**16. 1X TE buffer with RNase 0.002%**

| | | |
|---|---|---|
| 100 mg/ml RNase | 2 | µl |
| 1X TE buffer | 998 | µl |

Combine the solutions and mix thoroughly. Store at -20 °C.

**17. 50X TAE buffer**

| | | |
|---|---|---|
| Tris base | 242 | g |
| Glacial acetic acid | 57.1 | ml |
| EDTA.2Na | 37.2 | g |
| $dH_2O$ | 1,000 | ml |

Dissolve EDTA.2Na 37.2 g and Tris-base 242 g in $dH_2O$. Add glacial acetic acid 57.1 ml and adjust volume to 1,000 ml. Autoclave at 121 °C for 15 min.

**18. 1X TAE buffer**

| | | |
|---|---|---|
| 50X TAE | 20 | ml |
| $dH_2O$ | 980 | ml |

Combine the solutions and mix thoroughly.

**19. 10 µg/ml Ethidium bromide**

| | | |
|---|---|---|
| 10 mg/ml Ethidium bromide | 10 | ml |
| 1X TAE buffer | 1,000 | ml |

Combine the solutions and mix thoroughly. Store in dark at room temperature.

**20. 100 mg/ml Ampicillin** (Sigma-Aldrich, USA)

| | | |
|---|---|---|
| Ampicillin | 0.1 | g |
| Deionized water | 1 | ml |

Dissolve ampicillin 0.1 g in deionized water to 1 ml final volume. Filter sterilize and store at -20 °C.

**21. 0.1 M Isopropylthiogalactoside (IPTG)** (Vivantis, Malaysia)

| | | |
|---|---|---|
| IPTG | 1 | g |
| Deionized water | 42 | ml |

Dissolve IPTG 1 g in deionized water to 42 ml final volume. Filter sterilize and store at -20 °C.

**22. 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal)** (Vivantis, Malaysia)

| | | |
|---|---|---|
| X-Gal | 100 | mg |
| N,N'-dimethyl formamide | 2 | ml |

Disslove X-Gal 100 mg in N,N'-dimethyl formamide to 2 ml final volume. Cover with aluminum foil and store at -20 °C.

**23. 10% Ammonium persulfate (APS)** (BioRad, USA)

| | | |
|---|---|---|
| APS | 0.1 | g |
| Dionized water | 1 | ml |

Dissolved APS 0.1 g in dionized water to 1 ml final volume and stored at 4 °C up to 1 week.

**24. Gel check leak solution**

| | | |
|---|---|---|
| 0% denaturing solution | 2 | ml |
| 10% APS | 20 | µl |
| Tetramethylethylenediamine (TEMED) | 2 | µl |

Combine the solutions and mix thoroughly.

**25. Stacking gel**

| | | |
|---|---|---|
| 0% denaturing solution | 3 | ml |
| 10% APS | 30 | µl |
| TEMED | 3 | µl |

Combine the solutions and mix thoroughly.

**26. 100 mM Calcium Chloride ($CaCl_2$)**

| | | |
|---|---|---|
| $CaCl_2$ | 7.35 | g |
| $dH_2O$ | 500 | ml |

Dissolve $CaCl_2$ 7.35 g in $dH_2O$ to 500 ml final volume and mix thoroughly.

**27. 50 mM $CaCl_2$**

| | | |
|---|---|---|
| 100 mM $CaCl_2$ | 50 | ml |
| $dH_2O$ | 50 | ml |

Combine the solutions and mix thoroughly. Autoclave at 121 °C for 15 min.

**28. 20% Glycerol**

| | | |
|---|---|---|
| 100% Glycerol | 20 | ml |
| 100 mM $CaCl_2$ | 80 | ml |

Combine the solutions and mix thoroughly. Autoclave at 121 °C for 15 min.

# APPENDIX B

## Culture media

**1. Luria Bertani (LB) Agar**

| | | |
|---|---|---|
| Tryptone | 10 | g |
| Yeast extract | 5 | g |
| Sodium chloride | 5 | g |
| Agar | 15 | g |
| Distilled water ($dH_2O$) | 1,000 | ml |

Add components to $dH_2O$ and bring volume to 1,000 ml. Mix thoroughly. Gently heat and bring to boiling. Autoclave at 121°C for 15 min.

**2. LB Broth**

| | | |
|---|---|---|
| Tryptone | 10 | g |
| Yeast extract | 5 | g |
| Sodium chloride | 5 | g |
| $dH_2O$ | 1,000 | ml |

Add components to $dH_2O$ and bring volume to 1,000 ml. Mix thoroughly. Autoclave at 121°C for 15 min.

**3. LB plate with ampicillin**

Prepare LB agar and autoclave at 121°C for 15 min**.** Allow the medium to cool to 50 °C before adding ampicillin to a final concentration of 100 µg/ml. Pour 30-35 ml of medium into petri dishes. Let the agar harden. Store at 4°C for up 1 month or at room temperature for up to 1 week.

**4. LB plate with ampicillin/IPTG/X-Gal**

Make the LB plates with ampicillin as above, then spread 100 µl of 100 mM IPTG and 20 µl of 50 µg/ml X-Gal over the surface of a LB-ampicillin plate and allowed to absorb for 30 min at 37 °C prior to use.

# APPENDIX C

## Denaturing gradient gel electrophoresis (DGGE)

**Table C.1** The percentage acrylamide/bis needed for a particular size range.

| Gel percentage (%) | Base pair separation (base pair) |
|---|---|
| 6 | 300-1,000 |
| 8 | 200-400 |
| 10 | 100-300 |

**Table C.2** 0% denaturing solution

| Reagents | 6% gel | 8% gel | 10% gel | 12% gel |
|---|---|---|---|---|
| 40% acrylamide/bis (37:5:1) | 15 ml | 20 ml | 25 ml | 30 ml |
| 50X TAE buffer | 2 ml | 2 ml | 2 ml | 2 ml |
| Distilled water | 83 ml | 78 ml | 73 ml | 68 ml |

**Table C.3** 100% denaturing solution

| Reagents | 6% gel | 8% gel | 10% gel | 12% gel |
|---|---|---|---|---|
| 40% acrylamide/bis (37:5:1) | 15 ml | 20 ml | 25 ml | 30 ml |
| 50X TAE buffer | 2 ml | 2 ml | 2 ml | 2 ml |
| Formamide | 40 ml | 40 ml | 40 ml | 40 ml |
| Urea | 42 g | 42 g | 42 g | 42 g |
| Distilled water | 83 ml | 78 ml | 73 ml | 68 ml |

Note: for other denaturing solution, use the volumes in the 100% denaturing solution with the exception of the formamide and urea.

**Table C.4** Denaturing solution per 100 ml solution

| Reagents | 10% | 20% | 30% | 40% | 50% | 60% | 70% | 80% | 90% |
|---|---|---|---|---|---|---|---|---|---|
| Formamide (ml) | 4 | 8 | 12 | 16 | 20 | 24 | 28 | 32 | 36 |
| Urea (g) | 4.2 | 8.4 | 12.6 | 16.8 | 21.0 | 25.2 | 29.4 | 33.6 | 37.8 |

**Casting the parallel gradient gel and staring the run**

(for BioRad DCode™ Universal Mutation Detection System; BioRad, USA)

1. Feed 2 ml of gel check leak solution into assembled gel sandwich and let it stand for 15 min at room temperature.
2. Prepare DGGE gel solution.
3. Withdraw all of the high density solution into syringe I, carefully remove air-bubbles from syringe by turning the syringe upside down (plunger cap towards the bench) and gently tapping the syringe. Push the gel solution to the end of the tubing. Do the same for the low density solution into the syringe II.
4. Place the syringe I and II into the gradient delivery system syringe holder and rotate the cam wheel slowly steadily to deliver the gel solution. It is important to cast the gel solution at a steady pace to avoid any disturbances between the gel solutions within the gel sandwich.
5. Add small volume of 1X TAE buffer into the gel for protected the gel from the air. Let the gel polymerize overnight or at least for 4 hours.
6. After polymerization, discard the 1X TAE buffer, and insert the comb to the desired well depth and straighten.
7. Feed slowly of stacking gel. Let stacking gel polymerize for 20 min
8. Fill the electrophoresis tank with 7 L of 1X TAE running buffer and preheat the buffer to set temperature.
9. Flood 1X TAE buffer on the top of wells. Samples are load with special long tips into the wells. Check that all the wells should be well immersed in 1X TAE buffer before starting the run.
10. After the sample are loaded. The temperature controller is placed on the top of the tank and the temperature is set to 60 °C for electrophoresis run.

The electrophoresis tank is connected to a power pac where the desired volt and time of the run is set. Usually a higher voltage less time is need for the run. At 200 volts the electrophoresis is run for 5-6 hours.

# APPENDIX D

## Preparation of competent cell

(adapted from Sambrook and Russell, 2001)

1. Pick a single bacterial colony from plate that has been incubated at 37 °C for 16-20 hours. Transfer the colony into 3 ml of LB broth and incubated at 37 °C for 16-20 hours (pre-culture).
2. Pipette pre-culture 1 ml into 100 ml of LB broth. Incubate with 220 rpm at 37 °C for 3 hours, monitoring the growth of culture by measure the $OD_{600}$ of the culture every 15-20 min. Until the $OD_{600}$ of the culture is 0.4.
3. Transfer the bacterial cell to 50 ml centrifuge tube. Cool the cultures to 0 °C by storing the tubes on ice for 10 min.
4. Recover the cells by centrifugation at 3,500 rpm for 10 min at 4 °C.
5. Decant the medium from the cell pellets.
6. Resuspend each pellet by swirling or gentle vortexing in 25 ml of ice-cold-50 mM $CaCl_2$ for each 50 ml of original culture and incubated on ice for 30 min.
7. Recover the cells by centrifugation at 3,500 rpm for 10 min at 4 °C.
8. Decant the supernatant from the cell pellets.
9. Resuspend each pellet by swirling or gentle vortexing in 5 ml of ice-cold-50 mM $CaCl_2$ and incubated on ice for 15 min.
10. Add 5 ml of ice-cold-50 mM $CaCl_2$ and centrifuge at 3,500 rpm for 10 min at 4 °C.
11. Decant the supernatant from the cell pellets.
12. Add 5 ml of 20% glycerol and aliquots 200 µl into microcentrifuge tube. Store at -80 °C.

# APPENDIX E

## Jaccard's index similarity

**Table E.1** Jaccard's index similarity of bacterial population during operation of CD-UASB reactor co-digested with Napier grass at HRT 10 days

| **Lanes compared** | Seed | 3 d | 8 d | 13 d | 22 d | 27 d | 36 d | 45 d | 55 d | 66 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.78 | 1.00 | | | | | | | | |
| 8 d | 0.74 | 0.94 | 1.00 | | | | | | | |
| 13 d | 0.74 | 0.94 | 1.00 | 1.00 | | | | | | |
| 22 d | 0.74 | 0.94 | 1.00 | 1.00 | 1.00 | | | | | |
| 27 d | 0.74 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | | | | |
| 36 d | 0.74 | 0.94 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | |
| 45 d | 0.57 | 0.67 | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 | 1.00 | | |
| 55 d | 0.45 | 0.55 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.84 | 1.00 | |
| 66 d | 0.45 | 0.55 | 0.52 | 0.52 | 0.52 | 0.52 | 0.52 | 0.84 | 1.00 | 1.00 |

**Table E.2** Jaccard's index similarity of bacterial population during operation of CD-UASB reactor co-digested with Napier grass at HRT 20 days

| **Lanes compared** | Seed | 3 d | 8 d | 13 d | 22 d | 27 d | 36 d | 45 d | 55 d | 66 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.63 | 1.00 | | | | | | | | |
| 8 d | 0.63 | 1.00 | 1.00 | | | | | | | |
| 13 d | 0.63 | 1.00 | 1.00 | 1.00 | | | | | | |
| 22 d | 0.63 | 1.00 | 1.00 | 1.00 | 1.00 | | | | | |
| 27 d | 0.63 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | | |
| 36 d | 0.63 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | |
| 45 d | 0.32 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 1.00 | | |
| 55 d | 0.32 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 1.00 | 1.00 | |
| 66 d | 0.40 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.73 | 0.73 | 1.00 |

**Table E.3** Jaccard's index similarity of bacterial population during operation of CD-UASB reactor co-digested with Napier grass at HRT 30 days

| **Lanes compared** | Seed | 3 d | 8 d | 13 d | 22 d | 27 d | 36 d | 45 d | 55 d | 66 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.50 | 1.00 | | | | | | | | |
| 8 d | 0.56 | 0.79 | 1.00 | | | | | | | |
| 13 d | 0.48 | 0.95 | 0.84 | 1.00 | | | | | | |
| 22 d | 0.50 | 0.86 | 0.76 | 0.90 | 1.00 | | | | | |
| 27 d | 0.50 | 0.86 | 0.76 | 0.90 | 0.90 | 1.00 | | | | |
| 36 d | 0.55 | 0.85 | 0.75 | 0.90 | 0.90 | 0.90 | 1.00 | | | |
| 45 d | 0.48 | 0.43 | 0.46 | 0.46 | 0.51 | 0.48 | 0.46 | 1.00 | | |
| 55 d | 0.48 | 0.43 | 0.46 | 0.46 | 0.51 | 0.48 | 0.46 | 1.00 | 1.00 | |
| 66 d | 0.43 | 0.39 | 0.42 | 0.43 | 0.50 | 0.45 | 0.43 | 0.95 | 0.95 | 1.00 |

**Table E.4** Jaccard's index similarity of bacterial population during operation of CSTR co-digested with Napier grass at HRT 10 days

| **Lanes compared** | Seed | 3 d | 8 d | 13 d | 22 d | 24 d | 36 d | 50 d | 55 d | 62 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.90 | 1.00 | | | | | | | | |
| 8 d | 0.82 | 0.75 | 1.00 | | | | | | | |
| 13 d | 0.64 | 0.60 | 0.79 | 1.00 | | | | | | |
| 22 d | 0.60 | 0.67 | 0.73 | 0.93 | 1.00 | | | | | |
| 24 d | 0.53 | 0.50 | 0.47 | 0.65 | 0.61 | 1.00 | | | | |
| 36 d | 0.23 | 0.22 | 0.21 | 0.33 | 0.32 | 0.52 | 1.00 | | | |
| 50 d | 0.23 | 0.21 | 0.20 | 0.32 | 0.31 | 0.50 | 0.95 | 1.00 | | |
| 55 d | 0.23 | 0.21 | 0.20 | 0.32 | 0.31 | 0.50 | 0.95 | 1.00 | 1.00 | |
| 62 d | 0.23 | 0.21 | 0.20 | 0.32 | 0.31 | 0.50 | 0.95 | 1.00 | 1.00 | 1.00 |

**Table E.5** Jaccard's index similarity of bacterial population during operation of CSTR co-digested with Napier grass at HRT 20 days

| **Lanes compared** | Seed | 3 d | 8 d | 13 d | 22 d | 24 d | 36 d | 50 d | 55 d | 62 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.50 | 1.00 | | | | | | | | |
| 8 d | 0.35 | 0.57 | 1.00 | | | | | | | |
| 13 d | 0.33 | 0.64 | 0.79 | 1.00 | | | | | | |
| 22 d | 0.47 | 0.42 | 0.61 | 0.58 | 1.00 | | | | | |
| 24 d | 0.47 | 0.42 | 0.53 | 0.58 | 0.89 | 1.00 | | | | |
| 36 d | 0.33 | 0.30 | 0.37 | 0.36 | 0.56 | 0.56 | 1.00 | | | |
| 50 d | 0.38 | 0.23 | 0.26 | 0.25 | 0.44 | 0.50 | 0.81 | 1.00 | | |
| 55 d | 0.38 | 0.23 | 0.26 | 0.25 | 0.44 | 0.50 | 0.81 | 1.00 | 1.00 | |
| 62 d | 0.38 | 0.23 | 0.26 | 0.25 | 0.44 | 0.50 | 0.81 | 1.00 | 1.00 | 1.00 |

**Table E.6** Jaccard's index similarity of bacterial population during operation of CSTR co-digested with Napier grass at HRT 30 days

| **Lanes compared** | Seed | 3 d | 8 d | 13 d | 22 d | 24 d | 36 d | 50 d | 55 d | 62 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.50 | 1.00 | | | | | | | | |
| 8 d | 0.50 | 0.54 | 1.00 | | | | | | | |
| 13 d | 0.50 | 0.43 | 0.82 | 1.00 | | | | | | |
| 22 d | 0.46 | 0.50 | 0.75 | 0.91 | 1.00 | | | | | |
| 24 d | 0.35 | 0.32 | 0.47 | 0.56 | 0.63 | 1.00 | | | | |
| 36 d | 0.33 | 0.20 | 0.30 | 0.36 | 0.35 | 0.40 | 1.00 | | | |
| 50 d | 0.33 | 0.20 | 0.30 | 0.36 | 0.35 | 0.40 | 1.00 | 1.00 | | |
| 55 d | 0.35 | 0.21 | 0.32 | 0.38 | 0.36 | 0.42 | 0.95 | 0.95 | 1.00 | |
| 62 d | 0.35 | 0.21 | 0.32 | 0.38 | 0.36 | 0.42 | 0.95 | 0.95 | 1.00 | 1.00 |

**Table E.7** Jaccard's index similarity of bacterial population during operation of CD-UASB reactor co-digested with food waste at HRT 10 days

| **Lanes compared** | Seed | 3 d | 6 d | 10 d | 15 d | 22 d | 36 d | 45 d | 55 d | 62 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.91 | 1.00 | | | | | | | | |
| 6 d | 0.75 | 0.83 | 1.00 | | | | | | | |
| 10 d | 0.62 | 0.69 | 0.83 | 1.00 | | | | | | |
| 15 d | 0.62 | 0.69 | 0.83 | 1.00 | 1.00 | | | | | |
| 22 d | 0.53 | 0.60 | 0.71 | 0.85 | 0.85 | 1.00 | | | | |
| 36 d | 0.53 | 0.60 | 0.71 | 0.85 | 0.85 | 1.00 | 1.00 | | | |
| 45 d | 0.47 | 0.53 | 0.63 | 0.73 | 0.73 | 0.87 | 0.87 | 1.00 | | |
| 55 d | 0.60 | 0.67 | 0.79 | 0.79 | 0.79 | 0.93 | 0.93 | 0.81 | 1.00 | |
| 62 d | 0.60 | 0.67 | 0.79 | 0.79 | 0.79 | 0.93 | 0.93 | 0.81 | 1.00 | 1.00 |

**Table E.8** Jaccard's index similarity of bacterial population during operation of CD-UASB reactor co-digested with food waste at HRT 20 days

| **Lanes compared** | Seed | 3 d | 6 d | 10 d | 15 d | 22 d | 36 d | 45 d | 55 d | 62 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.80 | 1.00 | | | | | | | | |
| 6 d | 0.73 | 0.91 | 1.00 | | | | | | | |
| 10 d | 0.58 | 0.62 | 0.69 | 1.00 | | | | | | |
| 15 d | 0.58 | 0.62 | 0.69 | 1.00 | 1.00 | | | | | |
| 22 d | 0.64 | 0.67 | 0.75 | 0.91 | 0.91 | 1.00 | | | | |
| 36 d | 0.58 | 0.75 | 0.83 | 0.83 | 0.83 | 0.91 | 1.00 | | | |
| 45 d | 0.58 | 0.75 | 0.83 | 0.83 | 0.83 | 0.91 | 1.00 | 1.00 | | |
| 55 d | 0.58 | 0.75 | 0.83 | 0.83 | 0.83 | 0.91 | 1.00 | 1.00 | 1.00 | |
| 62 d | 0.58 | 0.75 | 0.83 | 0.83 | 0.83 | 0.91 | 1.00 | 1.00 | 1.00 | 1.00 |

**Table E.9** Jaccard's index similarity of bacterial population during operation of CD-UASB reactor co-digested with food waste at HRT 30 days

| **Lanes compared** | Seed | 3 d | 6 d | 10 d | 15 d | 22 d | 36 d | 45 d | 55 d | 62 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.64 | 1.00 | | | | | | | | |
| 6 d | 0.57 | 0.64 | 1.00 | | | | | | | |
| 10 d | 0.57 | 0.64 | 1.00 | 1.00 | | | | | | |
| 15 d | 0.69 | 0.44 | 0.69 | 0.69 | 1.00 | | | | | |
| 22 d | 0.69 | 0.44 | 0.69 | 0.69 | 1.00 | 1.00 | | | | |
| 36 d | 0.69 | 0.44 | 0.69 | 0.69 | 1.00 | 1.00 | 1.00 | | | |
| 45 d | 0.63 | 0.47 | 0.63 | 0.63 | 0.94 | 0.94 | 0.94 | 1.00 | | |
| 55 d | 0.67 | 0.50 | 0.67 | 0.67 | 0.88 | 0.88 | 0.88 | 0.93 | 1.00 | |
| 62 d | 0.67 | 0.50 | 0.67 | 0.67 | 0.88 | 0.88 | 0.88 | 0.93 | 1.00 | 1.00 |

**Table E.10** Jaccard's index similarity of bacterial population during operation of CSTR co-digested with food waste at HRT 10 days

| **Lanes compared** | Seed | 3 d | 8 d | 12 d | 17 d | 22 d | 36 d | 43 d | 52 d | 64 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.47 | 1.00 | | | | | | | | |
| 8 d | 0.38 | 0.72 | 1.00 | | | | | | | |
| 12 d | 0.35 | 0.68 | 0.93 | 1.00 | | | | | | |
| 17 d | 0.36 | 0.65 | 0.67 | 0.63 | 1.00 | | | | | |
| 22 d | 0.25 | 0.53 | 0.63 | 0.69 | 0.77 | 1.00 | | | | |
| 36 d | 0.29 | 0.59 | 0.60 | 0.56 | 0.91 | 0.83 | 1.00 | | | |
| 43 d | 0.29 | 0.59 | 0.60 | 0.56 | 0.91 | 0.83 | 1.00 | 1.00 | | |
| 52 d | 0.29 | 0.59 | 0.60 | 0.56 | 0.91 | 0.83 | 1.00 | 1.00 | 1.00 | |
| 64 d | 0.21 | 0.44 | 0.53 | 0.50 | 0.67 | 0.62 | 0.73 | 0.73 | 0.73 | 1.00 |

**Table E.11** Jaccard's index similarity of bacterial population during operation of CSTR co-digested with food waste at HRT 20 days

| **Lanes compared** | Seed | 3 d | 8 d | 12 d | 17 d | 22 d | 36 d | 43 d | 52 d | 64 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.50 | 1.00 | | | | | | | | |
| 8 d | 0.50 | 1.00 | 1.00 | | | | | | | |
| 12 d | 0.50 | 1.00 | 1.00 | 1.00 | | | | | | |
| 17 d | 0.50 | 1.00 | 1.00 | 1.00 | 1.00 | | | | | |
| 22 d | 0.50 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | | |
| 36 d | 0.50 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | | |
| 43 d | 0.50 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | | |
| 52 d | 0.50 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | |
| 64 d | 0.67 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 0.78 | 1.00 |

**Table E.12** Jaccard's index similarity of bacterial population during operation of CSTR co-digested with food waste at HRT 30 days

| **Lanes compared** | Seed | 3 d | 8 d | 12 d | 17 d | 22 d | 36 d | 43 d | 52 d | 64 d |
|---|---|---|---|---|---|---|---|---|---|---|
| Seed | 1.00 | | | | | | | | | |
| 3 d | 0.71 | 1.00 | | | | | | | | |
| 8 d | 0.63 | 0.82 | 1.00 | | | | | | | |
| 12 d | 0.67 | 0.76 | 0.93 | 1.00 | | | | | | |
| 17 d | 0.59 | 0.88 | 0.93 | 0.87 | 1.00 | | | | | |
| 22 d | 0.67 | 0.76 | 0.80 | 0.86 | 0.87 | 1.00 | | | | |
| 36 d | 0.67 | 0.76 | 0.93 | 0.86 | 0.87 | 0.86 | 1.00 | | | |
| 43 d | 0.71 | 0.71 | 0.86 | 0.92 | 0.80 | 0.92 | 0.92 | 1.00 | | |
| 52 d | 0.71 | 0.71 | 0.86 | 0.92 | 0.80 | 0.92 | 0.92 | 1.00 | 1.00 | |
| 64 d | 0.71 | 0.71 | 0.86 | 0.92 | 0.80 | 0.92 | 0.92 | 1.00 | 1.00 | 1.00 |

**Table E.13** Jaccard's index similarity of bacterial population at steady state in reactors co-digested with Napier grass

| **Lanes compared** | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 1.00 | | | | | |
| 2 | 0.52 | 1.00 | | | | |
| 3 | 0.48 | 0.93 | 1.00 | | | |
| 4 | 0.79 | 0.43 | 0.45 | 1.00 | | |
| 5 | 0.48 | 0.50 | 0.52 | 0.59 | 1.00 | |
| 6 | 0.40 | 0.63 | 0.67 | 0.50 | 0.80 | 1.00 |

Lane 1, 2 and 3; CD-UASB reactor at HRT 10, 20 and 30 days, respectively
Lane 4, 5 and 6; CSTR at HRT 10, 20 and 30 days, respectively

**Table E.14** Jaccard's index similarity of bacterial population at steady state in reactors co-digested with food waste

| **Lanes compared** | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 1 | 1.00 | | | | | |
| 2 | 0.89 | 1.00 | | | | |
| 3 | 0.75 | 0.75 | 1.00 | | | |
| 4 | 0.48 | 0.48 | 0.52 | 1.00 | | |
| 5 | 0.52 | 0.52 | 0.57 | 0.71 | 1.00 | |
| 6 | 0.52 | 0.52 | 0.57 | 0.71 | 1.00 | 1.00 |

Lane 1, 2 and 3; CD-UASB reactor at HRT 10, 20 and 30 days, respectively
Lane 4, 5 and 6; CSTR at HRT 10, 20 and 30 days, respectively

**Table E.15** Jaccard's index similarity of bacterial population at steady state in reactors co-digested with Napier grass or food waste

| **Lanes compared** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.00 | | | | | | | | | | | |
| 2 | 0.65 | 1.00 | | | | | | | | | | |
| 3 | 0.65 | 1.00 | 1.00 | | | | | | | | | |
| 4 | 1.00 | 0.65 | 0.65 | 1.00 | | | | | | | | |
| 5 | 0.63 | 0.81 | 0.81 | 0.63 | 1.00 | | | | | | | |
| 6 | 0.58 | 0.87 | 0.87 | 0.58 | 0.82 | 1.00 | | | | | | |
| 7 | 0.57 | 0.48 | 0.48 | 0.57 | 0.48 | 0.50 | 1.00 | | | | | |
| 8 | 0.59 | 0.50 | 0.50 | 0.59 | 0.50 | 0.52 | 0.95 | 1.00 | | | | |
| 9 | 0.54 | 0.46 | 0.46 | 0.54 | 0.46 | 0.48 | 0.87 | 0.91 | 1.00 | | | |
| 10 | 0.54 | 0.39 | 0.39 | 0.54 | 0.40 | 0.42 | 0.67 | 0.63 | 0.58 | 1.00 | | |
| 11 | 0.57 | 0.41 | 0.41 | 0.57 | 0.42 | 0.43 | 0.63 | 0.65 | 0.60 | 0.95 | 1.00 | |
| 12 | 0.57 | 0.41 | 0.41 | 0.57 | 0.42 | 0.43 | 0.63 | 0.65 | 0.60 | 0.95 | 1.00 | 1.00 |

Lane 1, 2 and 3; CD-UASB reactor co-digested with Napier grass at HRT 10, 20 and 30 days, respectively

Lane 4, 5 and 6; CSTR co-digested with Napier grass at HRT 10, 20 and 30 days, respectively

Lane 7, 8 and 9; CD-UASB reactor co-digested with food waste at HRT 10, 20 and 30 days, respectively

Lane 10, 11 and 12; CSTR co-digested with food waste at HRT 10, 20 and 30 days, respectively

# CURRICULUM VITAE

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2. Microbial community structure in anaerobic co-digestion system of mixed waste in lab-scale bioreactor. Commission on Higher Education Congress III: University Staff Development Consortium (CHE-USDC Congress III), 9-11 September, 2010, Royal Cliff Grand Hotel and Spa, Chon buri, Thailand.
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**PUBLICATIONS**

1. Singka, D. and Pathom-aree, W. 2011. Denaturing Gradient Gel Electrophoresis :Principle and Application in Environmental Microbiology. KKU Science Journal. 39: 321-333.
2. Singka, D., Kumdhitiahutsawakul, A., Rekkriangkrai, P. and Pathom-aree, W. A Simple Method for DNA Extraction from Activated Sludge. 2012. Chiang Mai Journal of Science. 39: 111-118. (impact factor 0.516)