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APPENDIX A
Results data

1. Microbial population

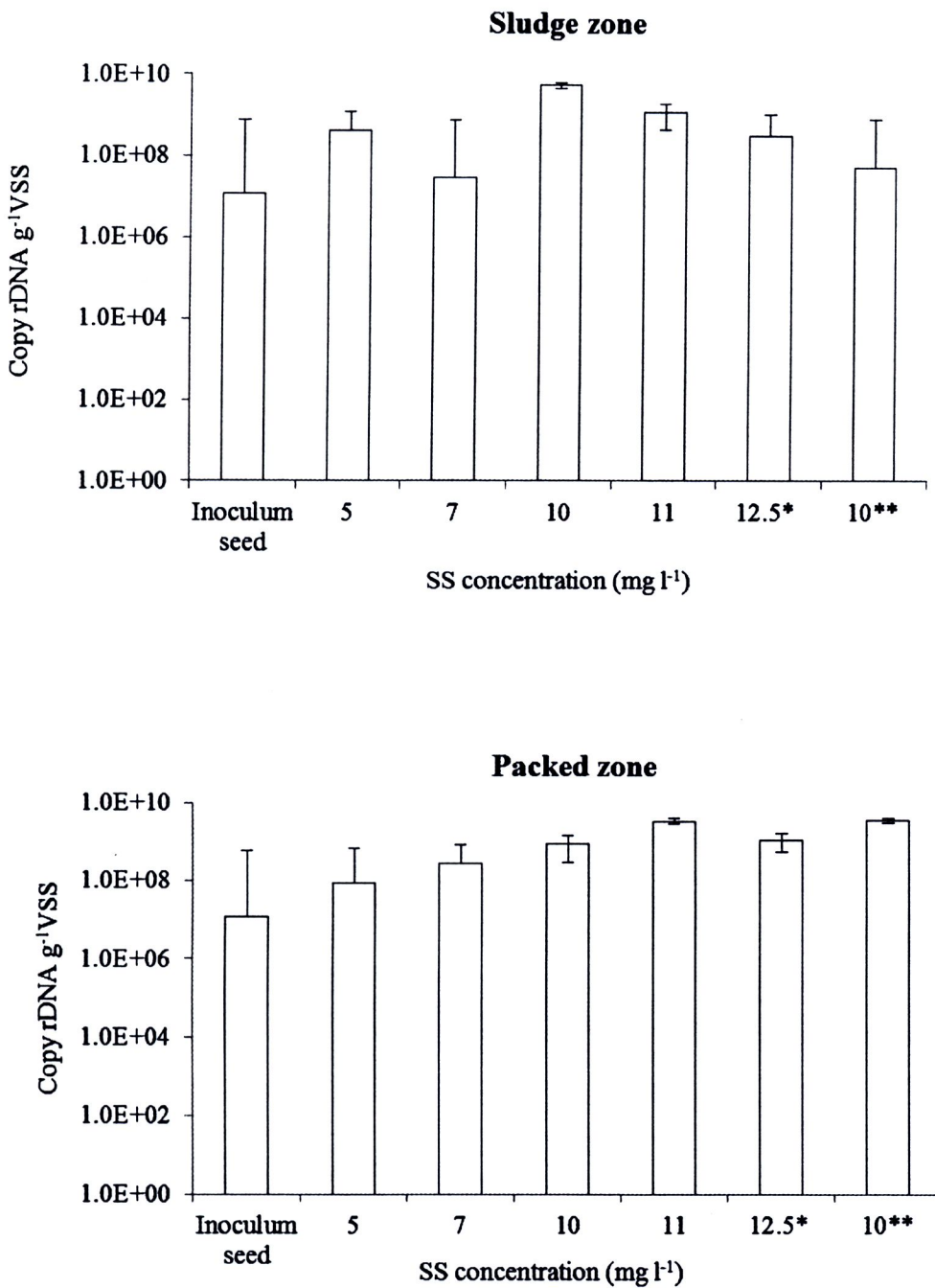


Figure A1. Eubacterial populations in AHR of POME;
 Remark: * shock load, ** recovery and operational back to 10 g l⁻¹

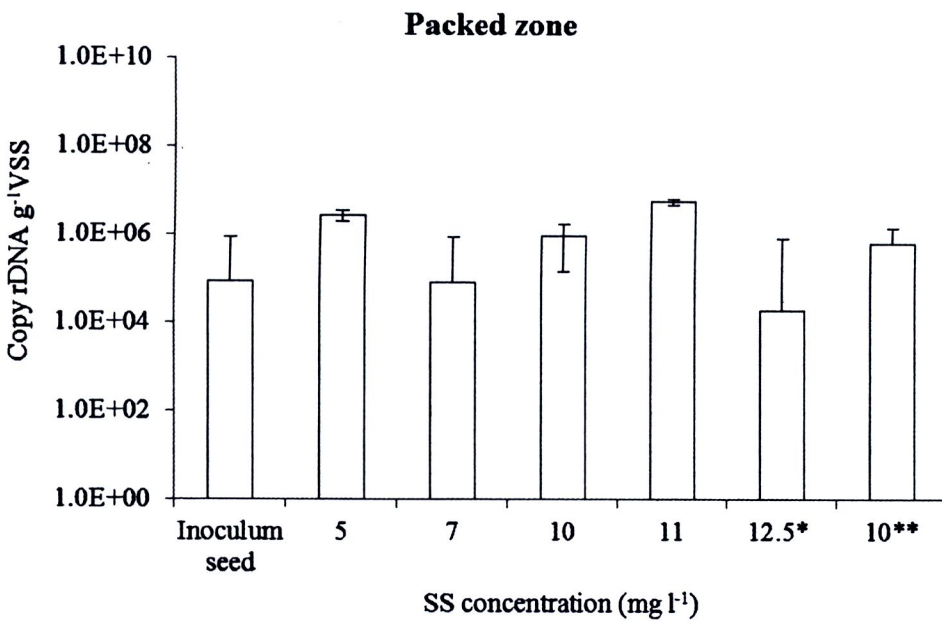
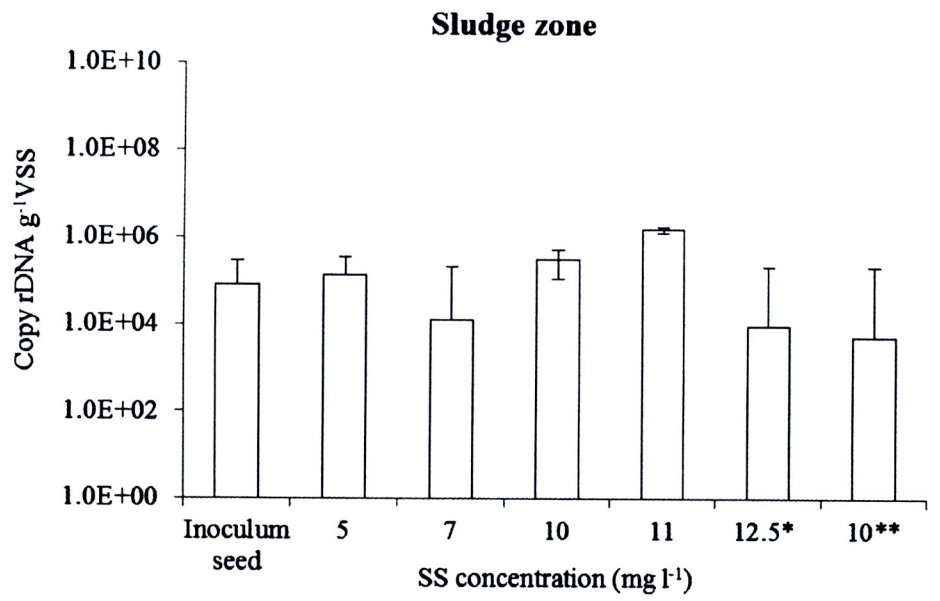


Figure A2. Archaeal populations in AHR of POME
 Remark: * shock load, ** recovery and operational back to 10 g l⁻¹

1. Suspended solid removal

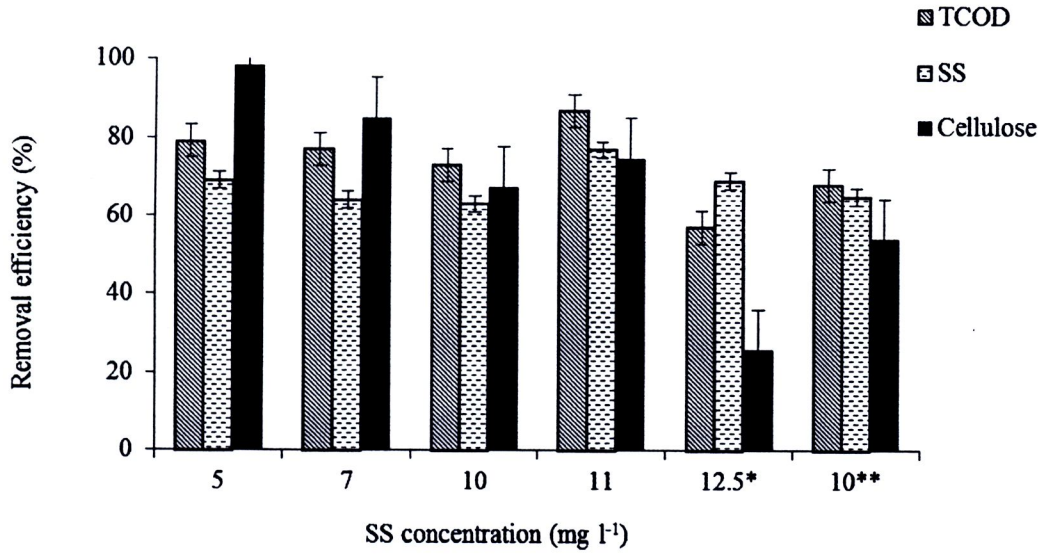


Figure A3. Suspended solid organic matters removal efficiency

Table A1. Effluent characteristic and overall organic removal of reactor operations

Zone	O&G (mg l ⁻¹)	SS (mg l ⁻¹)	TCOD (mg l ⁻¹)	SCOD (mg l ⁻¹)	Organic removal (%)		
					O&G	TCOD	SCOD
5 g SS l⁻¹							
Sludge	171 ± 0.3	1500 ± 0.1	2700 ± 0.4	1080 ± 0.2	81 ± 13.5	82 ± 11.5	88 ± 10.6
Packed	266 ± 0.1	1200 ± 0.1	2580 ± 0.3	810 ± 0.3	70 ± 14.5	83 ± 3.0	91 ± 6.0
Overall	300 ± 0.1	1560 ± 0.1	3120 ± 0.5	820 ± 0.2	67 ± 13.0	79 ± 4.5	91 ± 9.5
7 g SS l⁻¹							
Sludge	634 ± 0.1	11800 ± 0.6	27500 ± 1.0	1324 ± 0.3	50 ± 18.5	50 ± 4.8	92 ± 8.8
Packed	285 ± 0.1	2450 ± 0.2	2406 ± 0.5	1004 ± 0.2	70 ± 10.0	89 ± 9.9	90 ± 6.6
Overall	700 ± 0.1	2800 ± 0.2	4600 ± 0.9	2100 ± 0.4	58 ± 24.5	77 ± 9.7	88 ± 8.7
10 g SS l⁻¹							
Sludge	1100 ± 0.1	6800 ± 0.5	10500 ± 0.4	2800 ± 0.1	41 ± 12.5	50 ± 10.4	77 ± 8.4
Packed	1470 ± 0.1	1630 ± 0.4	2300 ± 0.2	540 ± 0.2	60 ± 11.4	77 ± 8.9	81 ± 4.5
Overall	2300 ± 0.1	4300 ± 0.4	5300 ± 0.4	2070 ± 0.6	56 ± 8.1	73 ± 5.5	80 ± 9.5
11 g SS l⁻¹							
Sludge	1200 ± 0.1	2780 ± 0.3	4550 ± 0.3	1900 ± 0.2	48 ± 11.0	81 ± 6.0	85 ± 4.0
Packed	340 ± 0.1	970 ± 0.1	1720 ± 0.3	240 ± 0.1	68 ± 9.7	70 ± 5.6	89 ± 8.2
Overall	1030 ± 0.1	2400 ± 0.3	3120 ± 0.3	1200 ± 0.1	55 ± 9.6	87 ± 12.5	89 ± 8.5
12.5 g SS l⁻¹							
Sludge	1788 ± 0.1	6357 ± 0.4	13907 ± 1.0	4070 ± 0.2	34 ± 9.7	54 ± 3.9	72 ± 3.2
Packed	1043 ± 0.1	1990 ± 0.2	4098 ± 0.2	1110 ± 0.2	43 ± 5.6	71 ± 5.7	73 ± 7.0
Overall	1180 ± 0.1	2464 ± 0.2	10662 ± 1.0	2290 ± 0.4	30 ± 10.2	57 ± 2.0	68 ± 6.5
10 g SS l⁻¹							
Sludge	1170 ± 0.1	4672 ± 0.2	10320 ± 0.8	3130 ± 0.3	39 ± 10.0	48 ± 5.0	61 ± 16.0
Packed	452 ± 0.1	1582 ± 0.2	3154 ± 0.2	1280 ± 0.1	60 ± 4.4	70 ± 5.0	84 ± 10.2
Overall	670 ± 0.1	2740 ± 0.3	4860 ± 0.3	2010 ± 246.6	52 ± 9.5	68 ± 4.0	80 ± 8.5

Remark: Values are the mean of n values ± standard deviation (%); whereby n in the range of 20-30 values.

Table A2. Overall process stability of reactor operations

Zone	TVA (mg l ⁻¹)	Alkalinity (mg l ⁻¹)	TVA/Alk	pH
5 g SS l⁻¹				
Sludge	700 ± 0.1	2593 ± 0.4	0.27 ± 0.07	7.2 ± 0.5
Packed	320 ± 0.1	1702 ± 0.4	0.19 ± 0.06	7.4 ± 0.4
Overall	570 ± 0.1	2818 ± 0.4	0.2 ± 0.06	7.3 ± 0.4
7 g SS l⁻¹				
Sludge	1380 ± 0.6	2141 ± 0.5	0.64 ± 0.12	6.6 ± 0.4
Packed	953 ± 0.3	1842 ± 0.4	0.52 ± 0.17	6.9 ± 0.2
Overall	1200 ± 0.5	2800 ± 0.3	0.43 ± 0.17	7.1 ± 0.3
10 g SS l⁻¹				
Sludge	2100 ± 0.2	3200 ± 0.5	0.66 ± 0.18	6.5 ± 0.2
Packed	1600 ± 0.4	3100 ± 0.3	0.52 ± 0.14	7.0 ± 0.3
Overall	1980 ± 0.5	3060 ± 0.5	0.64 ± 0.15	6.2 ± 0.3
11 g SS l⁻¹				
Sludge	1450 ± 0.1	2700 ± 0.1	0.91 ± 0.07	5.4 ± 0.3
Packed	1250 ± 0.3	2300 ± 0.3	0.54 ± 0.12	6.7 ± 0.3
Overall	1300 ± 0.2	2520 ± 0.3	0.55 ± 0.10	6.9 ± 0.3
12.5 g SS l⁻¹				
Sludge	2337 ± 0.3	1963 ± 0.1	1.17 ± 0.22	4.42 ± 0.2
Packed	1284 ± 0.4	2032 ± 0.1	0.63 ± 0.30	6.41 ± 0.2
Overall	1850 ± 0.3	2008 ± 0.2	0.88 ± 0.28	5.02 ± 0.3
10 g SS l⁻¹				
Sludge	2000 ± 0.2	2750 ± 0.2	0.72 ± 0.14	6.3 ± 0.5
Packed	1200 ± 0.1	2425 ± 0.2	0.5 ± 0.12	7.1 ± 0.3
Overall	1450 ± 0.2	2550 ± 0.1	0.57 ± 0.16	6.8 ± 0.3

Remark: Values are the mean of n values ± standard deviation (%); whereby n in the range of 20-30 values.

APPENDIX B
Analytical methods

Quantitative real-time PCR (qPCR) analysis of EUB & ARC

1. Standard curve generation of eubacteria (EUB)

1. Measurement initial DNA concentration of standard EUB (*E. coli* DH5 α clone no. ES1) by using Nano drop machine at 260 nm. Initial EUB DNA concentration was obtained at 244.3 ng/ μ l
2. Calculation DNA copy using the following equation (1):

$$\text{DNA copy} = \frac{(6.02 \times 10^{23} \text{ copy/mole}) \times \text{DNA amount (g)}}{\text{DNA length (bp)} \times 660 \text{ (g/mol/bp)}} \quad (1)$$

Result was obtained at

$$\begin{aligned} \text{DNA copy} &= \frac{(6.02 \times 10^{23} \text{ copy/mole}) \times 244.3 \times 10^{-9} \text{ (g)}}{4,500 \text{ (bp)} \times 660 \text{ (g/mol/bp)}} \\ &= 4.9 \times 10^{10} \text{ copy/ } \mu\text{l} \end{aligned}$$

3. Serial dilution of EUB DNA

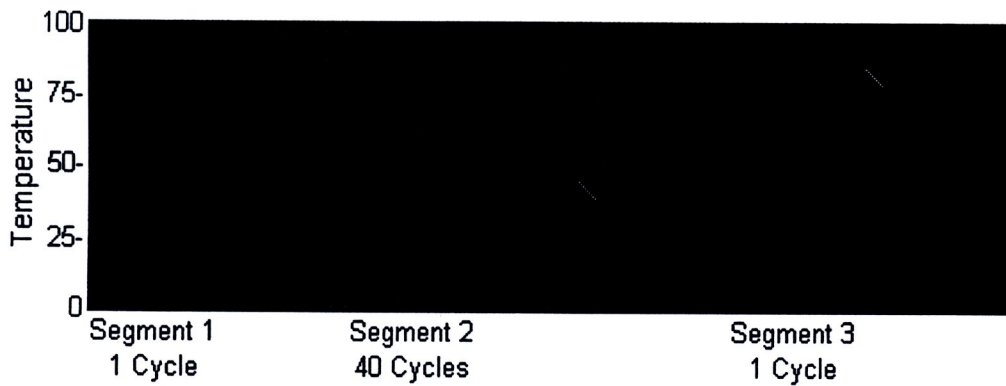
Dilution No.	Preparation				DNA amount (Copy / 2 μ l)
	Template	DNA (μ l)	H ₂ O (μ l)	Total volume (μ l)	
D1	D0	5	45	50	9.8×10^9
D2	D1	10	90	100	9.8×10^8
D3	D2	10	90	100	9.8×10^7
D4	D3	10	90	100	9.8×10^6
D5	D4	10	90	100	9.8×10^5
D6	D5	10	90	100	9.8×10^4
D7	D6	10	90	100	9.8×10^3
D8	D7	10	90	100	9.8×10^2

4. KAPA SYBR® Fast qPCR Kit was used for real-time reactions (KAPA, Brazil). The qPCR assay was performed using a fluorescence-detecting thermocycler (Stratagene Mx3005P). Sample was prepared using the following ratio:

KAPA Master MIX	10	μl
Forward primer (8F)	0.4	μl
Reverse primer (1492R)	0.4	μl
qPCR water	7.2	μl
Template DNA	2	μl
Total volume	20	μl

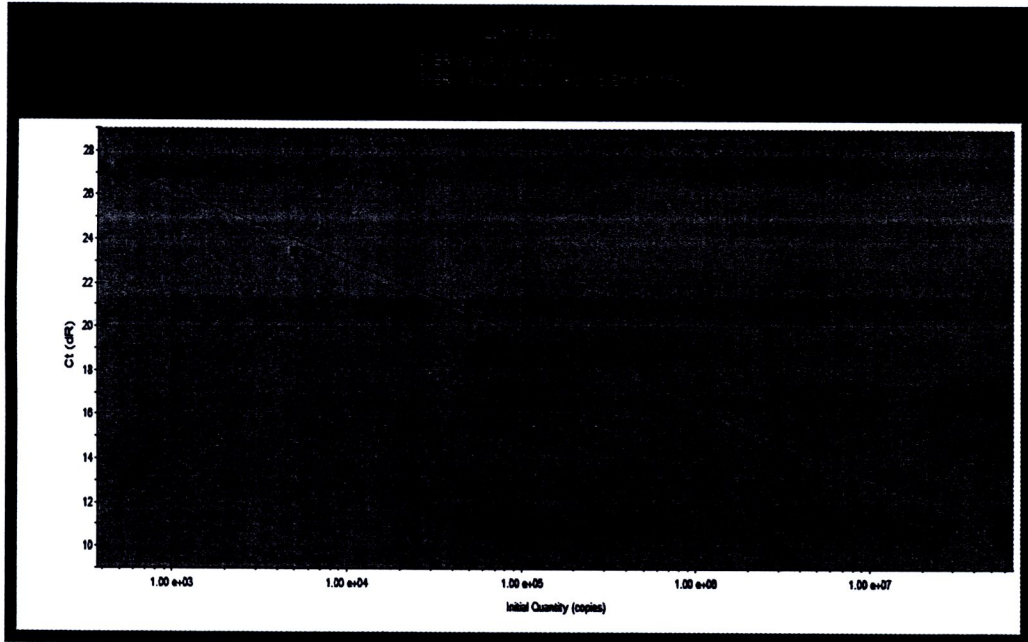
5. Thermal profile of Standard curve generation was shown in figure below:

Thermal Profile
(Estimated Run Time: 02:39:54)



6. Results of EUB standard curve generation

	Linear rang (copy/2 μl)	Slope	R ² of slope	Source strain
EUB	$9.8 \times 10^7 - 9.8 \times 10^2$	-3.286	0.996	ES1



2. Standard curve generation of archaea (ARC)

1. Measurement initial DNA concentration of standard ARC (*Methanosaeta* sp. clone no. POME_ARC54) by using Nano drop machine at 260 nm. Initial ARC DNA concentration was obtained at 38.9 ng/ μ l
2. Calculation DNA copy using the following equation (1). Result was obtained at

$$\text{DNA copy} = \frac{(6.02 \times 10^{23} \text{ copy/mole}) \times 38.9 \times 10^{-9} \text{ (g)}}{4,500 \text{ (bp)} \times 660 \text{ (g/mol/bp)}}$$

$$= 7.8 \times 10^9 \text{ copy/} \mu\text{l}$$

3. Serial dilution of EUB DNA

Dilution No.	Preparation				DNA amount (Copy / 2 μ l)
	Template	DNA (μ l)	H ₂ O (μ l)	Total volume (μ l)	
D1	D0	5	45	50	1.58×10^9
D2	D1	10	90	100	1.58×10^8
D3	D2	10	90	100	1.58×10^7
D4	D3	10	90	100	1.58×10^6
D5	D4	10	90	100	1.58×10^5
D6	D5	10	90	100	1.58×10^4
D7	D6	10	90	100	1.58×10^3
D8	D7	10	90	100	1.58×10^2

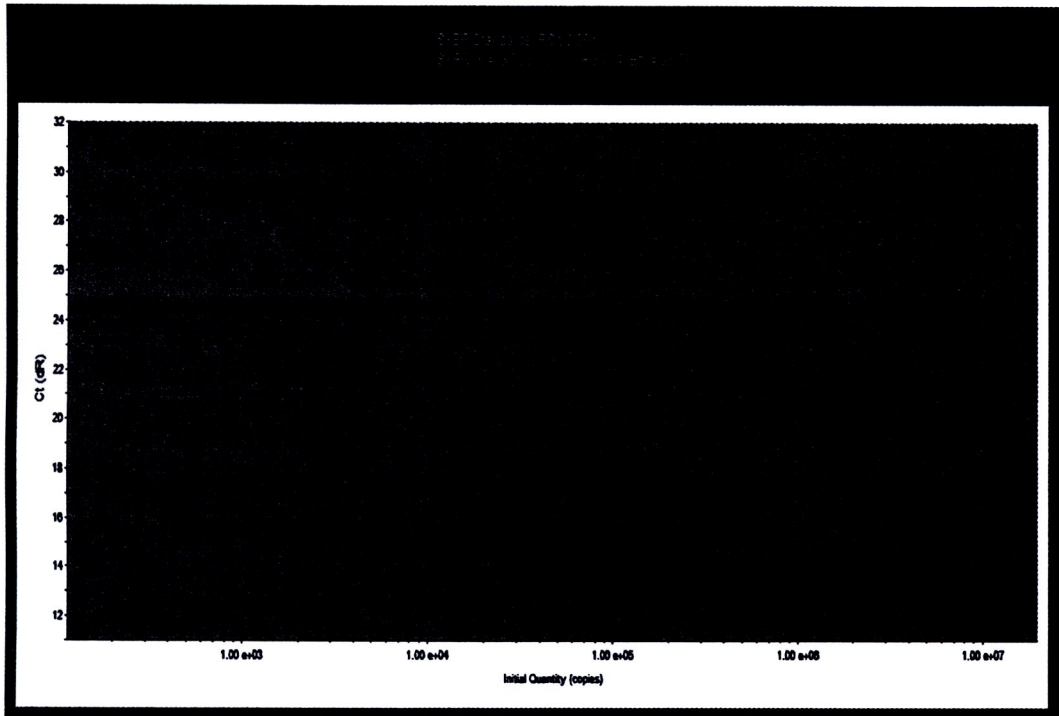
4. KAPA SYBR® Fast qPCR Kit was used for real-time reactions (KAPA, Brazil). The qPCR assay was performed using a fluorescence-detecting thermocycler (Stratagene Mx3005P). Sample was prepared using the following ratio:

KAPA Master MIX	10 μ l
Forward primer (A1F)	0.4 μ l
Reverse primer (1492R)	0.4 μ l
qPCR water	7.2 μ l
Template DNA	2 μ l
<u>Total volume</u>	20 μl

5. Thermal profile of Standard curve generation was similar to EUB

6. Results of ARC standard curve generation

	Linear rang (copy/2 μ l)	Slope	R ² of slope	Source strain
ARC	$1.58 \times 10^7 - 1.58 \times 10^2$	-3.709	0.991	ARC54



3. Quantitative real-time PCR study of microbial population in AHR of POME

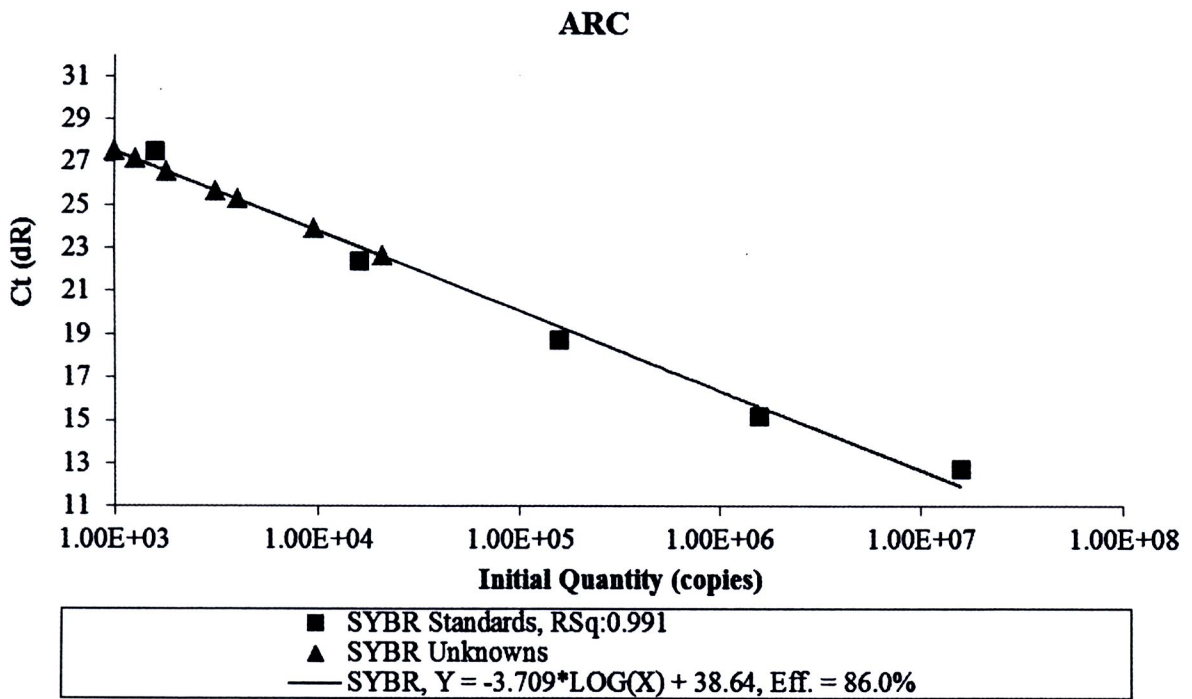
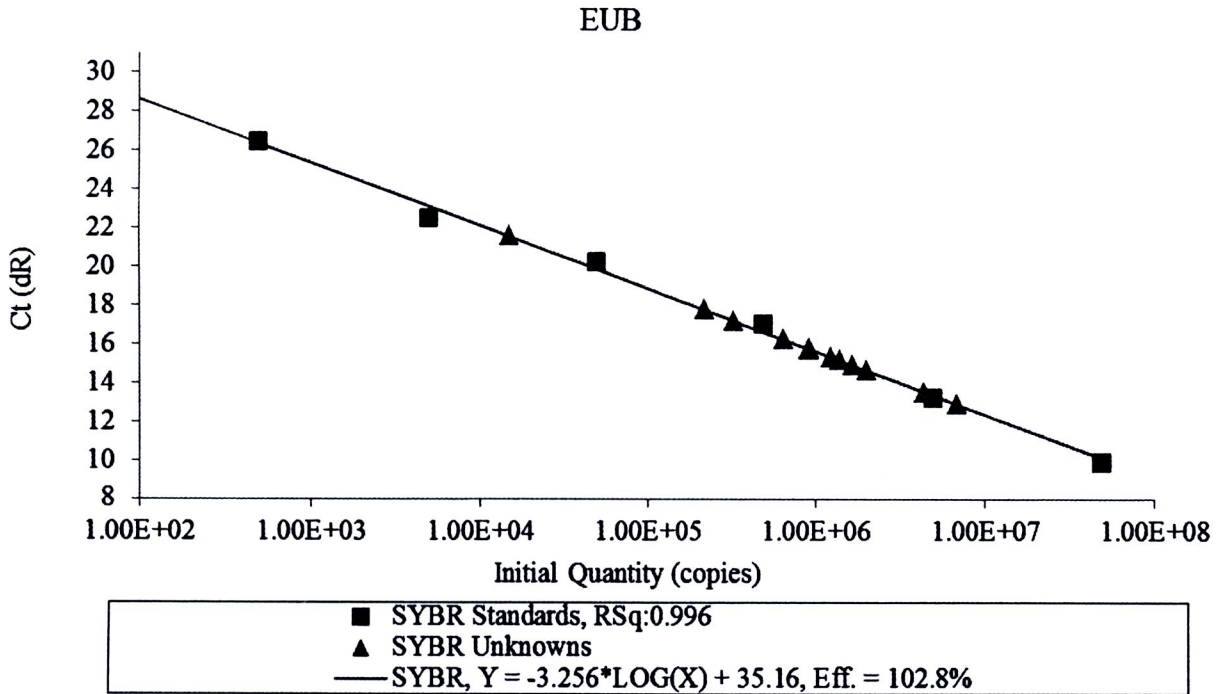


Figure B. Eubacterial (EUB) and archaeal (ARC) populations' analysis by qPCR

Gas composition

Methane in gas was analyzed by GC (Shimadzu, Class-GC 9A) using the following parameters:

Column ID and length:	Porapak-N-80/100, 1/8 inch
Detector:	TCD
Current bridge:	100 mA
Carrier gas:	Helium
Column temperature:	70°C
Detector temperature:	120°C
Injection temperature:	120°C

Biogas was collected by syringe (gas type) 250 µl from U-tube. Gas was injected to GC and then gas composition was reported by integrator of Shimadzu model C-R3A

VFA concentration

VFA in sample was analyzed by GC (Shimadzu, Class-GC 14B) using the following parameters:

Column:	Carbo-pack B-DA/4%carbowax 20M
Detector:	FID
Carrier gas:	Nitrogen
Column temperature:	170°C
Injection temperature:	200°C

VFA in samples was prepared by centrifuging at 10,000 rpm for 10 min to remove the suspended solid. The supernatants were acidified by oxalic acid 0.2 N of 200 µl to convert the VFA into the free acid form. Then the sample was ready for VFA examination with the same method as methane gas analysis. Concentrations of ethanol, acetic acid, butyric acid and propionic acid were reported by integrator of Shimadzu model C-R3A

Alkalinity and Total volatile acid

Alkalinity effluent was determined by titration with 0.05 N H₂SO₄ to the end-point when pH changes to 4.0. Volume of sample and H₂SO₄ were calculated to alkalinity value. The sample with pH 4.0 was then continuously titrated with 0.05 N NaOH to the end-point when pH changes to 7.0. Final volume of NaOH was calculated the TVA value (Kalayanee, 2000).

TCOD and SCOD

Filtrated and infiltrated samples were analyzed SCOD and TCOD, respectively. A fixed volume is added to a sample of the solution being analyzed as potassium permanganate (KMnO₄). After a refluxing digestion step, the initial concentration of organic substances in the sample is using spectrophotometric determination of the oxidant still remaining in the sample.

Total solid, Suspended solid and Volatile suspended solid

Total solid was determined both influent and effluent POME by placing the sample in the 105°C oven for 24 h. Let cool to room temperature in a desiccators. Weigh the solid and record. This is the total solid in influence and effluence. For suspended solid, mixed sample is filtered and dried at 105° C represents the amount of solids suspended in the sample solution. The amount of suspended solids in a water sample may be used as a general indicator of the overall quality of the sample. Suspended solids analyses are important in the control of biological and physical wastewater treatment processes and for assessing compliance with discharge regulations. The residue remaining after drying at 103° C is weighed and placed in a muffle furnace at 550° C. The weight loss from ignition determines "volatile suspended solids". These parameters will were analyze twice times per week.

Oil and Grease

Oil and grease represents a group of substances which have similar physical characteristics defined by their common solubility in an organic extracting solvent. It is essential that this extraction solvent be standardized for each method, because it partially defines the parameters that will be measured in the oil and grease analysis. This study analyzes oil and grease by hexane extraction method.

