Novel bioaugmentation strategy with a syntrophic enrichment for enhanced digestion (SEED) system for maximizing methane yield from municipal sludge

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Municipal sludge management: Major challenge in wastewater treatment

Higher sludge production

Canadian wastewater treatment facilities produce more than **660,000 dry tons (2.5 million wet tons).***

*CCME (Canadian Council of Ministers of the Environment), 2012. Canada-wide approach for the management of wastewater biosolids.

High sludge disposal costs

~40% capital cost and **~50% of operating cost**

Social and environmental concerns

Higher organic matter

Odor

Pathogens

Micropollutant

https://www.pinclipart.com

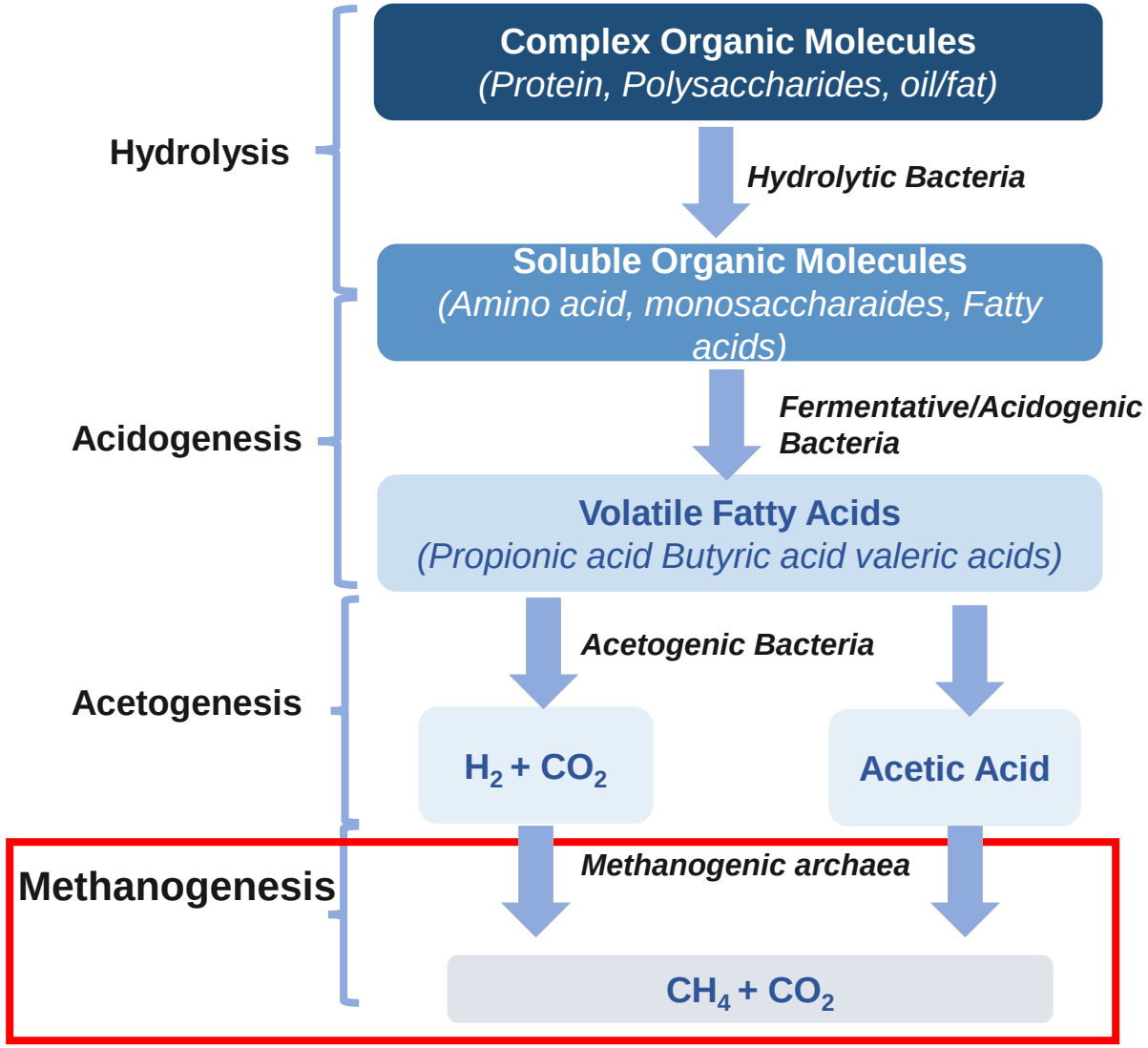
Anaerobic digestion as a sustainable solution

Long retention time (**20-30 days**)

Sensitive to physicochemical stress
(such as pH, temperature, toxins etc.)

http://www.metrovancouver.org/services/liquid-waste/consultations/annacis-island-wwtp/about-project/Pages/default.aspx

Anaerobic digestion: Challenges and approach



Doubling time (~**1-30** days)

Extremely sensitive to environmental stress, O_2, pH, temperature, shear, toxins etc.

All stages of engineered AD system ranging from **1 to 8%** only*

*Płaza G, Jałowiecki Ł, Głowacka D, Hubeny J, Harnisz M, Korzeniewska E. Insights into the microbial diversity and structure in a full-scale municipal wastewater treatment plant with particular regard to Archaea. Plos one. 2021 Apr 26;16(4):e0250514.

Microbial syntrophy: co-existing together

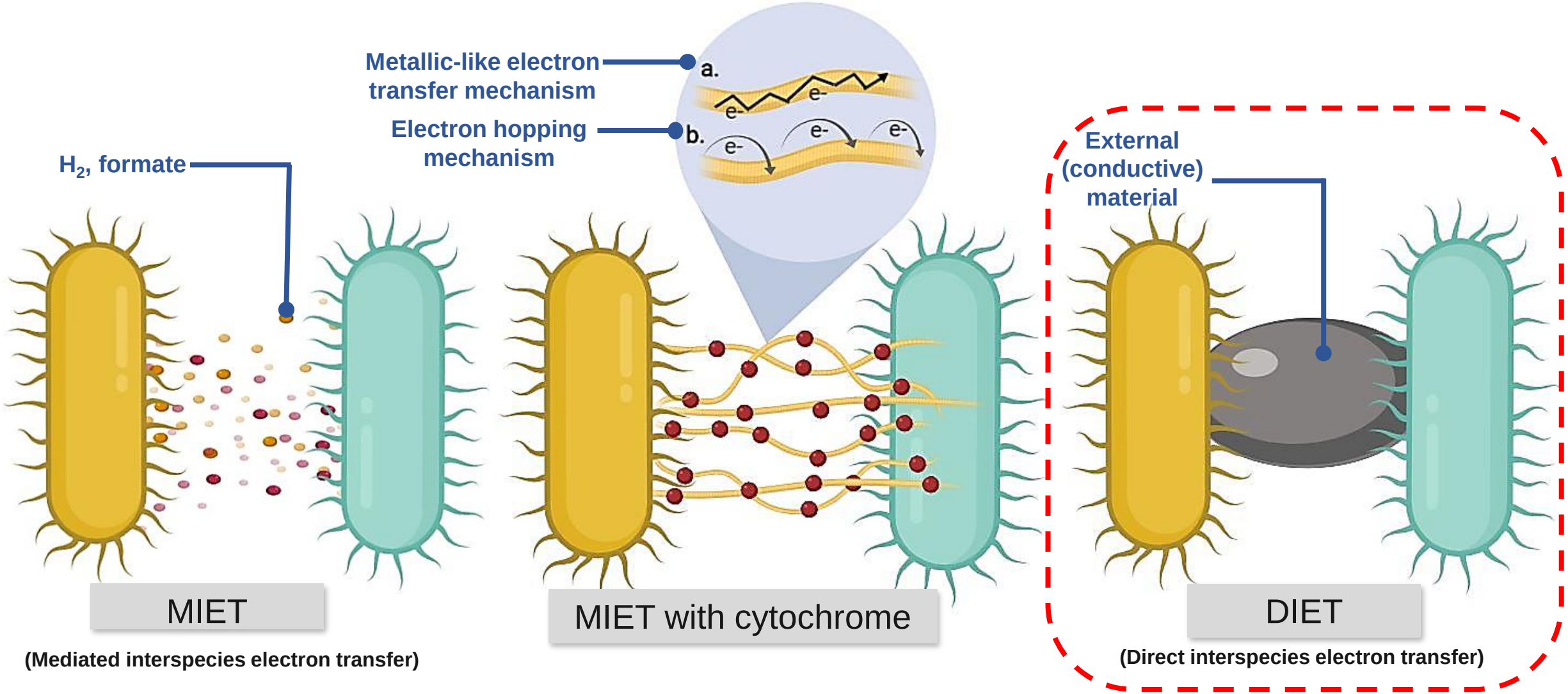
Syntrophic bacteria

Methanogenic archaea

Better communication brings better microbial syntrophy

https://www.pinclipart.com

Microbial Syntrophy: Concept of DIET (Direct interspecies electron transfer)

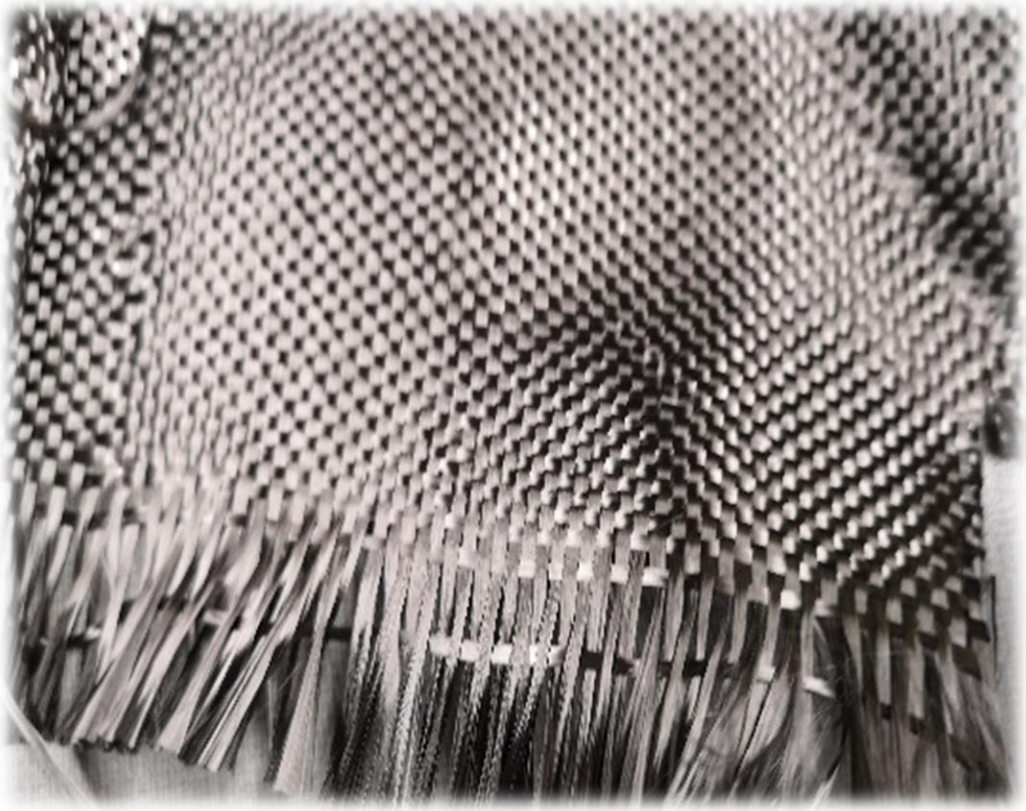


Kumar, V., Nabaterega, R., Khoei, S. and Eskicioglu, C., 2021. Insight into interactions between syntrophic bacteria and archaea in anaerobic digestion amended with conductive materials. *Renewable and Sustainable Energy Reviews*, *144*, p.110965.

Motivation for Research

Suitability of carbon cloth for enhanced anaerobic digestion performance

- Non toxic
- Biocompatible
- Inexpensive
- Conductive
- Retained in the reactor without loss due to washout
- Shows better performance in terms of organic removal compared to graphite rod and biochar*



Commercial carbon cloth represented as untreated carbon cloth (U-CC)

*Zhao Z, Zhang Y, Woodard TL, Nevin KP, Lovley DR. Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. Bioresour Technol 2015;191:140–5.

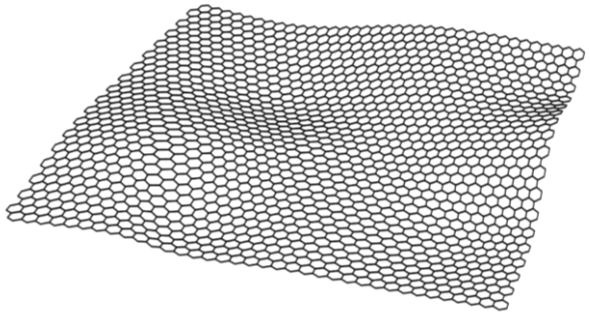
Research Questions

Is it possible to **develop a more biocompatible surface using carbon cloth** for the attachment of methanogens that promotes microbial growth leading to higher methane yield utilizing municipal sludge?

Is it possible to **build a side-stream reservoir of an active microbial culture** rich in methanogenic archaea grown on **carbon cloth** for bioaugmentation of conventional anaerobic digesters?

Methodology and approach

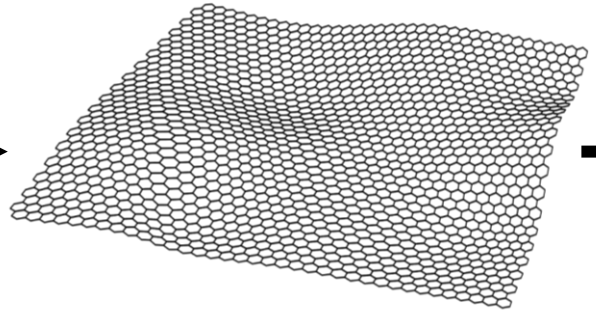
Activation of carbon cloth



Untreated carbon cloth
(U-CC)

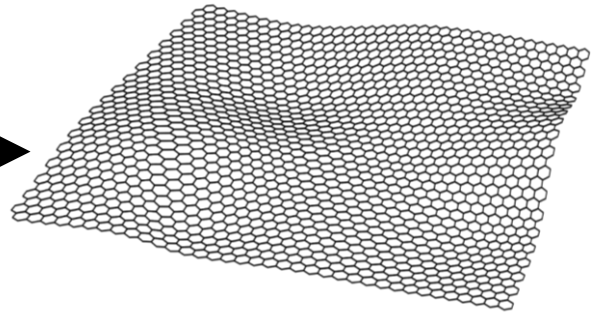
Step 1:

2 M H_2SO_4/ 20% HNO_3 (24 hours)

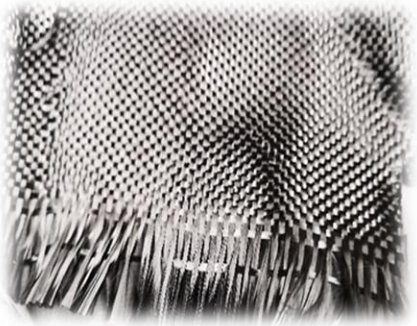


Step 2:

Air, Δ 550°C (3 hours)



Activated carbon cloth
(**A-CC**)



Methodology and approach

Biochemical methane potential assays with/without carbon cloth

Conditions:
Food to microorganism ration (F/M ratio) 1.0 (g VS_{feed}/g $VS_{inoculum}$); Temperature: 55°C;
Substrate: Thickened screened primary sludge (TSPS);
Inoculum: Thermophilic anaerobic reactor (55°C, 20 days SRT)

- ✓ Control [no CC]
- ✓ Blank-1 [with inoculum only]
- ✓ Blank-2 [inoculum with U-CC (1 g)]
- ✓ Blank-3 [inoculum with A-CC (1 g)]

Total BMP = 152 bottles

Batch-1

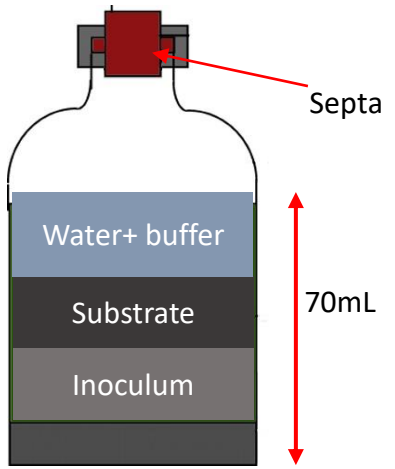
Contribution of each CC pretreatment step on methane improvement

Batch-2/Batch-3

Optimization of CC dose on rate/extent of methane improvement

Effect of CC on microbial shift (included to journal articles only)

- ✓ Blank-3 [inoculum with A-CC (1 g)]
- ✓ (0.20, 0.40. 0.80, 1.00 and 1.20 g) U-CC
- ✓ (0.20, 0.40. 0.80, 1.00 and 1.20 g) A-CC

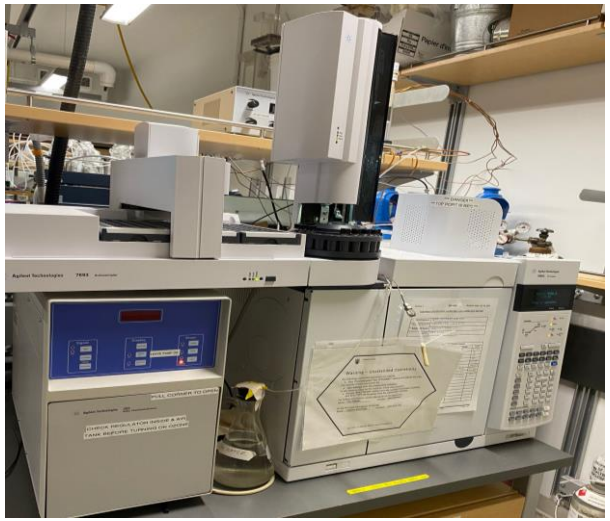


CC: Carbon cloth, A-CC: Activated carbon cloth, NiPcS: Nickel (II) phthalocyanine-tetrasulfonic acid tetrasodium salt complex, VS: Volatile solids

Methodology and approach

List of BTG equipment used for the experimental studies

Agilent 7820A gas chromatography with thermal conductivity detector



Agilent 7890A gas chromatography with flame ionization detector

Homogenizer

Incubator shaker

Spectrophotometer

Thermotron (Temperature controlled chamber)

Hydrothermal reactor

Methodology and approach

Facilities utilized outside BTG for the experimental studies

(for carbon cloth characterization)

- **BET analysis**: Micromeritics ASAP 2000 equipment at the Catalysis and Chemical Reaction Engineering Laboratories at the University of Saskatchewan, Canada
- **Raman spectroscopy**: LabRam spectrometer for Raman spectroscopy at UBC Okanagan
- **FTIR spectroscopy**: Nicolet Magna 850 Fourier transform spectrometer
- **XRD analysis**: Bruker D8-Advance X-ray diffractometer at UBC Vancouver
- **FTIR analysis**: Nicolet iS20 instrument at NPNL UBC Okanagan
- **SEM analysis**: Tescan Mira 3 XMU Scanning Electron Microscope at FiLTER (Fipke Laboratory for Trace Element Research) Laboratory at UBC Okanagan
- **Edx analysis**: Oxford Instruments X-126 at FiLTER Laboratory at UBC Okanagan.
- **Carbon cloth electrical conductivity:** UBC Okanagan, Physics laboratory

Surface characterization of various carbon cloth supplement

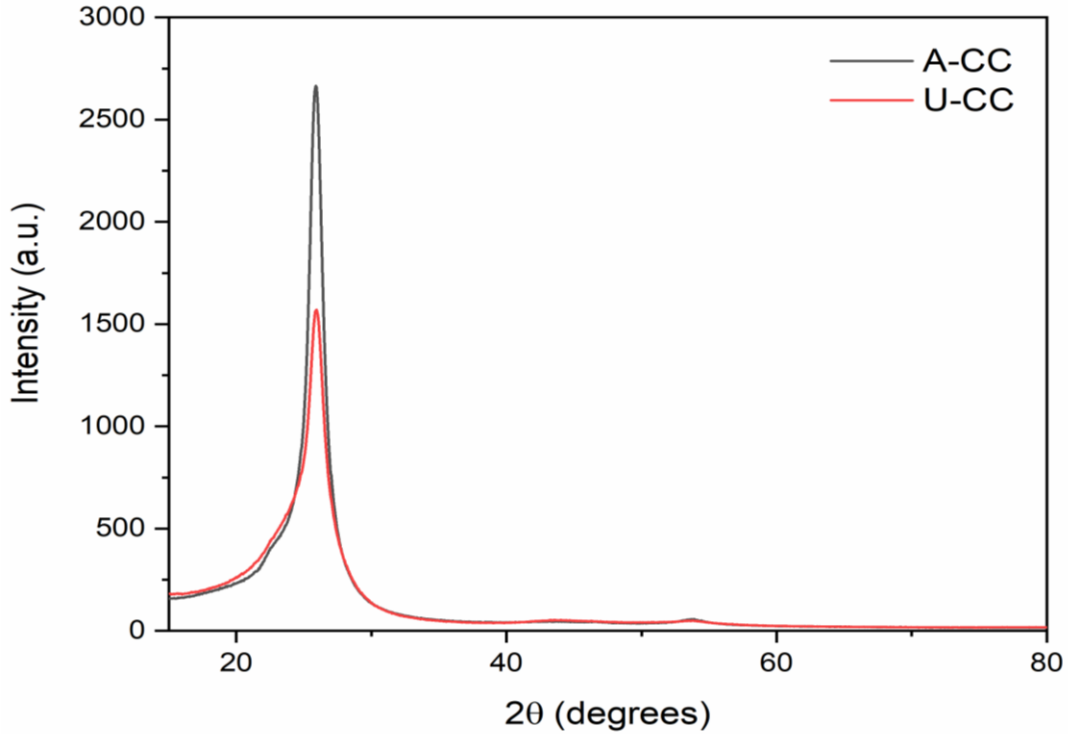


Fig. XRD (X ray diffraction) analysis of CC

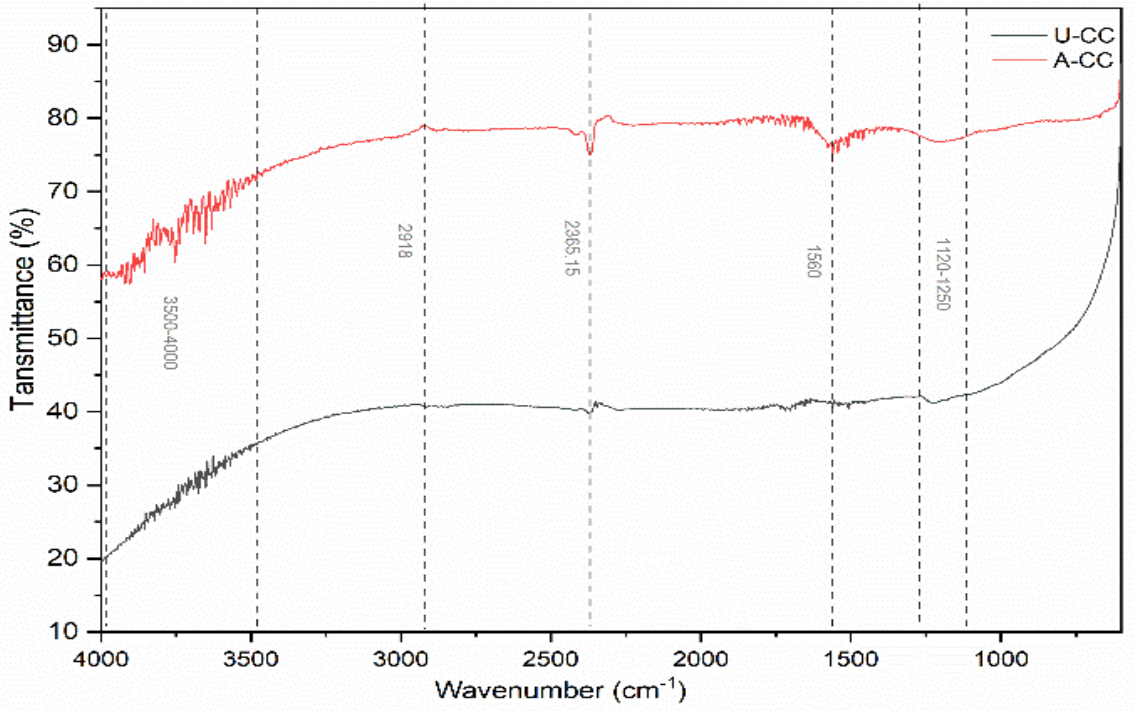


Fig. FTIR (Fourier transform Infrared spectroscopy) analysis of CC

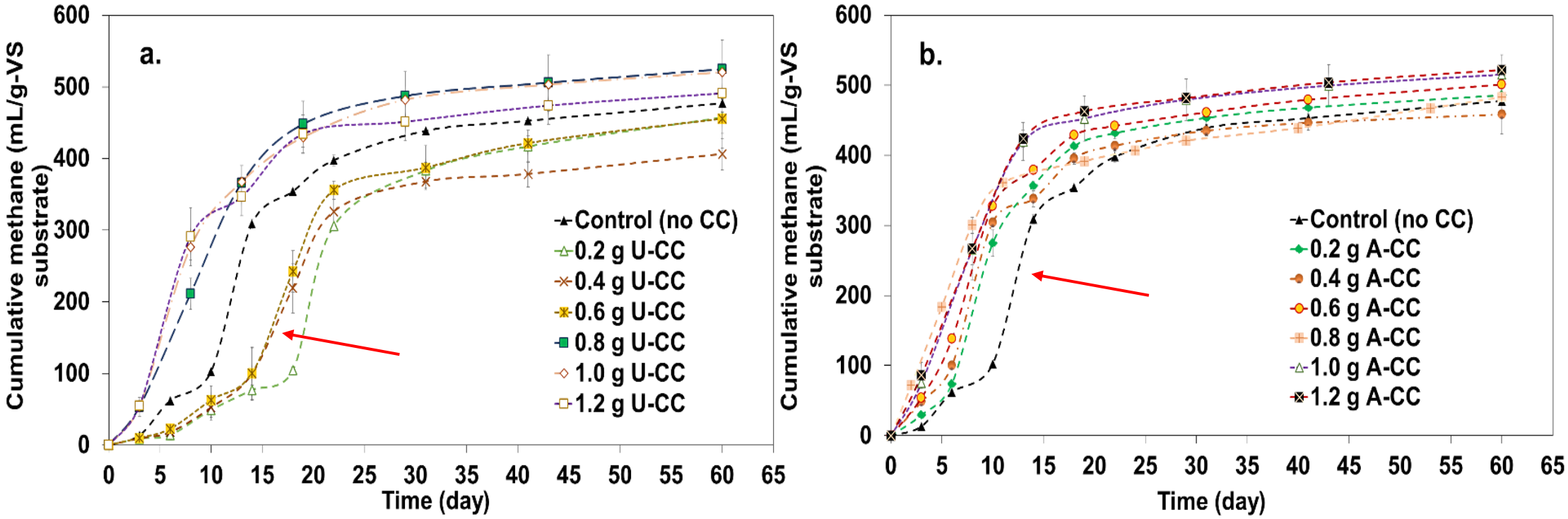
Table BET and BJH analysis for U-CC and A-CC

	Surface area (m^2/g)	Pore volume (BJH) (cm^3/g)	Pore size (nm)
U-CC	0.45 (BJH)	0.001	16.13 (BJH)
A-CC	386.15 ± 6.88 (BET); 195.60 (BJH)	0.151	3.08 (BJH)

BJH: Pore size determination
BET: Surface area analysis

U-CC: Untreated carbon cloth, A-CC: Activated carbon cloth, BJH: Barrett, Joyner, and Halenda, BET: Brunauer-Emmett-Teller

Performance of carbon cloth as a potential high performance AD supplement using BMP assays



Effect of carbon cloth dosing on **(a, b)** specific cumulative at standard temperature and pressure (STP) (0ºC, 1 atm) from municipal sludge. U-CC: untreated carbon cloth, A-CC: activated carbon. Data represent average and error bars represent standard deviations of triplicates.

Kinetic modeling results of U-CC and A-CC for dose optimization BMP assays

Modified Gompertz model

$$Y = P \times exp\left[-exp\left\{\frac{Rm \times e}{P}(\lambda - t) + 1\right\}\right]$$

Y = specific cumulative methane production (ml/g-$VS_{substrate}$)
t = time (days)
P = ultimate specific cumulative methane production (ml/g-$VS_{substrate}$)
R_m = maximum methane production rate (ml/g-$VS_{substrate}$/d),
e = natural logarithm constant
λ = lag phase time (days)

0.8 g A-CC

Table: Kinetic model results

BMP amendment conditions	Rate of methane production (R_m) (ml/g-$VS_{substrate}$/d)	Lag phase (λ) (day)
Control (no CC)	25.46 ± 0.33	4.85 ± 0.05
0.2 g U-CC‡	15.40 ± 1.51	9.10 ± 0.97
0.4 g U-CC	15.70 ± 2.60	8.09 ± 1.69
0.6 g U-CC	17.25 ± 3.15	7.34 ± 1.12
0.8 g U-CC	32.99 ± 0.87	2.03 ± 0.44
1.0 g U-CC	40.71 ± 1.91	2.11 ± 0.10
1.2 g U-CC	42.49 ± 6.11	2.07 ± 0.06
0.2 g A-CC	32.37 ± 0.24	3.36 ± 0.11
0.4 g A-CC	31.03 ± 1.64	2.69 ± 0.81
0.6 g A-CC	35.24 ± 3.00	2.10 ± 0.19
0.8 g A-CC	37.03 ± 11.94	0.31 ± 0.34
1.0 g A-CC	38.17 ± 3.25	1.37± 0.34
1.2 g A-CC	36.82 ± 3.79	1.01 ± 0.39

P<0.05 (Rm: 0.2 g U-CC – 0.6 g U-CC)
P>0.05 (Rm: 0.8 g U-CC – 1.2 g U-CC)
P<0.05 (Rm: 0.2 g A-CC – 0.6 g A-CC)
P<0.05 (λ: 0.2 g U-CC – 0.6 g U-CC)
P<0.05 (λ: 0.2 g A-CC – 0.6 g A-CC)

‡VS: volatile solids, CC: carbon cloth, U-CC: untreated carbon cloth, A-CC: activated carbon cloth. BMP: Biochemical methane potential ±The dosages of 0.20, 0.40, 0.60, 0.80, 1.00, and 1.20 g carbon cloth correspond to 0.37, 0.74, 1.11, 1.48, 1.85 and 2.22 g U-CC or A-CC/g-$VS_{substrate}$, respectively. *mean ± standard deviation of triplicate BMP bottles

Bio-augmentation strategy schematic diagram

Thermophilic: 55°C
Substrate: MS
SRT: 20 and 10 days

Control (baseline) digester

Mixed sludge

Digestate

Control, 55°C

Test (bioaugmented) digester

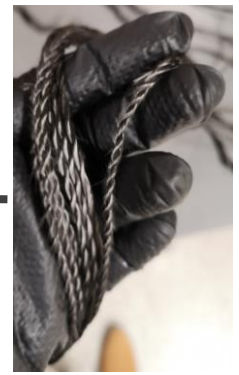
Mixed sludge

Digestate

TEST, 55°C

Microbial recycling (centrifuged pellets) to prevent microbial loss

SEED reactor
Thermophilic: 55°C
Substrate: Centrifuged AP effluent
HRT: 20; 16; 8 and 5 days



Archaea enriched stream (pellet)

Centrifugation#

Centrate (discard)

Acid phase (AP) reactor

Thickened screened primary sludge

AP, 55°C

Centrifugation#

Centrifuged AP effluent (volatile fatty acids rich stream)

solids (discard)

Acid phase (AP)
Thermophilic: 55°C
Substrate: TSPS
SRT: 3 days

RPM: 3500
Time: 15 min

SEED: Syntrophic enrichment for enhanced digestion, VFA: Volatile fatty acids, HRT: Hydraulic retention time, SRT: Solid retention time, MS: Mixed sludge, TSPS: Thickened screened primary sludge #Centrifugation condition: 3500 revolution per minutes for 10 minutes

Methodology and approach

The SEED, Test and Control reactor pictures

Bench-scale reactor vessels at BTG laboratory

SEED reactor (5 L*)

BTG: Bioreactor technology group, SEED: Syntrophic enrichment for enhanced digestion, *working volume

Methodology and approach

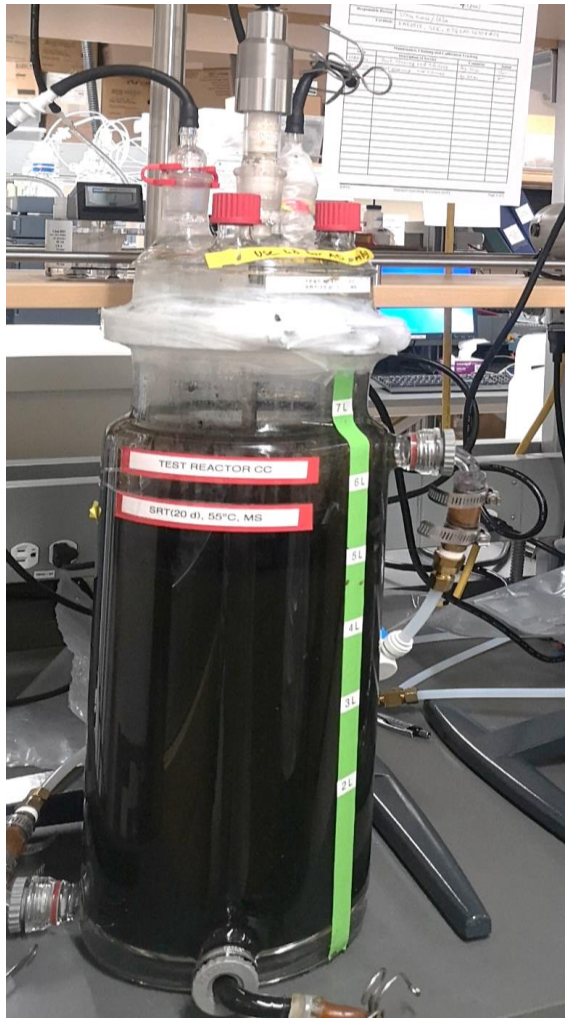
Bio-augmentation 1: Liquid bioaugmentation (LB) strategy

SEED reactor (5 L*)

Increase mixing speed (120 rpm 1 h) in SEED for sloughing off the microbial biomass before bioaugmentation

Frequency of **bio-augmentation 1 (LB)**: 8 days

1500 ml effluent from Test reactor replaced with 1500 ml effluent from SEED reactor.



Test reactor (5 L*)

*working volume

SEED: Syntrophic enrichment for enhanced digestion, rpm: revolution per minutes

Methodology and approach

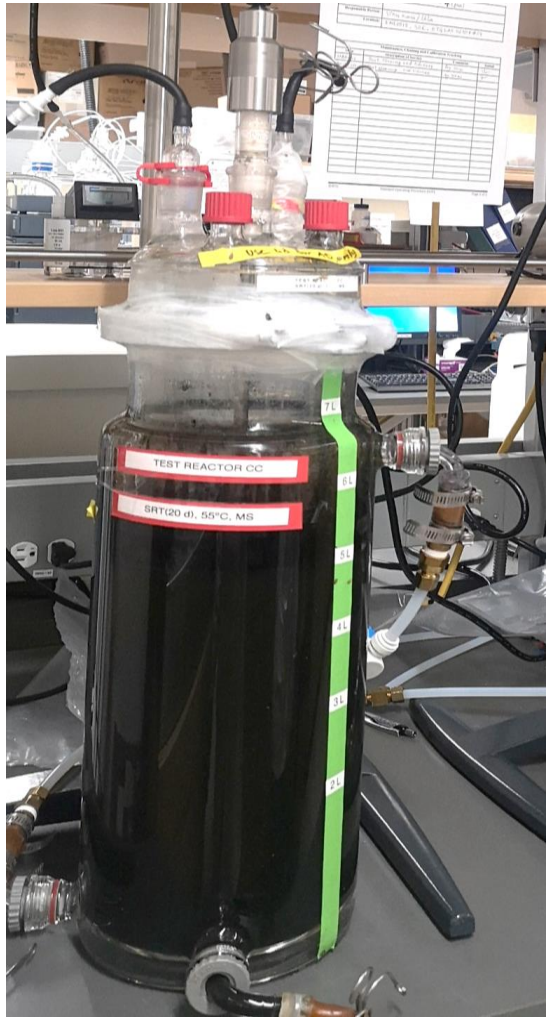
Bio-augmentation 2: Pellet bioaugmentation (PB) strategy

SEED reactor (5 L*)

Increase mixing speed (120 rpm 1 h) in SEED for sloughing off the microbial biomass before bioaugmentation

Frequency of **bio-augmentation 2 (PB)**: 7-15 days

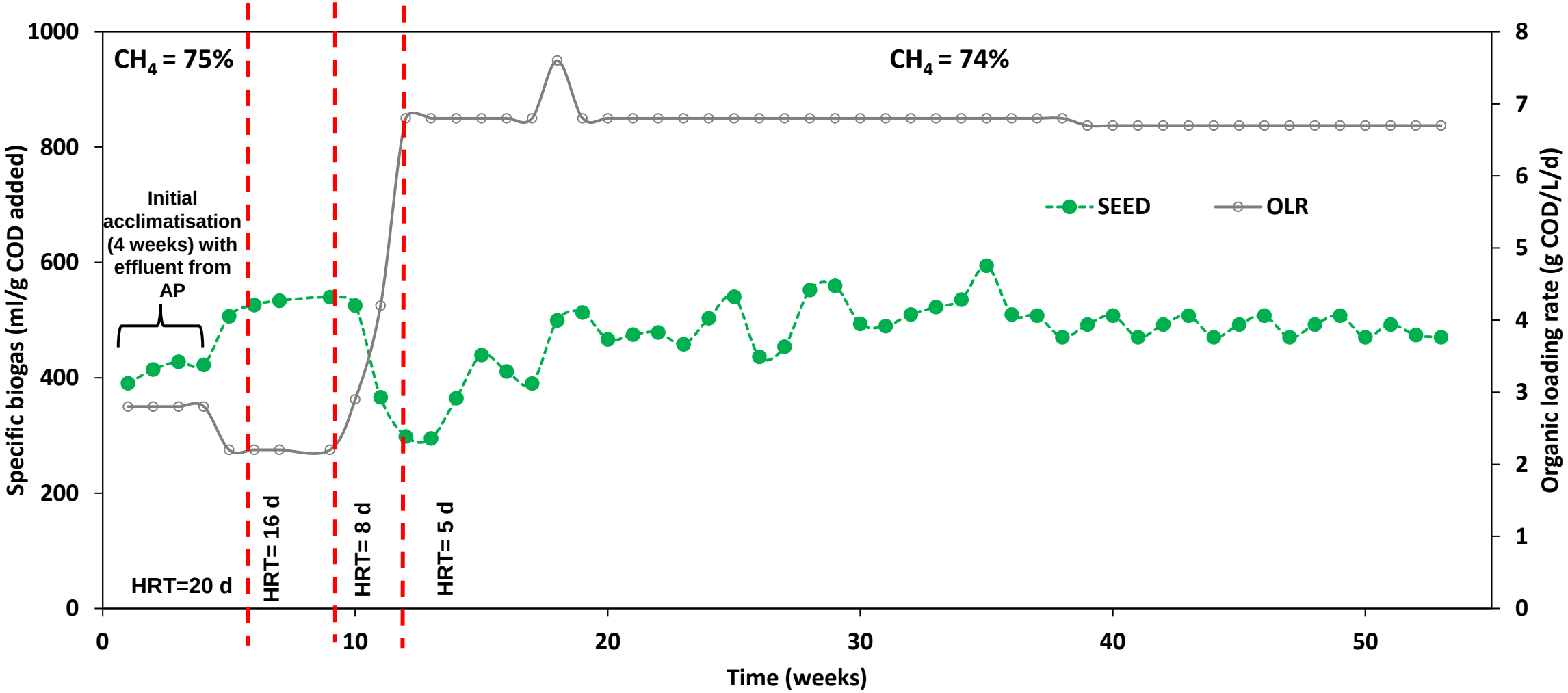
Test reactor effluent was replaced with SEED effluent centrifuged (pellet).



Test reactor (5 L*)

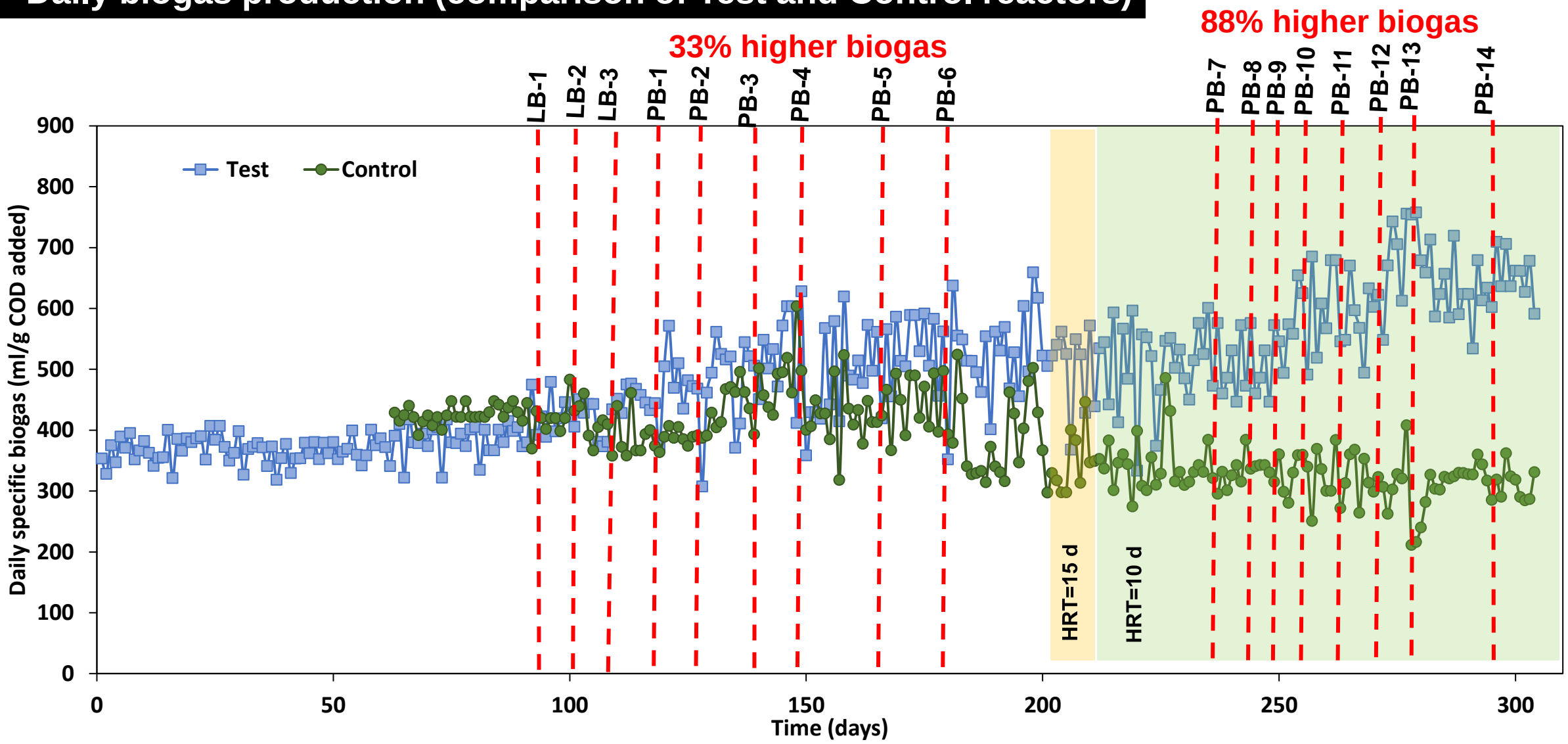
*working volume SEED: Syntrophic enrichment for enhanced digestion, rpm: revolution per minutes

Specific biogas production for SEED reactor



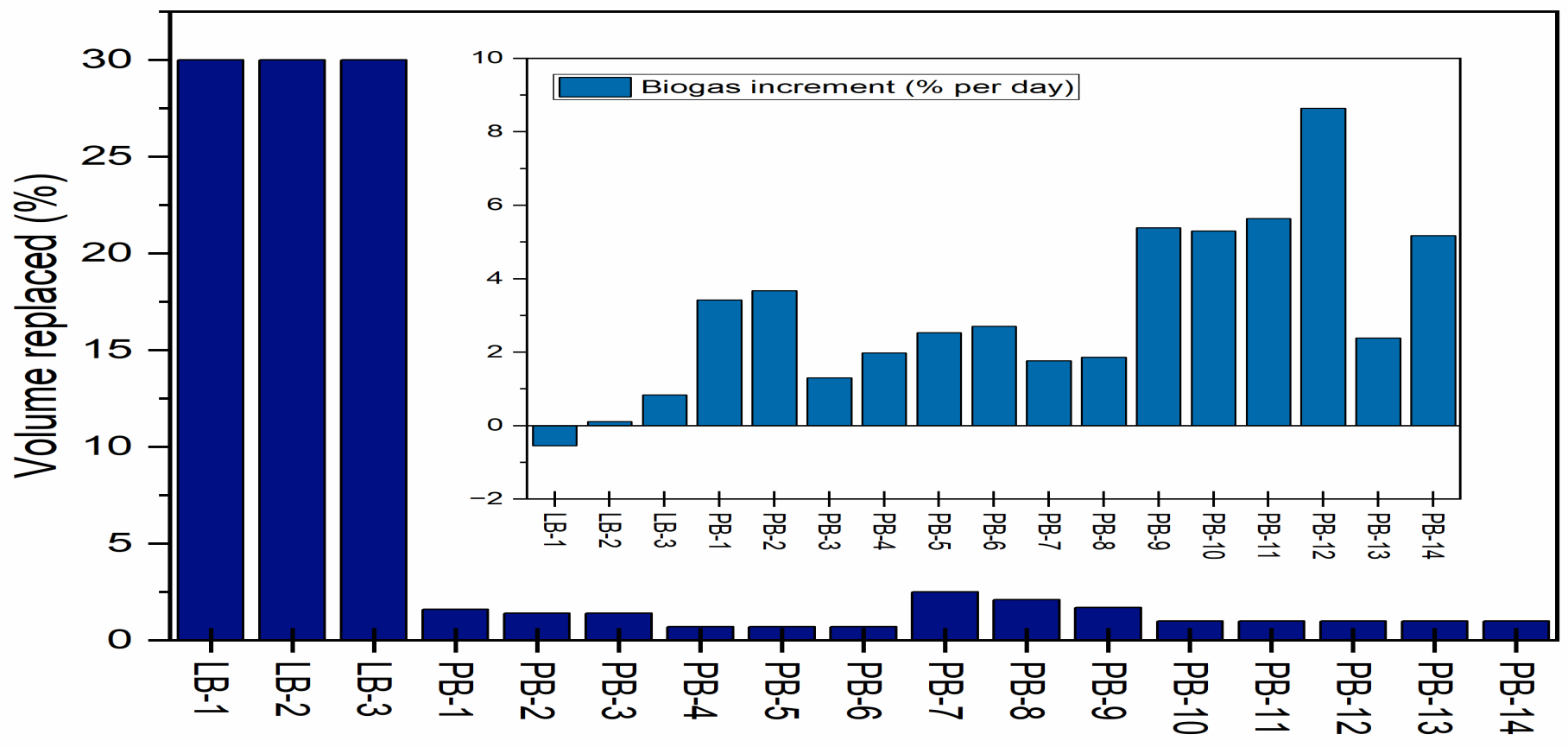
COD: Chemical oxygen demand, OLR: Organic loading rate, SEED: Syntrophic enrichment for enhanced digestion, AP: acid phase reactor, HRT: hydraulic retention time

Daily biogas production (comparison of Test and Control reactors)



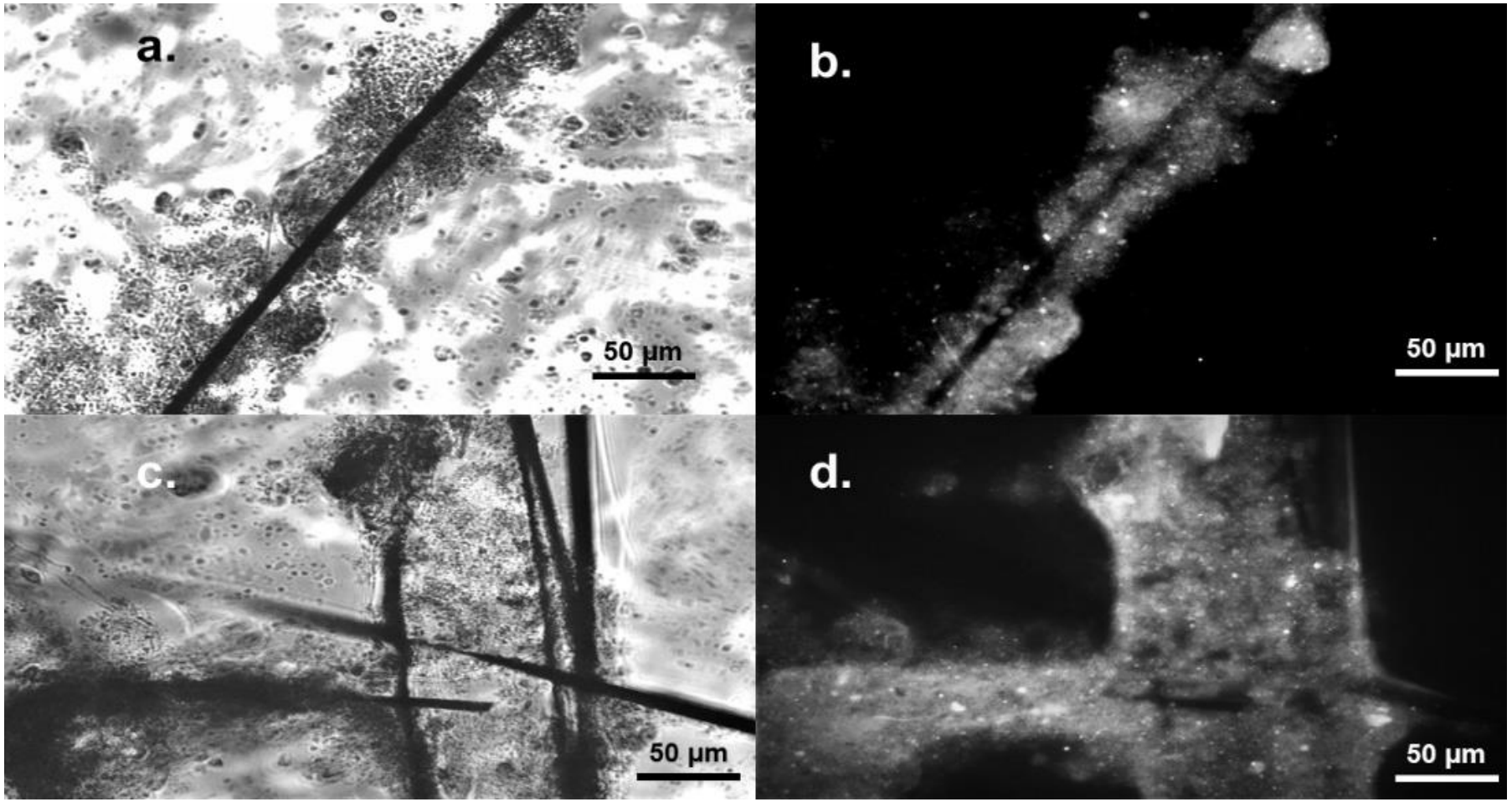
COD: Chemical oxygen demand, HRT: Hydraulic retention time, LB: Liquid bioaugmentation, PB: Pellet bioaugmentation

Impact of bio-augmentation on methane increment at varying hydraulic retention time (HRT)



LB: liquid bioaugmentation, PB: pellet bioaugmentation, COD: Chemical oxygen demand

Fluorescent microscopy for microbial biofilm analysis



Microscopy images of activated carbon cloth from SEED reactor (at COD of ~3,500 rpm, 20 min) (**a. and c.**) bright field images and (**b. and d.**) flourscent images with FilmTracer™ SYPRO® Ruby biofilm and DAPI (4′,6-diamidino-2-phenylindole) cell staining

Conclusions

Advantages of CC

- **Superior performance in terms of COD removal** and microbial adhesion.
- **Retained within the reactor without washout loss**

Two-step activation of CC

- Improves **surface area and biocompatibility.**
- **Optimized dosage 1.48 g A-CC/g-$VS_{substrate}$**

Novel SEED reactor with A-CC

- **Pellet bioaugmentation** allows active microbial transfer and improved higher methane at **shorter HRT.**
- Side-stream SEED incubator can be acts a retrofit model in existing treatment plant without modifications.

CC: Carbon cloth, A-CC: Activated carbon cloth, COD: Chemical oxygen demand SEED: Syntrophic enrichment for enhanced digestion, rpm: revolution per minutes

Thank you

BIOREACTOR TECHNOLOGY GROUP

metrovancouver

NSERC CRSNG

Science and Engineering Research Board (SERB)
Department of Science and Technology (DST)
Govt. of India

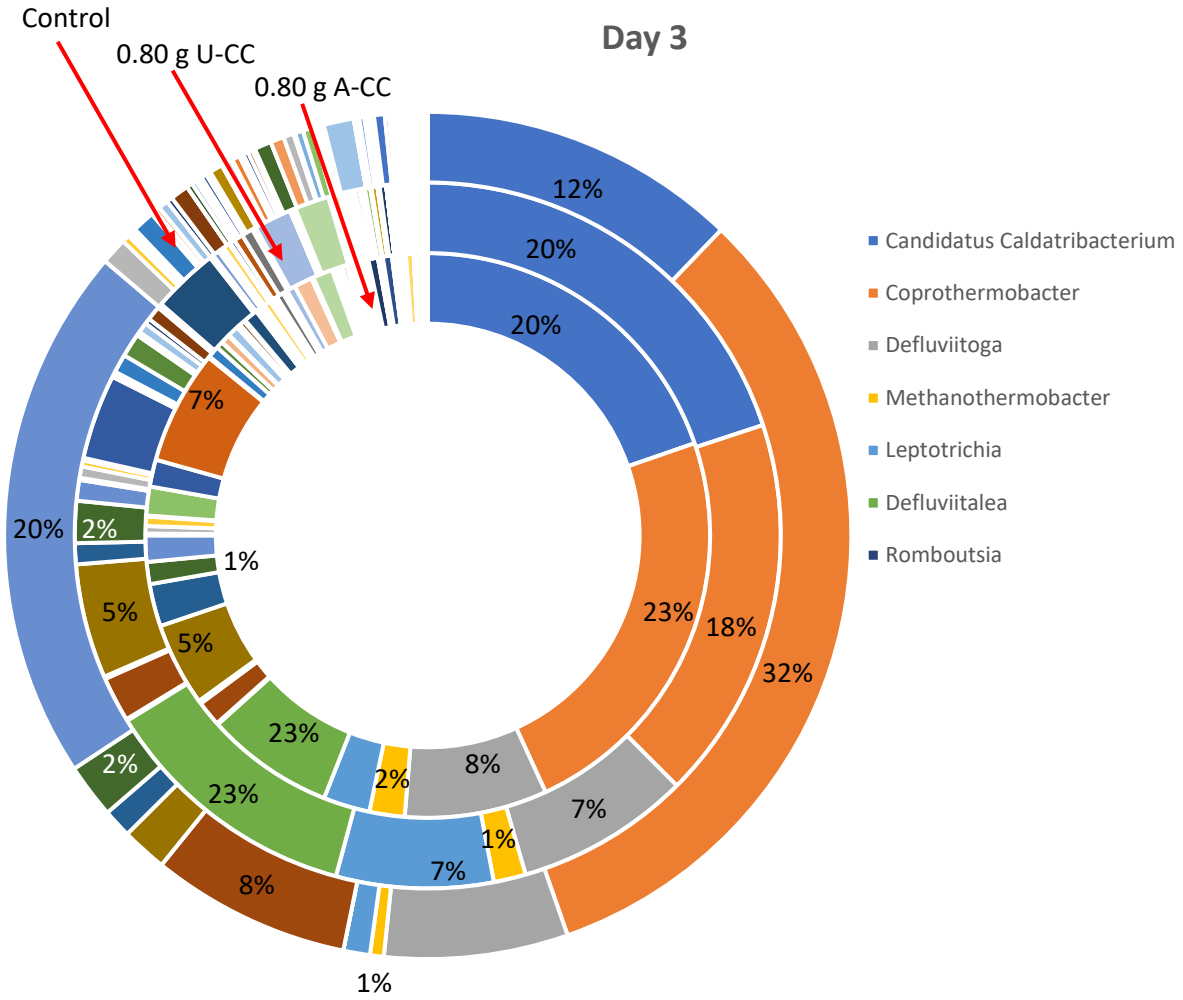
Continuous flow digester testing with carbon cloth medium (before and after to bioaugmentation)

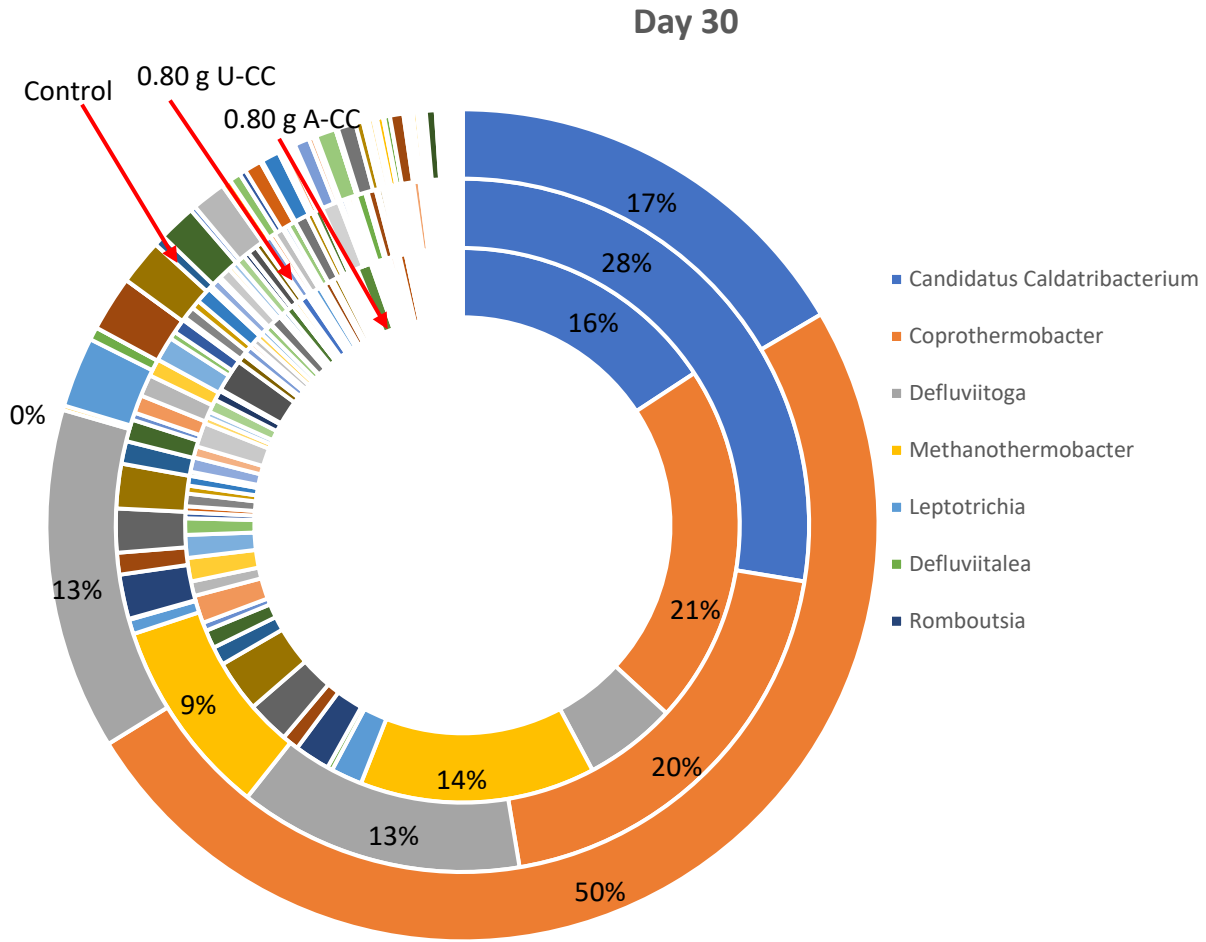
Parameters	Test			Control		SEED	AP reactor
	SRT 20 days (No bio-augmentation)	Bio-augmentation (HRT 20 days)	Bio-augmentation (HRT 10 days)	SRT 20 days	SRT 10 days		
% CH_4	63 (1; 27)*	65 (2; 15)	65 (1; 12)	63 (1; 28)	63 (1; 6)	74 (1.22; 54)	NA
pH	7.8 (0.1; 47)	7.6 (0.1; 51)	7.6 (0.1; 13)	7.8 (0.1; 60)	7.7 (0.1; 8)	7.6 (0.1; 30)	NA
Total ammonium nitrogen (mg/l)	1379 (229; 12)	1401 (100; 14)	1499 (199; 10)	1467 (203; 20)	1500 (303; 8)	1083 (0.09; 34)	915 (24; 60)
Total COD removal (%)	67 (1; 12)	74 (2; 14)	70 (1; 10)	66 (1; 20)	64 (1; 8)	88 (2.9; 34)	NA
TS removal (%)	58 (1; 12)	57 (2; 16)	56 (2; 8)	58 (1; 20)	58 (2; 8)	NA	NA
VS removal (%)	68 (0.3; 12)	64 (1; 16)	63 (1; 8)	67 (1; 20)	66 (2; 8)	NA	NA
Alkalinity (mg $CaCO_3$/l)	4905 (198; 12)	4395 (300; 14)	3985 (298; 10)	5189 (199; 20)	3889 (204; 8)	3566 (99; 34)	1450 (42; 20)
VFA (mg/l)	<100	<100	<100	<100	<100	<100	4020 (66; 33)
VFA/Alkalinity ratio (-)	~0.02	~0.02	~0.02	~0.02	~0.02	~0.02	NA
Average biogas (ml/g COD_{added})	373 (23; 90)	508 (71; 81)	576 (88; 95)	383 (43; 137)	306 (37; 103)	478; 58	NA

TS: Total solids, VS: Volatile solids, COD: Chemical oxygen demand, VFAs: Volatile fatty acids (acetic, butyric and propionic acids), OLR: Organic loading rate, SEED: Syntrophic enrichment for enhanced digestion, MS: Mixed sludge, AF: Acid fermenter

Results and discussion

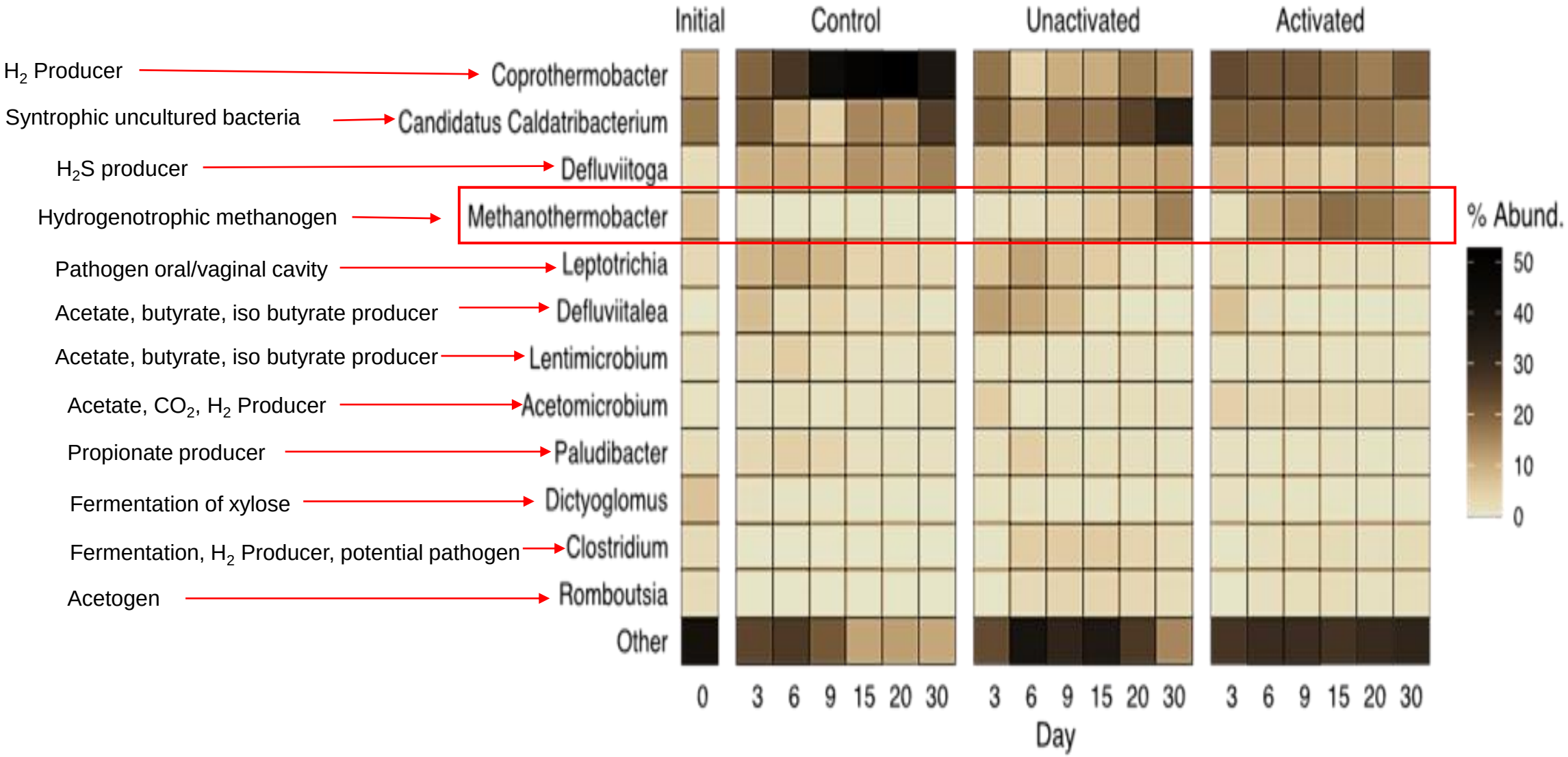
Variation in microbial diversity in BMP reactors utilizing U-CC and A-CC respectively





Supplementary slides

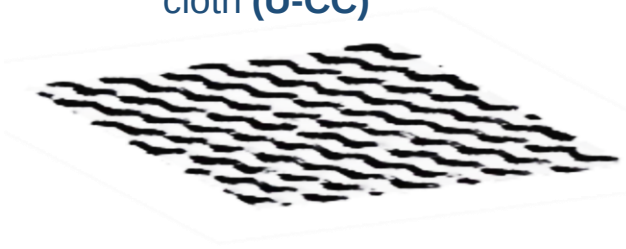
Microbial fingerprinting analysis of BMP bottles (Batch-4) utilizing A-CC supplement



Supplementary slides

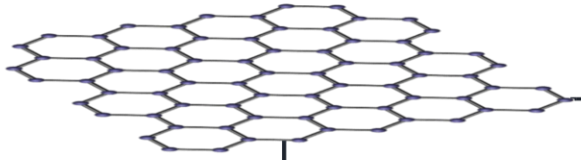
Synthesis of activated carbon cloth

Untreated carbon cloth **(U-CC)**

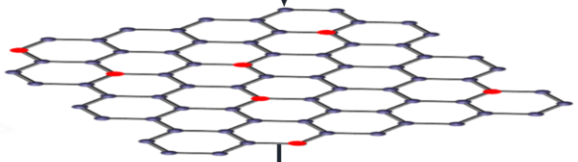


Carbon Cloth (CC)

Untreated Carbon Cloth (U-CC)
- hydrophibic
- low surface area

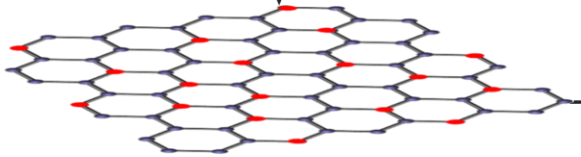


STEP 1
2M H_2SO_4/20% HNO_3



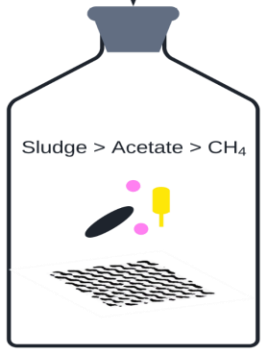
STEP 2
Air, 500°C, 3 Hours

O_2 doping



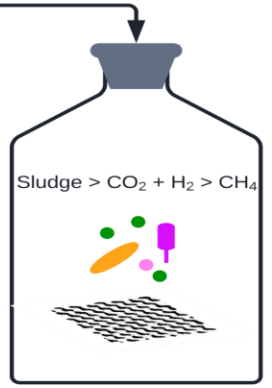
Activated Carbon Cloth (A-CC)
- hydrophilic
- increased surface area

Biochemical Methane Potential (BMP)



BMP U-CC

- higher CC dose
- reduced CH_4 yield
- reduced kinetic rate
- acetoclastic indicators

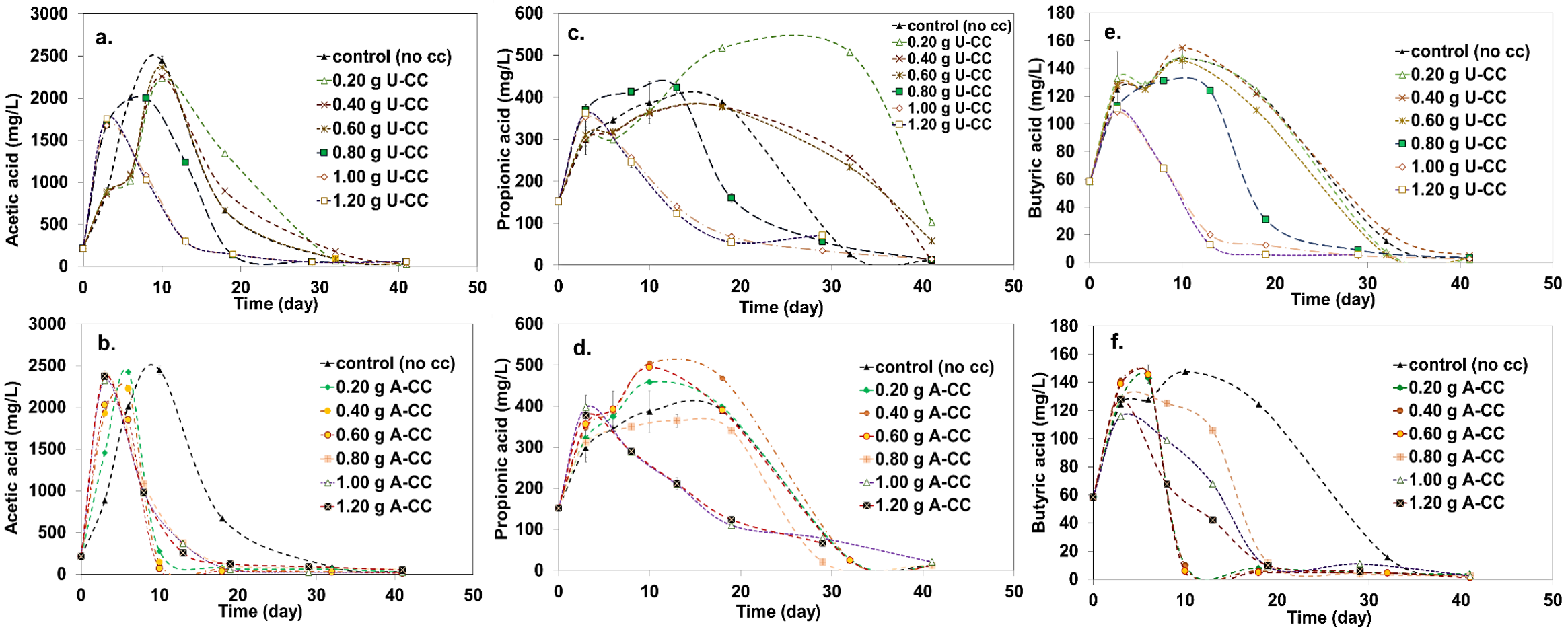


BMP A-CC

- lower CC dose
- increased CH_4 yield
- increased kinetic rate
- hydrogenotrophic indicators

Supplementary slides

Characterization of carbon cloth as a potential high performance AD supplement using BMP assays

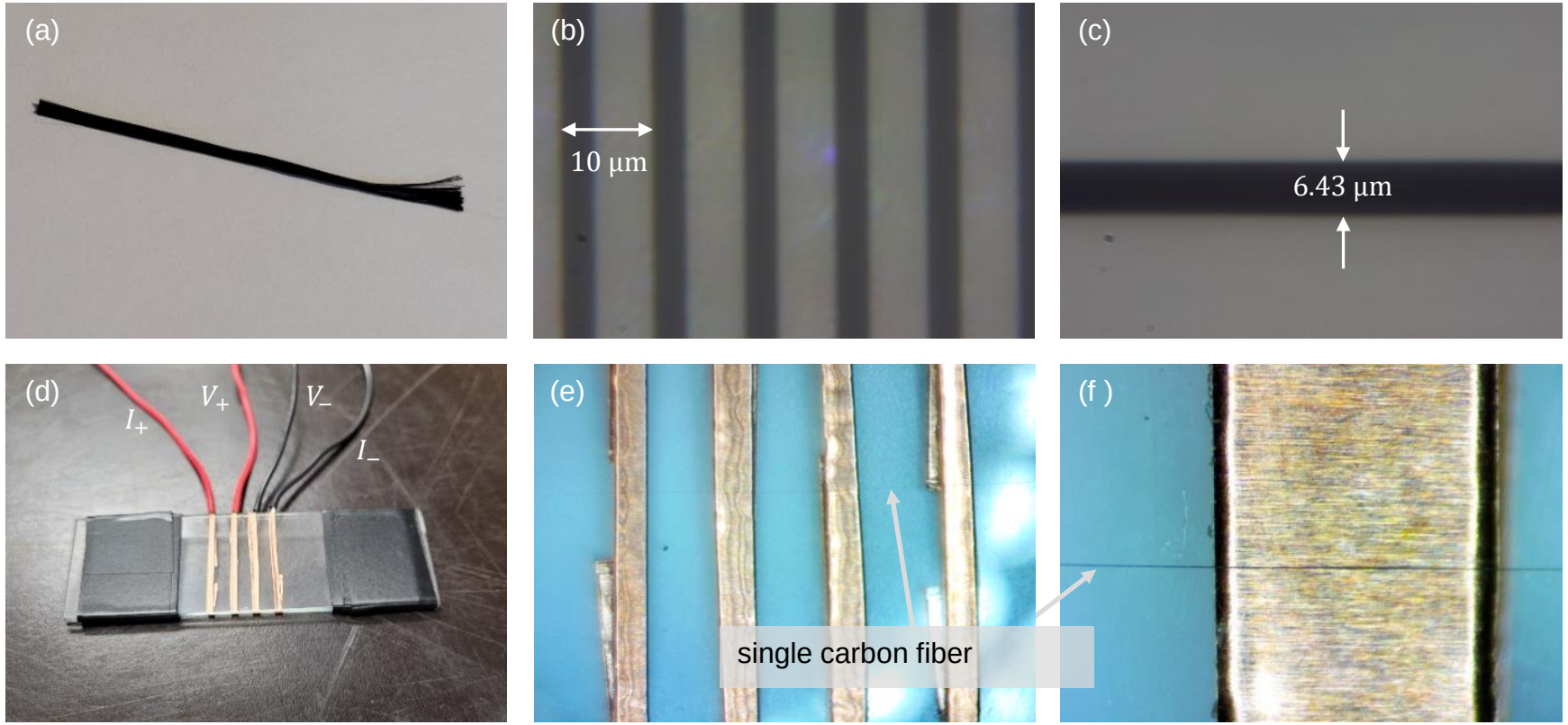


Effect of untreated carbon cloth (U-CC) and activated carbon cloth (A-CC) addition on **(a, b)** acetic acid, **(c, d)** propionic acid, and **(e, f)** butyric acid accumulation in BMP bottles, respectively. Data represent average and error bars represent standard

Summary of bioaugmentation

Bioaugmentation	Transferred COD from SEED to Test (mg)	Theoretical biogas produced from transferred COD (ml)	Surplus biogas produced after bioaugmentation (ml) [in days]	Biogas increment from bioaugmentation (–theoretical biogas) (ml)	Biogas increment from bioaugmentation (% per day)
bio-Augmentation 1 **[BL (I)]**	8578	4765	3558.65 [in 7 days]	**-1206**	**-0.55%**
bio-Augmentation 1 **[BL (II)]**	8070	4483	4779 [in 7 days]	**296**	**0.11%**
bio-Augmentation 1 **[BL (III)]**	7620	4033	6100 [in 7 days]	**2067**	**0.84%**
bio-Augmentation 2 **[BP-1 (I)]**	8561	3277	16,942 [in 9 days]	**13,665**	**3.42%**
bio-Augmentation 2 **[BP-1 (II)]**	7302	2795	12,636 [in 7 days]	**9,841**	**3.68%**
bio-Augmentation 2 **[BP-1 (III)]**	7260	2779	20,226 [in 15 days]	**16,663**	**1.30%**
bio-Augmentation 2 **[BP-1 (IV)]**	6346	2429	27,713 [in 16 days]	**25,284**	**1.98%**
bio-Augmentation 2 **[BP-1 (V)]**	5464	2091	26,031 [in 12 days]	**23,940**	**2.53%**
bio-Augmentation 2 **[BP-1 (VI)]**	4715	1805	57,733 [in 21 days]	**55,928**	**2.71%**
bio-Augmentation 3 **[BP-2 (I)]**	11,283	4319	11,515 [in 7 days]	**8,216**	**1.77%**
bio-Augmentation 3 **[BP-2 (II)]**	8618	3299	11,125 [in 7 days]	**21,394**	**1.87%**
bio-Augmentation 3 **[BP-2 (III)]**	7339	2809	23,929 [in 7 days]	**21,120**	**5.39%**
bio-Augmentation 3 **[BP-2 (IV)]**	6636	2540	23,794 [in 7 days]	**21,254**	**5.30%**
bio-Augmentation 3 **[BP-2 (V)]**	4534	1735	24,425 [in 7 days]	**22,690**	**5.64%**
bio-Augmentation 3 **[BP-2 (VI)]**	3694	905	30,736 [in 7 days]	**29,831**	**8.64%**
bio-Augmentation 3 **[BP-2 (VII)]**	3654	1399	47,054 [in 17 days]	**58,941**	**2.39%**
bio-Augmentation 3 **[BP-2 (VIII)]**	3414	1306	30,499 [in 9 days]	**29,193**	**5.18%**

Electrical conductivity (RC) of carbon cloth



$$\sigma\ (\mathrm{EC}) = R^{-1}\ell/A$$

R = resistance (ohm)
A = cross-sectional area (m^2)
L = length of the section of fiber measured (m)

a. A ribbon of carbon fibers extracted from a woven carbon cloth**, b.** Photograph of the microscope calibration slide. Each division marked on the slide is 10 □m wide, **c.** Photograph of a single carbon fiber using the same magnification used in (b), **d.** The sample holder and electrodes used to make the four-probe resistance measurements. The electrodes are made from copper-foil tape, **e.** Photograph of a single carbon fiber sandwiched between two glass slides while lying perpendicularly across the four copper electrodes. Under this magnification, the fiber is difficult to resolve, **f.** Same as (e), but at a higher magnification.

Summary of bioaugmentation

	Bioaugmentation date	Date	Volume transferred from SEED to Test (ml)	Transferred COD from SEED to Test (mg)	% Volume of Test reactor replaced	Status
SRT= 20 days	bio-Augmentation 1 **[BL (I)]**	Feb 24, 2022	1500 ml (liquid SEED Effluent)	8578	30%	✓
	bio-Augmentation 1 **[BL (II)]**	Mar 02, 2022	1500 ml (liquid SEED Effluent)	8070	30%	✓
	bio-Augmentation 1 **[BL (III)]**	Mar 09, 2022	1500 ml (liquid SEED Effluent)	7620	30%	✓
	bio-Augmentation 2 **[BP-1 (I)]**	Mar 23, 2022	2000 ml centrifuged (82 g) SEED pellets	8561	1.6%	✓
	bio-Augmentation 2 **[BP-1 (II)]**	April 1, 2022	2000 ml centrifuged (68 g) SEED pellets	7302	1.4%	✓
	bio-Augmentation 2 **[BP-1 (III)]**	April 8, 2022	2000 ml centrifuged (67 g) SEED pellets	7260	1.4%	✓
	bio-Augmentation 2 **[BP-1 (IV)]**	April 23, 2022	2333 ml centrifuged (40 g) SEED pellets	6346	0.7%	✓
	bio-Augmentation 2 **[BP-1 (V)]**	May 9, 2022	2333 ml centrifuged (34 g) SEED pellets	5464	0.7%	✓
	bio-Augmentation 2 **[BP-1 (VI)]**	May 23, 2022	2333 ml centrifuged (33 g) SEED pellets	4715	0.7%	✓
SRT= 10 days	bio-Augmentation 3 **[BP-2 (I)]**	July 18, 2022	2000 ml centrifuged (88 g) SEED pellets	9283	2.5%	✓
	bio-Augmentation 3 **[BP-2 (II)]**	July 25, 2022	2000 ml centrifuged (72 g) SEED pellets	8618	2.1%	✓
	bio-Augmentation 3 **[BP-2 (III)]**	August 1, 2022	2000 ml centrifuged (60 g) SEED pellets	7339	1.7%	✓
	bio-Augmentation 3 **[BP-2 (IV)]**	August 8, 2022	2000 ml centrifuged (36 g) SEED pellets	6636	1%	✓
	bio-Augmentation 3 **[BP-2 (V)]**	August 15, 2022	1400 ml centrifuged (34 g) SEED pellets	4534	1%	✓
	bio-Augmentation 3 **[BP-2 (VI)]**	August 22, 2022	1400 ml centrifuged (34 g) SEED pellets	3694	1%	✓
	bio-Augmentation 3 **[BP-2 (VII)]**	August 30, 2022	1200 ml centrifuged (36 g) SEED pellets	3654	1%	✓
	bio-Augmentation 3 **[BP-2 (VIII)]**	Sept 15, 2022	1200 ml centrifuged (36 g) SEED pellets	3414	1%	✓

Supplementary slides

Calculation COD basis for bioaugmentation 1

(SRT = 20 days)

Bio-augmentation 1 (BL)

BL (I) COD of SEED-CC effluent: 5719 mg/L

BL (II) COD of SEED-CC effluent: 5380 mg/L

BL (III) COD of SEED-CC effluent: 5080 mg/L

Volume of SEED effluent transferred to Test: 1500 ml

Total COD added to Test reactor: 5719 mg/L X 1.500 L = 8578.5 mg

All transferred COD are biodegradable

1g COD → 0.35 L CH_4 8.578 g → 3.00 L CH_4

Theoretical biogas generated from COD transferred:
3 X 100/63 = 4762 ml biogas

Extra biogas generated from Test reactor = 3917 ml (in 6 days)

Biogas produced from bioaugmentation process = (3917-4762) = **-844 ml**

Calculation COD basis for bioaugmentation 1

(SRT = 20 days)

Bio-augmentation 1 (BL)

BL (I) COD of SEED-CC effluent: 5719 mg/L

BL (II) COD of SEED-CC effluent: 5380 mg/L

BL (III) COD of SEED-CC effluent: 5080 mg/L

Volume of SEED effluent transferred to Test: 1500 ml

Total COD added to Test reactor: 5719 mg/L X 1.500 L = 8578.5 mg

All transferred COD are biodegradable

1g COD → 0.35 L CH_4 8.578 g → 3.00 L CH_4

Theoretical biogas generated from COD transferred:
3 X 100/63 = 4762 ml biogas

Extra biogas generated from Test reactor = 3917 ml (in 6 days)

Biogas produced from bioaugmentation process = (3917-4762) = **-844 ml**

Calculation COD basis for bioaugmentation 2

Supplementary slides

Bio-augmentation 2 (BP-1) **(SRT = 20 days)**

BP-1 (I) COD of SEED-CC effluent: 5719 mg/L; Weight of SEED pellet: 82 g/2000 ml

BP-1 (II) COD of SEED-CC effluent: 5100 mg/L; Weight of SEED pellet: 68 g/2000 ml

BP-1 (III) COD of SEED-CC effluent: 5080 mg/L; Weight of SEED pellet: 67 g/2000 ml

BP-1 (IV) COD of SEED-CC effluent: 4200 mg/L; Weight of SEED pellet: 40 g/2333 ml

BP-1 (V) COD of SEED-CC effluent: 3800 mg/L; Weight of SEED pellet: 34 g/2333 ml

BP-1 (VI) COD of SEED-CC effluent: 3500 mg/L; Weight of SEED pellet: 33 g/2333 ml

Total COD (pellets) added to Test reactor = COD of SEED effluent – COD of centrifuged SEED (filtrate)

= (5719 mg/L X 2.000 L) – (1500 mg/L X 1.918 L)

= (11438 mg) – (2877 mg)

= 8561 mg

If 70% is biodegradable: 0.7 X 8561 = 6000 mg

1g COD → 0.35 L CH_4 6.000 g → 2.10 L CH_4

Theoretical biogas generated from COD transferred:

2.10 X 100/64 = 3281 ml biogas

Extra biogas generated from Test reactor = 17236 ml (in 9 days)

Biogas produced from bioaugmentation process = (17,236-3281) = **13,955 ml**

Calculation COD basis for bioaugmentation 2

Supplementary slides

Bio-augmentation 2 (BP-2)

(SRT = 10 days)

BP-2 (I): COD of SEED-CC effluent: 5719 mg/L; Weight of SEED pellet: 88 g/2000 ml
BP-2 (II): COD of SEED-CC effluent: 5100 mg/L; Weight of SEED pellet: 72 g/2000 ml
BP-2 (III):COD of SEED-CC effluent: 5080 mg/L; Weight of SEED pellet: 66 g/2000 ml
BP-2 (IV): COD of SEED-CC effluent: 4200 mg/L; Weight of SEED pellet: 36 g/2000 ml
BP-2 (V): COD of SEED-CC effluent: 3800 mg/L; Weight of SEED pellet: 34 g/1400 ml
BP-2 (VI): COD of SEED-CC effluent: 3500 mg/L; Weight of SEED pellet: 34 g/1400 ml
BP-2 (VII): COD of SEED-CC effluent: 4500 mg/L; Weight of SEED pellet: 36 g/1200 ml
BP-2 (VIII): COD of SEED-CC effluent: 4300 mg/L; Weight of SEED pellet: 36 g/1200 ml

Total COD (pellets) added to Test reactor = COD of SEED effluent – COD of centrifuged SEED (filtrate)

= (5719 mg/L X 2.000 L) – (1500 mg/L X 1.918 L)

= (11438 mg) – (2877 mg)

= 8561 mg

If 70% is biodegradable: 0.7 X 8561 = 6000 mg

1g COD → 0.35 L CH_4 6.000 g → 2.10 L CH_4

Theoretical biogas generated from COD transferred:

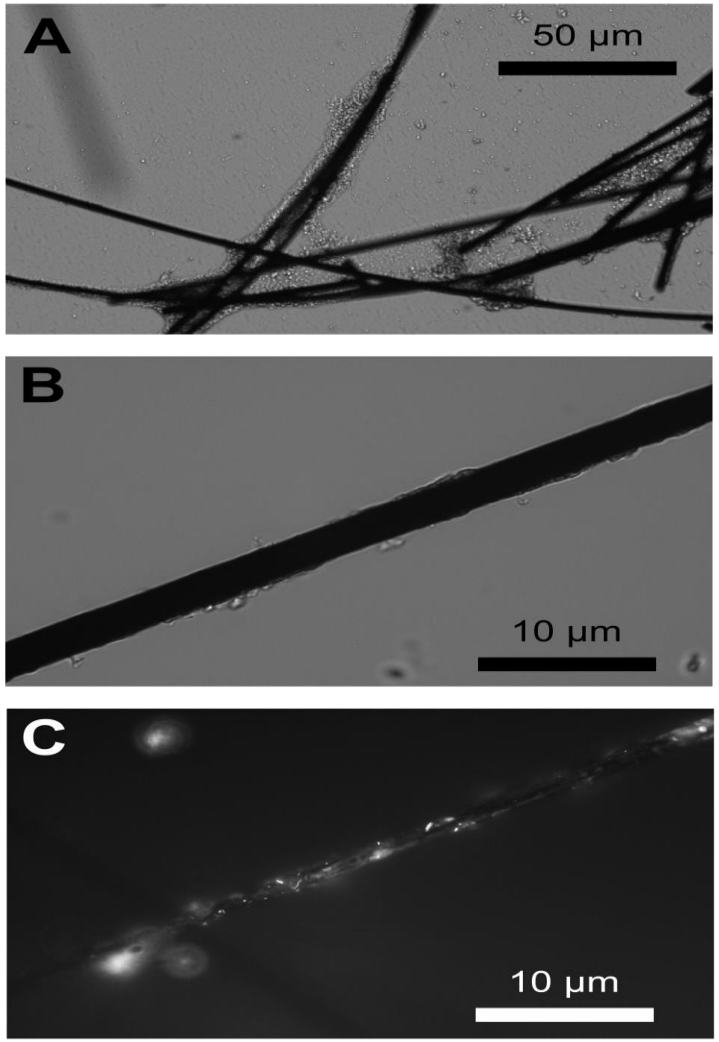
2.10 X 100/64 = 3281 ml biogas

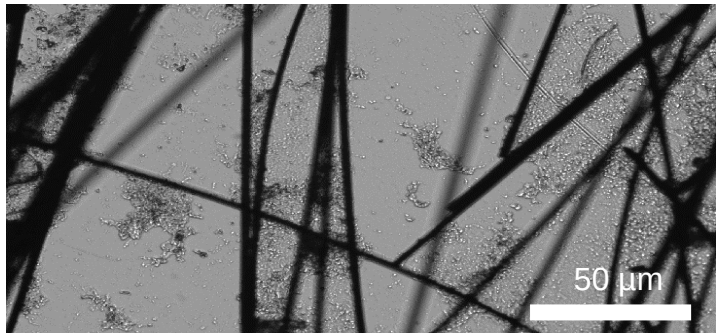
Extra biogas generated from Test reactor = 17236 ml (in 9 days)

Biogas produced from bioaugmentation process = (17,236-3281) = **13,955 ml**

Supplementary slides

Microbial colonization of carbon cloth medium





Microscopy images of activated CC after 30 days of bioreactor incubation by unfiltered light micrograph (A, B) and 461nm-filtered light micrograph for DAPI cell staining (C).

DAPI: 4′,6-diamidino-2-phenylindole