Successful enrichment novel anammox bacteria from eutrophic Lake Koto Baru, Indonesia

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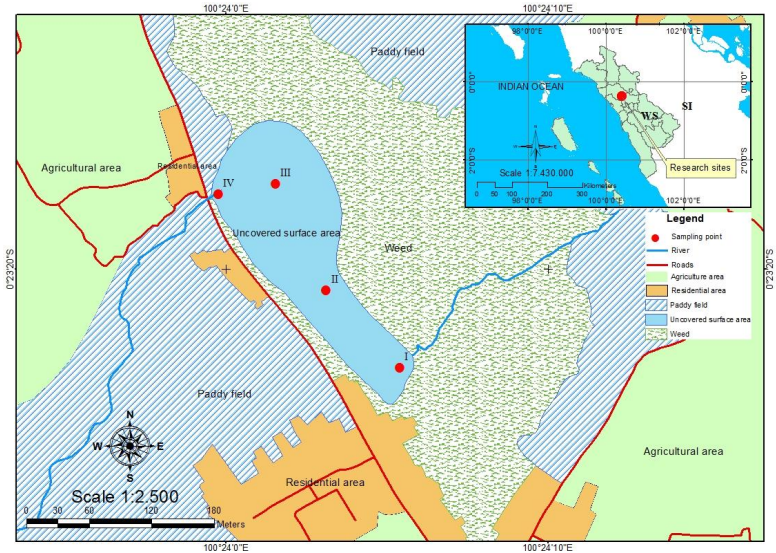
INTRODUCTION

Excess of nitrogen in the environment from agriculture (fertilizer) together with other nutrients promoted Eutrophication



The higher ammonium concentration in the environment could be an indicator of existence of anammox bacteria (convert ammonium to nitrogen gas using nitrite as electron acceptor in anaerobic condition)

Purpose : enrichment anammox bacteria in FtBR from Lake Koto Baru, Indonesia

MATERIALS AND METHODS

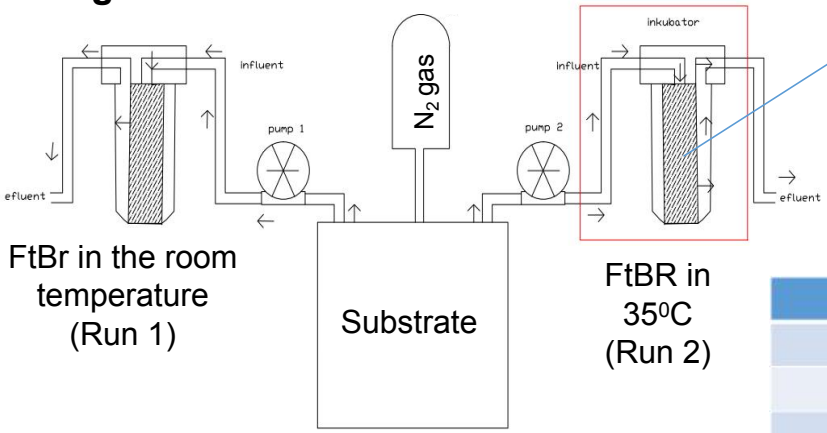
A novel Filter Bioreactor (FtBR) conducted for cultivation

Reactor configuration

V: 1.8 L,
sludge 2/3 volume

Reactor operation

HRT 24 h



FtBr in the room temperature (Run 1)

FtBR in 35^{0}C (Run 2)

String wound filter

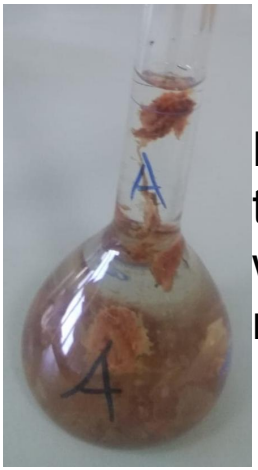
Housing 10”

Substrates	Concentration (mg/L)
$(NH_4)_2SO_4$	70 – 150 (mg-N/L)
$NaNO_2$	70 – 150 (mg-N/L)
$MgSO_4 \cdot 7H_2O$	300
$CaCl_2 .7H_2O$	180
KH_2PO_4	27,2
$KHCO_3$	500
Trace Element 1 and II*	1 ml
* Graaf et al., 1996	

The abundance of microbes was analyzed using Illumina MiSeq sequencing.

RESULTS AND DISCUSSION

Growth of anammox biofilm in 200 days



Red biofilm covered the filter in Run 1 where operated in room temperature

Run 1 **Run 2**

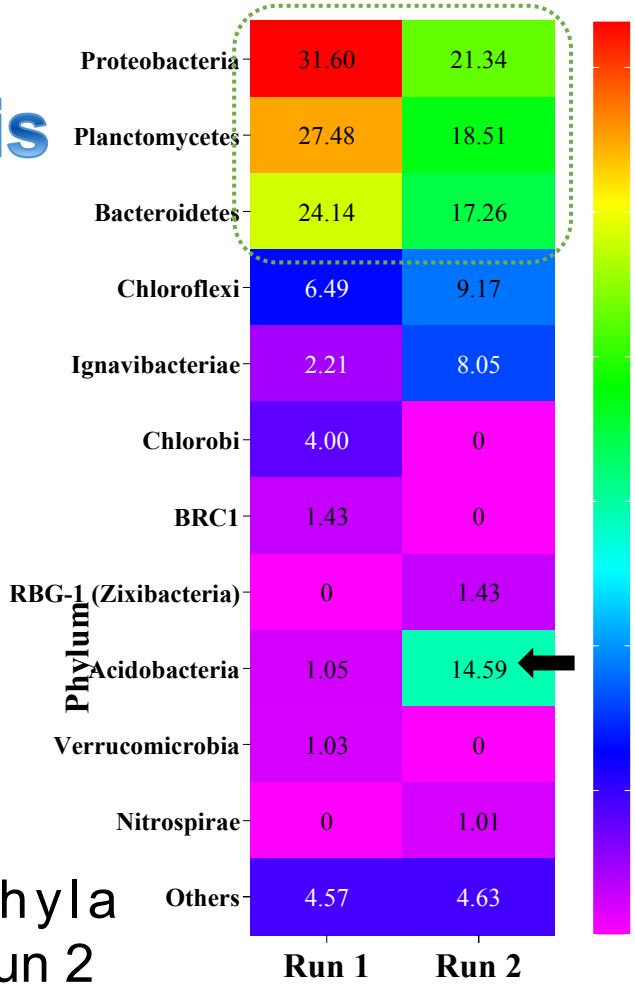
Conversely, in the Run 2 the biomass color in the filter was the same as the seeding sludge

Biofilm Run 2 Biofilm Run 1

Microbial community analysis

At phylum level

Proteobacteria
Planctomycetes
Bacteroidetes



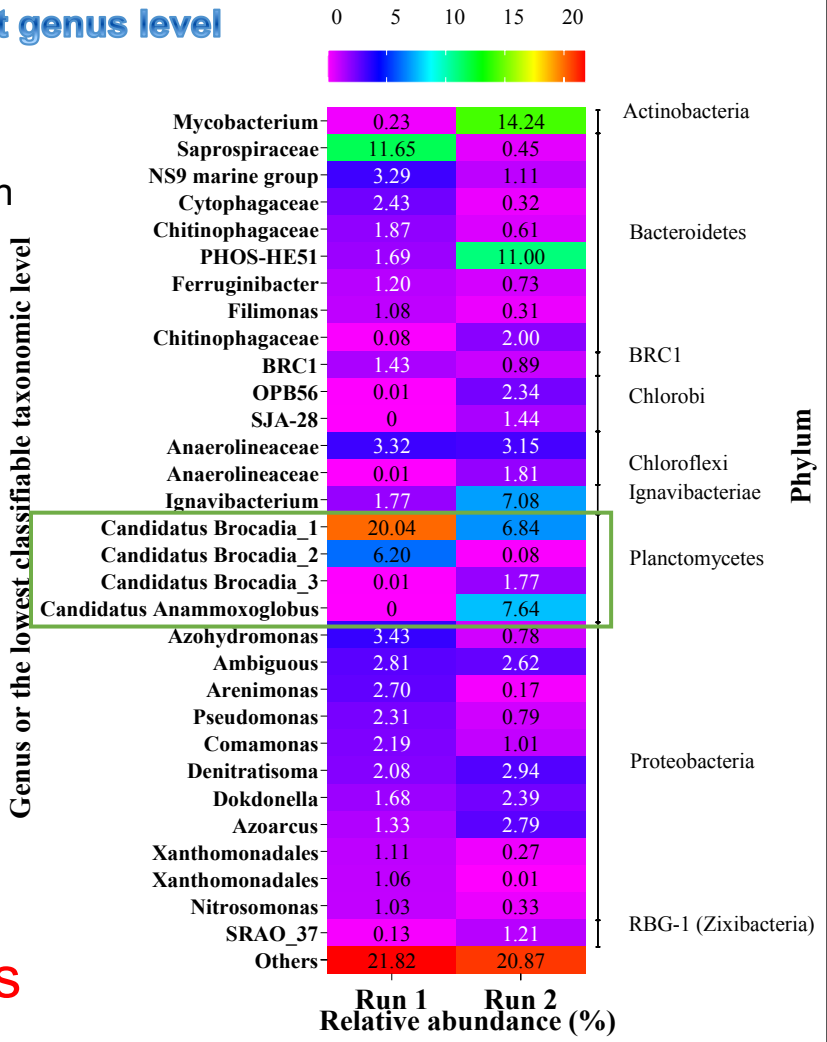
The most abundance Phyla similar between Run 1 and Run 2

Microbial community analysis at genus level

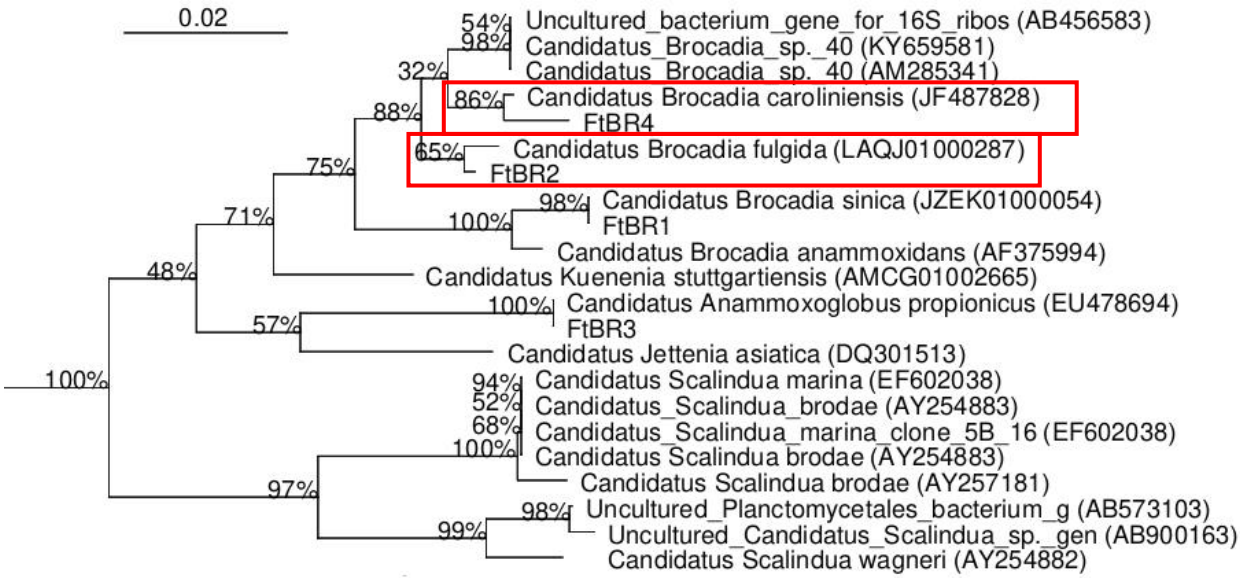
- ✓ Mycobacterium unclassified is unexpectedly predominant in Run 2.
- ✓ PHOS-HE51 might contribute to TP removal

Four Anammox species enriched in FtBR

Ca. Brocadia-1
Ca. Brocadia-2
Ca. Brocadia-3
Ca. Anammoxoglobus



Phylogenetic Tree analysis



Phylogenetic tree constructed by NJ methods using ARB software based on 16S rRNA gene

Two dominant Brocadia-like enriched in Run 1 as novel anammox bacteria

CONCLUSIONS

- Temperature cultivation in FtBR affected to diversity and abundance of anammox spesies.
- Two novel anammox species belong to Genus Ca. Brocadia predominant (20.04% and 6.20%) in Run 1.
- Rare Ca. Anammoxoglobus propionicus growth and predominant (7.64%) in Run 2 only.

CONTACT FOR FUTURE DISCUSSION

1D_11

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Successful enrichment novel anammox bacteria from eutrophic Lake Koto Baru, Indonesia

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INTRODUCTION

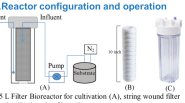
Distribution of anammox bacteria is found mostly in sub-tropical regions; there is very little information about anammox bacteria in the tropics. As a tropical country, Indonesia has great potential in the exploration of anammox bacteria in the tropical environment. However, information and research on this topic are limited. The first exploration of anammox bacteria conducted in the Lake Koto Baru where is experiencing eutrophication due to the accumulation of nutrients from the surrounding agricultural area. The surface is covered by mainly Salvinia and a little *Eichhornia crassipes* and the floor of the lake is shallow because of the decayed plants that have settled for years. Hence, Lake Koto Baru was chosen as a potential ecosystem for exploring and cultivation anammox bacteria from tropical environments. The purpose of this study was to cultivate and identify anammox bacteria in a tropical environment using a Filter Bioreactor (FtBR). FtBR is a novel tool constructed from a string wound filter as a supporting media on cartridge housing filters, which are commonly used for drinking water filtration.

MATERIALS & METHODS

Location of Lake Koto Baru

The sampling points were representative of the lake's condition. I, inlet; II, plant-surface covered area; III, surface-free area; IV outlet channel of the lake; WS: West Sumatera Province, Indonesia; SI: Sumatera Island.

1.4.Reactor configuration and operation



A 1.5 L Fiber Bioreactor for cultivation (A), string wound filter as carrier (B), housing filter (C).

Period	Time (d)	Concentration (mg/N-L)		HR T (h)	Temperature °C	Note
		NH_4^+-N	NO_2-N			
I	0-62	70	70	24		
Transition	63-91	70	70	24	Run 1 18.5-28 Run 2 35°C	Seeding sludge removed at day 63*
	92-105	100	100	24		
II	106-200	150	150	24		

Seeding sludge

- FtBR was inoculated with seeding sludge, filled 75% in reactor volume. The total solid (TS) and volatile suspended solids (VSS) of the seeding sludge were 40.79 and 26.235 mg/L respectively. The seeding sludge contained 3.163 mg/L total nitrogen and 1.660 mg/L total phosphor.

Composition of substrates

Substrate (mg/L destillate water)						Trace Element (mL)*	
NH_4^+-N	NO_2-N	KH_2PO_4	$MgSO_4.7H_2O$	$CaCl_2$	$KHCO_3$	I	II
70-150	70-150	27.2	300	135.88	500	1	1

*Van De Graaf et al. 1996.

Analytical methods

- The influents and effluents were sampled twice a week. Ammonia and nitrite were measured by colorimetric method and nitrate by ultraviolet spectrophotometric method with a UV-Vis spectrophotometer (Shimadzu 1800, Kyoto, Japan), according to the standard method (APHA, 2017). The temperature and pH were monitored using a thermo-hygrometer (Corona gl-89, China) and pH meter (STARTER300, Ohaus, USA), respectively.

Microbial community analysis

- The abundance of microbes in the samples were analyzed using Illumina MiSeq sequencing.

RESULTS & DISCUSSION

Water quality of Lake Koto Baru at sampling points.

Sampling points	NH_4-N (mg/N-L)	NO_2-N (mg/N-L)	NO_3-N (mg/N-L)	TC (mg-C/L)	TOC (mg-C/L)	TSS (mg/L)	pH	T (°C)	DO (mg/L)
I	5.55	0.26	2.70	42.11	37.35	14.645	6.6	25.2	4.2
II	5.20	0.25	2.10	44.32	41.01	14.085	7.1	25.6	3.1
III	5.15	0.26	2.15	44.43	39.51	14.875	7.4	25.5	3.4
IV	4.70	0.26	2.10	41.50	38.61	15.185	7.3	25.2	7.4

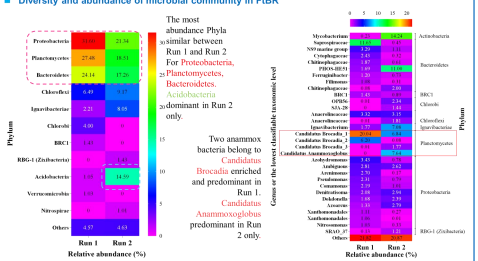
- Both ammonium and nitrite available in the lake for supporting the growth of anammox bacteria.

1.2. Nitrogen removal in FtBR

- In the beginning operation, ammonium concentration higher in effluent than influent due to degradation of sludge seeding. It rapid in Run 2.
- Clear anammox process observed after removal seeding sludge on 63th day.

- The performance of nitrogen removal in Run 1 (room temperature) higher then Run 2 (35°C)
- The maximum ACE, NRE and NRR were 98.19%, 92.95%, and 0.303 kg-N/m³.d, respectively for Run 1 and 88.61%, 68.93%, 0.214 kg-N/m³.d for Run 2.

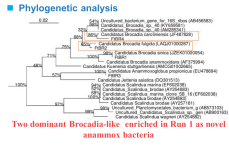
Diversity and abundance of microbial community in FtBR



The most abundance Phyla similar between Run 1 and Run 2 For Proteobacteria, Planctomycetes, Bacteroidetes, Acidobacteria dominant in Run 2 only.

Two anammox bacteria belong to Candidatus Brocadia enriched and predominant in Run 1. Candidatus Anammoxoglobus predominant in Run 2 only.

Phylogenetic analysis



Two dominant Brocadia-like enriched in Run 1 as novel anammox bacteria

Biofilm growth

- Red biofilm covered the filter in Run 1 where operated in room temperature
- Conversely, in the Run 2 the biomass color in the filter was the same as the seeding sludge

CONCLUSIONS

- Novel Filter Bioreactor (FtBR) was suitable for enrichment anammox bacteria in tropical environment.
- The performance of nitrogen removal in room temperature higher then in 35°C where ACE, NRE and NRR were 98.19%, 92.95%, and 0.303 kg-N/m³.d for Run 1 and 88.61%, 68.93%, 0.214 kg-N/m³.d for Run 2.
- Temperature cultivation FtBR affected to diversity and abundance of anammox spesies where two novel anammox species belong to Genus Ca. Brocadia predominant (20.04% and 6.20%) in Run 1 and *Ca. Anammoxoglobus propionicus* predominant (7.64%) in Run 2.

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November 8th, 2020

This is to certify that

Dr. Zulkarnaini
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had participated in the Water and Environment Technology Conference Online 2020 (WET2020-online) officially organized by Japan Society on Water Environment from 7th to 8th November, 2020, and had presented the presentation entitled "Diversity Anammox Bacteria in Eutrophic Koto Baru Lake, Indonesia".

井口晃徳

IGUCHI Akinori,
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Japan Society on Water Environment
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Ms. ZULFA, Mahdiah
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had participated in the Water and Environment Technology Conference Online 2020 (WET2020-online) officially organized by Japan Society on Water Environment from 7th to 8th November, 2020, and had presented the presentation entitled "Performance of Nitrogen Removal by Novel Anammox Bacteria From Talago Koto Baru at Room Temperature".

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November 8th, 2020

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