Spring 6-10-2014

Influence of one step temperature increase acclimation method on microbial community dynamics during thermophilic biochemical methane potential test of an industrial wastewater

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Impact of temperature increase on microbial community dynamics during thermophilic BMP test on an industrial wastewater

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Advanced Environmental Biotechnology Center

Wastewater and Biosolids Treatment and Use
Otranto, Italy

Water | Waste | Wastewater | Energy-Water Nexus

Introduction

Challenge 1
Industrial wastewater

- High strength (high organic content)
- Complex make-up of organics
- Presence of potential inhibitors

Challenge 2
Thermophilic process

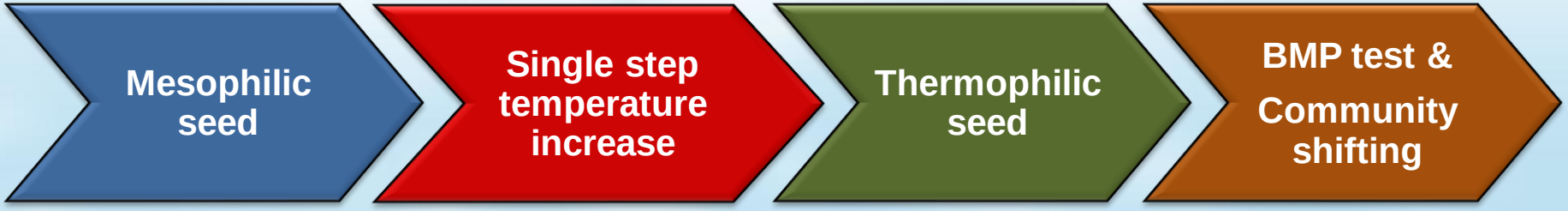
- Absence of readily available seed
- Sensitivity towards operational changes
- Bioconversion capability may increase

Challenge 3
Microbial community responses

- Understanding pattern of response (knowledge gap)
- Tolerance to complex organics + high temperature

Introduction

- Mesophilic seed in study subjected to 55°C
- Complex mesophilic seed contains thermophilic homologues
- Seed stressed for 10 days at 55°C without feeding of substrate



1. **Kobayashi T, et al. 2009**. Characterization of start-up performance and archaeal community shifts during anaerobic self-degradation of waste-activated sludge. Bioresour Technol 100: 4981–4988.
2. **Bousková A, et al. 2005.** Strategies for changing temperature from mesophilic to thermophilic conditions in anaerobic CSTR reactors treating sewage sludge. Water Res 39: 1481–1488.

Experimental methodology

Table 1 – Physico-chemical characterization of industrial wastewater

Wastewater parameters	Value
Total chemical oxygen demand (TCOD)	(343.12 ± 3.56) g/L
Soluble chemical oxygen demand (SCOD)	(294.35 ± 2.78) g/L
Volatile fatty acids (VFA's)	(3.6 ± 0.02) g/L
Acetic acid	0.83 g/L
Valeric acid	1.23 g/L
Other organic components present	Glutarate, adipate, succinate
pH	9.23
Sodium	44.1 g/L
Colour (visual)	Deep red

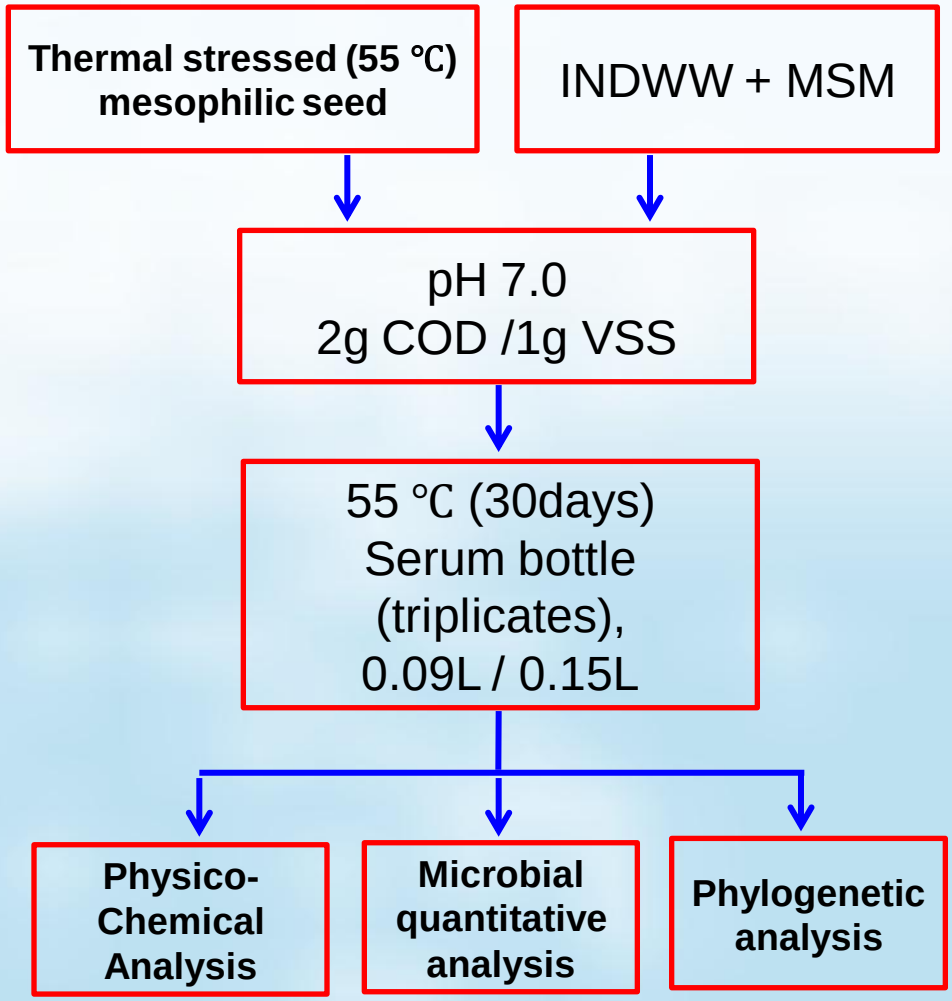


Fig. 1 Process flow chart of one step temperature increase on community shifting

Physicochemical analysis – VFA profile

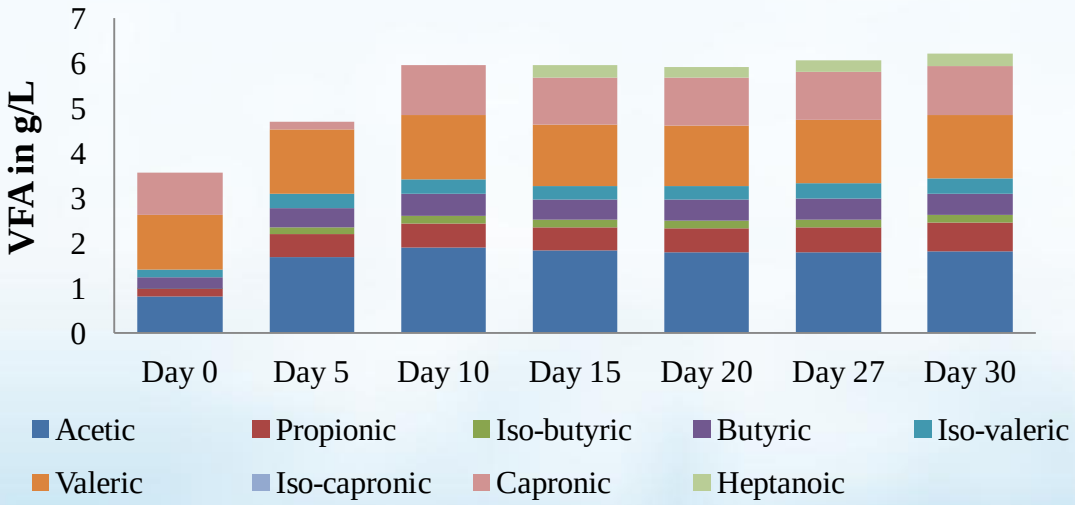


Fig. 2 – Volatile fatty acids profile of test group

- Accumulation of VFA intermediates
- Valerate not degraded completely
- Accumulation of acetic acid
- VFA accumulation can be attributed to improper seed acclimation to higher operating temperatures
- VFA in blank control below detectable levels

Table 2 – Residual chemical oxygen demand profile in test and control group

Group	Day 0	Day 15	Day 30
TCOD (AD/Control)	14.71 ± 0.03 g/L	12.62 ± 0.32 g/L	8.80 ± 0.01 g/L
TCOD (AD/IND-WW)	39.14 ± 0.10 g/L	38.37 ± 0.32 g/L	38.90 ± 0.29 g/L
SCOD (AD/Control)	N.D	1.22 ± 0.01g/L	N.D
SCOD (AD/IND-WW)	21.16 ± 0.08 g/L	26.11 ± 0.17 g/L	26.42 ± 0.12g/L

Physicochemical analysis – biogas profile

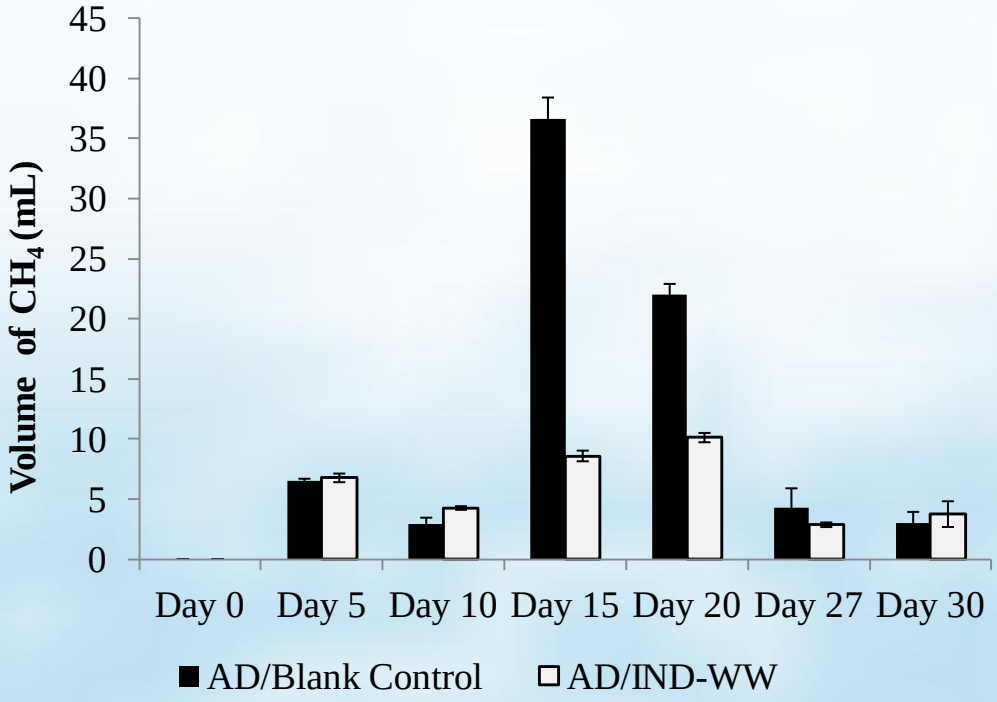


Fig. 3 Volume of methane produced over 30 days period

- Gas production calculated as volume produced every 5 days after equalizing headspace pressure to 1 atm
- Cumulative methane production in test groups – 36mL at STP; control groups – 75.43mL at STP
- Highest recorded methane percentage in test groups – 32.03% of total gas; control – 61.49%

Microbial quantitative analysis

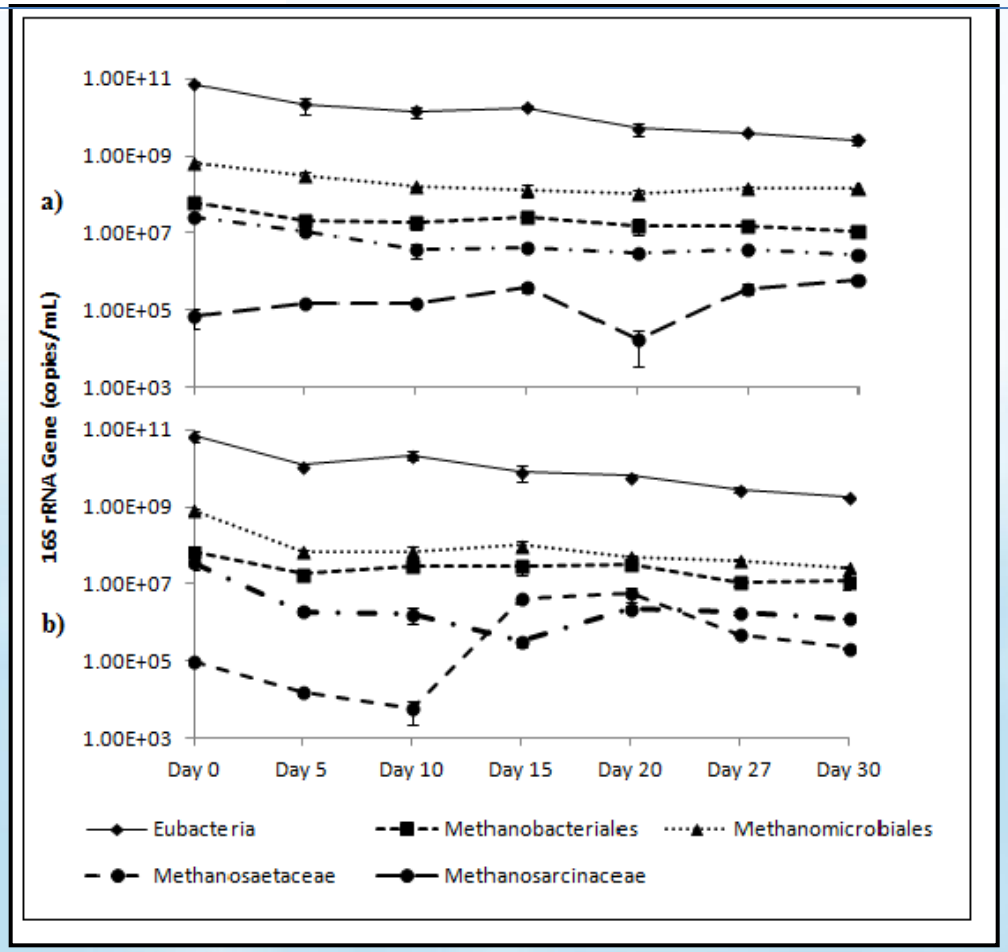


Fig. 4 Differences in shifts of microbial populations a) AD/INDWW and b) AD/Control

- Target – quantifying Eubacteria, Methanomicrobiales, Methanobacteriales, Methanosaetaceae and Methanosarcinaceae
- Eubacteria predominate over the methanogenic groups though with a steady decline in numbers over the test period
- Methanomicrobiales (H_2 + CO_2 utilizing methanogen) most dominant methanogenic group
- Even with substantial numbers of Methanosarcinaceae, acetic acid accumulation is observed

3. Yu, Y., et al. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol. Bioeng. 89(6),670–679.

Microbial phylogenetic analysis

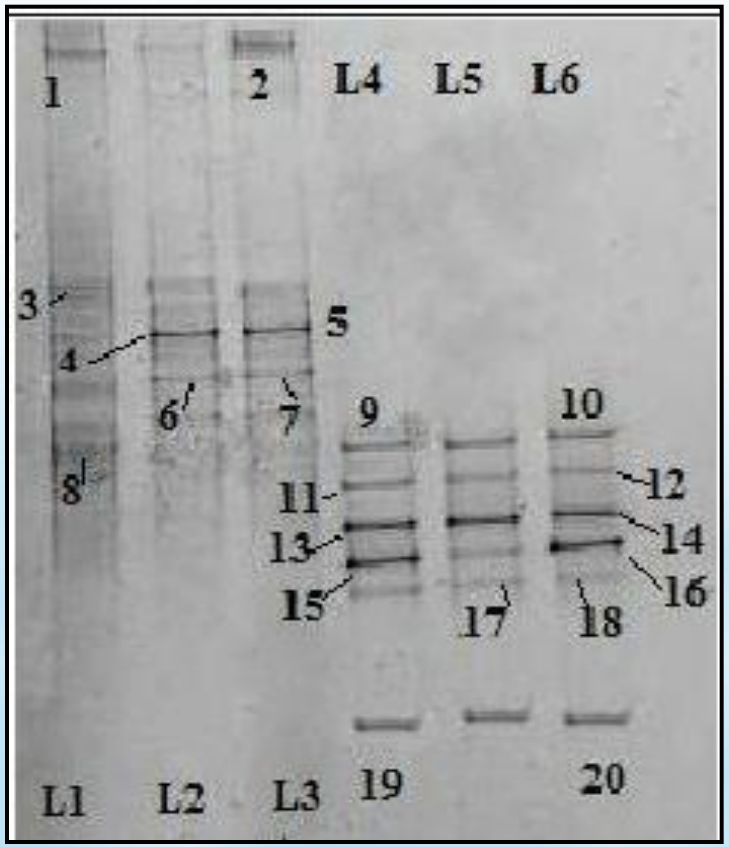


Fig. 5 Denaturing gradient gel electrophoresis (DGGE) profile of test group degrading wastewater at 55ºC. Eubacterial profile is highlighted in lanes 1-3 and Archaeal profile in lanes 4-6

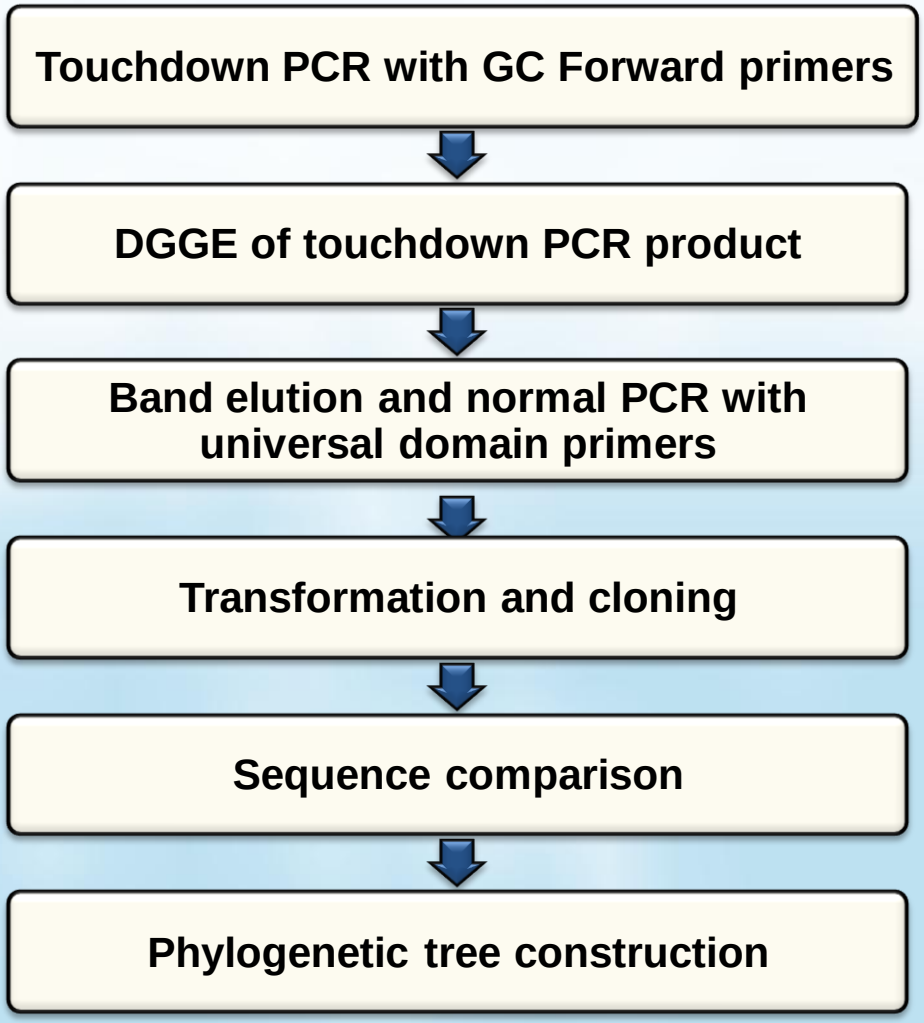
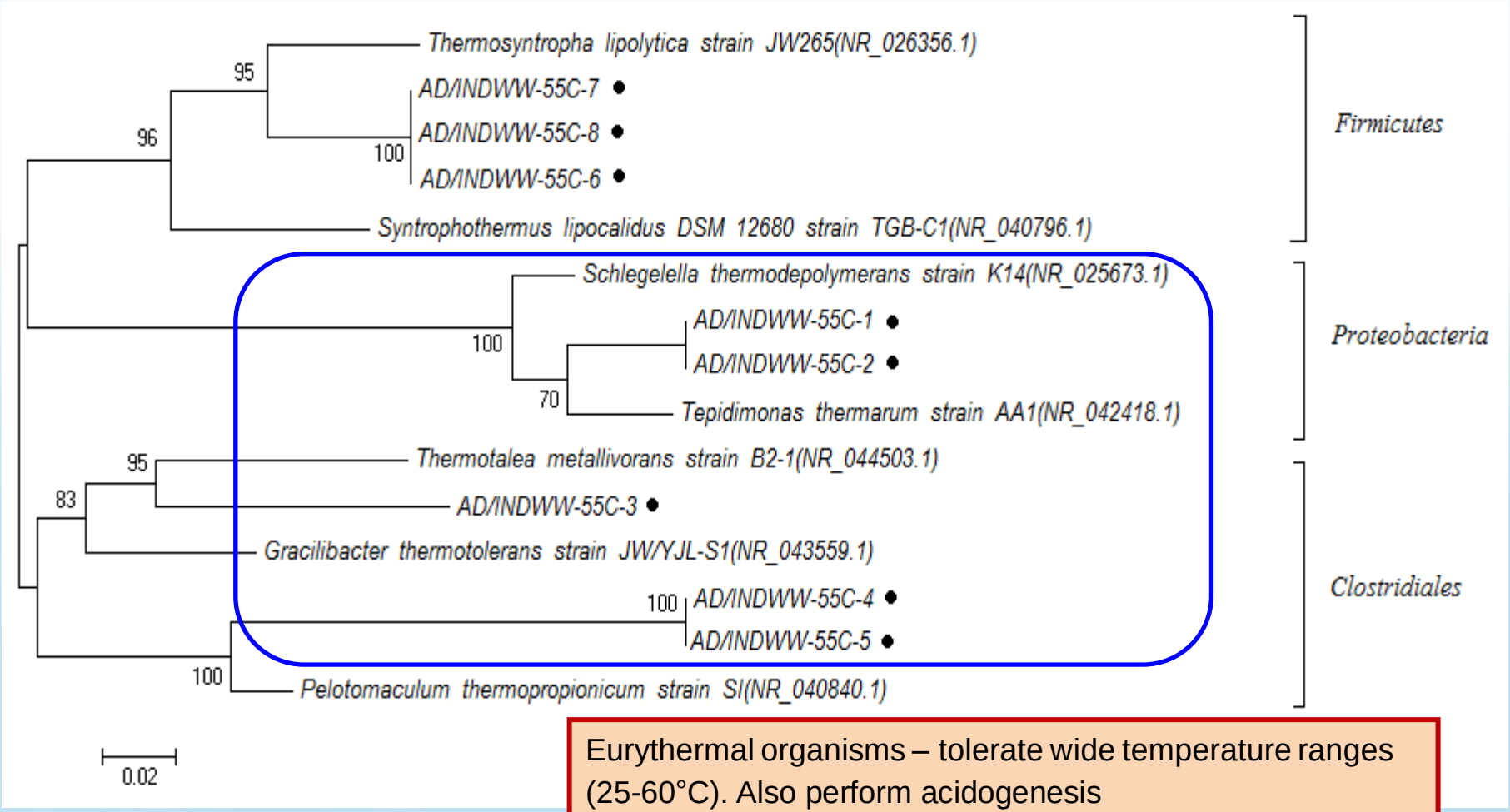


Fig. 1 Process flow chart of microbial analysis

Microbial phylogenetic analysis



Eurythermal organisms – tolerate wide temperature ranges (25-60°C). Also perform acidogenesis

Fig. 6 Eubacterial phylogenetic tree of test group constructed using the neighborhood joining method. Number at nodes indicate bootstrap analyses of 100 replicates

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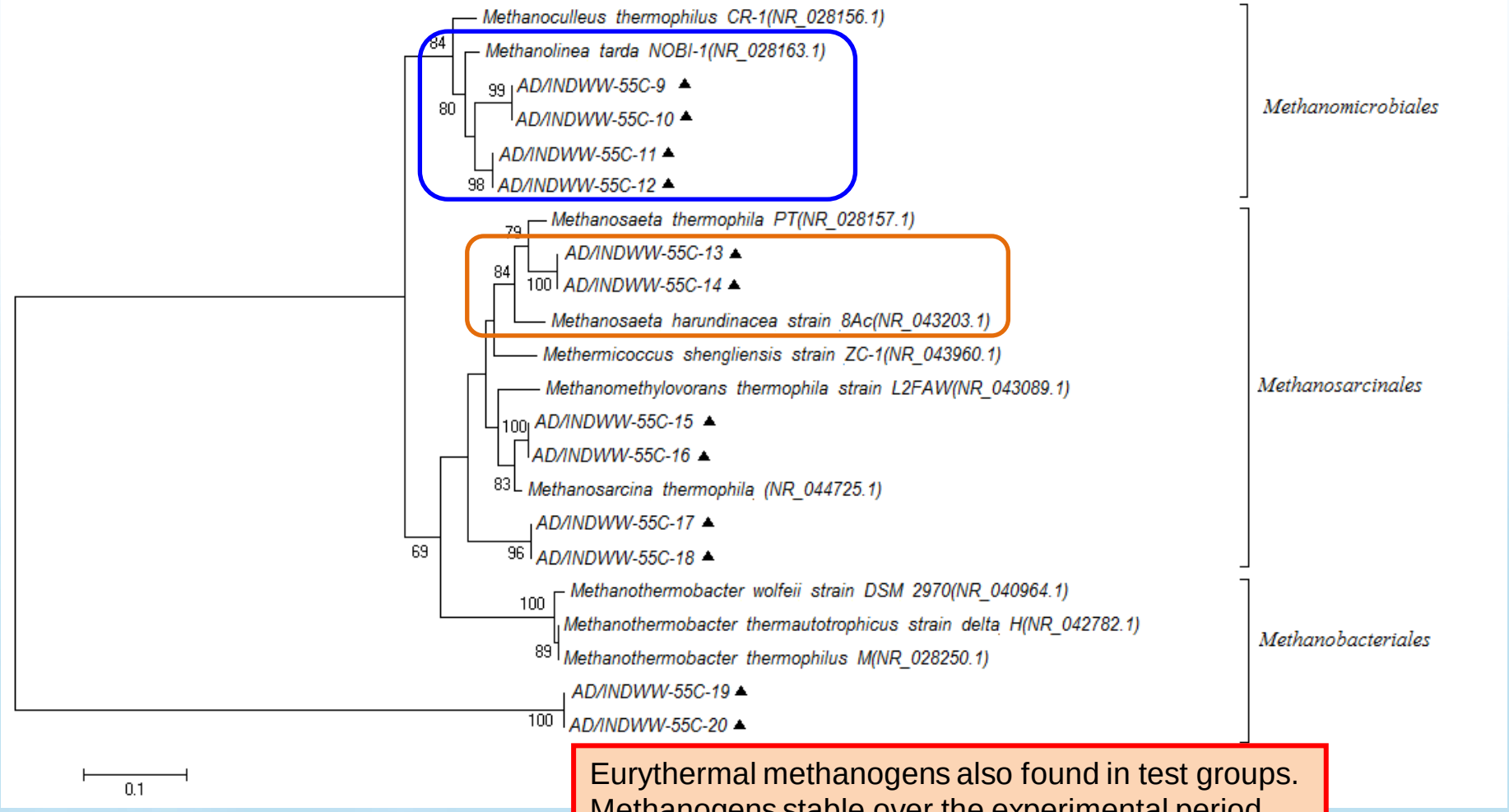
Microbial phylogenetic analysis



Eurythermal methanogens also found in test groups.
Methanogens stable over the experimental period

Fig. 7 – Archaeal phylogenetic tree of test group constructed using the neighborhood joining method. Number at nodes indicate bootstrap analyses of 100 replicates

Discussions

Obtaining a functional thermophilic seed

- Can be done by two basic methods
- Multistep temperature increase
- Single step temperature increase for a short time

Microbial community dynamics

- VFA accumulation → undefined community structure
- Wastewater organics imparts some form of inhibition
- Complex seed microbial community → incomplete picture of microbial process

Reasons for incomplete process/poor process performance

- Eurythermal organisms cause imbalance
- One step temperature increase knocks out niche community members tolerant to wastewater organics
- Methanogens experience loss of function (*Methanosarcinaceae*)

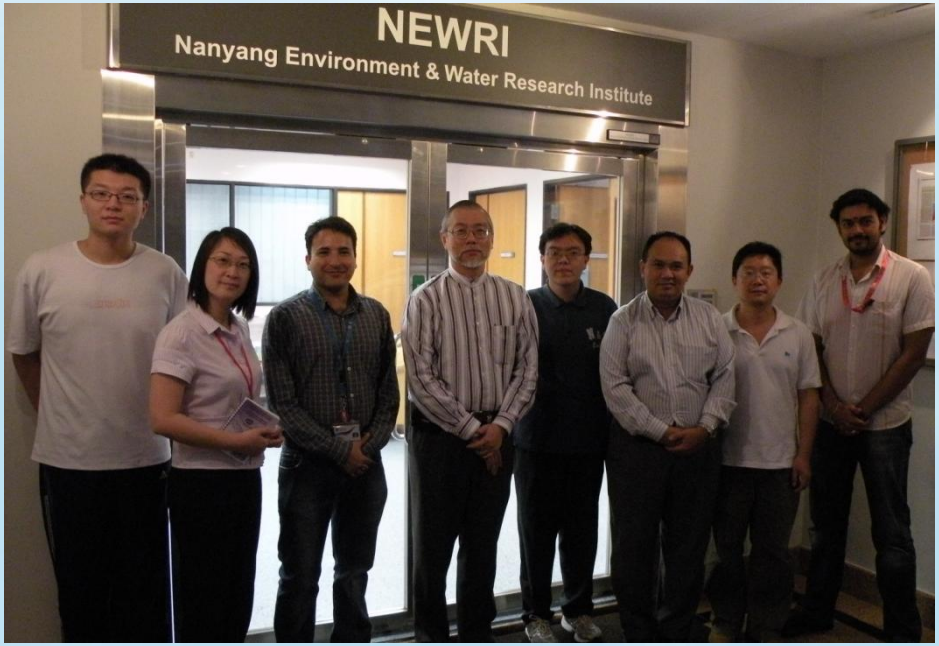
Conclusions

- One step temperature increase may not be right way to obtain a fully functional thermophilic seed
- Multistep process → wider temperature gradients or shorter time intervals
- Selection pressures on microbial communities may be mitigated with multistep process
- Seed sensitivity → Microbial community analysis, Lower organic loading during full scale process startup
- Continuous process control monitoring to avoid overload

Acknowledgement

- Funding agency: Energy Market Authority Singapore through Smart Energy Challenge research funding
- Industrial partner: Sembcorp Industries Ltd.

- Team members



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Thank You